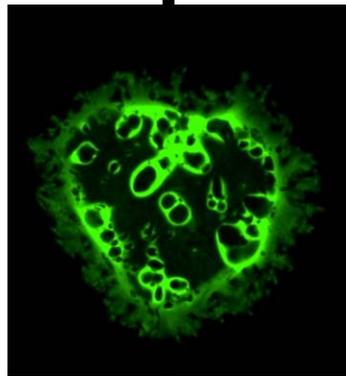


**17th Annual Infectious
Diseases Research Day
And
4th Annual Canadian Center for
Vaccinology Symposium**

April 23 & 24, 2012

Halifax



Sponsored by

Canadian Center for Vaccinology *and*

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

Dalhousie Infectious Diseases Research Alliance

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Dalhousie University Continuing Medical Education

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This program has not received any commercial support.



Cover photo by Michael Ha PhD, Dalhousie Medical Student
Cell monolayer infected with a type of measles virus
which expresses a fluorescent protein in the infected cells.
The infected cells grouped into the shape of a heart.



PROGRAM & ABSTRACTS



❖ Program

Monday April 23

1:00–2:00	Presentation by Mark Steinhoff M.D. <i>Maternal Immunization: a 3-for-1 strategy</i>	IWK Health Centre O.E. Smith auditorium
2:00–2:30	Presentation by Chris Richardson Ph.D. <i>Measles Virus: a potential therapeutic cancer vaccine?</i>	
2:30–3:00	Presentation by Matthew Herder L.L.B. <i>Against the Appropriation of Vaccine Assays</i>	
3:00–4:30	Poster judging (posters on display 1:00 – 5:30)	IWK Health Centre Gallery
4:30–5:30	Public presentation by Robbin Lindsay Ph.D. <i>Ticks and tick-borne diseases in Nova Scotia</i>	IWK Health Centre O.E. Smith auditorium
6:30–8:30	Dinner and presentation by Mark Steinhoff M.D. <i>Vaccine trials and serendipity</i>	VG Hospital Dickson Bldg Weather Watch Rm

❖ Program

Tuesday April 24

- | | | |
|------------|--|---|
| 8:00-9:00 | TJ Marrie Lecture
(Grand Rounds)
by Robbin Lindsay Ph.D
<i>Challenges associated with
detection, prevention and
control of Lyme disease in
Canada</i> | Halifax Infirmary
RB Theatre |
| 9:30-12:30 | Oral Presentations (10) | VG Hospital
Dickson Bldg
Weather Watch Rm |
| 12:30-2:30 | Buffet lunch and
presentation by
Pauline Dakin,
CBC Health/Medical
Reporter
<i>Ready for Primetime:
interpreting research for
the public</i> | VG Hospital
Dickson Bldg
Weather Watch Rm |

❖ Welcome



Lynn Johnston MD, FRCPC
Head, Div of Infectious
Diseases, Dept of
Medicine, Dalhousie U



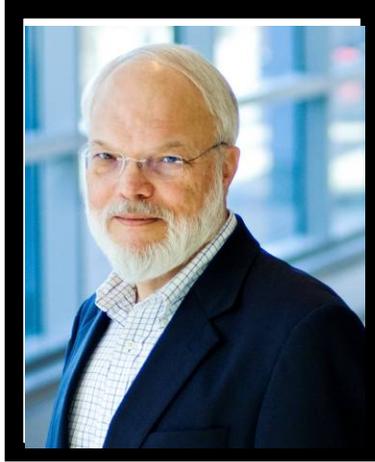
Scott Halperin MD, FRCPC
Director, Canadian Center
for Vaccinology



Rafael Gorduno PhD
Director, DIDRA

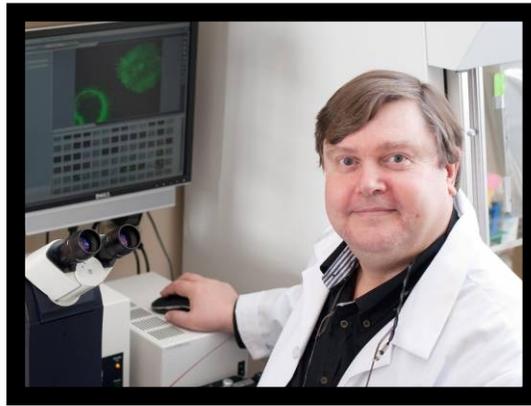
Welcome to the 17th Annual Infectious Diseases Research Day and 4th Annual CCfV Symposium. This event is a highlight for us each year as we bring together seasoned investigators, new investigators, researchers from different disciplines, students, front line health providers and the public, to learn more about infectious diseases that affect the health of Canadians. We are pleased to offer a variety of presentations and posters from international, national and local experts, addressing a range of topics from bench research to policy and programs. Researchers love what they do, and they make important contributions to our knowledge base, but it is equally important that their research is eventually translated into practices that improve our quality of life. As you watch and listen over the next two days we encourage you to ask questions of the presenters and share your new knowledge and insights with colleagues and friends. Also let us know what you gained from this event, and how we can make it better in the future. Thank you for joining us.

❖ The Speakers



Mark Steinhoff MD

Dr. Steinhoff is a pediatrician with infectious disease sub-specialization, and currently Professor of Pediatrics and Director of the Children's Global Health Center at Cincinnati Children's Hospital in Ohio. Following his undergraduate degree and MD from the University of Chicago, he was a pediatric resident, chief resident, and pediatric infectious diseases fellow at University of Rochester. He was faculty in the Dept of Child Health, CMC Hospital, Vellore, India 1980-1985. He has held faculty positions in the Depts of Public Health and Pediatrics at the University of Michigan. Since 1986 he has been faculty at the School of Medicine and the Bloomberg School of Public Health at Johns Hopkins University in Baltimore. His major research interest is in assessing the burden of preventable infectious diseases and the effectiveness of vaccines in low resource settings, having conducted research in South & East Asia, Africa, South America, and Europe. He has authored over 160 peer-reviewed research papers, and He has authored over 160 peer-reviewed research papers, and over 20 textbook chapters in pediatric and tropical medicine. He has been a consultant to CDC, NIH, FDA, WHO, the Rockefeller Foundation, the Ford Foundation, and an advisor to the Bill and Melinda Gates Foundation. He is currently conducting a large antenatal influenza vaccine trial in Nepal, with Gates Foundation support, and a NIH-funded post-partum influenza vaccine trial in the US over 20 textbook chapters in pediatric and tropical medicine.



Chris Richardson PhD

Dr. Chris Richardson is Professor and Canada Research Chair (Tier I) in the Departments of Microbiology & Immunology and Pediatrics at Dalhousie University. He received undergraduate (B.Sc.) and graduate (M.Sc., Ph.D.) training at the University of British Columbia under Dr. Dennis E. Vance. Dr. Richardson pursued postdoctoral studies in the laboratories of Dr. Purnell W. Choppin (Rockefeller University) and Dr. Robert A. Lazzarini (NIH, Bethesda) where he studied measles and influenza viruses. He held Senior Research Officer and faculty positions at NRC Canada (BRI) and McGill University where he developed the BlueBac baculovirus expression system and identified the first receptor (CD46) for vaccine strains of measles virus. Moving to Toronto in 1994 he joined the Amgen Research Institute/Ontario Cancer Institute at the University of Toronto and shifted his studies to include hepatitis B and C viruses. His laboratory also contributed to the discovery of CD150/SLAM as the lymphocyte/dendritic cell receptor for measles virus and developed transgenic mouse models containing this receptor. Dr. Richardson joined Dalhousie University/IWK Health Sciences Centre in 2006 where he continues to study measles, hepatitis B, and hepatitis C viruses. He recently discovered the elusive epithelial cell receptor (Nectin 4) for measles virus, which turned out to be a highly expressed tumour cell marker on many adenocarcinomas.



Matthew Herder LL.M., J.S.M.

Matthew Herder is a member of the Health Law Institute, appointed to the Faculty of Medicine and cross-appointed to the Schulich School of Law at Dalhousie. He holds a Master of the Science of Law degree from Stanford Law School, LL.B. and LL.M. degrees from Dalhousie University, and a science degree from Memorial University. Prior to his appointment at Dalhousie, Matthew completed his articles at McCarthy Tétrault LLP and is a member of the Ontario Bar. He clerked at the Federal Court, served as a policy consultant to Health Canada, was a Visiting Professor of Law at Loyola University Chicago, and the Kauffman Fellow at New York University School of Law. Matthew's research focuses on how intellectual property rights (especially patent rights) and the emphasis placed upon commercializing early-stage, publicly funded research impacts knowledge sharing, wealth distribution, and human health. He is interested in better understanding how intellectual property rights and management practices relate to other elements of an innovation system, especially regulatory frameworks, as well as how those rights and practices can shape new fields of scientific inquiry like stem cell research or new health interventions such as biopharmaceuticals and vaccines.



Robbin Lindsay, PhD

Robbin Lindsay received his Bachelor's degree from the University of Winnipeg in 1986, a Master's degree from the University of Manitoba in 1989, and his PhD from the University of Guelph in 1995. He currently is employed with the Public Health Agency of Canada at the National Microbiology Laboratory (NML) in the Zoonotic Disease and Special Pathogens section. The focus of his work is laboratory and field-based surveillance for various zoonotic disease agents including: Lyme borreliosis, hantaviruses and West Nile virus. His program balances reference diagnostic services, surveillance activities and applied research projects and ultimately the information gained from these core activities are used to better define the risk factors, geographic distribution and intervention strategies available to minimize human exposure to zoonotic disease agents.



Pauline Dakin,
Health/Medical Specialist Reporter

Pauline Dakin is a national health reporter for CBC News, and the host of the regional documentary program *Maritime Noon*. Her work has been recognized with awards from the National Science Writers Association, the Canadian Association of Journalists, The Canadian Medical Association/Canadian Nurses Association, the Registered Nurses Association of Ontario, the international Investigative Reporters and Editors, the Radio-Television News Directors Association, and the Society of Obstetricians and Gynecologists of Canada. She was also nominated for Canada's top public service journalism prize, The Michener Awards for a collaborative series on adverse drug reactions in children. She is a three-time recipient of fellowships from the National Press Foundation in Washington. Pauline sits on the boards of the Canadian Science Writers Association and the Gordon Foundation for Children and Youth. She has worked as a producer, on-air host, assignment editor and reporter in various media including film, television, radio and print. Originally from North Vancouver, B.C., she has also lived in Manitoba, New Brunswick and is now based in Halifax, Nova Scotia.



POSTER PRESENTATIONS



❖ Poster Presentations

(Presenter's name is in bold face type)

Abstract	Page
1 Sarah M. McAlpine , S. Lee, T. Hatchette, S A. Halperin "Evaluating Immunity Induced by Influenza and <i>Bordetella Pertussis</i> Vaccines Formulated Using the Depovax™ Platform"	21
2 Amanda Wisner , J. LeBlanc, T. Hatchette, M. El-Sherif, S. McNeil Molecular Detection of <i>Streptococcus pneumoniae</i> in Nasopharyngeal Swabs and Deduction of "Sero" Types by Polymerase Chain Reaction (PCR)"	22
3 Joanne Langley L. Frenette, R. Jeanfreau, L. Chu, S. McNeil, S. Halperin, M. Dramé, L.Fries, D. Vaughn (TBC) "Immunogenicity of a Heterologous H5N1 Influenza Booster Vaccine 6 or 18 Months after Primary Vaccination in Adults"	23
4 Turkiya Al-Siyabi , K. Forward, K. BinKhamis "Spice Bacteria in Blood Cultures; Ten Years Experience in a Large Tertiary Care Centre"	24
5 Rahwa Osman , M. Snider, P. Griebel "The Role of Interferon Resistance in BHV-1 Virulence"	25
6 Oren Krajden , S.Gajic, M. El-Sherif, K. Green, A. McGeer, D. MacKinnon-Cameron, C. Wang, S. McNeil "Performance Characteristics of Influenza-Like-Illness Case Definitions in Predicting Influenza in Hospitalized Adults"	26
7 Jessica Connors , S. Halperin, J. Wang "B-1 Cells Modulate Host Responses to Chlamydia Trachomatis Via IL-10 Production"	27

❖ Poster Presentations Continued...

Abstract	Page
8 Caitlyn D. Carpenter , T. E. O'Neill, K.T. Ellsworth, K. T., C.A. Gray, J.A. Johnson, D. Webster "Isolation of Anti-Micobacterial Natural Products from <i>Juniperus Communis</i> "	28
9 David Haldane , T.E. O'Neill, K.T. Ellsworth, C.A. Gray, J.A. Johnson, D. Webster, J.J. Pettipas, C. Cizik, PPHLN "Screening for Community Acquired MRSA in Nova Scotia"	29
10 Irene Ma , E. Brouwers, N.A.Thomas "Characterization of an Extended -10 Promoter for the Type III Secretion Chaperone Gene <i>cesT</i> of Enteropathogenic <i>Escherichia Coli</i> "	30
11 Ian D. Haidl , J.Marshall "Mouse Peritoneal Leukocyte Recruitment in Response to Bacterial Products and Reovirus Infection is Independent of Mast Cells, but Dependent on TLR2 and NOD2"	31
12 Liliana Portales Cervantes , I. Haidl, J.Marshall "The Regulation of Type III Interferons Production by Human Mast Cells"	32
13 Carly Schofield , S.A. Halperin, & M. Appleton "Understanding Privacy Regulation of Health Information"	33
14 Khalifa Binkhamis , T. Al-Siyabi, K. Forward "A Study of the Microbial Burden of Non-Sterile Gloves. Is it Adequate to Contribute to Blood Culture Contamination Rates?"	34
15 Maram Hulbah , L. Davey, S.Halperin, S.Lee "Auxotrophic complementation as an effective means of vaccine antigen gene delivery in <i>Streptococcus gordonii</i> "	35
16 Farhan Khan , S.Halperin, S.Lee "Anti- <i>Fusobacterium Nucleatum</i> Single-Chain Antibody Library: Construction and Adhesin Identification"	36

❖ Poster Presentations Continued...

Abstract	Page
17 Lauren Davey, S. A. Halperin, S. F. Lee "Biological Functions of the <i>Streptococcus Gordonii</i> Oxidoreductase SGBDBD"	37
18 Vanessa Meier-Stephenson, S. McNeil, A. Kew, J. Sweetapple, D. Mombourquette and K. Slayter "Vaccination of Splenectomized Adult Patients in the Capital District Health Authority—An Audit of Our Results After Implementation of a Peri-Splenectomy Program/Vaccine Kit"	38
19 Sanela Gajic, A. Ambrose, S. McNeil "Outlining Healthcare Utilization for Invasive Meningococcal Disease to Quantify Economic Burden of Disease"	39
20 Donna MacDougall, BA Halperin, D Janowitz, D MacKinnon-Cameron, J Langley, SA McNeil, S Halperin "Universal Tdap Vaccination: What Healthcare Providers Need and Want to Know"	40
21 Marcia Taylor, C. Barden, D. Byers, A. Henneberry, E. Lu, C. McMaster, D. Weaver, F. Wu "Effect of ACYL Carrier Protein Synthase Inhibitors on MRSA Metabolism"	41
22 Beth Halperin, DM MacDougall, D Janowitz, D MacKinnon-Cameron, J Langley, SA McNeil, S Halperin "Universal Tdap Vaccination: What Adults Need and Want to Know"	42
23 Marta Ciechonska, R. Duncan "Human Annexin A1 Interacts With Reovirus Fusion Associated Small Transmembrane (FAST) p14 During Cell-to-Cell Fusion"	43
24 Saima Sidik, JJR Benjamin, RJ Ryu, A. Jarra, JR Rohde " <i>Shigella flexneri</i> Virulence Plasmid (pWR100) Deletion Collection"	54

❖Poster Presentations Continued...

- 25 Michael MacNeil**, BL Johnston, P. Povah, D. haase, K. Thompson, K. Slayter "Characterization of Renal Dysfunction in a Cohort of Adult HIV-Infected Patients: A Retrospective, Observational Case-Control Study" 45
- 26 Yassein Shamout**, B. Coleman, S. Halperin, J. Langley, A. Taddio, V. Shah, A. McGeer, S. McNeil "Immunogenicity, Safety, and Preference for Self –Versus Nurse- Administered Intradermal Influenza Vaccine" 46
- 27 Mary Halpine**, J. Salmon, L. Frechette "Preliminary assessment of needs for a refugee and immigrant clinic in Saint John, New Brunswick" 47
- 28 Taryn E O'Neill**, D. Webster, J.A. Johnson, C.A. Gray "Optimization of the Microplate Resazurin Assay as a Screening tool for Natural Products with Anti-Tuberculosis Activity" 48
- 29 Rachelle Blackman**, S. El-Bailey, S. Hull, D. Webster "Potential Pathogens Cultured from Healthcare Worker Pagers, Cell Phones, Stethoscopes, Laboratory Coats and Identification Cards on a Medical Ward in New Brunswick: Analysis of a Brief Infection Control Education Program" 49
- 30 Shelly McNeil**, A. McGeer, K. Green, A. Ambrose, J. Johnstone, T. Hatchette, J. Leblanc, D. Webster, M. Loeb, A. McCarthy, G. Boivin, S. Trottier, H. Wang, D. MacKinnon-Cameron, M. ElSherif, S. Halperin, J. Langley, M. Andrew, L. Valiquette, G. Stiver, T. Marrie "Seasonal Versus Pandemic Vaccine Effectiveness in Prevention of Influenza-Related Hospitalization in Canadian Adults" 60
- 31 Cory Stevens**, S. El-Bailey, H. MacKenzie, D. Webster "Utilization of the Interferon-Gamma Release Assay (IGRA) in Maritime Canada: A Quality Assurance Project"



ORAL PRESENTATIONS



❖ Oral Presentations

Presenter's name in **bold**

		abstract	page
9:30-9:45	H. B. Parmar , C. Barry, F.B. Kai, R. Dunca "Directed Trafficking Through the ER/Golgi Pathway to the Plasma Membrane by a Novel Polybasic Motif in the Reovirus p14 FAST Protein"	30	50
9:45-10:00	H. Abdelhady , Emma-Jean Slobodesky, Jungmin Kim, Rafael Garduño "Characterization of <i>Legionella</i> <i>Pneumophila</i> Mature Infectious Forms (MIFs) From Different Hosts"	31	51
10:00-10:15	M. Graven , P. Allen, I. Smith, N. MacDonald "Major Country-Wide Reduction in Mortality with the Belize Health Information System (BHIS) with Program Management"	32	52
10:15-10:30	R. Junkins , A. MacNeil, S. Carrigan, C. McCormick, Tong-Jun-Lin "A Novel Role for the Down Syndrome Critical Region Gene RCAN1 During <i>P. aeruginosa</i> Lung Infection"	33	53
10:30-10:45	Todd F Hatchette , JJ. LeBlanc, C. Roberts, DeLong, J. MacDonald, C. Heinstein "Detection of Neisseria Gonorrhoea Using Viper XTR; Confirmation is Necessary to Avoid False Positive Results"	34	44

10:45-11:00	Break		
11:00-11:15	H. Li , T. O'Neill, D. Webster, J. Johnson, C. Gray "Anti-TB Natural Products from <i>Aralia nudicaulis</i> "	35	55
11:15-11:30	C. Van Zoost , A. Ghaly, P. Morrison, K. Babb, H. Flemming, S. McNeil "Vaccination Rates of Halifax's Homeless: Are the Barriers to Vaccine Delivery and Uptake?"	36	56
11:30-11:45	J. Rosenroll , A. Stadnyk, J. Wang "The Role of Early IL-17A Production in Regulating Host Resistance Against <i>Chlamydia</i> Infection"	37	57
11:45-12:00	M. Matheson-Orchard , L. Frechette, J. Salmon "Intestinal Parasitic Infection in Bhutanese Refugees Resettled in Saint John, New Brunswick in 2010"	38	58
12:00-12:15	J.J. LeBlanc , T. Hatchette, C. Heinstein "Monitoring for PCR Inhibition Following Homogenization ANS Heat Treatment"	39	59



ABSTRACTS



❖❖1. EVALUATING IMMUNITY INDUCED BY INFLUENZA AND *BORDETELLA PERTUSSIS* VACCINES FORMULATED USING THE DEPOVAX™ PLATFORM

S. McAlpine, S.Lee, T.Hatchette, S.Halperin

Introduction: *Bordetella pertussis* (Bp) and influenza A virus (IAV) cause morbidity and mortality, particularly in the young and elderly. While current vaccines protect against these pathogens, high IAV mutation rates prompting re-formulation each year and waning immunity to Bp highlight the need for more effective vaccines. DepoVax™, can enhance antigen uptake and drive Th1 immunity, and is currently in clinical trials as a cancer vaccine. We aim to understand how DepoVax™ may protect from Bp and IAV.

Methods: Mice were vaccinated i.m. with DepoVax™ vaccine containing either heat-inactivated IAV strain PR8 or Bp antigens, or with saline or standard IAV or Bp vaccines. Antigen-specific antibodies in sera were quantified by ELISA and hemagglutination inhibition assays. Cellular immunity was assessed by antigen restimulation of splenocytes followed by analysis of the production of the Th1 cytokine IFN- γ and the Th2 cytokine IL-13 by ELISA. A subset of mice were challenged on day 29 with the appropriate pathogen (PR8 or Bp) and monitored for clinical score and survival.

Results: Vaccination of mice with DepoVax™ vaccines induced significantly higher antigen-specific antibody titres compared to vaccination with standard IAV or Bp vaccines. Antigen restimulation of splenocytes from mice vaccinated with DepoVax™-Bp demonstrated strong IFN- γ production with little IL-13 compared to splenocytes from mice vaccinated with currently-licensed DTaP. Upon challenge with a lethal dose of infectious IAV, mice vaccinated with a single dose of DepoVax™-IAV were protected from infection.

Conclusions: Vaccines formulated with the DepoVax™ platform can induce improved immunity to IAV and Bp that result in balanced Th1 and Th1 immune responses in mouse models of lung infection. These preliminary findings may ultimately lead to the introduction of new vaccines in the clinic that prevent influenza and pertussis.

❖❖2. MOLECULAR DETECTION OF STREPTOCOCCUS PNEUMONIAE IN NASOPHARYNGEAL SWABS AND DEDUCTION OF "SERO" TYPES BY POLYMERASE CHAIN REACTION (PCR)

A. Wisner, J. LeBlanc, T. Hatchette, M. El-Sherif, S. McNeil

Introduction: The major virulence factor in *S. pneumoniae* is the capsular polysaccharide, upon which serotyping is based. Today, 93 different serotypes, belonging to 46 serogroups, have been described. The traditional method for serotype determination (Quellung reaction) is technically and financially challenging; however, PCR-based "sero"typing represents a simple, economic and promising alternative method. The goals of this study were two-fold: 1) evaluate the PCR-based typing method developed by the Centers for Disease Control and Prevention (CDC); and 2) develop a screening method using real-time PCR for the detection of *S. pneumoniae* from nasopharyngeal swabs collected for respiratory testing.

Methods: The CDC protocol for the multiplex assay is composed of 9 different PCR reactions which enable the detection of the most predominant *S. pneumoniae* serogroups causing invasive disease. For verification, the assay was tested using DNA extracted from a collection of *S. pneumoniae* isolates previously characterized by serotyping using the Quellung reaction (as part of the IMPACT study). To ensure specificity, DNA from each isolate was tested against all PCR multiplex reactions. Secondly, the DNA from each isolate was subjected to real-time PCR analysis using two different gene targets: *lytA* (autolysin) and *cpsA* (conserved capsule region). The analytical sensitivity of the two real-time PCR assays was determined using serial dilutions of an *S. pneumoniae* ATCC strain and its utility to detect the pathogen from nasopharyngeal swabs was validated using clinical specimens with or without invasive pneumococcal disease.

Results: During the experimental validation of the molecular typing assays, identification of each serotype was accurate and no cross-reactivity was observed. As for the real-time PCR screening assays, both targets were able to detect *S. pneumoniae* in several nasopharyngeal swabs from patients with invasive pneumococcal disease; however, the *cpsA* target was by far more sensitive.

Conclusion: The CDC multiplex is an accurate and reliable method which can be used in place of the conventional serotyping methods for *S. pneumoniae*. Furthermore, the ability to detect and "sero" type *S. pneumoniae* from nasopharyngeal swabs provides the framework for epidemiologic studies where serotype distribution could be monitored prior to and following implementation of pneumococcal vaccine programs.

❖❖3. IMMUNOGENICITY OF A HETEROLOGOUS H5N1 INFLUENZA BOOSTER VACCINE 6 OR 18 MONTHS AFTER PRIMARY VACCINATION IN ADULTS

J. Langley, L. Frenette, R. Jeanfreau, L. Chu, S. McNeil, S. Halperin, M. Dramé, L.Fries, D. Vaughn (TBC)

Introduction: One of the influenza pandemic preparedness strategies involves priming a population with a pre-pandemic subtype-specific vaccine, followed by boosting with a strain-matched vaccine during the pandemic.

Methods: In this phase II, observer-blind, multicenter trial, 841 adults aged ≥ 18 years were randomized to 7 groups to receive a single priming dose with a subclade 2.1 A/Indonesia/5/2005 (H5N1) vaccine (3.75 μg or 7.5 μg hemagglutinin antigen [HA]) or placebo (P) at Day 0 (D0), followed 6 or 18 months later (M6 or M18) with a subclade 2.2 (A/turkey/Turkey/1/05) H5N1 booster vaccine or P (table). Vaccines were adjuvanted with AS03_A or AS03_B (α -tocopherol/squalene o/w emulsion [11.9 mg or 5.9 mg tocopherol, respectively]). A/turkey/Turkey/1/05 HI antibodies were measured before administration, and at 10 and 42 days after the priming and booster vaccinations. Solicited and unsolicited adverse events (AEs) were collected for 7 and 42 days, respectively, after each dose. Serious AEs (SAEs) were collected during the reported study period (D0 to M18).

Results: 10 days after the M18 injection, the lower limit of the 97.5% confidence interval for the difference in seroconversion rates (SCRs) and adjusted geometric mean antibody titer (GMT) ratios between primed (A–F) and H5N1-naïve subjects (G) was $>15\%$ and >2.0 , respectively (primary endpoint based on hemagglutination inhibition antibody). SCRs/GMTs were higher in primed subjects, compared with H5N1-naïve subjects for subjects boosted at M6 and M18 (Table). There were no differences between groups in terms of solicited or unsolicited AEs or SAEs.

Conclusions: All adjuvant and antigen combinations tested conferred significant priming, and H5N1-subtype specific priming persisted for 18 months. All vaccines had a clinically acceptable safety profile.

❖❖4. SPICE BACTERIA IN BLOOD CULTURES; TEN YEARS EXPERIENCE IN A LARGE TERTIARY CARE CENTRE

T. Al-Siyabi, K. Forward, K. BinKhamis

Introduction: *Serratia sp.*, *Providencia sp.*, indole positive *Proteus sp.*, *Citrobacter sp.*, and *Enterobacter sp.* (SPICE) all possess inducible *ampC* beta-lactamase genes. Stable derepression and subsequent clonal spread may be promoted by overuse of extended-spectrum penicillins and cephalosporins. We reviewed ten years of blood culture results to determine whether SPICE bacteria represent a growing proportion of isolates.

Methods: Retrospective analysis of our existing microbiology database was performed between January 2001 and December 2011. We reviewed the total number of positive blood cultures and looked into the proportion of SPICE isolated relative to members of enterobacteriaceae during the ten year period.

Results: Between 2001 and 2011, we had total of 129,029 blood cultures, 3399 (2.6 %) of which were isolates of different members of the enterobacteriaceae. Overall, the total number of SPICE bacteria isolated was 819 (0.63%). A slight increase was noted in the total number of enterobacteriaceae isolated over the study period (263 in 2001 to 361 in 2011) but not in the percent positive. The proportion of SPICE isolated relative to enterobacteriaceae did not significantly change over the study period (21.7% in 2001 to 19.7% in 2011).

Conclusions: SPICE bacteria are important nosocomial pathogens responsible for various infections, including bacteremia, which can lead to significant morbidity and mortality. Although one would expect these isolates might continue to grow and spread with the extensive use of extended-spectrum penicillins and cephalosporins, our data showed that over the last decade SPICE bacteria did not cause bacteremia at increasing rates.

❖❖5. THE ROLE OF INTERFERON RESISTANCE IN BHV-1 VIRULENCE

R. Osman, M. Snider, P. Griebel

Introduction:

Evasion of the innate immune system is important for viral replication during a primary infection. An important antiviral response by the innate immune system is the production of interferon (IFN), including IFN- α and IFN- γ . IFN binds receptors on uninfected cells and induces antiviral effector molecules that inhibit viral replication. Bovine herpes virus -1 (BHV-1) is a potent inducer of IFN responses but IFN has little effect on BHV-1 replication. Therefore, we hypothesized that BHV-1 virulence is linked to evasion of IFN-induced anti-viral effector responses. To test this hypothesis, BHV-1 isolates that varied in virulence were screened to determine if they varied in IFN resistance. Virus was also plaque-purified from individual BHV-1 isolates to determine if there was significant variation in IFN-resistance within a single viral population.

Methods:

Vesicular Stomatitis virus (VSV) was used as a positive control for IFN sensitivity and Immunohistochemistry was used to detect Infectious foci in a plaque-inhibition assay using bovine epithelial cells pre-treated with either recombinant bovine IFN- α and - γ .

Results:

When three BHV-1 isolates, P8-2, 108 and Cooper strain, were compared to VSV we observed that all BHV-1 isolates were 100 to 1000 times more resistance to IFN inhibition than VSV. Furthermore, there were significant differences in resistance to both IFN α and γ when comparing BHV-1 isolates. Plaque purification of virus from Cooper and 108 isolates also revealed significant variation in IFN γ resistance within each isolate.

Conclusions:

These observations support the conclusion that there is significant variation in resistance to IFN-induced anti-viral effector mechanism both within and among BHV-1 isolates. Future work will use isogenic strains of BHV-1 to investigate the relationship between interferon resistance.

❖❖6. PERFORMANCE CHARACTERISTICS OF INFLUENZA-LIKE-ILLNESS CASE DEFINITIONS IN PREDICTING INFLUENZA IN HOSPITALIZED ADULTS

O. Kraiden, S.Gajic, M. El-Sherif, K. Green, A. McGeer, D. MacKinnon-Cameron, C. Wang, S. McNeil

Introduction: Influenza-like-illness (ILI) can be caused by a variety of respiratory viral infections, including influenza. While many cases of ILI are not caused by influenza, ILI activity in the community is a useful surrogate for influenza activity. Several case definitions of ILI exist. We examine the performance characteristics of the PHAC and CDC ILI case definitions for predicting influenza among adults hospitalized with respiratory disease in Canada.

Methods: The Public Health Agency of Canada/Canadian Institutes of Health Research (PCIRN) Serious Outcomes Surveillance (SOS) Network comprises 8 adult hospitals and ~5500 beds in 6 provinces. We conducted active surveillance for influenza during the 2009/10 and 2010/11 influenza seasons. Presenting signs and symptoms were recorded for all patients tested for influenza and test-negative controls.

Results: In 2009/10, 373 cases of influenza (pH1N1) were enrolled; in 2011/12 319 cases of seasonal influenza were enrolled. The mean age of cases was 45.9yrs (range 16-87) in 2009/10 and 68.7yrs (18-99) in 2010/11. Overall, 128 (34.3%) of patients with pandemic influenza and 82 (25.7%) of those with seasonal influenza met the Public Health Agency of Canada (PHAC) ILI case definition (fever + cough + one or more of sore throat, athralgia, myalgia, prostration), respectively. Overall, 210 (30.3%) of patients with PCR-confirmed influenza met the PHAC ILI case definition. Thus, the PHAC ILI case definition has a positive predictive value (PPV) of 67% and a negative predictive value (NPV) of 57% in the diagnosis of influenza in hospitalized adults. Although the PPV is somewhat better in patients <65yrs than those over 65yrs (69% vs 64%), the NPV is lower in younger adults than older adults (52% vs 63%).

Conclusions: The PHAC ILI case definition is not sufficiently sensitive nor specific to predict influenza in adults hospitalized with respiratory illness. Given a NPV of only 57%, use of the ILI case definition to guide testing, infection control measures or treatment of hospitalized adults is inappropriate and may lead to nosocomial transmission or outbreaks when patients with influenza are not appropriately isolated. During the influenza season, all patients admitted with respiratory disease should be tested for influenza and isolated until test results become available.

❖❖7. B-1 CELLS MODULATE HOST RESPONSES TO CHLAMYDIA TRACHOMATIS VIA IL-10 PRODUCTION

J. Connors, S. Halperin, J. Wang

Introduction: *Chlamydia trachomatis* is an intracellular bacteria that primarily infects mucosal epithelial cells lining the ocular, respiratory and urogenital tract causing a variety of human and animal disease. Despite major efforts, there is currently no vaccine against *Chlamydia*. The development of an effective antichlamydial vaccine requires complete understanding of how optimum protective immunity to *Chlamydia* is generated. T helper type 1 (Th1) responses, particularly interferon- γ (IFN- γ) production, are critical to host protection against *Chlamydia* whereas production of interleukin-10 (IL-10) during the immune response is believed to dampen Th1 induction and impair host resistance. Recent work has shown B cells to be an important source of IL-10 in multiple models of inflammatory and infectious disease but the role of B cell-derived IL-10 in *Chlamydia* infection is unknown.

Methods: IL-10-producing B cells were investigated in a mouse model *C. trachomatis* infection using the mouse-specific strain *C. muridarum* (*Cm*). The regulatory role of IL-10-producing B cells on Th1 induction was assessed via *in vitro* co-culture with activated T cells and *in vivo* during pulmonary *Cm* infection.

Results: We have identified B cells, in particular the B-1 subset, as a major cellular source of IL-10 produced during *Chlamydia* infection. *Cm*-stimulated B-1 cells potently produce IL-10 capable of suppressing T cell proliferation and IFN- γ production *in vitro*. During pulmonary *Cm* infection, B-1 cells accumulate in the lung and local draining lymph node. Adoptive transfer of wild-type, but not IL-10-deficient, B-1 cells into B cell-deficient mice dampens host resistance to pulmonary *Cm* infection.

Conclusions: Our data indicate that B-1 cells are an important and previously unappreciated source of counter-regulatory IL-10 produced during *Chlamydia* infection. Modulating IL-10 produced by B-1 cells during immunization may be an important consideration in antichlamydial vaccine design.

❖❖8. ISOLATION OF ANTI-MYCOBACTERIAL NATURAL PRODUCTS FROM *JUNIPERUS COMMUNIS*

C. Carpenter, T.E. O'Neill, K.T. Ellsworth, C.A. Gray, J.A. Johnson, D. Webster

Introduction: *Juniperus communis* has been used in treating consumption by the First Nations of the Canadian Maritimes, which made it an attractive plant to investigate for compounds with anti-mycobacterial properties. The objective of this study was to identify if there are natural products within *J. communis* that show activity against tuberculosis by investigating both the plant material (needles and braches) and the endophytic fungi associated with the plant.

Methods: Using the bioassay guided fractionation process on the plant material; compounds will be isolated that are active against *Mycobacterium tuberculosis* strain H37Ra using the microplate resazurin assay. Solvent partitioning, silica column chromatography and high performance liquid chromatography will be used to fractionate the crude extract. The molecular structure of active compounds will be determined through application of standard spectroscopic techniques. The endophytes that have been extracted from the needles will be tested using the microplate resazurin assay as well to identify any potential endophytes that contain anti-mycobacterial compounds.

Results: Isocupressic acid, communic acid, and deoxypodophyllotoxin were isolated from the *J. communis* methanolic extract of the needles and branches. Isocupressic acid and communic acid displayed MICs of 78 μM and 31 μM and IC₅₀s of 45.7 μM and 15.0 μM respectively. The MICs and IC₅₀s for deoxypodophyllotoxin are currently being determined.

Conclusions: Isocupressic acid, communic acid and deoxypodophyllotoxin were identified to be the major compounds within *J. communis* that were responsible for the anti-mycobacterial activity of the plant material. Further work will be done to determine if the endophytes are active, and if the endophytes are producing the same compounds that were isolated from the plant material.

❖❖9. SCREENING FOR COMMUNITY ACQUIRED MRSA IN NOVA SCOTIA

D.J.M. Haldane, J.J. Pettipas, C. Cizik and the PPHLN

Introduction: Community acquired methicillin resistant *Staphylococcus aureus* (caMRSA) is associated with skin and soft tissue infections and increased virulence. It has become endemic throughout Canada and has caused outbreaks of infection. Two strains, designated caMRSA10 and caMRSA7 (corresponding to USA300 and USA400) cause community acquired MRSA infections. Previous studies on selected populations have indicated that these isolates have become prevalent in Nova Scotia. The proportion of caMRSA amongst MRSA in the province was determined in a consecutive sample, and the strain found locally determined.

Methods: District health authority laboratories were asked to send all of their isolates of MRSA that were cultured during the months of November to January 2010/2011. Isolates were cultured from clinical samples or from screening swabs for MRSA. Screening was done using chromogenic media (Demin Blue, Oxoid, Napean ON) Isolates were stored at -70C after receipt. They were confirmed as MRSA using PCR for the *mecA* gene, *pvl* gene PCR (panton valentine leukocidin toxin gene) was used to screen isolates as possible caMRSA. A subset of isolates underwent pulsed field gel electrophoresis to determine if their patterns corresponded to caMRSA7 or caMRSA10.

Results: 110 isolates were received from CDHA. 108 were confirmed as MRSA. 40 were from clinical specimens and 68 from screens. The clinical specimens included 23 superficial sites, 8 respiratory, 4 urinary, 2 from blood and 1 from bone. 18 isolates were PVL positive (isolated from 10 superficial sites, 6 screens, and 1 urine and 1 respiratory). Of these 18, 11 had PFGE and all resembled caMRSA10 (USA300). 106 isolates were received from DHA 3 to 8. Of these 85 were clinical specimen isolates and 19 from screens (2 unknown). 28 of the 106 were positive for PVL (26.4%). 26 of 28 were from clinical specimens and 1 from a screen, and 1 unknown

Conclusions: caMRSA 10 is established locally. 16.66% isolates were screen positive for caMRSA in CDHA and 26% screen positive in the rest of the province. Further typing of isolates would be useful to determine if caMRSA7 is present in other parts of the province or at lower levels of endemicity. Confirmation of the numbers of MRSA isolated may be useful to determine if this sample represents a subset of the MRSA isolated over this period, and whether isolates detected on screening are underrepresented.

❖❖10. CHARACTERIZATION OF AN EXTENDED -10 PROMOTER FOR THE TYPE III SECRETION CHAPERONE GENE *cesT* OF ENTEROPATHOGENIC *ESCHERICHIA COLI*.

I. Ma, E. Brouwers, N.A.Thomas

Introduction: Our lab studies the multivalent chaperone, CesT, which is a significant contributor to Enteropathogenic *Escherichia coli* (EPEC) pathogenesis. *cesT* is part of the locus of enterocyte effacement (LEE) – 5 operon. Since the exact role of the *cesT* promoter (*cesTp*) is not known, we functionally characterized the *cesTp* in this study.

Methods: Luciferase reporter assays were used to measure promoter activity, where end point and real-time luciferase reporter assays were performed with DH5 α or EPEC strains harbouring *luxCDABE* plasmid constructs. HT-29 colonic epithelial cells were infected with various EPEC strains at a multiplicity of infection of 50 for 3 hours. Finally, precipitated proteins were subjected to SDS-PAGE for the time course *in vitro* secretion assay.

Results: By creating mutations that altered *cesTp* activity, it was confirmed that *cesTp* has features that are consistent with $\sigma 70$ promoters containing an extended -10 element. In stark contrast to the *LEE2-5* transcriptional operons, *cesTp* did not require Ler for transcriptional activity, which was confirmed by *lux* assays. Furthermore, *cesTp* activity was not dependent on the presence of GrlA or GrlR, which are two regulators associated with *LEE* gene expression. A *cesTp*-*lux* fusion was used in a real-time assay that demonstrated that *cesTp* is initially activated before the *LEE5* promoter activation, when EPEC was grown under conditions that support *LEE* gene expression. *cesTp* was also shown to be active during *in vitro* infection of HT-29 cells. Inactivation of *cesTp* reduced CesT protein levels at early growth time points.

Conclusions: Unlike the *LEE5* promoter that requires Ler for transcriptional activity our results show that *cesTp* does not require Ler, which indicates a different and temporal regulatory mechanism for *cesT* gene expression. We suggest that attaching and effacing pathogenic *E. coli* have evolved a mechanism to ready the cell for early CesT protein expression in support of infection prior to Ler and GrlA activity.

❖❖11. MOUSE PERITONEAL LEUKOCYTE RECRUITMENT IN RESPONSE TO BACTERIAL PRODUCTS AND REOVIRUS INFECTION IS INDEPENDENT OF MAST CELLS, BUT DEPENDENT ON TLR2 AND NOD2

I. D. Haidl and J. S. Marshall

Introduction: Mast cells use pattern recognition receptors to respond to bacterial or viral stimuli. This response produces cytokines and chemokines that mediate the *in vitro* chemotaxis of multiple cell types including human NK cells and CD56+ve T cells. We have utilized a mouse model system to study the *in vivo* role of mast cells and the pattern recognition receptors TLR2 and NOD2 in leukocyte recruitment in response to bacterial products and virus infection.

Methods: Mice were injected intraperitoneally with peptidoglycan (PGN), FSL-1 (a bacterial lipopeptide), or reovirus (serotype 3 Dearing). Strains of mice utilized included wild-type C57BL/6, mast cell deficient W^{sh} , TLR2 deficient, NOD2 deficient, and mice lacking both TLR2 and NOD2. Following 4, 16, or 24 hours, the peritoneal contents were harvested. The lavage fluid was analyzed for the presence of cytokines and chemokines by ELISA and protein arrays. Peritoneal cells were analyzed by flow cytometry for neutrophils, macrophages, eosinophils, CD4⁺ and CD8⁺ T cells, B1 and B2 B cells, and NK cells.

Results: PGN and FSL-1 induced recruitment of eosinophils, NK cells, and neutrophils in a mast cell independent manner. Leukocyte recruitment in response to PGN was reduced in the absence of TLR2 and NOD2 in combination, whereas FSL-1 activation was strictly dependent on TLR2. TLR2 activation resulted in the production of cytokines/chemokines including CXCL13 and CCL9. The recruitment and activation of leukocytes in response to reovirus infection was not mast cell dependent and not dependent on TLR2 or NOD2 alone. However, some chemokine responses, such as CCL9, were decreased in mice lacking both TLR2 and NOD2.

Conclusion: Leukocyte recruitment *in vivo* in response to PGN, FSL-1, and reovirus is not dependent on mast cells. Since PGN and FSL-1 induce eosinophil and NK cell recruitment, these cell types may play a role in bacterial clearance. The reduced level of chemokines in reovirus infected mice lacking TLR2 and NOD2, suggests a regulatory role for these pattern recognition receptors in viral infection.

❖❖12. THE REGULATION OF TYPE III INTERFERONS PRODUCTION BY HUMAN MAST CELLS

L. Portales-Cervantes, I. D. Haidl, J. S. Marshall

Introduction: Mast cells (MCs) can be infected by several viruses, producing cytokines and chemokines including interferons (IFNs). Type III IFNs (IL-29, IL-28A, IL-28B) have similar antiviral functions to Type I IFNs, but their target cells are limited to those that express the IL28R α /IL10R β receptor complex. MCs could contribute to IFN regulation either through recruiting plasmacytoid dendritic cells, recognized as a major source of Type III IFNs, or by direct production at sites of viral infection. MC can also recruit NK cells, a potent source of IFN- γ which may enhance Type III IFN production. The purpose of this project is to elucidate the mechanisms whereby human MCs may regulate local Type III IFN responses during viral infection.

Methods: Cord blood-derived mast cells (CBMC), human MC line (HMC-1), and the basophil/MC line KU812 were used. MCs were infected with Reovirus at 20 MOI; 24 h post infection, cell-free supernatants were harvested and the production of type III IFNs was analyzed by ELISA. Total RNA was extracted from cells to determine the induction of Type III IFNs by quantitative PCR. The expression of the type III IFN receptor was determined by flow cytometry.

Results: The expression of type III IFNs mRNA was induced in MCs after reovirus infection. However, CBMC produced only IL-29 at the protein level while HMC-1 produced both IL-29 and IL-28B. KU812 cells, differentiated with IFN- γ into a more mast cell-like phenotype in culture, showed a higher production of IL-29. Furthermore, we found that HMC-1 and KU812 cells expressed high levels of IL10R β (>80%) but IL-28R α expression was low (< 1-2% of cells).

Conclusions: Expression of Type III IFN genes was enhanced in the MCs studied after reovirus infection. IL-29 protein was produced by CBMC, KU812 and HMC-1 while IL-28B was produced only by HMC-1 cells. At sites of infection MCs could serve as a source of IL-29 that would induce antiviral responses in neighboring cells. Our results indicate that IFN- γ may enhance the production of type III IFNs, specifically IL-29. At sites of active viral infection, NK cells, which can be recruited by MCs, would be an important source of IFN- γ suggesting further possible interaction between these cell populations.

❖❖13. UNDERSTANDING PRIVACY REGULATION OF HEALTH INFORMATION

C. Schofield, S.A. Halperin, & M. Appleton

Introduction: Given the amount of health research conducted within Canada, there is a growing relationship between the availability of health data and the right and regulation to access such information. In an attempt to understand the research implications for the application of privacy legislation to health information, current studies have examined the relationship between privacy legislation and the interpretations made by decision making bodies. This literature review aims to recognize the current practices associated with health data retention in Canada and to uncover whether variability exists among privacy review officers and its effects on the access to health data needed for population health research. Since the sharing and access of health data are controlled by privacy officials, this paper aims to examine whether the current public perceptions available are similar to those of the legislation or whether these would support an opportunity to control health information in a more systematic method. The literature review was prepared to inform the design of a prospective study to further elucidate differences in interpretation of privacy laws, and public perceptions and preferences.

Methods: Keyword searches were conducted on MEDLINE, and EBSCOhost databases for relevant peer-reviewed literature. Additional searches were completed through national and provincial government databases for legislation and policy documentation.

Results: It is evident within the literature that there is a high degree of variability among privacy review officers, leading to inconsistencies and uncertainties around appropriate legislative regulation. These inconsistencies are creating significant implications for researchers requiring access to health data for population health research. As well, public perceptions around health data sharing have been examined, and there is evidence of perceived variation in privacy regulation for different types of health information.

Conclusion: In order to effectively track and monitor vaccination rates and uptake, data sharing policies must be more fluid to allow for information sharing. It is suggested that a more systematic method for health data sharing could benefit such health issues. By creating a tiered level of privacy regulation for health information, immunization data could be extracted to a lower privacy standard to enhance record tracking and sharing among healthcare providers and public health bodies.

❖❖14. A STUDY OF THE MICROBIAL BURDEN OF NON-STERILE GLOVES. IS IT ADEQUATE TO CONTRIBUTE TO BLOOD CULTURE CONTAMINATION RATES?

K. Binkhamis, T. Alsiyabi, K. Forward

Introduction: Blood culture contamination can lead to unnecessary antibiotic use, prolonged inpatient stay and increased health care costs. A recent study (Kim et al. Ann Int Med. 2011;154:145-151) suggested that the use of sterile gloves can reduce blood culture contamination rates. The objective of this study was to determine the frequency of contamination of non-sterile gloves in use in our facility.

Methods: We studied gloves (Medline Advantage, Accutouch Chemo and Sencare Nitrile) from Medline Industries, Inc. Mundelein, Illinois. 110 gloves were sampled. After hand sanitization was performed, the top glove from previously opened packages was donned and each of the digits was applied to the surface of a trypticase soy blood agar plate. Plates were incubated for 48hrs at 35°C in ambient air. Colony forming units (CFUs) were counted; representative colonies of different morphotypes were gram stained and identified.

Results: In all, 45% of gloves were culture negative. The average number of CFUs on the 60 positive gloves was 2.17 per glove. The average number of CFUs on nitrile gloves was not different from that on the polyvinyl chloride (PVC) gloves (0.95 vs 1.2). 59 of 97 morphotypes (60%) were gram positive rods. 9/9 of those were identified as *Bacillus* sp.; 34 of 97 (35%) of the colonies were gram positive cocci. Those that were further identified were coagulase negative staphylococci. Four isolates were Gram negative rods.

Conclusions: Although more than half of the sample gloves had bacterial growth, the average number of colonies was very low. There were no significant differences between the types of gloves sampled. The very low microbial burden on these gloves makes them an implausible source of blood culture contamination.

❖❖15. AUXOTROPHIC COMPLEMENTATION AS AN EFFECTIVE MEANS OF VACCINE ANTIGEN GENE DELIVERY IN *STREPTOCOCCUS GORDONII*

M. J. Hulbah, L. Davey, S.A. Halperin , S. Lee

Introduction: Delivery of vaccine antigens by live bacterial carriers has the potential of eliciting both mucosal and systemic immune responses. *Streptococcus gordonii*, a commensal oral bacterium, is considered as a good candidate for a live oral vaccine vector. The introduction of vaccine antigen genes into *S. gordonii* relies on the use of antibiotic resistance genes as selectable markers, which is undesirable. In this study, we used auxotrophic complementation (deletion of an essential gene from the chromosome and insertion into a plasmid) as a means to create an antibiotic marker-free gene delivery system in *S. gordonii*.

Methods: *thyA* gene, encoding for thymidylate synthase, is an essential gene for the synthesis of DNA precursors. *S. gordonii* Δ *thyA* was created by inserting the *ermAM* gene into *thyA* and the *ermAM* was subsequently deleted using the *cre/loxP* system. The *thyA* mutation was complemented by cloning *thyA* onto pDL276. The kanamycin resistance gene was then removed from pDL276/*thyA* to obtain an antibiotic markerless expression plasmid, pDL276/*thyAdelkan*.

Results: *S. gordonii* Δ *thyA* is a thymidine auxotroph and only grew in media supplemented with thymidine. The *ermAM* cassette was successfully deleted from the *thyA* mutant which showed sensitivity to erythromycin. The resulting markerless *thyA* mutant when transformed with pDL276/*thyAdelkan* gave an unexpected increase in transformation efficiency as compared to pDL276. The pDL276/*thyAdelkan* transformants arose from single and double crossing over. The increase in transformation efficiency suggests that a highly efficient antibiotic marker-free system to deliver genes to the chromosome has been created using *thyA* complementation.

Conclusions: An efficient antibiotic marker-free delivery system has been successfully created for testing vaccine antigen gene delivery and expression in *S. gordonii*.

❖❖16. ANTI-FUSOBACTERIUM NUCLEATUM SINGLE-CHAIN ANTIBODY LIBRARY: CONSTRUCTION AND ADHESIN IDENTIFICATION

F. M. Khan S. Halperin, and S. Lee

Introduction: Dental plaque forms through a sequential process of bacterial accretion. A key bridge organism capable of coaggregating with both early and late colonizers is *Fusobacterium nucleatum*. However, the lack of an effective tool to identify *F. nucleatum* adhesins has limited the study of bacterial coaggregation. We hypothesize that a single-chain variable fragment (scFv) antibodies library will enable the identification of *F. nucleatum* adhesins and help elucidate the molecular mechanism of coaggregation between *F. nucleatum* and other bacteria.

Methods: scFv M13 phage display library was created using spleen RNA from a mouse immunized with *F. nucleatum*. The library was enriched by biopanning against *F. nucleatum* and individual clones were analyzed by ELISA to identify *F. nucleatum* specific scFvs. scFvs that inhibit *Streptococcus sanguinus* interaction with *F. nucleatum* were identified by coaggregation assays. Selected scFvs were analyzed by Western blotting against *F. nucleatum* outer membrane proteins, *Bst*OI restriction analysis and DNA sequencing.

Results: The library consisted of 4×10^8 clones and was enriched by biopanning 6 times. All 292 individual clones tested reacted strongly to *F. nucleatum* by ELISA. Sixty-two of the 292 clones inhibited *F. nucleatum* interaction with *S. sanguinis*. The 62 scFvs were further grouped into 5 categories based on reactivity with *F. nucleatum* outer membrane proteins by Western blotting. Analysis of 11 representative clones from the 5 groups revealed differences in coaggregation inhibition, recognition of outer membrane proteins, and *Bst*OI restriction pattern. DNA sequencing showed 6 unique scFvs and of them 3 strongly inhibited interaction with 5 *Streptococcus* species.

Conclusions: A phage display scFv library against *F. nucleatum* was successfully constructed and from it several scFvs that inhibit coaggregation were identified, opening the door to the identification of *F. nucleatum* adhesins involved in coaggregation.

❖❖17. BIOLOGICAL FUNCTIONS OF THE *STREPTOCOCCUS GORDONII* OXIDOREDUCTASE SGBDBD

L. E. Davey, S. A. Halperin, S. F. Lee

Introduction: Disulphide bonds are important for the activity and stability of many extracellular proteins, including bacterial virulence factors. However, information about disulphide bond formation in Gram-positive bacteria is extremely limited. We used the Gram-positive commensal bacterium *Streptococcus gordonii*, a vaccine delivery candidate, as a model organism to investigate disulphide bond formation by a predicted thiol-disulfide oxidoreductase, SgBdbD.

Methods: Experiments were carried out in *S. gordonii* SecCR1, which secretes a single chain variable fragment antibody that contains two disulphide bonds (anti-CR1 scFv). A SgBdbD deficient mutant was constructed and disulphide bond formation in anti-CR1 scFv was tested by reaction with maleimide-PEG-biotin and Western blotting. SgBdbD mutants were analyzed for biofilm formation, genetic competence, copper sensitivity, and extracellular DNA release. Potential substrates of SgBdbD were identified using an *in silico* approach.

Results: Anti-CR1 scFv produced in a SgBdbD mutant lacked disulphide bonds, supporting our hypothesis that SgBdbD is an oxidoreductase. The mutants showed enhanced biofilm formation ($p < 0.05$), a 12-fold increase in genetic competence, sensitivity to copper, impaired autolysis, and failed to release DNA. In addition, the extracellular protein profile of the mutant differed markedly from the parent. Complementation with a functional *sgbdbD* gene eliminated the mutant phenotype. To understand how SgBdbD produces these effects, we sought to identify natural substrates of the enzyme. An *in silico* screen identified 38 potential substrates, including the major autolysin AtIS. Analysis of AtIS revealed that it was non-functional in the SgBdbD mutant, and reaction with maleimide-PEG-biotin showed that AtIS in the mutant lacked a disulphide bond that was present in the parent. This indicates that AtIS is a substrate of SgBdbD, and validates our screening approach.

Conclusions: Our results demonstrate that SgBdbD is involved in disulphide bond formation, and that the enzyme affects numerous biological processes. This suggests that oxidoreductases may play a more important role in Gram-positive bacteria than previously thought.

❖❖18. VACCINATION OF SPLENECTOMIZED ADULT PATIENTS
IN THE CAPITAL DISTRICT HEALTH AUTHORITY—AN AUDIT OF
OUR RESULTS AFTER IMPLEMENTATION OF A PERI-
SPLENECTOMY PROGRAM/VACCINE KIT

V. Meier-Stephenson, S. McNeil, A. Kew, J. Sweetapple, D. Mombourquette, K. Slayter

Introduction: Overwhelming post-splenectomy infection (OPSI) is a serious potential outcome in patients who have had their spleens removed and is associated with a high mortality. The most common bacterial causes for this are encapsulated organisms—*Streptococcus pneumoniae*, *Neisseria meningitidis* and *Hemophilus influenzae*, type B—all of which are vaccine-preventable. Current guidelines recommend vaccination against these three bacteria. Adherence to these guidelines, however, is less than ideal. In 2007, a "splenectomy kit" was introduced at our institution that involves the many critical components to allow proper adherence and education, including the vaccines themselves, a wallet card, a standardized letter for the family physician and patient education pamphlets. Four years have passed since the implementation of this program. This work sought to evaluate and compare the current vaccination rates of splenectomized patients in CDHA with that of the rates prior to the introduction of the "splenectomy kit". Secondly, non-vaccinated patients were evaluated for any patterns/characteristics which may identify them as less likely to receive appropriate post-splenectomy vaccinations.

Methods: This was an observational study performed at the QEII Health Sciences Centre in Halifax, Nova Scotia. Patients were identified through Health Records via the surgical code for splenectomy between 2008-2011. Charts were reviewed for information relating to vaccination. When documentation was lacking, information was sought from their respective family physicians. Vaccination rates as well as other descriptive statistics were determined and comparisons were made to rates at our institution for a 3-year period prior to the program's implementation.

Results: Prior to the introduction of the "splenectomy kit", vaccination rates were 91% for *S. pneumoniae*, 75% against *N. meningitidis* and 68% against *H. influenzae*, type B. Since implementation of this kit/program, the rates are now 100%, 97% and 93%, respectively. Some characteristics of non-vaccinated patients will be discussed.

Conclusions: The introduction of a pharmacy-driven "splenectomy kit" program can improve rates of appropriate vaccination in splenectomised patients.

❖❖19. OUTLINING HEALTHCARE UTILIZATION FOR INVASIVE MENINGOCOCCAL DISEASE TO QUANTIFY ECONOMIC BURDEN OF DISEASE

S. Gajic, A. Ambrose, S. McNeil

Introduction: The intent of this study is to provide a comprehensive guidance document for an appropriate cost collection methodology for Invasive Meningococcal Disease (IMD). More specifically to identify healthcare utilization associated with IMD and to provide sufficient tools to ascertain all direct, indirect, and intangible costs related to IMD.

Methods: Prospective, multi-institutional, longitudinal data will be collected from all identified cases of IMD across sentinel Serious Outcomes Surveillance Network participating hospitals in Canada. In order to capture the necessary data we will look at costs per case. This study outlines all costs that need to be collected in order to determine total cost of a case of IMD in Canada. Tools outlining healthcare utilizations and assigning costs have been developed primarily through comprehensive review of the literature and through consultations with experts. Healthcare utilizations are assigned into direct or indirect costs. Quality of Life questionnaires are incorporated as a measure for intangible costs to the survivor and the caregiver.

Results: We present a comprehensive data set that captures lifetime healthcare utilization and associated costs; direct, indirect, and intangible, of a case of IMD in Canada. Costs to the patient, society, and the healthcare system are considered. We provide specific cost collection tools which take a societal perspective on collection of healthcare utilization optimized by direct participation from patients and caregivers in cost collection. Pilot testing, validation and assessment of feasibility of data collection tools will be presented

Conclusions: This study will provide important information on the extent of healthcare utilization and provide a guidance document for collection of complete economic burden of IMD to help inform evidence-based decision-making around Provincial and Territorial publicly-funded immunization programs in Canada.

❖❖20. UNIVERSAL TDAP VACCINATION: WHAT HEALTHCARE PROVIDERS NEED AND WANT TO KNOW

DM MacDougall, BA Halperin, D Janowitz, D MacKinnon-Cameron, J Langley¹ SA McNeil, S Halperin

Introduction: Currently the Canadian and US immunization advisory committees recommend that all adults receive a single dose of the adult-formulation tetanus, diphtheria, and acellular pertussis vaccine (Tdap). There are very little published data on the proportion of Canadian adults vaccinated with Tdap; however anecdotal reports indicate that uptake is very low. Similarly, vaccination coverage amongst US adults reached just 6.6% four years after Tdap became available.

Methods: A national survey was distributed to a representative sample of Canadian health care providers to evaluate knowledge, attitudes and beliefs (KAB) regarding the delivery of Tdap to adults. National focus groups were also conducted in order to explore in-depth KAB about the Tdap vaccine.

Results: Most providers surveyed (n=1167) were family physicians (42.8%) followed by pharmacists (34.3%), and nurses (15.5%). 83.9% lived in a urban/suburban area with 15.9% being from a rural area. 69.4% were aware of the national recommendation and agreed with it, 58.5% routinely offer Tdap to adults and 47.5% received Tdap themselves. 59.6% stated that their patients were not at risk for serious pertussis disease; 68.4% indicated that the Tdap program was not promoted adequately and one quarter said the recommendations were confusing. National focus group data suggested that it is important to inform HCPs about the Tdap guidelines as many have very little information on this matter. HCPs also stated that they do not feel sufficiently informed to discuss or recommend Tdap to their patients and that they wish to have tools to help them.

Conclusions: Understanding provider knowledge and attitudes regarding pertussis and pertussis vaccines is critical to inform the development and implementation of interventions in order to achieve the goal of improved Tdap vaccine uptake in the adult population.

❖❖21. EFFECT OF ACYL CARRIER PROTEIN SYNTHASE INHIBITORS ON MRSA METABOLISM

M. Taylor, C. Barden, D. Byers, A. Henneberry, E. Lu, C. McMaster, D. Weaver, F. Wu

Introduction: Using antibiotics as tools for fighting disease became widespread in 1946. Shortly thereafter, during a Japanese outbreak of *Shigella* in 1953, a multidrug resistant strain of *Shigella dysenteriae* was isolated. In 2002, an estimated 1.7 million infections were acquired United States hospitals resulting in 99 000 deaths.¹ Many of these infections were caused by bacteria resistant to one or more antibiotics. Antibiotic resistance occurs when bacterial growth is no longer inhibited by an antibiotic. If antibiotics have similar mechanisms of action, bacteria resistant to one may also be resistant to the other. The need for alternative antibiotic targets is paramount. Acyl carrier protein synthase (AcpS) is a widely conserved, essential bacterial enzyme with no homologue in mammals. Small molecule inhibitors of AcpS have been developed at the IWK Health Centre's Cheminformatics and Drug Discover Laboratory utilizing a combination of computational drug design, synthetic organic chemistry, enzymology, and microbiology. Several novel anti-infective candidates have been shown to effectively inhibit growth of a variety of *Staphylococcus sp.* including MRSA and VRE strains as well as *Streptococcus sp.* Work is ongoing to definitively determine the mechanism of action of this class of compounds. The research presented here will include changes in metabolic labeling in response to addition of candidate anti-infectives.

Methods: MRSA metabolism was followed by addition of ³H-labelled precursors. Leucine, glycerol, thymidine, and uracil were used as markers for protein, lipid, DNA, and RNA synthesis, respectively. Cells were grown to early log phase and then incubated with radiolabelled precursors in the presence or absence of a candidate AcpS inhibitor. Samples were taken over time and total internalized radiolabel counts were compared.

Results: Incubation with sub-MIC concentrations of compounds tested resulted in a decrease in lipid and protein incorporation of radiolabel with respect to cells exposed to vehicle control only. There was no significant change in the amount of radiolabel incorporated into DNA and RNA.

Conclusions: A family of compounds has been synthesized capable of potent inhibition of growth of *Staphylococcus sp.* and *Streptococcus sp.* The effect of sub-MIC concentration of these compounds on MRSA *in vivo* is a decrease in lipid and protein synthesis. Further experiments are required to more specifically determine the anti-infective mode of action.

❖❖22. UNIVERSAL TDAP VACCINATION: WHAT ADULTS NEED AND WANT TO KNOW

BA Halperin, DM MacDougall, D Janowitz, D MacKinnon-Cameron, J Langley, SA McNeil, S Halperin

Introduction: A single dose of the adult-formulation tetanus, diphtheria, and acellular pertussis vaccine (Tdap) is recommended for all adults by both the Canadian and US immunization advisory committees. There are few existing data on the proportion of Canadian adults vaccinated with Tdap, however anecdotal reports indicate that uptake is very low. This study aimed to explore knowledge, attitudes and beliefs of Canadian adults in an attempt to identify potential barriers and facilitators to Tdap uptake in this population.

Methods: Canadian adults were surveyed and a geographic and age representative sample was obtained (N=4023). In addition, eight focus groups were conducted nationwide.

Results: 81.4% of respondents did not know that there was a Tdap vaccine; 77.3% did not know that Tdap was publicly funded, and 88.8% either did not know or would not receive the vaccine. Public knowledge was very poor as the majority of respondents answered "do not know" to many of the questions. For example, 63.4% of respondents did not know that protection from the childhood whooping cough vaccine diminishes over time leaving adults at risk for the disease. Attitudinally, respondents were indifferent towards the Tdap vaccine which may be attributed to the low knowledge scores. Focus group data support the survey results and indicate that the public wish to receive information about whooping cough and the Tdap vaccine in order to make a decision whether or not to be vaccinated.

Conclusions: Understanding public knowledge and attitudes regarding pertussis and pertussis vaccines is critical to inform the development and implementation of interventions in order to achieve the goal of improved Tdap vaccine uptake in the adult population.

❖❖23. ANNEXIN A1 INTERACTS WITH REOVIRUS FUSION ASSOCIATED SMALL TRANSMEMBRANE (FAST) P14 DURING CELL-TO-CELL FUSION

M. Ciechonska and R. Duncan

Introduction: Membrane fusion is a process involved diverse biological processes including muscle and bone formation, granuloma generation, sperm-egg fertilization, viral entry into host cells, and virally induced syncytium formation. Despite the ubiquitous nature of this reaction, little is known about the cellular protein machinery involved. The non-enveloped reovirus fusion-associated small transmembrane (FAST) proteins are the smallest known membrane fusogens, ranging from 10 to 22kDa in size. Unlike other known viral fusion proteins, the FAST proteins are non-structural and therefore not involved in virus entry. Their sole function is to induce cell-to-cell fusion and syncytium formation following infection. Our team has identified several cellular partners recruited by the FAST proteins during syncytium formation. We report that the host protein Annexin A1 interacts with the p14 FAST protein endodomain to facilitate syncytium formation in human cell lines.

Methods: Annexin A1 was cloned from PC3 cDNA library. HT-1080 cells were co-transfected with FLAG-Annexin A1 and p14 FAST, Anti-FLAG co-immunoprecipitation was performed and analyzed by Western blotting, probing for p14. Annexin A1 knock down studies were performed by transfecting Annexin A1 siRNA followed by a p14 DNA transfection. The extent of fusion was assessed by syncytial nuclei indexing.

Results: We report that Annexin A1 interacts with the endodomain of p14 as shown by co-immunoprecipitation studies. This interaction is essential for syncytiogenesis, as knocking down the expression of Annexin A1 greatly reduces p14-mediated fusion.

Conclusions: This is the first report of a cellular protein that directly interacts with a viral fusion protein to promote cell-to-cell fusion. It has previously been reported that Annexin A1 is essential for myoblast fusion, leading us to suggest that p14 may access an innate cellular fusion pathway in order to generate multinucleated syncytia during reovirus infection.

❖❖24. DETECTION OF NEISSERIA GONORRHOEA USING VIPER XTR;
CONFIRMATION IS NECESSARY TO AVOID FALSE POSITIVE RESULTS

JJ LeBlanc, C. Roberts, T. DeLong, T. MacDonald, C. Heinstein, TF Hatchette'

Introduction: Molecular methods for the diagnosis of *Neisseria gonorrhoea* (NG) have the potential for detection of non-NG *Neisseria* species, a problem that leads to false positive results. Here we describe our experience with false positive tests using the Viper XTR.

Methods: 1) Genital and throat specimens collected over a 10 week period were tested using the Viper XTR. Positive specimens were confirmed using the EraGen MultiCode®-RTx assay directed at conserved regions of the NG 16s rDNA. 2) To assess cross reactivity, a 10-fold dilution series of 6 different *Neisseria* species was tested with the Viper XTR. NG dilutions were tested in parallel with the EraGen kit.

Results: 1) Of the 6694 specimens tested, 13 tested positive for NG. Three of the 10 genital specimens positive for NG on the Viper XTR failed to confirm using the EraGen PCR. All 3 throat specimens positive for NG failed to confirm. 2) The LOD of the EraGen and Viper XTR assays are equivalent at 500 copies/ml. The Viper XTR NG assay cross reacted with *N. cinerea*, *N. lactamica*, and *N. meningitidis* but did not cross react with *N. sicca* or *N. subflava*. No cross reactivity was observed with the EraGen assay.

Conclusions: Cross-reactivity with non-NG *Neisseria* species does occur with the Viper XTR. Confirmation with a second molecular assay is necessary and particularly important for all non-genital specimens.

❖❖25. CHARACTERIZATION OF RENAL DYSFUNCTION IN A COHORT OF ADULT HIV-INFECTED PATIENTS: A RETROSPECTIVE, OBSERVATIONAL CASE-CONTROL STUDY

M. MacNeil, B.L. Johnston, P. Poyah, D. Haase, K. Thompson, K. Slayter

Introduction: The incidence and prevalence of kidney disease are rising among HIV-infected individuals. The primary objective of our study was to identify risk factors for renal dysfunction and/or renal damage. Our secondary objective was to develop a tool that could identify and manage those HIV-infected patients at increased risk for renal dysfunction.

Methods: This was a retrospective, observational, case-control study comparing HIV-infected patients with renal dysfunction and/or damage to those without dysfunction/damage. Renal dysfunction was defined as a Glomerular Filtration Rate (GFR) < 60 ml/min. Renal damage was defined as the presence of microalbuminuria. Chi square tests and multivariate analysis were used to analyze relationships between study groups.

Results: 72-patients with renal dysfunction and/or damage were compared to 105 controls. Factors shown to be associated with renal dysfunction and/or damage included the use of non-antiretroviral medications (55.6% vs. 34.3%, P = 0.0050); hypertension (43.1% vs. 20%, P = 0.0009), moderate-to-severe liver disease (4.2% vs. 0%, P = 0.0656), diabetes (with or without end-organ damage (6.9% vs. 1%, P = 0.0415; 15.3% vs. 3.8%, P = 0.0071)), and nadir CD4 cell count (182 vs. 220, P = 0.0769). After multivariate analysis, hypertension (OR = 2.28; p = 0.0332) remained as an independent risk factor for renal dysfunction or damage.

Conclusions: Our study demonstrates the impact that chronic disease states have on the risk for developing renal dysfunction and/or renal damage in patients infected with HIV. A tool for identifying and managing patients with early signs of renal dysfunction was developed that stresses the importance of both chronic disease management and the appropriate monitoring of potentially nephrotoxic medications.

❖❖26. IMMUNOGENICITY, SAFETY, AND PREFERENCE FOR SELF – VERSUS NURSE- ADMINISTERED INTRADERMAL INFLUENZA VACCINE

Y. Shamout, B. Coleman, S. Halperin, J. Langley, A. Taddio, V. Shah, A. McGeer, S. McNeil

Background: Intradermally administered influenza vaccine is as immunogenic as intramuscular vaccine at a lower unit dose. New microinjection systems could allow self administration of vaccine, potentially reducing the cost and inconvenience. We compared the immunogenicity, reactogenicity, success rate, and acceptability of self- versus nurse-administered intradermal trivalent seasonal influenza vaccine.

Methods: Adults (18-59 years old) were randomized to either self- or nurse-administered intradermal vaccine. Prior to vaccination, participants completed a questionnaire and had serology drawn for haemagglutination inhibition titres. Participants in the nurse-administered group were vaccinated by study personnel. The self-administered group were given an instruction sheet and administered their own vaccine. All participants completed a questionnaire and adverse event diaries for 21 days post vaccine, at which time serum was again collected.

Results: 228 participants were randomized: 115 to self-administration and 113 to nurse administration. Groups did not differ by sex, age, or levels of seroprotection at baseline. Of the 114 who completed self-administration, 106 (92%) were successful on the first attempt. There were no group differences in measures of immunogenicity for any of the strains. Self-administering participants reported a lower mean pain rating at vaccination but had larger areas of redness post-vaccine. Seventy percent of all participants said they would prefer intradermal over intramuscular vaccinations in future, if given the choice.

Conclusion: Compared to nurse-administered intradermal influenza vaccine, self administered vaccine was immunologically non-inferior and reached all the EMA criteria for the A strains, was highly successful and preferred by study participants. Together, these data provide preliminary evidence of feasibility for this method of influenza vaccine administration, which may improve vaccine uptake in adults and increase efficiency of vaccine delivery during outbreaks

❖❖27. PRELIMINARY ASSESSMENT OF NEEDS FOR A REFUGEE AND IMMIGRANT CLINIC IN SAINT JOHN, NEW BRUNSWICK

M. Halpine, J. Salmon, L. Frechette

Introduction: Despite the increasing numbers of refugees and immigrants to Saint John, New Brunswick there have been no formal initiatives to address their unique health care needs with comprehensive screening or treatment. A few healthcare practitioners have been working on an ad hoc basis to meet the health needs of refugees and immigrants but this is neither sustainable nor sufficient. This study was undertaken to assess what healthcare priorities for the refugees and immigrants should be and what Saint John is equipped to offer immediately and in the long-term.

Methods: Using the Canadian Center for Immigrant and Refugee Health Guidelines as the standard for refugee and immigrant healthcare we reviewed what health needs are being met in the current system; what could be met in the near future by the initiation of a dedicated clinic; and what requires long term investment. Healthcare practitioners currently offering screening, treatment and advanced care to refugees and immigrants were surveyed to discover what healthcare is and is not currently available. We also reviewed the issues with leaders in the social organizations serving refugees and immigrants to ensure we were not missing any active practitioners.

Results: Saint John currently meets the full standards for only two of the 20 conditions under the four parameters of: infectious diseases, chronic conditions, mental health and women's health in the CCIRH guidelines. With a focus on improved infrastructure and commitment to refugee and immigrant health, such as a dedicated clinic, Saint John will be able to meet standards for an additional eleven conditions, leaving seven conditions that require a longer-term commitment and investment.

Conclusions: While Saint John currently receives a relatively small number of refugees and immigrants each year, there is already a need to offer a more sustainable healthcare solution. By providing a dedicated clinic that caters to the unique healthcare needs of refugees and immigrants it is hoped their immediate health issues will be addressed and allow easier entrance into the Canadian Health care system. Utilizing the CCIRH guidelines, steps are currently being taken to sustainably offer comprehensive healthcare to refugees and immigrants arriving to Saint John.

❖❖28. OPTIMIZATION OF THE MICROPLATE RESAZURIN ASSAY AS A SCREENING TOOL FOR NATURAL PRODUCTS WITH ANTI-TUBERCULOSIS ACTIVITY

T.E. O'Neill, D. Webster, J.A. Johnson, C.A. Gray

Introduction: Natural products chemistry has provided novel bioactive compounds for drug discovery through bioassay-guided fractionation techniques. The microplate resazurin assay (MRA) is used for the isolation of anti-mycobacterial compounds from phytochemical extracts. However, we found the MRA problematic, providing inconsistent results. In this study we optimize the efficiency of the MRA for both screening and bioassay-guided fractionation of phytochemical extracts to facilitate the discovery of potential anti-mycobacterial therapeutics.

Methods: MRA aspects evaluated include: DMSO effect on mycobacterial growth; optimization of resazurin indicator concentration; plate type used; and assay duration. All tests were conducted on avirulent *M. tuberculosis* strain H37Ra as it is more safe and accessible and has been demonstrated to be an acceptable surrogate strain for H37Rv.

Results: The optimal DMSO solvent concentration is 2% as test samples were fully dissolved with minimal impact on mycobacterial growth. The ideal resazurin indicator concentration was 12.5 µg/mL in 10% Tween 80 delivering optimal fluorescence and colourimetric readings. Black plates delivered optimal results when read fluorometrically and clear plates when observing colour change. Traditionally, the MRA is a 7-day assay, however, may be completed in 2 days. The assay was verified using methanolic extracts of *Heracleum maximum*. Through bioassay-guided fractionation using the optimized MRA, the natural product falcarindiol was identified as the primary constituent conferring anti-mycobacterial activity in extracts of *H. maximum*.

Conclusions: We have optimized the MRA to deliver consistent results more rapidly than the standard 7-day protocol thus increasing the efficiency of both extract screening and bioassay-guided fractionation for the better facilitation of lead compounds for anti-mycobacterial drug discovery.

❖❖29. POTENTIAL PATHOGENS CULTURED FROM HEALTHCARE WORKER PAGERS, CELL PHONES, STETHOSCOPES, LABORATORY COATS AND IDENTIFICATION CARDS ON A MEDICAL WARD IN NEW BRUNSWICK: ANALYSIS OF A BRIEF INFECTION CONTROL EDUCATION PROGRAM

R. Blackman, S. El-Bailey, S. Hull, D. Webster

Introduction: Nosocomial infections are a major cause of morbidity and mortality within the healthcare system. Unfortunately, health care workers (HCWs) act as potential vectors in the spread of infectious organisms with personal items and equipment as important reservoirs and vehicles. Use of an effective cleaning agent may reduce bacterial colonization of items, however, use by HCWs is not generally consistent. This study aims to identify potential pathogens on HCW items on a medical ward at a tertiary care centre. In addition, this study will assess the impact of a brief education program encouraging the use of 0.5% accelerated H₂O₂ wipes.

Methods: On the medical teaching unit at the Saint John Regional Hospital, swabs of pagers, cell phones, stethoscopes, laboratory coats and identification cards belonging to physicians, residents, medical students and nurses were obtained and plated onto blood and MacConkey agar. Following incubation, potential pathogens were identified and results were presented on the ward maintaining anonymity. A brief education program encouraging the use of 0.5% accelerated H₂O₂ wipes was also employed and a second set of swabs was obtained two weeks subsequent to the first round of swabbing with repeat microbiological analysis. Results will be reviewed to determine the impact of the education program on potential pathogen colonization of HCW items.

Results: With the first round of swabbing, 32 of the 35 items sampled harbored micro-organisms, with a total of 105 identified isolates. The vast majority of isolates were coagulase-negative *Staphylococcus spp.* Two methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, five *Pantoea sp.* isolates and nine streptococcal isolates were also identified. The MRSA isolates were cultured from two different cell phones. The streptococcal isolates were found on all item types. The *Pantoea sp.* isolates were cultured from items belonging to five different individuals. At the time of abstract submission, microbiological work-up of the second round of swabs is ongoing.

Conclusions: The isolation of potential pathogens from HCW items on the medical ward highlights the importance of HCW education and strong infection control practices. The effectiveness of the education program and utilization of 0.5% accelerated H₂O₂ wipes will be determined.

❖❖30. DIRECTED TRAFFICKING THROUGH THE ER/GOLGI PATHWAY TO THE PLASMA MEMBRANE BY A NOVEL POLYBASIC MOTIF IN THE REOVIRUS P14 FAST PROTEIN

H. B. Parmar, C. Barry, F.B. Kai and R. Duncan

Introduction: The reovirus fusion-associated small transmembrane (FAST) proteins are the smallest known membrane fusion proteins. All FAST proteins are single-pass transmembrane proteins that traffic through the ER-Golgi pathway to the cell surface where they induce cell-cell membrane fusion. Protein traffic to the plasma membrane has important implications on human disease and virus replication, and the pathways and signals involved remain unclear. Here, we have investigated the role of the polybasic motif in the endodomain of the p14 FAST protein in terms of its functionality and trafficking properties. It is now clear that the p14 polybasic motif contains novel sorting signals required for Golgi export to the plasma membrane, one of the least understood steps in cellular protein trafficking.

Methods: Various p14 polybasic mutant proteins were created by site-directed mutagenesis. The fusion activity of all constructs was analyzed by syncytia formation assays, and cell surface expression was determined by flow cytometry. Subcellular localization of these p14 constructs was determined by confocal microscopy and endoglycosidase assays.

Results: Alanine substitution of the p14 polybasic motif led to a loss of plasma membrane localization and p14 accumulation in the trans-Golgi network (TGN), indicating the polybasic motif acts as a Golgi export signal. Further extensive mutagenesis indicated that the polybasic region contains redundant Golgi export signals, including a Basic-X-Basic motif and additional sorting signals based on basic amino acids.

Conclusions: The p14 polybasic motif is involved in protein export from Golgi bodies. Furthermore, multiple redundant signaling sequences in the p14 polybasic motif appear to mediate protein trafficking to the cell surface. This is the first example of such basic sorting signals, which presumably interact with cellular trafficking machinery to mediate protein export from the Golgi bodies to the cell surface. 50

❖❖31. CHARACTERIZATION OF *LEGIONELLA PNEUMOPHILA* MATURE INFECTIOUS FORMS (MIFS) FROM DIFFERENT HOSTS

H.Abdelhady, E.J.Slobodesky, J.Kim, R.Garduño

Introduction: *L. pneumophila* is a Gram-negative bacterium that replicates intracellularly in freshwater amoebae, and accidentally infects susceptible humans causing the atypical pneumonia known as Legionnaires' disease (LD), which is not transmitted from person to person. *L. pneumophila* has a developmental cycle and alternates between replicative forms and resilient, metabolically dormant mature infectious forms (MIFs). MIFs are only produced *in vivo*, and thought to be responsible for LD transmission. We have previously shown that *L. pneumophila* MIFs produced in human macrophages have some fitness and infectivity disadvantages compared to *L. pneumophila* MIFs produced in protozoa, which provides a clue for why LD is not transmissible. Here, we are trying to understand the molecular mechanisms behind such differences.

Methods: We performed proteome and transcriptome comparisons of *L. pneumophila* growing inside the amoeba *A. castellanii*, the ciliate *Tetrahymena tropicalis*, and human U937 macrophages, using 1-dimensional protein analysis and quantitative RT-PCR respectively.

Results: We generated a list of 18 *L. pneumophila* genes that were upregulated during amoebae infections compared to human macrophages, which create a potential target for future studies. This list included hypothetical genes, dot/icm effectors, genes involved in lipid and carbohydrate metabolism. Genes coding for amylase (*lpg1669*) and malate dehydrogenase (*lpg2971*) enzymes were particularly interesting. They were upregulated in amoebae, ciliates, and *in vitro* differentiation models. Interestingly, *lpg1669* showed the most dramatic upregulation in amoebae. Current work is focused on these genes trying to understand their importance in *L. pneumophila* differentiation.

Conclusions: *L. pneumophila* upregulates a different set of genes during infections of freshwater protozoa compared to human macrophages. Genes that are involved in carbohydrate metabolism are of a particular interest for future studies. These findings may further increase our understanding of why LD is not communicable.

❖❖32. MAJOR COUNTRY-WIDE REDUCTION IN MORTALITY WITH THE BELIZE HEALTH INFORMATION SYSTEM (BHIS) WITH PROGRAM MANAGEMENT

M. Graven, P. Allen, I. Smith, N. MacDonald

Introduction: Belize deployed the world's first country-wide, fully integrated health information system in 2007 at a cost of \$4 (Can) per citizen. Within 1 year the BHIS was being used for >90% of patient health encounters. The BHIS includes 8 disease management protocols; 5 directed at children under 5 years: neonatal care, vaccines, management of acute respiratory illness (ARI), management of acute diarrhea and prevention of mother to child HIV transmission.

Objective: To assess the impact of the BHIS with the management protocols on child and adult mortality.

Methods: De-identified data for 2005-2010 were used to study primary and secondary causes of death, selected for causes of death either among the 8 with BHIS protocols, or 3 without BHIS protocols -diabetes, homicide, motor vehicle crashes. Belize population data were obtained from the Statistical Institute of Belize for 2005-2009, and from the Belize census for 2010. All mortality comparisons were made as deaths/1,000 population.

Results: The BHIS was introduced in 2007. Overall, the summed decrease in crude mortality rates was a dramatic country wide drop of 1.011deaths /1000 population from 2005 to 2010. The mortality rates for the causes of death associated with the 8 BHIS protocols demonstrated significant reductions for most causes studied. Mortality rate per 1000 due to acute respiratory illness decreased from 0.223 to 0.092 between 2005 and 2010. Reductions were also observed for HIV, diarrhea, hypertension, and maternal death (.021 to .00, 0.33 to .13, .55 to .24, and .03 to .01, respectively). In contrast the mortality for diabetes, diseases of pulmonary circulation and MVAs, all lacking BHIS protocols, rose over the study period.

Conclusions: The BHIS has been well accepted and saves lives in Belize for modest implementation costs. The country wide mortality decrease for the 8 protocols associated with the BHIS is 10 times greater than for stopping a famine in a country(1/1000 vs 1/10,000).

❖❖33. A NOVEL ROLE FOR THE DOWN SYNDROME CRITICAL REGION GENE RCAN1 DURING *P. AERUGINOSA* LUNG INFECTION

R. Junkins, A. MacNeil, S.Carrigan, C. McCormick, T. Jun-Lin

Introduction: Down syndrome is the most common chromosomal anomaly and is caused by trisomy of chromosome 21. It is associated with cognitive and physical impairments as well as an increased risk of respiratory tract infections, however the gene(s) responsible for these immunological defects remain undefined. Recently, Regulator of Calcineurin 1 (RCAN-1), a gene located in the Down syndrome critical region on chromosome 21, has been implicated in signaling pathways which mediate the inflammatory response, however the biological contribution of the gene to the immune response remains unknown. The objective of this study was to determine the role of RCAN1 in the immune response using a mouse lung infection model.

Methods: A *P. aeruginosa* lung infection model was used to assess the immune response in wild-type and RCAN-1 deficient mice. Protein arrays, EMSA, ELISA, and luciferase assays were used to further compare the inflammatory response during infection *in vivo* and *in vitro*.

Results: RCAN-1 deficient mice did not survive beyond 24 hours after infection with a dose of *P. aeruginosa* that killed 50% of wild-type mice. Following infection with a sub-lethal dose of the bacteria the RCAN-1 deficient mice displayed greatly enhanced bacterial clearance, and elevated levels of numerous inflammatory cytokines. Furthermore, enhanced activation of the pro-inflammatory transcription factors NFAT and NFkB was observed both *in vivo* and *in vitro* following *P. aeruginosa* infection.

Conclusions: These findings demonstrate a novel negative regulatory role for RCAN1 during *P. aeruginosa* infection. This role is carried out through inhibition of the pro-inflammatory transcription factors NFAT and NFkB, leading to decreased production of inflammatory cytokines. The survival differences suggest that this negative regulatory function of RCAN1 is critical for preventing sepsis following *P. aeruginosa* infection. These results suggest that trisomy of RCAN1 could contribute to immunosuppression and recurrent infections in Down syndrome patients.

❖❖34. *SHIGELLA FLEXNERI* VIRULENCE PLASMID (PWR100) DELETION COLLECTION

S.Sidik, JJR Benjamin, RJ Ryu, A.Jarrar, JR Rohde

Introduction: Bacteria of *Shigella* spp. are the causative agent of bacillary dysentery, also known as shigellosis, which is a human disease transmitted by the fecal-oral route. Nearly all *Shigella flexneri* virulence factors are encoded on a 213 kb extra-chromosomal virulence plasmid (pWR100) which encodes ~110 open reading frames. A number of these genes are well characterized, including those encoding structural and regulatory components of the type III secretion system; however, many are not well characterized. To better understand the contribution that individual pWR100 genes make towards *Shigella* infection and virulence we have systematically deleted the majority of the genes on pWR100 in the commonly used *S. flexneri* strain M90T, resulting in a deletion collection consisting of 103 individual single-gene-deletion-mutants.

Methods: Each deleted ORF is replaced with a tetracycline resistance (Tet^R) gene flanked by flippase recognition target sites. Genes were targeted for deletion by gene specific sequences flanking this Tet^R cassette and homologous recombination was facilitated by the λ Red recombinase system. Congo red is a synthetic compound sequestered by virulent strains of *Shigella* more readily than avirulent strains. We developed a rapid quantitative assay for congo red binding and screened the deletion collection.

Results: Our screen identified previously characterized and novel gene deletion mutants with altered congo red binding efficiency. The ability of deletion mutants to elicit Interleukin-8 (IL-8) from U937 macrophages was also examined, leading to the identification of virulence factors that inhibit IL-8 production.

Conclusions: These experiments validate the use of the deletion collection as a tool for studying the functions of virulence factors using high-throughput screening techniques.

❖❖35. ANTI-TB NATURAL PRODUCTS FROM *ARALIA NUDICAULIS*

H. Li, T. O'Neill, D. Webster, J. A. Johnson, C.A. Gray

Introduction: *Aralia nudicaulis* L., wild sarsaparilla, is a member of the Araliaceae or ginseng family that is indigenous to North America and was used extensively as a traditional herbal medicine by the First Nation communities of Canada. The Mi'kmaq and Maliseet First Nation peoples use *Aralia* rhizomes to prepare medicines for the treatment of wounds and respiratory ailments such as coughs. Results of a previous study showed that the aqueous extract of *A. nudicaulis* rhizomes possessed moderate anti-mycobacterial activity. The objective of the present research was to isolate and identify the anti-mycobacterial constituents of *A. nudicaulis*.

Methods: The microplate resazurin assay was used to assess the anti-mycobacterial activity of methanolic extracts of *A. nudicaulis* against *Mycobacterium tuberculosis* (H37Ra) and facilitated the isolation of active compounds through bioassay guided fractionation. Extracts of *A. nudicaulis* were fractionated by liquid-liquid partitions, column chromatography and high performance liquid chromatography (HPLC). The structures of the active compounds were elucidated through analysis of one dimensional and two dimensional nuclear magnetic resonance (NMR) data, mass spectrometry and polarimetry.

Results: Two C17 diynes were found to be responsible for the anti-mycobacterial activity of *A. nudicaulis* rhizomes. The diynes were identified as falcarinol and panaxydol with MICs of 25.6 μM and 36.0 μM and IC₅₀ of 15.3 μM and 23.5 μM against *M. tuberculosis* (H37Ra), respectively.

Conclusions: The isolation of two natural products with significant anti-mycobacterial activity from the rhizomes of *A. nudicaulis* has validated one of the traditional medicinal uses of this plant by Canadian First Nations. This was also the first report of anti-TB activity of the C17 diyne panaxydol.

❖❖36. VACCINATION RATES OF HALIFAX'S HOMELESS: ARE THE BARRIERS TO VACCINE DELIVERY AND UPTAKE?

C. Van Zoost, A. Ghaly, P. Morrison, K. Babb, H. Flemming, S. McNeil

Introduction: Homeless persons are at high risk for morbidity and mortality from influenza and invasive pneumococcal disease. Limited data is available on vaccination rates and barriers to vaccination in this population.

Methods: From October 15, 2011 to January 15, 2012, a survey was administered to homeless persons in Halifax at both shelter and non-shelter locations. Information gathered included demographics, vaccination history and barriers to influenza vaccination. Inclusion criteria were age \geq 16 years and being homeless for more than 1 day in the past week.

Results: Of the 99 homeless persons surveyed 56% were male. Forty-nine percent received the influenza vaccine in the previous year (2010-2011) and 82% percent received the influenza vaccine at some point in their life. The vaccination rate for pneumococcus was 12%. Factors associated with influenza vaccination in the previous year included female sex ($p < 0.0001$), being born in Nova Scotia ($p 0.0061$), being on prescription medication ($p 0.0002$) and accessing primary health care at the North End Community Health Centre ($p 0.0191$) or from the Metro Outreach Street Health nurses ($p 0.0059$). Factors not associated with influenza vaccination included having a chronic medical condition ($p 0.3671$) or having a family doctor ($p 0.7016$). Eighty-nine percent of persons who were vaccinated against influenza were willing on getting the influenza vaccine if there their physician recommended it, compared with 62% in unvaccinated persons ($p 0.0025$).

Conclusions: Vaccination rates for influenza among homeless persons in 2010-2011 were higher than the general population. Potential explanations for the high vaccination rate include increased access to community based health clinics and outreach nurses. Vaccination rates to pneumococcus are suboptimal in the homeless. This is an important group to target as the current guidelines recommend the 23-valent pneumococcal polysaccharide vaccine for homeless persons.

❖❖37. THE ROLE OF EARLY IL-17A PRODUCTION IN REGULATING HOST RESISTANCE AGAINST *CHLAMYDIA* INFECTION

J. de Rosenroll, A. Stadnyk, J. Wang

Introduction: *Chlamydia trachomatis* is the most common cause of bacterial sexually transmitted disease in the USA, and the leading cause of preventable blindness in the world. Previous experiments in our lab showed that mice lacking the C5a receptor (C5aRKO) have a greater mortality rate than wild type mice (WT) upon *Chlamydia* infection. The increased mortality was associated with increased inflammation, increased lung pathology, and a higher bacterial load. The objective of this study is to address whether IL-17A production during early infection have an important role in modulating anti-*Chlamydia* immunity *in vivo*.

Methods: WT mice and C5aRKO mice were subjected to Chlamydia infection. Mice weights were monitored daily and sacrificed at 24 hours or 6 days post infection. In some experiments, rIL-17A was administered at early time points to a group of C5aRKO mice, while PBS was given to two control groups (WT & C5aRKO). Cytokine levels in bronchoalveolar lavage fluid (BAL), lung homogenate and *in-vitro* recall experiments were measured via ELISA. Cell types were measured via Flow Cytometry, and bacterial load was measured via inclusion forming unit assay (IFU).

Results: We found that IL-17 was induced in BAL fluids in WT mice but absent in the C5aRKO mice 24 hours post infection indicating early IL-17A production require C5aR signal. While C5aRKO mice lost significantly more body weight compare to WT mice, administration of rIL-17A in C5aRKO mice markedly protected C5aRKO mice from body weight loss. Of interest, IFU experiments indicated that C5aRKO mice treated with PBS or IL-17A had similar bacteria load. However, the proinflammatory cytokines IL-1 β , IL-6, TNF- α showed a reduced trend in C5aRKO mice receiving rIL-17A compared to that receiving PBS.

Conclusions: Our data suggest that C5aR-signalling is required for early IL-17A production during *Chlamydia* infection. However, IL-17A production doesn't appear to have a significant role in controlling *Chlamydia* infection, rather, the early IL-17A may play an anti-inflammatory role in modulating other pro-inflammatory cytokine productions, inflammation and pathology during *Chlamydia* infection.

❖❖38. INTESTINAL PARASITIC INFECTION IN BHUTANESE REFUGEES RESETTLED IN SAINT JOHN, NEW BRUNSWICK IN 2010

M. Matheson-Orchard, L. Frechette, J. Salmon

Introduction: Newcomers to Canada may have different health concerns relative to more established populations. There is limited data available about burden of potential communicable diseases in these groups. This study aimed to investigate the burden of intestinal parasites in Bhutanese refugees resettled in Saint John, New Brunswick in 2010 and suggest whether ongoing extensive testing should be encouraged.

Methods: A retrospective chart review of the selected cohort was conducted and cohort demographics and laboratory test results were extracted. Simple epidemiological analysis was then performed on the cohort data.

Results: Amongst this cohort, the prevalence of intestinal parasites was 44.4%. The most prevalent intestinal parasite was *Giardia intestinalis* (9%) with rates being higher in females and those under 15 years of age. *Dicrocoelium dendriticum* was another parasite of significance detected. The majority of other intestinal parasites detected were not clearly pathogenic and burden of parasites did not seem to correlate with symptoms.

Conclusions: Intestinal parasites were detected in approximately 45% of Bhutanese refugees resettled in Saint John in 2010 although most of the parasites were not considered pathogenic, nor did they cause symptoms. The relatively high burden of *Giardia intestinalis* in the stool amongst this population might suggest that perhaps screening would be indicated both from a public health and patient care point of view, however, given the lack of symptoms an argument could be made for symptomatic testing only.

❖❖39. MONITORING FOR PCR INHIBITION FOLLOWING HOMOGENIZATION AND HEAT TREATMENT

TF Hatchette, C. Heinstejn, JJ LeBlanc

Introduction: Most laboratories use a nucleic acid extraction to remove PCR inhibitors. Homogenization followed by heat treatment was recently shown to be a rapid and cost effective method to recover DNA from swabs submitted for herpes simplex virus (HSV) real-time PCR. Homogenization uses multidirectional motion to disrupt cells through contact with silica beads. Despite a subsequent heat treatment, this crude method to recover DNA may not inactivate PCR inhibitors. To assure its optimal performance after introduction, this study established a quality control system and interpretive criteria to monitor for PCR inhibition using an exogenous internal control (IC).

Methods: Following 48 consecutive experiments, crossing point (Cp) values of the IC in 750 HSV-negative specimens were analyzed to assess: 1) the distribution within and between experiments; 2) the deviation from the control in each experiment; and 3) the deviation from the average obtained for all HSV-negative specimens in each experiment (intra-experimental average).

Results: Cp values were affected by intra- and inter-experimental variation; however, when normalized to the control or to the intra-experimental average, the deviations displayed a normal Gaussian distribution. Using a cut off of Cp +/- 2 standard deviations, normalization to the control identified potential inhibition in a single specimen. When data was normalized to the intra-experimental average, an additional 15 specimens were identified with possible PCR inhibition. No additional HSV positive specimens were identified following repeat processing by homogenization and heat treatment or following nucleic acid extraction.

Conclusions: This study describes a rigorous method to monitor for potential PCR inhibition after sample homogenization and heat treatment that would have otherwise been masked by the intra- and inter-experimental variation.

❖❖40. SEASONAL VERSUS PANDEMIC VACCINE EFFECTIVENESS IN PREVENTION OF INFLUENZA-RELATED HOSPITALIZATION IN CANADIAN ADULTS

S.McNeil, A.McGeer, K.Green, A.Ambrose, J.Johnstone, T.Hatchette, J.Lebanc, D.Webster, M. Loeb, A. McCarthy, G.Boivin, S.Trottier, H.Wang, D.MacKinnon-Cameron, M.EISherif, S.Halperin, J.Langley, M.Andrew,L.Valiquette,G.Stiver,T.Marrie

Introduction: Following its launch in 2009, the PCIRN Adult Serious Outcomes Surveillance (SOS) Network has continued to conduct hospital-based surveillance for influenza to characterize clinical features and serious outcomes (SO), and to measure vaccine effectiveness (VE).

Methods: In 2010-2011, the PCIRN SOS Network operated in 8 acute care facilities in 6 Provinces, encompassing approximately 5500 beds. Surveillance for influenza among hospitalised adults was active from 15/10/10 to 30/04/11. An NP swab for influenza PCR or viral culture, was obtained from all patients with an admitting diagnosis of respiratory infection, exacerbation of COPD/asthma, CAP, unexplained sepsis, or cardiac/respiratory diagnosis with fever ($\geq 37.5^{\circ}\text{C}$). The next two consecutive test-negative, age-matched patients were controls for calculation of VE. Using univariable and multivariable analysis adjusted for age, gender, current smoker, and comorbidities, VE was estimated as $(1-OR) \times 100$.

Results: 318 cases of lab-confirmed influenza were enrolled, most admitted from a private house, with a mean Frailty Scale of 4.8 at baseline. 54% were female, with mean age 68.7y. The admitting diagnosis was influenza or Influenza-like illness (ILI) in 20%, while other diagnoses included CAP (38%), exacerbation of chronic disease (14%), and sepsis (6%). 2% were pregenant; 94% had ≥ 1 comorbidity, 16% were obese ($\text{BMI} \geq 30$) and 2% were Aboriginal. 34% had received the seasonal vaccine. Collectively, 47% had ≥ 1 complication; 19% were admitted to ICU and 13% required ventilation. Mean length of stay was 12d (1-93d), while 24 (8%) died within 30 days from discharge; only cases and controls with confirmed immunization were included in the VE assessment; VE was 50% (95% CI 30-64) in univariate analysis.

Conclusions: The PCIRN SOS Network is a foundation for characterization of serious influenza outcomes and VE assessment in hospitalized adults. Alike the preceding pandemic season, over 2/3 of lab-confirmed cases did not meet the PHAC definition for ILI, making it a weak criterion to identify influenza disease burden. The 2010 seasonal influenza vaccine was modestly effective in preventing hospitalization due to influenza, much less effective though, than the adjuvanted vaccine in the prevention of pandemic influenza. Mean age was greater than the pandemic year, with consequently more comorbidities.



Acrylic painting by Madelaine Gordon
1st year medical student Dalhousie University NB campus
Interpretation of the innate and adaptive immune responses
to an infectious pathogen, inspired by Dr. Tim Lee's enthusiasm
for teaching immunology to first year medical students.