21st Annual Infectious Diseases Research Day

&

8th Annual Canadian Center for Vaccinology Symposium

April 25 & 26, 2016

Halifax

Sponsored by

Canadian Center for Vaccinology

Dalhousie Divisions of Infectious Diseases
of the Departments of Pediatrics and Medicine

Dalhousie Infectious Diseases Research Alliance

Educationally co-sponsored by
Dalhousie University Continuing Medical Education

As an accredited provider, Dalhousie University, CME designates this continuing medical education activity for up to 10 credit hours for MAINPRO M-1 of the College of Family Physicians of Canada. This event is an accredited group learning Section 1 activity as defined by the Maintenance of Certification Program of the Royal College of Physicians and Surgeons of Canada.

Educationally cosponsored by
DALHOUSIE UNIVERSITY
Continuing Medical Education

Welcome

Shelly McNeil MD, FRCPC
Chief
Div of Infectious Diseases,
Dept of Medicine, Dalhousie U

Scott Halperin MD, FRCPC
Director
Canadian Center for Vaccinology

Welcome to the 21st Annual Infectious Diseases Research Day and 7th Annual CCfV Symposium

Our program this year is filled with a variety of presentations and posters, from basic science to trial participant surveys. The study of infectious diseases and vaccines requires a spectrum of research skills to examine the microbes that cause disease, the biotechnology to prevent disease, and the behaviour needed to implement proven strategies. All of this research is interrelated and necessary to provide the evidence that will lead to improved health outcomes.

Research is conducted by experienced investigators working with highly qualified graduate and post graduate trainees, as well as skilled technicians, analysts and support staff, to ensure their research is developed and executed at the highest standards. Peer review is essential to this process, which is another reason we provide this annual opportunity to showcase local research to peers and the public. Your 'peer' evaluation is important, so when you receive an email later this week requesting your evaluation of these sessions, please give us your feedback so we can continue to improve this learning experience for everyone.

We know that these two half days will be filled with excellent presentations from local and international experts, and hope that you will take advantage of this exposure to new and exciting research. Ask questions, talk with colleagues, and learn as much as you can about the process and results of research.

On behalf of all of you, we thank the dedicated planning committee and the financial support from our corporate sponsors which makes this educational opportunity possible.

With thanks to….

This program is supported in part by educational grants provided by:

astellas

Pfizer

GILEAD

MERCK
Be well

sanofi pasteur

The Planning Committee

Joanne Langley, Chair
Glenn Campbell
Michael Fleming
Shelly McNeil
Audrey Steenbeek
Susan Brushett
Allison Young

Program

Monday April 25

1:00–2:00pm	Presentation: John T Schiller, National Cancer Institute "Why Prophylactic HPV Vaccines Work so Well: Implications for Future Vaccines"	IWK Health Centre O.E. Smith auditorium
2:00–2:30pm	Presentation: Melissa Andrew, Dalhousie "Frailty in relation to influenza vaccine effectiveness and clinical outcomes: Experience from the Serious Outcomes Surveillance Network"	
2:30-3:00pm	Presentation: Craig McCormick, Dalhousie "Discovery and Development of New Host-Targeted Antiviral Drugs"	
3:00–4:30pm	Poster judging (posters on display 1:00 – 5:30)	IWK Health Centre Gallery
4:30-5:30	Panel – Refugee Health Hour: "New to Canada 2015-2016 – Infectious Disease Screening and Immunization in Refugees" *Mandi Irwin* *Alkesh Patel* *Ian Davis* Moderated by: Todd Hatchette	O.E. Smith Auditorium

Tuesday April 26

8:00–9:00am	TJ Marrie Lecture (Grand Rounds) – Andrew Morris, Mt. Sinai Hospital "Love in the time of antimicrobials"	Halifax Infirmary RB Theatre
9:20–12:30pm	Oral Presentations (11)	
12:30–2:00pm	Buffet lunch and presentation by John T Schiller "Therapeutic Vaccines Against STIs: Getting T Cells to Where They Are Needed"	

Speakers

John T Schiller Ph.D.
Chief, Neoplastic Disease section of the Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, Maryland

Dr. Schiller received his bachelor's degree in Molecular Biology from the University of Wisconsin, Madison in 1975, and his Masters and Ph.D. degrees in Microbiology from the University of Washington, Seattle, in 1978, and 1982, respectively. He is currently a NIH Distinguished Investigator and the Chief of the Neoplastic Disease section of the Laboratory of Cellular Oncology, Center for Cancer Research, National Cancer Institute, Bethesda, MD.

In his 30 years at the NCI, Dr. Schiller has studied various aspects of papillomavirus molecular biology, immunology and epidemiology. The laboratory headed by Dr. Schiller and Dr. Lowy led in the discovery, characterization, and clinical testing of virus-like particle (VLP) vaccines to prevent the HPV infections that cause cervical and other cancers. They have received numerous awards for this work including the 2007 Sabin Gold Medal Award and the 2014 National Medal of Technology and Innovation from President Obama. Dr. Schiller's current interests include basic studies of papillomavirus virion assembly and infection, and development of 2nd generation HPV vaccines and vaccines and therapies for other infectious diseases and cancers.

Andrew Morris MD, SM(Epi), FRCPC
Medical Director, Antimicrobial Stewardship Program, Mount Sinai Hospital, Toronto, Ontario

Dr. Morris obtained his BSc (1990) and MD (1994) degrees from the University of Toronto. He trained in Internal Medicine from 1994 to 1997 at the University of Toronto, where he subsequently completed sub-specialty training in Infectious Diseases in 1999. He went on to complete a Master of Science degree in Epidemiology from the Harvard School of Public Health in 2000, while completing a Bayer Healthcare-Canadian Infectious Diseases Society (now AMMI Canada) research fellowship under the supervision of Dr. Allison McGeer.

Dr. Morris has been with the Division of Infectious Diseases in the Department of Medicine at Mount Sinai Hospital and University Health Network since 2007. He is an Associate Professor, works as a consultant in Infectious Diseases and General Internal Medicine, and is the founding Director of the Mount Sinai Hospital-University Health Network Antimicrobial Stewardship Program, formed in 2009. Dr. Morris is the Chair of the Specialty Committee of Infectious Diseases with the Royal College of Physicians and Surgeons of Canada, Chair of the Antimicrobial Stewardship and Resistance Committee for the Association of Medical Microbiology and Infectious Diseases Canada, and is a member of the Society for Healthcare Epidemiology of America Antimicrobial Stewardship and Resistance Committee.

Melissa Andrew MD, MSc Public Health, PhD, FRCPC
Assistant Professor, Geriatric Medicine,
Dalhousie University
Halifax, Nova Scotia

Dr. Andrew is Assistant Professor of Medicine and a consultant in Geriatric Medicine at the QEII Health Sciences Centre in Halifax. She completed her MD as well as residency training in Internal Medicine and Geriatrics at Dalhousie University. She did a Masters of Public Health at the London School of Hygiene and Tropical Medicine on a Commonwealth Scholarship and completed her PhD in Interdisciplinary Studies at Dalhousie University. Her research focuses on frailty and social vulnerability in relation to older people's health. In her work with the CCfV, she studies how frailty impacts both vaccine effectiveness and clinical outcomes of infections in older people.

Craig McCormick BScN, PhD
Associate Professor, Microbiology and Immunology,
Dalhousie University
Halifax, Nova Scotia

Dr. McCormick obtained a B.Sc. (Hons.) in Biology/Chemistry from the University of New Brunswick and a Ph.D. in Microbiology and Immunology from the University of British Columbia under the supervision of Dr. Frank Tufaro. At UBC, in the course of studying herpesvirus entry mechanisms, he discovered the function of the human tumour suppressor genes *EXT1* and *EXT2*, which are linked to Hereditary Multiple Exostoses (HME). Following this, he pursued postdoctoral training at the University of California, San Francisco under the supervision of Dr. Don Ganem. At UCSF, in the course of studying pro-tumourigenic gene products of the Kaposi's sarcoma-associated herpesvirus (KSHV) he discovered a new viral mechanism for controlling the turnover and translation of pathogenetically important pro-inflammatory cytokines and angiogenic factors. Currently, he holds an appointment as an Associate Professor in the Department of Microbiology and Immunology, Dalhousie University. His lab studies cellular antiviral stress responses to KSHV and Influenza virus infection (e.g. stress granules, autophagy), and viral gene products that are able to overcome these responses.

Poster Presentations

(Presenter's name **in bold**) Page

Oral Presentations

(Presenter's name **in bold**) Abstract Page

Abstracts

(Presenter's name **in bold**)

1. GENOMIC SUBTYPING ADVANCES OUR UNDERSTANDING OF THE EPIDEMIOLOGY OF CAMPYLOBACTERIOSIS: A STUDY FROM NOVA SCOTIA, CANADA

Authors: D Haldane, E. Schliehauf, E Taboada, B Billard, S Mutschall
Affiliation: Provincial Public Health Laboratory Network of Nova Scotia; Public Health Agency of Canada; Pathology and Laboratory Medicine**,** Laboratory for Foodborne Zoonoses; Lethbridge site**,** Department of Health and Wellness; Government of Nova Scotia, Dept of Pathology, Dalhousie University

Introduction: Campylobacteriosis is a common cause of diarrhea in Nova Scotia (18/100,000 population). Campylobacter is not typed, limiting the ability to understand case etiology, monitor trends and detect outbreaks. Comparative Genomic Fingerprinting (CGF40) typing is a recently described method for typing Campylobacter spp. We used CGF40 typing of Campylobacter isolates and compared the results with epidemiological data to determine the utility of this genomic tool for routine public health surveillance of campylobacteriosis.

Methods: Campylobacter isolates from stool specimens collected from January 1, 2012 to March 31, 2015 were typed by CGF40, using 5 multiplex PCR to determine the presence or absence of 40 genes. Fingerprints were compared to those in a national CGF database to assess source associations. Case report form data was compared with typing results. Case clusters were defined as clusters of cases reported by public health officials as being epidemiologically-linked or clusters of two or more isolates with matching CGF40 results with symptom onset dates within a 30 day period. A case-case study design was used to calculate odds ratios for exposures related to cases of a common CGF40 subtype versus cases with CGF40 subtypes which were detected only once in the study period (sporadic cases).

Results: 299 isolates comprised 140 distinct subtypes. There were three CGF40 subtypes comprising more than 10 strains, and 71% of isolates sharing fingerprints with one or more isolate. Some subtypes were detected in multiple years with exposures in local and overseas locations, while others were rare. All 4 Campylobacter jejuni case clusters identified by public health were confirmed by CGF40 and CGF40 identified >20 additional case clusters. Statistically significant associations between types and exposures were detected.

Conclusions: This study validated CGF typing of Campylobacteriosis with routinely collected public health data. CGF40 identified isolates from patients with known epidemiologic links, and dentified additional clusters of cases. A small number of commonly occurring widespread subtypes require additional typing methods to further differentiate between strains. Routine CGF may play a useful role in public health surveillance and outbreak investigations of campylobacteriosis.

2. HCV CARE BY DESIGN: RAPID IMPLEMENTATION OF A COMPREHENSIVE HCV MODEL OF CARE THROUGH INNOVATIVE PARTNERING AND COLLABORATION

Authors: R Rankin[1], J Beck[2], C Hoare[1], R Khan[1], DJ Smyth[3,4], GJ German[2], **L Barrett**
Affiliation: [4,51]Health PEI, PEI, Canada [2]QEII Hospital, Charlottetown, PEI, Canada [3]Horizon Health Network, Moncton, NB, Canada [4]Dalhousie University, Halifax, NS, Canada [5]Nova Scotia Health Authority, Halifax, NS

Introduction: In many provinces, HCV care is fragmented with little province-wide coordination, and limited real time evaluation. The result is delayed and inefficient care with frequent care gaps and service duplication. With new curative but high cost HCV medications, it is critical to develop and implement a sustainable, comprehensive HCV model of care with innovative cost containment solutions and excellent access in a publicly funded system.

Methods: Between September 2014 and February 2015, care providers, government, industry, and HCV community groups partnered to review available data and design a province-wide HCV model of care in Prince Edward Island, Canada. A hybrid effectiveness-implementation type I mixed methods study design will be used to evaluate the treatment intervention, as well as program implementation.

Results: The PEI HCV program was implemented in April 2015, and includes centralized referral and triage, HCV treatment specialists, public access to direct acting antiviral therapy, patient education and individualized follow-up with both public and industry-affiliated nursing and pharmacist support.

In the first 8 months of the program, 150 HCV referrals were received from 24 providers (family doctors, nurse practitioners, internists, addictions services, other physicians) compared with only 5 in the same period last year. All HCV genotype 1 infections were assessed for treatment readiness (including transient elastography) and started on HCV treatment in a nurse-led, physician-oversight, pharmacist-supported provider paradigm. Before program implementation, there were only 2 on-treatment persons in the same time frame last year.

Conclusions: In just 8 months, initial success of the provincial PEI HCV model supports an implementation science approach for similar programs in other publicly funded systems. This model also highlights the value of public and private partnerships to rapidly introduce highly effective programs for timely access to quality HCV care.

3. STRATEGIES TO INCREASE IMMUNIZATION UPTAKE AMONG 4-6 YEAR OLDS IN ATLANTIC CANADA FIRST NATIONS COMMUNITIES

Authors: C Langlois
Affiliation: Health Canada, First Nations and Inuit Health Branch-Atlantic Region/MPH practicum student, Lakehead University

Introduction: Vaccine preventable diseases continue to pose a threat to the health of Canadians - including First Nations populations. Between 2009 and 2014, immunization rates for 4-6 year olds in Atlantic First Nations communities were consistently lower than rates for 1 and 2 year olds in the same communities. The 2013-2014 reporting period showed rates of 73% for 4-6 year olds compared with 82% for 1 year olds and 83% for 2 year olds. There is a need to identify strategies to increase immunization rates in 4-6 year olds in order to increase disease protection and as a result, enhance the overall health of communities.

Methods: A preliminary literature review was conducted to identify relevant research. An interview tool and process were developed to collect information from Atlantic First Nations Community Health Nurses (CHNs) on successful immunization strategies and challenges for 4-6 yr olds in their communities. Email invitations were sent to 43 Atlantic First Nations CHNs requesting participation in a short telephone interview. Two follow-up emails were also sent to those who did not initially respond. All interviews occurred between 08-Feb and 26-Feb 2016. Interview results will be anonymized and shared with communities and key stakeholders. The results will be used to inform future program planning aimed at increasing childhood immunization rates within Atlantic First Nations communities. Where appropriate, explicit permission will be sought from First Nations communities to share immunization success stories.

Results: Of 43 CHNs contacted, 23 completed the interview, yielding a response rate of 54%. Compilation and analysis of results is currently underway. Current information suggests that many similarities exist between both Atlantic First Nations childhood immunization successes and challenges, and those identified in the literature review. Synthesis of interview results is expected to produce a deeper understanding of those successes and challenges particularly in regard to 4-6 year olds in First Nations communities.

Conclusions: The literature describes several strategies that have been previously identified as effective in increasing childhood immunization rates. Among these, the most frequently discussed intervention was the use of parental reminders. Another key factor was health care provider competence, particularly in regard to relationship building and communication skills. However, very little research was found that addresses strategies for increasing childhood immunization rates in First Nations populations, or within specific age-groups - which suggests that an opportunity exists for future research.

4. INTERFERON-OMEGA (IFN-ω) EXPRESSION BY VIRUS-INFECTED CELLS AND DIFFERENTIAL EFFECTOR FUNTIONS

Authors: L Portales-Cervantes, JS Marshall
Affiliation: Dalhousie Inflammation Group. Department of Microbiology and Immunology, Dalhousie University, Halifax, NS

Introduction: Human type I IFNs including IFN-α, -β, -κ, -ω and –ε are mainly induced during viral infections. Although type I IFNs bind to a common receptor (IFNAR), different immunoregulatory activities have been attributed to IFN-β and IFN-α. In the current study we investigated the expression pattern of the less well-studied IFN-ω during viral infection and assessed its role in the induction of key immune factors.

Methods: Peripheral blood-derived T cells (n=4), monocytes (n=3) and NK cells (n=2) as well as cord blood-derived mast cells (CBMC n=4) were infected with the mucosal pathogen reovirus type 3 Dearing for 24 h. Peripheral blood mononuclear cells (PBMC n=2) were stimulated with 10 ng/ml IFN-α, 10000 U/ml IFN-β or 10000 U/ml IFN-ω for 6 or 24 h. mRNA gene expression was analyzed by qPCR. In some experiments, antibody blockade of IFNAR was included during reovirus infection.

Results: We have previously shown that human epithelial cell lines and fibroblasts do not express IFN-ω in response to reovirus. Primary T and NK cells expressed IFN-ω and IFN-β genes, but no IFN-α subtypes (1 through 21). Similar to CBMC, IFNAR blockade on both reovirus-infected T and NK cells decreased IFN-ω gene expression. IFN-ω stimulation in PBMC showed a higher upregulation of SOCS3, IL-6 and IL-10 genes at 6 h, while the expression of other genes such as Mx1 was similar in response to IFN-α, IFN-β or IFN-ω.

Conclusions: In addition to CBMC, other immune cells such as NK cells, T cells and monocytes express IFN-ω in response to reovirus. However, T and NK cells preferentially expressed IFN-ω and -β in a positive feedback loop via IFNAR. We observed that IFN-ω has the ability to induce the expression of immune genes (IL-6, IL-10, SOCS3) not seen after IFN-α or -β stimulation, despite the fact that these three IFNs signal via IFNAR. Such responses might be the result of different affinities to IFNAR which could lead to the activation of specific signaling pathways. Overall, our results show that IFN-ω production is differentially regulated or absent in distinct cell types. Importantly, IFN-ω can induce distinct immunomodulatory activities to those described for IFN-β and –α, while inducing antiviral mediators (Mx1) at similar levels to these IFNs.

5. IDENTIFICATION AND CHARACTERIZATION OF A NOVEL ENDOCYTOSIS PATHWAY FOR MEASLES VIRUS ENTRY

Authors: S Delpeut[1,2], G Sisson[1], KM Black[1], CD Richardson[1,2,3]
Affiliation: [1]Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 1X5, [2]Canadian Centre for Vaccinology, IWK Health Centre, Goldbloom Pavilion, Halifax, Nova Scotia, Canada B3H 1X5, [3]Department of Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada

The author requests that this abstract not be published.

6. IDENTIFICATION AND CHARACTERIZATION OF REDOX PARTNERS OF THE THIOL-DISULFIDE OXIDOREDUCTASE SDBA IN *STREPTOCOCCUS GORDONII*

Authors: **NA Jalal,** L Davey, SA Halperin, SF Lee
Affiliation: Department of Microbiology and Immunology, Dalhousie University; Canadian Center for Vaccinology, IWK Health Centre

Introduction: The formation of disulfide bonds via thiol-disulfide oxidoreductases (TDOR) is crucial for the proper folding and activity of many extracytoplasmic proteins. *Streptococcus gordonii* is a pioneer organism in the human oral cavity and a potential live oral vaccine vector. We recently identified a novel TDOR named SdbA in *Streptococcus gordonii*. In this study, we identify and characterize two redox partners of SdbA, named SdbB and SdbC, together with their associated CcdA proteins in *S. gordonii.*

Methods: Single and double mutants of s*dbB, sdbC*, and *ccdA* genes were constructed via an allelic replacement strategy. Mutants were analyzed for autolysis, extracellular DNA (eDNA) release, bacteriocin production, genetic competence, and zymographic analysis of AtlS, the natural substrate of SdbA. The redox status of AtlS was also determined in the mutants and parent strains. Recombinant SdbA, SdbB, and SdbC proteins were prepared from *E. coli*, and oxidase activity of SdbB and SdbC was analyzed using RNase A refolding assay. The disulfide exchange reactions between SdbA and SdbB or SdbD were performed using equimolar concentrations of the recombinant proteins.

Results: The results demonstrate that inactivation of *sdbBccdA2* replicated the *sdbA* mutant phenotype and that SdbC and CcdA1 could compensate for the loss of SdbB and CcdA2. The *sdbBccdA2* mutant produced a non-functional AtlS protein that lacked an intramolecular disulfide bond. Using the RNase A refolding assay, we showed that both SdbB and SdbC exhibited oxidase activity. We also showed that both SdbB and SdbC were able to reoxidize SdbA.

Conclusions: Taken together, our results revealed that both SdbB and CcdA2 are required as redox partners of SdbA. We propose that SdbA introduces disulfide bonds in protein substrates, which results in a disulfide-bonded substrate protein and a reduced SdbA. SdbB, with the assistance of CcdA2, reoxidized SdbA to allow SdbA to perform another round of reaction. This SdbA-SdbB-CcdA2 pathway is the main oxidative pathway affecting autolysis, bacteriocin production, genetic competence, and eDNA release. The reduced SdbA can also be reoxidized by SdbC-CcdA1; this is a minor pathway affecting only bacteriocin production. The two pathways appear to be interconnected, with SdbB interacting with CcdA1 and SdbC with CcdA2.

7. TRANSLATIONAL EFFICIENCY OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) DURING LYTIC REPLICATION

Authors: **ES Pringle**[1,2], N Crapoulet[3], AM Leidal[4], J Fawcett[5], S Lewis[1,2,3] J Uniacke[6], C McCormick[1,2]
Affiliation: [1]Department of Microbiology and Immunology, Dalhousie University. [2]Beatrice Hunter Cancer Research Institute. [3]Atlantic Cancer Research Institute. [4]Department of Pathology, University of California, San Francisco. [5]Department of Pharmacology. [6]Department of Molecular and Cellular Biology, University of Guelph

The author requests that this abstract not be published

8. B1 CELLS MIGRATE TO THE DRAINING LYMPH NODE AND MODULATE HOST RESPONSES TO CHLAMYDIA INFECTION IN A SEX-SPECIFIC MANNER

Authors: **RM Kampen**[1,2], JM Moore-Connors[1], C Tram[1,2], J Wang[1,2,3]
Affiliation: [1]Canadian Center for Vaccinology, [2]Department of Microbiology & Immunology, [3]Department of Pediatrics, Faculty of Medicine, Dalhousie University, Halifax, NS

Introduction: *Chlamydia trachomatis* (*Ct*) is an intracellular bacterium that infects the mucosal epithelium of the ocular, respiratory, and genital tract leading to severe outcomes. Female *Ct* infections are reported twice as often as male infections but the cause of this dichotomy is unknown. It has been shown that the immune responses differ between the sexes, with females favouring a humoral response. Since cell-mediated, not humoral, immune responses are needed to clear *Ct* infection, it is possible that males have a more protective immune response to *Ct*. However, the impact of sex in controlling *Ct* is unknown. B1 cells are a B cell subset that have innate sensing properties and are able to produce immune modulatory cytokines to regulate immune responses to systemic infections. While B1 cells are activated by *Chlamydia* stimulation in *vitro*, it is unclear how B1 cells are involved in *Ct* infection *in vivo*. In this study, we analyzed sex-specific immune responses to respiratory *Ct* infection, focusing on the role of B1 cells.

Methods: Intranasal infection with C. *muridarum* (*Cm*) was used to model *Ct* infection in mice. Prior to infection, B1 cells were tracked by injecting fluorescent dye into the peritoneal cavity. After infection, flow cytometry was used to identify the location of the labelled cells. In another experiment, B and T cell responses in the spleen and lymph nodes of infected and uninfected male and female mice were analyzed by flow cytometry.

Results: In the tracking experiment, we observed migration of B1 cells from the peritoneal cavity to the lung and mediastinal lymph node (MLN) following *Cm* infection. Furthermore, we noted that female mice had a significantly higher number of B1 cells in the MLN and an increase in body weight loss compared to male mice following *Cm* infection.

Conclusions: Together our data suggests that following *Chlamydia* infection B1 cells migrate to the MLN, which may modulate host responses to *Chlamydia* infection in a sex-specific manner.

9. REGULATION OF CELL GROWTH CONTROL BY THE KSHV vGPCR PROTEIN

Authors: **S O'Brien**[1,2], C McCormick[1,2]
Affiliation: [1]Department of Microbiology and Immunology, Dalhousie University. [2]Beatrice Hunter Cancer Research Institute

The author requests that this abstract not be published.

10. A PROTEOMIC APPROACH TO ELUCIDATE MECHANISM OF ACTION OF INFLUENZA VIRUS HOST SHUTOFF PROTEIN PA-X

Authors: **B Porter**[1], D Khaperskyy[1], C McCormick[1]
Affiliation: [1]Department of Microbiology and Immunology, Dalhousie University

The author requests that this abstract not be published.

11. STRESS GRANULE-INDUCING ANTIVIRAL DRUGS TO CONTROL INFLUENZA

Authors: **PD Slaine**, D Khaperskyy, S McAlpine, M Warhuus, T Hatchette, C McCormick
Affiliation: Department of Microbiology and Immunology, Dalhousie University

The author requests that this abstract not be published.

12. A VITAL CONTROL OF INTRACELLULAR *CHLAMYDIA MURIDARUM* INFECTION REQUIRES INTERLEUKIN-17 RECEPTOR-MEDIATED SIGNALING

Authors: **C Tram**, RM Kampen, SA Halperin, J Wang
Affiliation: Department of Microbiology and Immunology, Dalhousie University
Canadian Centre for Vaccinology, IWK Health Centre, Department of Microbiology & Immunology and Department of Pediatrics, Dalhousie University

Introduction: IL-17A is a pleiotropic proinflammatory cytokine that has a vital role in host resistance against extracellular bacterial and fungal infections. However, its role in controlling intracellular bacterial infections remains elusive. Here, we examined the IL-17/IL-17 receptor-mediated signaling in controlling intracellular *Chlamydia muridarum* (*Cm*) infection in non-hematopoietic cells *in vivo*.

Methods: To study the impact of IL-17 signaling specifically in tissue structure cells, a respiratory *Cm* infection model was established in bone marrow (BM) chimeric mice using WT BM donor cells transferred into γ-irradiated WT, IL-17RAKO and IL-17RCKO mice.

Results: Both IL-17RAKO and IL-17RCKO BM chimeric mice had significantly elevated bacterial burden in the lung compared to WT mice at days 5 and 11 post infection. However, IL-17RCKO, but not IL-17RAKO, BM chimeric mice exhibited significant body weight loss compared to WT mice during early infection. While IL-17RAKO BM chimeric mice displayed an increased IL-17A response, IL-17RCKO mice showed an increased IFN-γ response in the lung. Interestingly, an increase in IL-17A production from a non T cell population was observed early during *Cm* infection in the IL-17RAKO BM chimeric mice. Upon further investigation, the majority of this population appears to be of the type 3 innate lymphoid cells (ILC3). ILC3s are an important subset of innate cells that regulate immunity, inflammation, and tissue repair. It is the first time ILCs have been linked to IL-17 signaling in the context of *Cm* infection.

Conclusions: Our data demonstrates an indispensable role of the IL-17RC-mediated signaling in controlling *Chlamydia* infection and suggests differential roles of IL-17RA- and IL-17RC-mediated signaling in shaping *Chlamydia*-induced host responses.

13. CHARACTERIZATION OF AN ANTIGEN-TARGETING FUSION PROTEIN (OVA-CD40LS) FROM *ESCHERICHIA COLI*

Authors: **Y Shi**, SA Halperin, SF Lee
Affiliation: Department of Microbiology and Immunology, Dalhousie University; Canadian Center for Vaccinology, IWK Health Centre

Introduction: Coupling an antigen to a molecule specific for a receptor on antigen-presenting cells (antigen-targeting) can elicit an immune response more efficiently. The approach, however, has not been attempted in the oral cavity. We hypothesized that antigen-targeting can induce a robust immune response in the oral cavity. An antigen-targeting fusion protein, a fragment of albumin from chicken egg white (OVA) fused to CD40LS (soluble extracytoplasmic domain) was constructed and expressed in *Escherichia coli*. The ability of OVA-CD40LS to target to CD40 *in vitro* and to elicit an immune response in the mouse oral cavity was tested.

Methods: The DNA coding for OVA-CD40LS and OVA were obtained by PCR and cloned into the *E. coli* expression plasmid, pQE30. Protein production was detected using anti-OVA antibody in Western blotting. Proteins were purified from *E. coli* cell lysates using His60 Ni gravity columns. The purified proteins were tested for binding to human CD40 in ELISA and stimulation of bone marrow-derived dendritic cells (BMDC). Mice were given OVA-CD40LS without adjuvant by subcutaneous (SC) injections, oral intramucosal (IMu) injections, or oral mucosal surface applications. Serum anti-OVA IgG titer was determined by ELISA.

Results: The OVA-CD40LS (31 kDa) and OVA (15 kDa) proteins were expressed and purified from *E. coli*. The purified proteins were recognized by the anti-OVA antibody in Western blotting. OVA-CD40LS showed strong binding to human CD40 and stimulated BMDC to produce TNF-□ and IL-6. Preliminary results showed that SC injections of OVA-CD40LS induced serum anti-OVA IgG production. Oral IMu injections and mucosal surface applications of OVA-CD40LS also induced an antibody response and IMu injections induced a higher antibody titer than SC injections.

Conclusions: OVA-CD40LS was functional *in vitro* as it stimulated cytokine production in BMDCs. The preliminary *in vivo* results suggested that OVA-CD40LS could induce an antigen specific antibody response without adjuvant in the oral cavity.

14. THE IMPACT OF BACTERIAL TYROSINE PHOSPHORYLATION AS A REGULATORY MECHANISM OF TYPE THREE SECRETION AND PATHOGENESIS

Authors: **C Runté**,[a] J Leblanc,[c] N Thomas,[a,b]
Affiliation: Dalhousie University, Department of Microbiology & Immunology,[a] Department of Medicine (Infectious Diseases)[b], Department of Pathology and Laboratory Medicine, Division of Microbiology, Capital District Health Authority (CDHA)[c]

Introduction: Pathogenic *E. coli* cause serious gastrointestinal disease and inflict a significant burden on healthcare systems. The *E. coli* type III secretion system (T3SS) is a needle-like complex that rapidly injects multiple effector proteins into host target cells. This results in bacterial infection and subversion of normal host signalling. Pathogenic *E. coli* tightly regulate T3SS activity, which involves a variety of bacterial chaperone proteins in the cytosol that bind to and stabilize effectors prior to their injection into a target cell. A recent phosphotyrosine proteome study of EHEC O157:H7 identified an unexpected abundance and diversity of tyrosine (Tyr)-phosphorylated proteins. This reversible form of post-translational modification was previously thought to be rare among prokaryotes, yet was discovered to occur among proteins involved in type III secretion-mediated virulence of *E. coli*. Specifically, two consecutive Tyr residues, located within the unique C-terminal domain of the type III secretion chaperone (T3SC) protein known as CesT, were identified as being phosphorylated. As the C-terminus of CesT is functionally required for efficient effector secretion, we set out to characterize the effect of its phosphorylation on regulation of type III secretion and EPEC virulence.

Methods: *E. coli* strains that are deficient for CesT phosphorylation were generated with recombinant DNA mutagenesis techniques on the *cesT* allele. These CesT variants express specific Tyr to phenylalanine (Phe) substitutions, a strategy that retains protein structure but prevents phosphorylation due to crucial absence of a necessary hydroxyl group on Phe. Additionally, with well-established infection assays we have been able to characterize the impact of phospho-deficiencies on CesT function.

Results: Phosphorylation of C-terminal Tyr residues within CesT appears to directly influence the secretion of different effectors. Certain Tyr to Phe sequence changes for CesT resulted in loss of specific effector injection, and loss of phenotypes associated with disease progression.

Conclusions: Tyr phosphorylation of CesT appears to contribute to efficient delivery of type III effectors during EPEC infection. EPEC strains deficient for CesT phosphorylation are attenuated in a variety of infection assays.

15. MULTILOCUS SEQUENCE TYPING ANALYSIS OF *STREPTOCOCCUS PNEUMONIAE* ISOLATES THAT ARE NON-TYPABLE BY QUELLUNG SEROTYPING

Authors: **C Brisseau**[1], M Warhuus[1], I Martin[2], M ElSherif[1], R Davidson[1], T Hatchette[1], S McNeil[1], J LeBlanc[1]
Affiliation: [1]Canadian Center for Vaccinology (CCfV), Izaak Walton Killiam (IWK) Health Centre, Nova Scotia Health Authority (NSHA), and Dalhousie University, Halifax, NS. [2]Streptococcus and STI Unit, National Microbiology Laboratory (NML), Public Health Agency of Canada (PHAC), Winnipeg, MB

Introduction: *Streptococcus pneumoniae* is a significant human pathogen that has 98 known serotypes. A subset of these serotypes are vaccine-preventable, therefore monitoring serotype distribution is important. Serotyping using the Quellung reaction is well recognized as the reference method, and uses capsule-specific antibodies to assign a serotype. Over the years, polymerase chain reaction (PCR)-based serotype deduction has gained much interest by targeting differences in the capsule biosynthesis genes but is limited to the detection of 70 serotypes and lack of discrimination between serotypes. However, using either Quellung or PCR-based serotyping, some *S. pneumoniae* strains remain non-typeable. Multilocus sequence typing (MLST), which relies on comparing the genetic sequences of seven housekeeping genes, has been extensively used in molecular surveillance to define genetic lineages of *S. pneumoniae*, and can potentially be associated to serotypes using international genetic databases.

Objectives: This study aimed to evaluate whether MLST or PCR could deduce serotypes of *S. pneumoniae* that were previous characterized as non-typeable by Quellung.

Methods: DNA extracted from *S. pneumoniae* strains were analyzed using PCR-based serotyping and MLST. Three non-typeable strains were evaluated, as well as 4 reference strains that could not be differentiated using PCR-based serotype deduction (serotypes 7F, 7A, and serotypes 22F and 22A).

Results: All 7 PCR targets were successfully amplified and sequenced from the 3 non-typeable and 4 references strains of *S. pneumoniae*. However, serotypes could not been deduced for any of the strains evaluated when compared to online databases.

Conclusions: While MLST has some merit in the repertoire of genetic techniques for *S. pneumoniae* surveillance, it cannot be used for deduction or discrimination of vaccine-preventable serotypes since the link between genetic lineages defined by MLST and capsular serotype (phenotype) does not yield useful results.

16. AN OBSERVATIONAL EXPLORATORY STUDY EXAMINING DEMOGRAPHICS, IMMUNIZATION STATUS AND TB TESTING AMONG REFUGEES IN HALIFAX, NOVA SCOTIA

Authors: **K Brownridge**, A Steenbeek, T Sampalli, G Kohler, A Sharpe
Affiliation: Dalhousie University, Transitional Health Clinic for Refugees

Introduction: In Canada, the refugee population experiences significant health disparities in comparison to other immigrant groups and the native born population. Since 2000, Canada has resettled approximately 11,000 refugees per year, representing 10-15% of all foreign-born people entering the country each year. Despite the increasing number of refugees entering Canada, there still exists a paucity of research and literature addressing their overall health and healthcare needs. In Halifax, Nova Scotia, refugees meet with a health professional for a post arrival health assessment, which includes a review of immunizations and tuberculosis (TB) status. Currently, there does not exist a systematic process that provides clinicians with an overview of these health indicators for those accessing the Transitional Health Clinic for Refugees. Therefore, the development of a dataset which clinicians can utilize to record and track immunizations, TB, and health statuses of their refugee clients is imperative in order to improve services provided and promote better health outcomes.

Methods: The proposed research is a descriptive observational study that includes the development of a health related dataset that will record immunization status, TB status and underlying health issues on the refugee population that access the Transitional Health Clinic for Refugees at the Mumford Professional Centre. This study is approved by the Nova Scotia Health Authority Research Ethics Board.

Results: Descriptive statistics will be completed on the data which includes information on age, ethnicity, biological sex, previous counties of residence, immunizations, TB status and underlying medical issues. Means and proportions will be presented in tables and figures.

Conclusions: The development of this dataset will help clinicians that provide care to refugees be more knowledgeable of the basic demographics and health issues of the refugee population that currently use the Transitional Health Clinic for Refugees at the Mumford Professional Centre, Halifax NS. Additionally, it will help staff improve tracking of their refugee client's health information to ultimately, provide more efficient and effective care.

17. IMPACT OF PHARMACISTS AS IMMUNIZERS ON INFLUENZA VACCINATION COVERAGE IN NOVA SCOTIA, CANADA: 2013-2015

Authors: **J Killen**, J Isenor, B Billard, S McNeil, D MacDougall, B Halperin, K Slayter, S Bowles
Affiliation: Dalhousie University, College of Pharmacy

Introduction: Vaccination is the most effective method of preventing influenza; however recommended Canadian coverage goals are not being achieved. The addition of additional immunization providers may improve accessibility and convenience. Pharmacists with appropriate training began administering influenza vaccines to those 5 years of age and older within the publicly funded universal influenza program in 2013.

Methods: Physician, pharmacy, and public health influenza immunization data, and population census data were obtained from the Department of Health and Wellness (DHW). Coverage rates were calculated as a proportion of vaccinations administered over the total population. Data included 3 years prior to pharmacists immunizing (2010-2013) and 2 years after pharmacists had authority to immunize (2013-2015).

Results: Overall vaccination coverage increased in the first year pharmacists were immunizing to 41.8%, however decreased in the second year to 39.9%. Prior to pharmacists immunizing, the highest coverage reached was 38% in 2010-2011, and below 36% in 2011-2012 and 2012-2013. Physicians have been experiencing an ongoing decrease in the number of immunizations they administer since 2010. Public health intentionally held fewer vaccination clinics with the addition of pharmacists, so there was an expected decrease in vaccines provided by public health noted. With the addition of pharmacists immunizing, coverage for patients 65 years of age or older increased to 71.6% in 2013-2014 and 73.3% in 2014-2015, from the highest noted coverage of 61.8% prior to the addition of pharmacists immunizing.

Conclusions: With the addition of pharmacists to the publicly funded universal influenzavaccination program, more Nova Scotia residents received their influenza vaccination compared to previous years without pharmacists immunizing. The impact may be greatest in community-dwelling adults 65 years of age or older.

18. THE CORRELATION BETWEEN INFLUENZA VIRAL LOADS AND DISEASE OUTCOMES

Authors: **A Lund**[1], M Petten[1], M Warhuus[1], M ElSherif[1], S McNeil[1], T Hatchette[1], J LeBlanc[1]
Affiliation: Dalhousie University, College of Pharmacy

Introduction: Influenza virus is a respiratory pathogen and a significant public health concern due to its ability to cause severe morbidity and mortality. It has been postulated that viral load at the time of diagnosis may be able to predict influenza disease severity. This study aims to evaluate the correlation of influenza A viral loads and disease outcomes in hospitalized adults who tested positive for influenza A (IA) or influenza B (IB) virus by reverse-transcription polymerase chain reaction (RT-PCR) using data collected by the Canadian Immunization Research network (CIRN) Severe Outcomes Surveillance (SOS) Network.

Methods: To generate a stranded curve that would allow for the estimation of viral load based on cycle threshold (Ct) values, total nucleic acids were extracted for influenza A virus H1N1 (California-like) and influenza virus B (Victoria-like), and IA/IB detection RT-PCR was performed. The resulting amplicons were cloned into pGEM-T Easy, generating plasmids pFluA and pFluB, respectively. These plasmids were transformed into *E. coli* for propagation, and confirmed using PCR and sequencing. Then, pFluA and pFluB were purified, quantified, and subjected to 10-fold serial dilutions prior to RT-PCR analyses. The concentration of each dilution of pFluA and pFluB was correlated to Ct values generated by the influenza A and B RT-PCR.

Results: ThepFluA and pFluB plasmids were confirmed to be identical to the expected target sequence generated by the IA /IB RT-PCR detection RT-PCR. The dilution series created a standard curve plotted against the Ct values creates a standard curve that will be used to estimate the influenza virus concentrations (i.e. viral load) in each of the CIRN SOS specimens.

Conclusions: We have successfully generated standard curves that will allow estimation of influenza A and B viral loads. With availability of patient demographic and clinical outcomes data in the CIRN SOS database, the link between influenza viral load and disease outcome will be assessed, including length of hospital stay, intensive care unit admission, requirement for ventilation, and mortality will be determined.

19. CARDIAC COMPLICATIONS OF COMMUNITY ACQUIRED PNEUMONIA AMONGST HOSPITALIZED CANADIAN ADULTS: A PUBLIC HEALTH AGENCY OF CANADA/CANADIAN INSTITUTES OF HEALTH RESEARCH (PCIRN) SERIOUS OUTCOMES SURVEILLANCE NETWORK STUDY

Authors: G Worthen[1], A Ambrose[1], M Andrew[1,2,7], G Boivin[3,4], W Bowie[5], M Elsherif[1], K Green[6], T Hatchette[1,2,7], J Johnstone[6], J Leblanc[1,2,7], M Loeb[6], D Mackinnon-Cameron[1], T Marrie[1,2,7], A McCarthy[8], A McGeer[6], M Semret[9], G Stiver[5], S Trottier[3], L Valiquette[10], D Webster[11], L Ye[1], S McNeil[1,2,7] on behalf of the Public Health Agency of Canada/Canadian Institutes of Health Research Influenza Research Network (PCIRN) Serious Outcomes Surveillance Network Investigators
Affiliation: [1]Canadian Center for Vaccinology, [2]Nova Scotia Health Authority, [3]Centre Hospitalier Universitaire de Québec/ Centre Hospitalier de l'Université Laval, [4]University of British Columbia, [5]Mt. Sinai Hospital, [6]McMaster University, [7]Dalhousie University, [8]The Ottawa Hospital, [9] McGill University, [10]Centre Hospitalier Universitaire de Sherbrooke Hopital Fleurimont, [11]Saint John Regional Hospital

Introduction: Community acquired pneumonia (CAP) is an important contributor to morbidity and mortality among Canadian adults. CAP can precipitate acute cardiac events. We sought to characterize the incidence, risk factors and outcomes of cardiac complications among Canadian adults hospitalized with CAP and to evaluate the effectiveness of pneumococcal immunization in the prevention of these complications.

Methods: The PCIRN Serious Outcomes Surveillance (SOS) Network conducted active surveillance for CAP in 9 SOS Network sites from 12/1/10 to 12/31/13. Surveillance monitors reviewed hospitalizations daily and enrolled consenting adults admitted with CAP. Detailed information regarding comorbidities, hospital course and outcomes were collected. In-hospital occurrence of unstable angina (UA), myocardial infarction (MI), incident congestive heart failure (CHF), and incident arrhythmias were recorded. Vaccine effectiveness was estimated as 1-OR of pneumococcal vaccination in CAP patients with cardiac complications compared to those without.

Results: 6833 cases of CAP were enrolled; 706 (10.3%) experienced a cardiac complication in hospital: 384 (5.6%) arrhythmia, 211 (3.1%) CHF, 178 (2.6%) MI, and 22 (0.3%) UA. Patients with cardiac complications were older (mean age 75.7y vs 68.4y; p=0.000) and were more likely to have underlying comorbidities (94.6% vs 92.0%; p=0.01) and were more likely to require ICU admission (36.4% vs 16.1%; p=0.000) and mechanical ventilation (23.7% vs 10.1%; p=0.000), had longer length of stay (median 11d vs 7d; p 0.000), and were more likely to die (25.2% vs 10.4%; p=0.000). Overall adjusted effectiveness of pneumococcal vaccine in the prevention of cardiac complications was 19% (3% - 32%).

Conclusions: Cardiac complications are common amongst adults admitted with CAP and are associated with increased morbidity, mortality, and healthcare utilization. Pneumococcal vaccination is associated with a reduced risk of cardiac complications. Evaluation of cost-effectiveness of immunization programs should address this. Improved prevention of pneumococcal disease may result in significant cost savings.

20. DEVELOPMENT AND PILOT OF AN ONLINE PEDIATRIC ANTIMICROBIAL STEWARDSHIP VIRTUAL PATIENT LEARNING MODULE

Authors: A Alshengeti, K Slayter, E Black, K Top
Affiliation: Dalhousie University

Introduction: Antimicrobial stewardship (AMS) programs are coordinated interventions to assess and improve the appropriate use of antimicrobials. AMS principles include optimization of diagnostic evaluation, antimicrobial selection, dosing, administration route, and treatment duration. A proposed method for prescriber education about AMS principles is the virtual patient (VP) learning module. VP modules are simulation-based computer programs that interact with learners to enhance clinical reasoning. The effectiveness of VP modules in AMS education has not been evaluated. We developed a VP learning module to educate pediatric residents regarding AMS principles.

Methods: A team of pediatric infectious disease (ID) physicians and ID clinical pharmacy specialists designed the VP module using the online platform DecisionSim™. The clinical scenario was complicated pneumonia. Decision points were based on AMS principles. The module was evaluated for content validity by 4 faculty reviewers using a reviewer checklist. The appropriateness of the case and ease of navigation were evaluated by 3 residents from other ID and pediatric programs using a validated survey.

Results: All 4 faculty reviewers agreed that the case represented a typical clinical scenario. Three of 4 reviewers agreed that the case triggered the learners' clinical reasoning. One reviewer felt that the case encouraged over investigation. The case was edited based on reviewers' comments. All 3 residents agreed that the module was easy to navigate and reflected a real life case.

Conclusions: AMS principles were incorporated into a clinically relevant VP learning module. Evaluation of the effectiveness of this module for improving learners' AMS knowledge is ongoing.

21. COMPARISON OF TWO AUTOMATED INSTRUMENTS FOR EPSTEIN BARR VIRUS (EBV) SEROLOGY TESTING

Authors: H Al Sidairi[1,2], C Jackson[1], C Roberts[1], C Heinstein[1], T Hatchette[1,2], J LeBlanc[1,2]
Affiliation: [1]Nova Scotia Health Authority (NSHA), and [2]Dalhousie University, Halifax, NS.
[2]Streptococcus and STI Unit, National Microbiology Laboratory (NML), Public Health Agency of Canada (PHAC), Winnipeg, MB

Introduction: Serology remains the mainstay for diagnosis of Epstein-Barr virus (EBV) infection, and EBV panels are now available for automated instruments to help streamline EBV testing. This study aimed to compare two automated serology platforms (BioRad Bioplex 2200 and the Abbott Architect i2000) for testing of three EBV serological markers: viral capsid antigen [VCA] IgM, VCA IgG, and EBV nuclear antigen-1 [EBNA-1] IgG.

Methods: Pedigreed specimens previously tested by Euroimmun IgM (n=37), IgG (n=38), and EBNA (n=40) were use to compare the automated instruments. Discrepant results were resolved with IgM immunofluorescence testing using Merifluor IFA VCA EBVM, or enzyme immunoassays using the Zeus EBV IgG and EBNA kits. Each automated method was compared to a modified gold standard defined as two of three concordant results between the automated system (Bioplex or Architect), Euroimmun, and the discrepant analysis.

Results: Compared to the modified gold standard, Bioplex testing resulted in 100% sensitivity and specificity for all targets. The overall concordance between the Bioplex and Architect was 98.2%, and there were two discordant results. First, a falsely reactive VCA IgG on the Architect i2000 (signal 1.10 vs. cutoff 1.0) was obtained in a patient who was IgM and IgG negative by all tests (including discrepant analysis), thus resulting in a specificity of 93.3%. Second, a false negative EBNA result was noted for Architect i2000, where a positive result was obtained for Euroimmune, Bioplex i2000, and Zeus EBNA, thus resulting in a sensitivity of 96.1%.

Conclusions: This study demonstrated that both automated systems for EBV serology had good performance; however, the Bioplex 2200 had better sensitivity for EBNA and specificity for VCA IgG. Since the patient population tested at our institution is primarily adults, an algorithm-based approach for Bioplex EBV testing was implemented, decreasing turnaround times and reducing test order errors.

22. COMPARISON OF MONOPLEX AND DUPLEX RT-PCR ASSAYS FOR THE DETECTION OF MEASLES VIRUS

Authors: K BinKhamis[1], J Hiebert[2], L Mendoza[2], JD Lafreniere[1], T Hatchette[1], J LeBlanc[1]
Affiliation: [1]Nova Scotia Health Authority (NSHA) and Dalhousie University, Halifax, NS. [2]Viral Exanthemata and STDs, National Microbiology Laboratory (NML), Public Health Agency of Canada (PHAC), Winnipeg, MB

Introduction: Measles virus (MeV) infection is of significant public health concern. Diagnostic methods for MeV include RT-PCR, and the National Microbiology Laboratory (NML) uses two monoplex reactions targeting the nucleoprotein (N) and hemagglutinin (H) genes. This study aimed to compare the analytical and clinical performance of the H and N gene monoplex RT-PCRs to a duplex RT-PCR reaction using both targets, in order to reduce testing cost.

Methods: The HN duplex RT-PCR used the same oligonucleotides as the NML monoplex reactions, but differed in reagents and amplification conditions. Analytical sensitivity was assessed using 10-fold serial dilutions of MeV strain Edmonston (ATCC VR-24). Analytical specificity was tested against MeV genotypes (A, B3, D8, D9, and H1) and various other viruses, which included paramyxoviruses (respiratory syncytial virus, parainfluenza viruses 1 to 4, mumps virus, and human metapneumovirus). Clinical performance was evaluated using 33 throat swabs, 43 nasopharyngeal swabs, and 53 urine specimens (of which 3, 13, and 23 were positive, respectively).

Results: Using a reference MeV strain, the analytical sensitivity was found to be equivalent for all assays, and no cross reactivity was observed. Concordance in clinical specimens was 100% between the duplex and N gene monoplex. The H gene monoplex failed to detect MeV genotype B3 in four urines, one throat, and one nasopharyngeal swab, resulting in a clinical sensitivity of 83%.

Conclusions: While the H gene monoplex failed to detect genotype B3, both the HN duplex and N gene RT-PCRs were able to accurately detect all MeV genotypes evaluated. Recently, the NML has validated an alternate H gene primer/probe combination that enables detection of genotype B3, yet the HN duplex RT-PCR described in this study provides a suitable alternative for MeV detection. Future analyses will evaluate the benefits of the novel NML H gene oligonucleotides in a duplex RT-PCR with N gene.

23. SEROPREVALENCE OF JAMESTOWN CANYON VIRUS IN NOVA SCOTIA

Authors: G Patriquin[1,2], E Schleihauf[6], BL Johnston[1], K Dimitrova[4], A Mask[6], M Traykova-Andonova[4], D Haldane[1,2,3], M Drebot[4], T Cole[5], R Lindsay[4] T Hatchette[1,2,3]
Affiliation: [1]Department of Medicine, Nova Scotia Health Authority and Dalhousie University, Halifax NS, [2]Department of Pathology and Laboratory Medicine, Nova Scotia Health Authority, Halifax NS, [3]Department of Pathology, Dalhousie University, Halifax NS, [4]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg MB, [5]Nova Scotia Department of Health and Wellness, Halifax NS, [6]Public Health Agency of Canada, Ottawa, ON

Introduction: Jamestown Canyon Virus (JCV) is a mosquito-borne arbovirus in the California serogroup (CSG) within the *Bunyaviridae* family. The primary reservoir host is believed to be the white-tailed deer. JCV is an emerging pathogen in North America, implicated in presentations ranging from subclinical illness to meningoencephalitis. Preliminary data demonstrated a high seroprevalence of JCV in white-tailed deer and humans in 2 district health authorities (DHAs) in Nova Scotia (NS). In this study we sought to determine the areas of highest seroprevalence of JCV among residents of NS.

Methods: Anonymized residual sera from specimens submitted for diagnostic testing in each DHA in 2012 were randomly selected and screened for JCV antibodies, then subsequently confirmed using plaque reduction neutralization assay (PRNT). A PRNT titre ≥1:20 was considered positive. Additional PRNT endpoint titrations were required in some cases to discriminate between JCV and other CSG viruses. Seroprevalence estimates and 95% confidence intervals (CIs) were calculated using the Clopper-Pearson Exact method. Design weights accounted for regional oversampling in the provincial estimate (SAS v9.4; SAS Institute, Inc., Cary, NC, USA). Population estimates for 2014 were based on Statistics Canada census data.

Results: There were 251 samples across 9 NS DHAs tested. The overall seroprevalence of JCV was 21.2% (95% CI 16.1-27.0). Seroprevalence by DHA ranged from 12.9% to 48.2%, with low rates in DHAs containing NS's two largest urban centres, Halifax and Sydney. DHA 1, located in southern NS had a significantly higher seroprevalence than all other DHAs, at 48.2% (CI 35.1-61.3) vs. 20.5% (CI 14.8-26.2), respectively, with a p-value <0.05.

Conclusions: The seroprevalence of JCV in NS is high, with DHA variation. As there have been no known clinical cases of JCV infection in the province, this suggests the possibility of an under-recognized zoonotic disease in NS.

24. HALIVAX PIIE- PHARMACY INITIATED IMMUNIZATIONS IN THE EMERGENCY DEPARTMENT

Authors: H Flemming, C Van Zoost, S Campbell, A Fry, J Isenor, K Thompson
Affiliation: Department of Emergency Medicine, Department of Internal Medicine

Introduction: Influenza is responsible for thousands of hospitalizations and deaths among Canadian adults. Estimations are that between 10-20% of the population becomes infected with influenza each year. Vaccination is the single most important intervention for preventing influenza associated morbidity and mortality, yet seasonal coverage rates repeatedly fail to meet established targets. Pharmacists play an important role in enhancing immunization services. A pilot project for influenza vaccine screening and delivery via the Emergency Department (ED) pharmacy team was initiated in the Charles V. Keating Emergency Department in Halifax, NS. Our study aimed to examine the feasibility of this pilot project, influenza immunization status at time of presentation and the patients' willingness to receive vaccination in the ED.

Methods: Questionnaires were administered to a convenience sample of adult patients visiting the ED who had contact with the pharmacy team. The questionnaire collected patient age, gender, chronic medical conditions, access to health care and vaccination history.

Results: Of the 85 total patients surveyed, 88% (n=75) were high risk for influenza-related complications by the National Advisory Committee on Immunization (NACI) criteria, yet only 43% (n=32) were aware of their high risk status. Approximately one third (33%) of patients were unimmunized prior to ED visit; the majority (64%) of unimmunized patients indicated they would be willing to receive immunization in the emergency department and were referred on to the department pharmacist for vaccination.

Conclusions: The majority of patients who encountered the ED pharmacy team were high risk for influenza related complications, yet a significant proportion had not received the influenza vaccine. The majority of unimmunized patients were willing to receive vaccination in the ED. Encounters with the ED pharmacy team represent opportunities for enhancing influenza immunization services and providing evidence-based advice to ED patients.

25. ELUCIDATING A ROLE FOR UFM1 IN KSHV INFECTION

Authors: C Robinson[1], C McCormick[1,2]
Affiliation: [1]Department of Microbiology and Immunology, Dalhousie University. [2]Beatrice Hunter Cancer Research Institute

The author requests that this abstract not be published.

26. IDENTIFICATION OF LOW FREQUENCY DRUG RESISTANCE AND IMMUNE EPITOPE MUTATIONS IN HCV VIA NEXT GENERATION SEQUENCING

Authors: K Arseneault[1], SA Oldford[1], L Barrett[1,2]
Affiliation: [1]Dalhousie University, Halifax, NS, Canada [2]Nova Scotia Health Authority, Halifax, NS, Canada

Introduction: Hepatitis C virus (HCV) infection is a global public health concern with no prophylactic vaccine and over 180 million people infected worldwide. New direct acting antiviral (DAA) agents are highly efficacious, however there are still treatment failures in some populations that are not well understood. It is unclear if virus variability related to drug resistance, immunologic escape variants, or the two factors combined contribute to virologic failure. We hypothesize that combined knowledge of baseline drug resistance mutations and immune epitope mutations might better predict treatment outcomes, particularly in cirrhotic and high viral load individuals.

Methods: We utilize next generation sequencing and a novel bioinformatics approach to evaluate circulating low frequency variants before treatment in cirrhotic and high HCV viral load individuals compared to low HCV viral load in 15 individuals with chronic HCV infection before initiation of HCV DAA treatment at a correctional facility in PEI, Canada. Virus variant model based clustering is used to reconstruct variance at the single base level.

Results: We describe baseline host HCV genome variance in both immune epitope and NS5a, NS3/4, NS5b drug resistance regions correlated with treatment outcome, liver disease, injection drug use, and estimated duration of HCV treatment, as well as in vitro HCV T cell responses.

Conclusions: A better understanding of baseline HCV variance may better predict treatment outcome for more difficult to treat populations and aid in the development of prophylactic HCV vaccines.

27. DEVELOPMENT OF PROCEDURES FOR TESTING LOW DOSE, PER OS CYCLOPHOSPHAMIDE IN COMBINATION WITH A POTENT CANCER VACCINE IN PRECLINICAL MOUSE MODEL

Authors: A West, A MacKay, G Weir, M Stanford, M Mansour
Affiliation: Immunovaccine, Inc.

Introduction: The efficacy of cancer vaccines may be enhanced by combination with immune modulating drugs. Immunovaccine Inc. has demonstrated clinically that cyclophosphamide (CPA), provided by daily oral low dose administration, can enhance the immunogenicity of a cancer vaccine, DPX-Survivac, in ovarian cancer patients. This treatment schedule was optimized and safety demonstrated using preclinical mouse models. To model CPA administration in our preclinical studies, we had to overcome two major challenges: first, establish a method of delivery that was safe and effective; second, develop a set of procedures for preparing, delivering, and discarding the chemotherapeutic drug that limited exposure to research and animal care staff.

Methods: To mimic the planned administration in humans, we provided CPA to mice per os, in drinking water (poCPA). C57BL6 mice were implanted with C3 tumours on study day 0 and poCPA provided for 7 days between study days 14-21. Concentration of drug was based on average water consumption of 3 mL/ mouse/ day in order to deliver 20 mg/kg/day. Blood was collected by submandibular venipuncture during the course of treatment and for two weeks after drug was stopped in order to monitor white blood cell levels. Safety was evaluated by regular body weight measurements and detailed clinical examinations. We then developed a series of standard operating procedures to ensure consistency in drug delivery and limit exposure to research and animal care staff. These procedures include safe handling and discarding practices, labelling requirements and special care regarding animal health and husbandry.

Results: We found that poCPA administration did not significantly reduce circulating white blood cells or reduce body weight compared to untreated controls, and no significant safety incidences were noted. We next evaluated poCPA in combination with vaccination using a schedule modeled after our clinical trial design. C3 tumour bearing mice were treated with poCPA every other week and vaccinated every three weeks with a peptide vaccine. The combination therapy provided a greater delay in tumour growth compared to either treatment alone, confirming that this treatment combination was efficacious. The combination treatment did not increase injection site reactions.

Conclusions:. We determined that poCPA is safe to administer at 20 mg/kg/day continuously for 7 days in drinking water. poCPA in combination with vaccine provides enhanced tumour control and no increase in site reactions. We developed standard procedures for handling, delivering and disposing of poCPA to streamline our experiments. Using our standardized procedures we were able to perform experiments efficiently with large numbers of mice. The results directly translated to clinical trials of DepoVax™ and CPA combination therapy.

28. BURDEN OF PNEUMOCOCCAL COMMUNITY-ACQUIRED PNEUMONIA AND INVASIVE PNEUMOCOCCAL DISEASE AMONG HOSPITALIZED CANADIAN ADULTS: A CANADIAN IMMUNIZATION RESEARCH NETWORK (CIRN) SERIOUS OUTCOMES SURVEILLANCE (SOS) NETWORK STUDY

Authors: M ElSherif[1], J Leblanc[1], M Warhuus[1], I Martin[2], M Andrew[1], L Ye[1], D MacKinnon-Cameron[1], A Ambrose[1], T Hatchette[1], S McNeil[1] on behalf of the Public Health Agency of Canada/Canadian Institutes of Health Research Influenza Research Network (PCIRN) Serious Outcomes Surveillance Network
Affiliation: [1]Canadian Center for Vaccinology, Nova Scotia Health Authority, Dalhousie University, Halifax, NS; [2]National Microbiology Laboratory (NML), Winnipeg, MB

Introduction: Pneumococcal community acquired pneumonia (CAP) and invasive pneumococcal disease (IPD) cause significant morbidity and mortality worldwide, particularly in children and elderly. Although childhood immunization programs have helped reduce the burden of pneumococcal disease, recent data suggests that adult immunization may also be beneficial. However, there is a paucity of data describing the burden of pneumococcal disease in adults to advise immunization policy.

Methods: Active population-based surveillance was performed nationally for CAP and IPD in hospitalized Canadian adults to collect patient demographics, describe clinical outcomes associated with pneumococcal disease, and characterize the *S. pneumoniae* serotype distribution.

Results: Ninety cases of IPD (non-CAP) were identified; of 6605 CAP cases, *S. pneumoniae* was detected in 552 (8.4%), 257 were bacteremic. The burden of pneumococcal CAP and IPD only was measured in terms of length of hospital stay, ICU admission, need for ventilation, and 30-day mortality. Using the PCV13-specific urinary antigen detection (UAD) assay alone, proportion of 13-valent pneumococcal conjugate vaccine (PCV13)-preventable *S. pneumoniae* serotypes among pneumococcal CAP, bacteremic CAP and IPD was 67.8%, 52% and 11%, respectively. *S. pneumoniae* isolates, colonization swabs, and UAD results demonstrated similar serotype distributions with predominance for vaccine-preventable serotypes 3, 7F, 19A, and 22F.

Conclusions: Overall, pneumococcal CAP and IPD remain significant causes of morbidity and mortality in hospitalized Canadian adults, with a large proportion of S. pneumoniae serotypes that remain vaccine-preventable.

29. A MODIFIED CARBA NP TEST FOR CARBAPENEMASE DETECTION

Authors: CM Phillips[1,2], D Haldane[2,3,4]
Affiliation: [1]Public Health Agency of Canada, Winnipeg, Manitoba, Canada; [2]Provincial Public Health Laboratory Network, Halifax, Nova Scotia, Canada; [3]Department of Pathology, Dalhousie University, Halifax, Nova Scotia, Canada; [4]Division of Microbiology, Department of Pathology and Laboratory Medicine, Nova Scotia Health Authority (NSHA), Halifax, Nova Scotia, Canada

Introduction: The incidence of Carbapenem-resistant *Enterobacteriaceae* (CRE) is increasing and outbreaks have occurred world-wide. The Carba NP test was developed by Nordmann *et al.*, (2012) to detect carbapenemase-producing *Enterobacteriaceae*, and in 2013, Pires *et al.* developed a modification called Blue Carba.

Methods: Using techniques from both of these assays, we designed an in-house test for detection of CRE that was cheap and ready-to-use. The Carba NP test was modified by: 1. using a cheaper pharmaceutical preparation, 2. freezing the solutions to improve agent stability, 3. standardizing the quantity of bacteria being tested, and 4. eliminating the detergent. The stability of Imipenem at 4°C and -80°C was assessed by disc diffusion.

Results: CREs were reliably detected in 3 h by the modified Carba NP test. For strains with stronger carbapenemase activity, the reaction was immediate and fewer bacteria were required for the modified Carba NP compared to Carba NP. Strains with weaker activity were not reliably positive by the Carba NP or Blue Carba.

Conclusions: The modified Carba NP test detected more CRE than the Carba NP test and was easier to interpret than the Blue Carba test. The modified Carba NP test is 99.2% sensitive and 100% specific. The only false negative was an OXA-48 strain which was consistently negative. No appreciable breakdown of Imipenem has occurred at -80°C after 40 weeks but breakdown at 4°C occurred rapidly.

30. PREVALENCE OF ANTIBIOTIC RESISTANCE GENES IN INFLUENT AND EFFLUENT WATER FROM MUNICIPAL WASTEWATER TREATMENT SYSTEMS IN ARCTIC CANADA

Authors: KD Neudorf, Y Huang, CK Yost, RC Jamieson, L Truelstrup Hansen
Affiliation:

Introduction: The discharge of treated or untreated wastewater can have major implications on human health and ecosystem quality, with the potential release antibiotic resistant microbes quickly attracting more attention. Wastewater treatment plants (WWTPs) have been identified as a major source of antibiotic resistant genes (ARGs) across the world.

Methods: Quantitative real-time PCR, using TaqMan Probes, was used to quantify nine different antibiotic resistant target genes (*int1, sul1, sul2, tet(O), erm(B), mecA,* $bla_{CTX\text{-}M}$*,* bla_{TEM}*,* and *qnr(S)*) in the influent and effluent water from three arctic WWTPs in Nunavut.

Results: This study provides novel evidence that bacteria residing in three types of northern WWTPs harbour various antibiotic resistant genes belonging to multiple different clinically-relevant classes of antibiotics. Furthermore, effluent water quality results ($CBOD_5$, ammonia, total suspended solids (TSS), *E. coli* and total coliforms) indicated limited effect of the treatment to possibly explain the high prevalence of the ARGs in the effluent. Moreover, the study suggests a trade-off between time spent in the wastewater treatment process to benefit removal of organic material, while potentially allowing the enrichment of ARGs.

Conclusions: Given the high abundance of ARGs that are detectable in the effluent and/or receiving waters, and the potential threat they may cause to ecological and human health, it appears that the design of current northern wastewater treatment processes is not appropriate to significantly reduce the release of antibiotic resistant bacteria into the environment.

30-B. POINT PREVALENCE SURVEY OF ANTIMICROBIAL USE IN WOMEN'S HEALTH AND PEDIATRIC PATIENTS AT THE IWK HEALTH CENTER IN HALIFAX, NOVA SCOTIA

Authors: M Harrison, M Losier, E Black, K Slayter, H Neville, K Abbass, BL Johnston, I Sketris
Affiliation: Dalhousie University, College of Pharmacy

Introduction: Point prevalence surveys (PPS) have been widely used to describe antimicrobial use within and between institutions. The objective of this PPS was to determine the prevalence of antimicrobial use in women and pediatric patients at the IWK Health Centre, and to assess adherence to select prescribing guidelines (surgical prophylaxis, urinary tract infection, and community acquired pneumonia).

Methods: A PPS design was used to assess prevalence of antimicrobial use. Paper charts were reviewed by the IWK infectious diseases pharmacist (KS) and two pharmacy students (MH and ML). All patients admitted for at least 24 hours by 0800 the day of the survey were included in the denominator and those patients with an active systemic antimicrobial prescription served as the numerator. The following data was collected: age, sex, antimicrobial drug, dose, doses per day, route, indication, intended duration, and adherence to local guidelines.

Results: The prevalence of antimicrobial use was calculated to be 28.6% (40/140). The most commonly prescribed antimicrobials were fluconazole (11.6%; 8/69) and cefazolin (11.6%; 8/69). The oral to parenteral ratio was calculated to be 14:55.

Conclusions: This PPS will provide local infectious disease specialists with important insight in regards to antimicrobial utilization and guideline adherence at the local pediatric and women's health tertiary care hospital. This information can be compared to national and international antimicrobial utilization data. Furthermore the results may be used to promote the importance of antimicrobial stewardship initiatives.

31. KSHV MODULATES THE IRE1-XBP1 AXIS OF THE UNFOLDED PROTEIN RESPONSE DURING LYTIC REPLICATION

Authors: BP Johnston[1,2], C McCormick[1,2]
Affiliation: [1]Department of Microbiology and Immunology, Dalhousie University. [2]Beatrice Hunter Cancer Research Institute

The author requests that this abstract not be published.

32. TRANSLATIONAL DEVELOPMENT OF A NOVEL THERAPEUTIC CANCER VACCINE FOR ADVANCED HPV-ASSOCIATED CANCERS

Authors: M Stanford, G Weir, M Mansour, E Reinherz, R Haddad, J Lorch
Affiliation: Immunovaccine, Inc, Dana Farber Cancer Institute

Introduction: Human papilloma virus (HPV) causes more cancers than any other virus. The incidence of HPV associated cancers in tissues for which no screening algorithms exist, including the oropharynx and anus, is increasing steadily. The preventive vaccines have no therapeutic effect. Because HPV proteins E6 and E7 are functionally required for disease initiation and persistence, they offer compelling targets for immune-based therapies. DepoVax^{TM} (DPX) is a lipid based vaccine adjuvanting platform that has been shown to be effective in raising robust and sustained antigen-specific immune responses. DPX based vaccines have demonstrated safety and immunogenicity in clinical cancer trials.

Methods: Preclinical testing was performed in C57BL/6 mice implanted with HPV16-E7 expressing tumors (C3). A murine H2D^{b} restricted peptide, HPV16-E7$_{49-57}$, was formulated in DPX to evaluate the efficacy of an immune response to HPV16E7 in this model. Poisson detection mass spectrometry analysis of peptides eluted from MHC complexes from HPV16^{+} tumor biopsy samples in HLA-A2^{+} cervical cancer subjects identified a human specific strain-invariant epitope, HPV16-E7$_{11-19}$ on the majority of patient's tumors. This peptide was formulated in DPX to create DPX-E7.

Results: The murine DPX-based E7 vaccine induced antigen-specific CD8^{+} T cells that were effective in providing significant long-term control of tumor growth in the murine C3 tumor model. The identified human MHC class I epitope, HPV16-E7$_{11-19}$ was able to effectively bind HLA-A2 in the in vitro T2 shift assay. Combined, these studies support the translation of the DPX-E7 vaccine to clinical testing in HLA-A2^{+} patients with histologically confirmed HPV related cervical, anal and oropharyngeal cancer that is metastatic or unresectable and for which standard curative measures do not exist or are no longer effective. .

Conclusions: The presumed primary mechanism of action of DPX-E7 is to elicit a cytotoxic T lymphocyte response against tumor cells presenting the HPV16-E7 peptide. The proposed clinical study is a single-center open label non-randomized trial of DPX-E7. This trial has received funding from the Stand Up 2 Cancer and the Farrah Fawcett Foundation.

33. PATIENT MOTIVATION TO PARTICIPATE IN PHASE I (EBOLA AND INFLUENZA) VACCINATION TRIALS

Authors: A Cattapan[1], K Browne[2], in collaboration with the Canadian Center for Vaccinology*
*Study conducted in collaboration with F Baylis, J Graham, B Halperin, SA Halperin, JM Langley, L Li, D MacDougall, D MacKinnon-Cameron, S McNeil, A Petropanagos
Affiliation: Novel Tech Ethics, Faculty of Medicine, Dalhousie University, Department of Philosophy, Langara College

Introduction: Little is known about the motivations of participants in Phase I vaccine trials, which require healthy volunteers and offer inoculation as the only health benefit to participants. This study investigated the motivations of participants in a Phase I Ebola vaccine trial and a Phase I influenza vaccine adjuvant (PAL) trial. Further, by comparing the motivations of participants in these two trials, this study examined whether participant motivations differ in high-profile and lower profile vaccine trials.

Methods: Participants in two Phase I vaccine trials were asked to complete an online questionnaire on their motivations to participate in the trial. Research ethics approval was obtained from the IWK REB. Participants were recruited directly by the Canadian Center for Vaccinology via email and completed an online survey created using Opinio software. Results were analyzed using SAS software.

Results: A total of 55 of a potential 88 participants responded to the survey. Participants generally indicated that they were primarily motivated by a combination of factors, such as contributing to the advancement of science, contributing to the health of others, wanting to participate in something important, wanting to receive an incentive, or curiosity about the study. While the main motivations of participants were largely the same across the two trials, the Ebola trial participants were substantially more likely to count media coverage and a desire to control the illness/infection amongst factors motivating their participation.

Conclusions: Motivations for healthy participants in Phase I vaccine trials will be both selfish and altruistic. While financial incentive ranks highly among the motivations of healthy participants, it is neither the sole nor main factor motivating people to volunteer and occurs in concert with seemingly altruistic motivations, including wanting to contribute to science, and wanting to contribute to the health of others.

34. THE ROLE OF MAST CELLS IN THE IMMUNE RESPONSE TO *CHLAMYDIA* INFECTION

Authors: E Shantz[1,3], JS Marshall[1], J Wang[1,2,3]
Affiliation: [1]Department of Microbiology & Immunology, [2]Department of Pediatrics and [3]Canadian Center for Vaccinology, Dalhousie University, Halifax, NS

Introduction: *Chlamydia trachomatis (Ct)* is an intracellular bacteria that represents the most frequently reported bacterial sexually transmitted infection as well as the leading cause of infectious blindness worldwide. Mast cells are sentinel immune cells located at tissues that interact with the external environment. Although traditionally implicated in allergic responses, mast cells can be activated by pathogen recognition receptors such as toll-like receptors (TLRs) to selectively release cytokines that recruit immune effector cells. The role of mast cells in the immune response to *Ct* infection of the genital tract has not been elucidated.

Methods: Murine bone marrow-derived mast cells (BMMCs) and human cord blood-derived mast cells (CBMCs) were incubated with *Chlamydia muridarum* (*Cm*) or *Ct*. Cytokine production was measured by enzyme-linked immunosorbent assay (ELISA) or Luminex array. Mast cell-deficient Wsh and wildtype (WT) C57BL/6 mice were infected intravaginally with *Cm*. Genital swabs were obtained to assess bacterial burden by qPCR. Splenocytes from infected mice were restimulated with *Cm* and cytokines were measured by ELISA. Dendritic cell (DC) subsets were analyzed by flow cytometry.

Results: BMMCs and CBMCs produced pro-inflammatory cytokines and chemokines such as IL-1β, IL-6 and CCL3 in response to *Chlamydia*, and this appeared to be TLR2-dependent. Both live and heat-killed *Ct* induced similar cytokines, and *Ct* was unable to replicate in human mast cells. Although Wsh and WT mice had comparable bacterial burdens during infection, Wsh mice had less severe oviduct pathology after 50 days as well as decreased IFN-γ, IL-13 and IL-17A memory responses. Similar data were obtained in $TLR2^{-/-}$ mice. At 3 days post-infection, Wsh mice had less infiltration of DCs in the draining lymph node compared to WT.

Conclusions: These data indicate that mast cells may have a role in the immediate response to *Ct* as well as the development of pathology and adaptive immunity.

35. RECEIPT OF PRIOR SEASONAL INFLUENZA VACCINATION REDUCES SUBSEQUENT INFLUENZA VACCINE EFFECTIVENESS IN YOUNG ADULTS

Authors: M Nichols-Evans[1,2], L Ye[2], M Andrew[2], T Hatchette[2], S McNeil[2] on behalf of the Serious Outcomes Surveillance (SOS) Network of the Canadian Immunization Research Network (CIRN)
Affiliation: [1] Department of Community Health and Epidemiology, Dalhousie University, [2]Canadian Center for Vaccinology, IWK Health Centre and Nova Scotia Health Authority, Dalhousie University

Introduction: Recent controversy has arisen from observational studies showing a negative association between prior influenza vaccination and subsequent influenza vaccine effectiveness (VE). As immunologic theories suggest that impact of prior season vaccination could be important in the 14/15 season, characterized by unchanged vaccine components and circulation of a drifted influenza A (H3N2), we investigated this association in Canada.

Methods: The CIRN SOS Network prospectively identified laboratory-confirmed influenza cases and influenza-negative controls admitted to participating hospitals. Using a test-negative control design, unconditional logistic regression modeling stratifying participants into 4 groups (not vaccinated current or prior season [referent], vaccinated prior season only, vaccinated current season only, and vaccinated both seasons) was used to calculate odds ratios (OR) to estimate the effect of vaccination status on influenza-related hospitalization.. VE= 1-OR X100. We report VE for overall effect and effect stratified by age (<65, ≥65y) and against influenza A (H3N2).

Results: In the overall final model (1123 cases, 1206 controls), vaccination in current season only (VE: 21.1%, 95% CI: -13.5, 45.1) and in both prior and current season (VE: 14.7%, 95% CI: -4.8, 30.6) yielded similar protection against influenza-related hospitalizations. However, in those <65y, vaccination in current season only yielded better protection than vaccination in both prior and current season (VE: 27.6%, 95% CI: -35.0, 61.1 versus -4.2%, 95% CI: -55.7, 30.2, respectively). This reduction in VE was not observed in those >65y. When restricted to influenza A in those <65y, vaccination in current season only yielded VE of 37.5%, 95% CI: -27.0, 69.2, but VE dropped substantially for vaccination in both current and prior season to -18.3%, 95% CI: -82.3, 23.3.

Conclusions: These findings provide evidence that prior season vaccination can have a negative impact on influenza VE under some circumstances, particularly in young adults. Further studies to examine this association and to explore contributing biological and immunological mechanisms are critical to inform immunization policy.

36. IMMUNE PHENOTYPE DURING DAA TREATMENT IN AN INCARCERATED POPULATION WITH HIGH RATES OF INJECTION DRUG USE ADULTS

Authors: SA Oldford[1], K Arseneault[1], L Barrett[1, 2]
Affiliation: [1]Dalhousie University, Halifax, NS; [2]Nova Scotia Health Authority, Halifax, NS

Introduction: Immune mechanisms of achieving sustained virologic response among HCV infected subjects are unclear. Previous work has demonstrated immunologic augmentation with HCV direct acting antiviral (DAA) treatment but not specifically in a population of persons who inject drugs where immune function is known to be altered.

Methods: 8 HCV GT-1 subjects (7 with active injection drug use within 3 months, 1 with noinjection drug use history) were treated with a triple DAA regimen for 12 weeks. Comprehensive peripheral blood mononuclear cell (PBMC) T cell (CD3, CD4, CD8, CD27, CD28, Tim-3, PD-1, CTLA-4, and CD57), B cell (CD10, CD19, CD20, CD21, CD27), and NK cell (CD16, CD27, CD56, CD158b, CD158e1/e2, CD159a, CD314, CD337) immunophenotyping was performed in these patients as well as age and sex matched controls at baseline, day 7, week 4, week 8 and end of treatment. Data comparing baseline and day 7 of treatment are described here.

Results: All patients had rapid decline of HCV viral load within 7 days of therapy, and all were undetectable at week 8. There were no significant differences between control individuals and HCV infected individuals at baseline or following 1 week of DAA therapy. B cell subsets and NK cell exhaustion markers did not significantly differ but there was a trend towards decreased cytotoxic NK cells and increased memory B cells in HCV. There were no significant differences in T, B, and NK cell phenotype between the persons who injected drugs and the 1 person who did not inject drugs.

Conclusions: Inhibition of HCV replication by a DAA regimen is rapidly associated with a less exhausted T cell immunophenotype in incarcerated injection drug users. Even in individuals with immune changes related to injection drug use, potent viral suppression rapidly alters T cell phenotype. These findings highlight the plasticity of immune phenotype and reversibility of immune exhaustion even in a population with lifestyle factors associated with altered immune function.

37. OXIDATIVE STRESS RESPONSES AT 20ºC: CLUES TO *LEGIONELLA PNEUMOPHILA* BIOFILM FORMATION AND ENVIRONMENTAL PERSISTENCE

Authors: M Dobrovolskis[2], C Leeks[2], KA Brassinga[3], J LeBlanc[1,2]
Affiliation: [1]Nova Scotia Health Authority (NSHA) and Dalhousie University[2], Halifax, NS.
[3]University of Manitoba, Winnipeg, MB

Introduction: *L. pneumophila* is the causative agent of legionellosis, a potentially lethal pneumonia. Outbreaks are associated with water sources, where *L. pneumophila* resides as an intracellular pathogen of amoebae or associated in biofilms. While the molecular mechanisms leading to environmental persistence remain ill defined, previous studies suggested involvement of peroxide scavenging enzymes called alkylhydroperoxide reductases (AhpC). The expression of AhpC is typically controlled by OxyR, a transcriptional regulator activated during oxidative stress. In *L. pneumophila*, two AhpCs have been identified (*ahpC1* and *ahpC2D*), and LpOxyR regulates *ahpC2D* expression. No signals for LpOxyR activation have yet been identified. Interestingly, marked up-regulation *ahpC2D* was noted *L. pneumophila* biofilms. Since biofilm formation is favored at 20°C compared to 37°C, this study compared the effects of temperature on *ahpC1* and *ahpC2D* expression and their response to oxidative stress.

Methods: Wild-type (WT) and *oxyR* mutants of *L. pneumophila* containing GFP reporter plasmids were used to monitor the expression of *ahpC1* and *ahpC2D* in broth cultures grown at 20°C and 37°C. Bacteria were harvested at different stages of growth for GFP analyses, and challenged with various concentrations of hydrogen peroxide (H_2O_2) or paraquat (a superoxide generator) for the induction experiments.

Results: At 37°C, *ahpC1* increased during exponential phase, whereas *ahpC2D* was absent past early exponential phase. At 20°C, both *ahpC1* and *ahpC2D* decreased over time. In the induction experiments, expression of *ahpC1* and *ahpC2D* were unaffected by H_2O_2. In contrast, *ahpC2D* (not *ahpC1*) showed an OxyR-dependent upregulation following exposure to paraquat. Optimal induction was seen during early exponential and exponential phases at 37°C and 20°C, respectively.

Conclusions: Temperature and oxidative stress might influence the expression of *ahpC2D* in biofilms, through an OxyR-dependent mechanism. This study may help delineate mechanisms involved in the environmental persistence of *L. pneumophila*, and resistance to oxidizing biocides.

38. LYSIS CENTRIFUGATION METHOD FOR THE DIRECT IDENTIFICATION OF POSITIVE BLOOD CULTURES USING MALDI-TOF MS

Authors: K BinKhamis, H Al Sidairi, J Head, RJ Davidson
Affiliation: Div of Microbiology, Dept. of Pathology & Laboratory Medicine, QE II HSC

Introduction: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) bacterial identification has revolutionized clinical microbiology. Typically, bacteria must be first cultured prior to identification; however, several techniques have emerged that allow the identification of bacteria directly from certain specimen types, including blood cultures. The aim of this study was to compare a direct MALDI-TOF MS identification technique of positive blood cultures with those having at least 4-6 hours of sufficient growth.

Methods: Only blood cultures flagged overnight as positive by the BD Bactec were included for study. Positive blood culture samples were sub-cultured onto agar plates as per standard laboratory practice, incubated for 4-6 hours and if sufficient growth was present, processed using MALDI-TOF. Cultures with insufficient growth are incubated overnight. An additional 1ml aliquot was drawn and immediately processed using a lysis centrifugation technique and analyzed using MALDI-TOF. Direct identifications were compared to those where sufficient growth was achieved.

Results: Between June 2015 to February 2016, 300 positive blood cultures were included for study. Of these there were 156 Gram positive cocci, 112 Gram negative bacilli, 15 anaerobic organisms, 11 Gram positive bacilli and 6 yeast. Using a confidence threshold of 99.9%, 69% of all organisms were correctly identified using the direct identification method. The identification of any organism with a confidence threshold <99.9% was not accepted. Approximately 81% of Gram negative bacilli were correctly identified compared with 64% of Gram positive cocci 36% of Gram positive bacilli.

Conclusions: The lysis-centrifugation direct identification method is a relatively inexpensive ($1.00) and rapid technique that will allow clinicians to receive the identification of organisms from approximately 70% of bacteremic patients 6 to 24 hours early than waiting for sufficient growth. This should allow clinicians to make better informed empiric antimicrobial choices to manage their patients.

39. STANDARDIZATION OF HEMAGGLUTINATION INHIBITION ASSAY FOR INFLUENZA SEROLOGY ALLOWS FOR COMPARABILITY BETWEEN REFERENCE LABORATORIES

Authors: M ElSherif[1], M Zacour[2], P Tang[3], G Boivin[4], Y Li[5], BJ Ward[6], M Warhuus[1], J LeBlanc[1], T Hatchette[1]
Affiliation: [1]Canadian Center for Vaccinology, Nova Scotia Health Authority & Dalhousie, Halifax, NS; [2]PCIRN Montreal and BioZac Consulting; [3]BC-CDC, Vancouver BC; [4]Laval, Quebec City, PQ; [5]NML, Winnipeg, MB; [6]McGill, Montreal, QC

Introduction: Quality assurance is fundamental to a quality laboratory system. Standardization of hemagglutination inhibition (HAI) assay for influenza serology is challenging. The PCIRN/CIRN reference laboratory network evaluated intra-laboratory and inter-laboratory variations in HAI titers of a standardized panel of samples repeatedly tested in five laboratories.

Methods: Following the same protocol, single operators at five PCIRN/CIRN sites tested blinded panels (spanning usual titre ranges) over six separate assays and days. Ten samples were included per influenza virus (H1 [A/California like] and H3 [A/Perth like]). Results were analyzed collectively by a third party. Within-lab precision included repeatability of duplicates within assays and reproducibility of titres between assays (titres within 2-fold of comparator(s) were considered not different). As the samples were not reference standards, accuracy was assessed using between-lab comparisons relative to reference lab (NML) results and/or consensus values (geometric mean titre across all test labs for a given sample). Seropositivity was defined as a titer > 1/40.

Results: Duplicate precision in laboratories and reproducibility between assays was 99.8% (99.2-100%) and 91.1% (60-100%) respectively. 90% of samples quantified at every lab were within 2-fold of their consensus titre. Compared to the NML results, the accuracy of non-NML labs was 90% (80% for H1N1; 100% for H3N2). Low-titre samples showed the greatest variability, both between assays and sites. Seropositive/negative classifications were identical in 74.4% of 500 tests done in all labs (70% of H1N1;80% of H3N2). When NML's non-equivocal results (n=19) were used, seropositivity/negativity matched the NML in 90.3%, 98.9% and 94.2% of analyses for H1, H3, and all viruses, respectively.

Conclusions: Poor reproducibility of HAI results from one lab to another is a long-standing concern, limiting comparisons between candidate vaccines in different clinical trials and posing challenges for licensing authorities. We show that with careful standardization processes, high reproducibility is achievable.

40. PCR-BASED DISCRIMINATION OF VACCINE-PREVENTABLE SEROTYPES OF *STREPTOCOCCUS PNEUMONIAE*

Authors: H Gillis[1]*, D Gaston[1], M Warhuus[1], I Martin[2], M ElSherif[1], T Hatchette[1], S McNeil[1], J LeBlanc[1]
Affiliation: [1]Canadian Center for Vaccinology (CCfV), Izaak Walton Killiam (IWK) Health Centre, Nova Scotia Health Authority (NSHA), and Dalhousie University, Halifax, NS. [2]Streptococcus and STI Unit, National Microbiology Laboratory (NML), Public Health Agency of Canada (PHAC), Winnipeg, MB.QC

Introduction: The reference for *Streptococcus pneumoniae* serotyping is the Quellung reaction, which uses serotype-specific antibodies to classify isolates based on differences in capsular antigens. PCR-based serotype deduction has been introduced as an alternative; however, the capsular biosynthesis (*cps*) genes on which it relies fails to discriminate certain serotypes, and thus limits its use for pneumococcal surveillance.

Objectives: This study aimed to identify and validate novel PCR targets located outside the *cps* loci that can accurately discriminate vaccine-preventable serotypes of *S. pneumoniae.*

Methods: Next generation sequencing and comparative genomics was used to identify unique PCR targets for each serotype within the "non-discriminated" groups of traditional PCR that contained a vaccine-preventable serotype (underlined): 6A/6B/6C/6D; 7F/7A; 9V/9A, 9N/9L, 11A/11D, 12F/12A/12B/44/46; 15B/15C; 18C/18F/18A/18B; 22F/22A, and 33F/33A/37. Each novel target was evaluated for its ability to discriminate the desired serotype, and specificity was tested against 82 *S. pneumoniae* serotypes characterized by Quellung and 32 other members of the *Streptococcaceae* family.

Results: To date, 16 of the 28 desired serotypes can accurately be discriminated: 6A, 6B, 6C, 9A, 9L, 9V, 11D, 12A, 12B, 12F, 18C, 18F, 44, and 46. No significant cross-reactions were observed.

Conclusions: This study provides the proof-of-principle that PCR targets outside the *cps* loci can be used to accurately discriminate vaccine-preventable serotypes of *S. pneumoniae*, and could be used following screening with traditional PCR-based serotyping. Since serotyping of *S. pneumoniae* is important to monitor its epidemiology and to determine the proportion of pneumococcal disease is vaccine-preventable, this study represents a significant technological advance in pneumococcal disease surveillance.

41. IMPLEMENTATION OF AN ANTIMICROBIAL STEWARDSHIP PROGRAM IN A PEDIATRIC AND WOMEN'S HEALTH CENTER

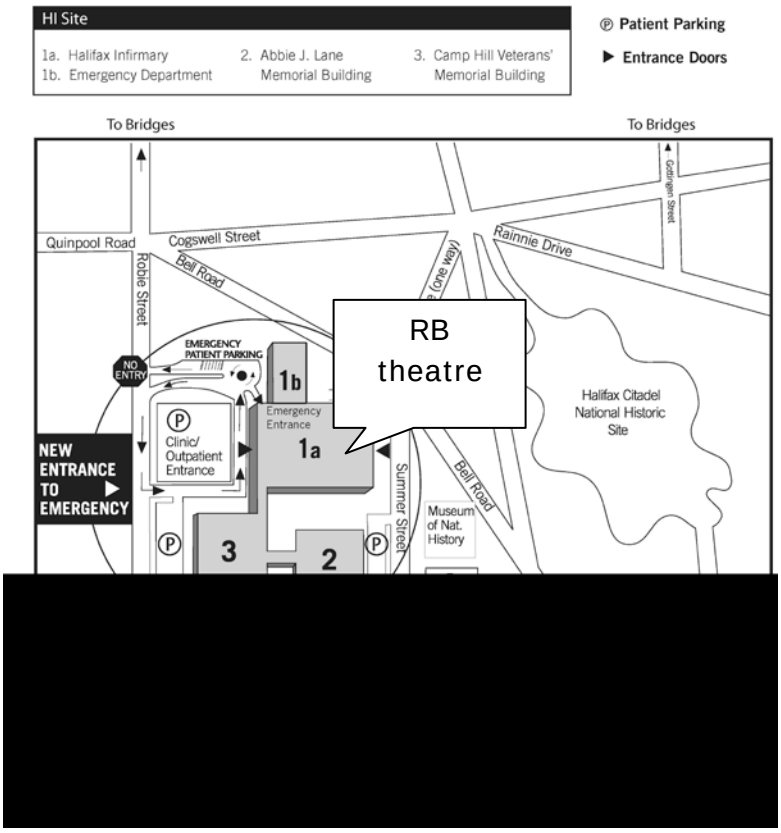
Authors: K Slayter, J Langley, K Top, A Ivany, L Huestis, T Mailman, S Halperin
Affiliation: IWK, CCfV, Dalhousie University

Introduction: Antimicrobials represent the main class of medications used in hospitalized children and constitute significant use in women's health. Recent literature suggests that approximately 30 % of antimicrobials prescribed in hospital are suboptimal or unnecessary. With increasing antibiotic resistance and diminishing antibiotic options, it is essential for Canadian pediatric and women's health programs to establish methods to measure, assess and optimize the use of antimicrobials.

Methods: The implementation of a formal antimicrobial stewardship program (ASP) was initiated in May 2015. The team was made up of representatives from Pharmacy, Pediatric Infectious Diseases (ID) and Infection Prevention and Control. The following processes were put into place prior to the implementation of a formal program: development of empiric antimicrobial guidelines, development of an antimicrobial stewardship (AMS) dashboard and standardized form to facilitate prospective audit and feedback (PAF), education on AMS principles, establishment of an AMS website and completion of a point prevalence survey (PPS) to establish baseline indicators of antimicrobial use. Additional measurable performance indicators were established at a CIHR Pan-Canadian Pediatric Collaborative meeting.

Results: PAF was implemented across the institution in December, 2015 to optimize antimicrobial therapy. On a daily basis, the AMS pharmacist screened a mean of 36 patients and reviewed a mean of 52 antimicrobials. The AMS pharmacist attended microbiology rounds to review positive cultures and consulted with the ID team regarding all complex patients or suggested a formal ID consult. Recommendations were communicated to clinicians and or written recommendations were placed on the patient's chart. A mean of 4.5 patient interventions and 5.4 antimicrobial interventions were completed per day. The recommendation acceptance rate was 89%.

Conclusions: We have established a formal ASP to conduct continuous and transparent monitoring of antimicrobial use. We plan to continue to measure antibiotic usage with serial PPS and to compare our findings with 14 other pediatric/women's health centers across Canada. We will also compare antimicrobial appropriateness rates at our institution with those of institutions utilizing our newly established Pan-Canadian Collaborative. Our comprehensive ASP will ensure that data is gathered, measured, acted upon and benchmarked for optimal use of antimicrobials within our institution.



IWK Health Centre