The Emergence of Zika Virus and Other Arboviruses: Public Health Impacts At Home and Abroad

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Presentation Summary:

1. An Introduction to Zika Virus and other Emerging Mosquito-Borne Arboviruses: Ecology, Epidemiology, and Clinical Aspects

2. Zika Virus Global Expansion and Outbreak Response

3. Zika / Arbovirus Diagnostic Test Complexities and Caveats


5. Other Arboviruses to Consider During the Canadian Mosquito Season

* I have No Conflict of Interests to Disclose
Mosquito-Borne Pathogens (MBP) Are Significant Contributors to Emerging Infectious Disease – ie. Arboviruses

MBP / Arbovirus associated disease is a concern both for travellers and “stay at home” Canadians
“Emerging” and Re-Emerging Arboviruses of Concern to Travelling Canadians:
Zika, Chikungunya, Dengue, Yellow Fever, Murray Valley, Japanese Encephalitis, etc
Vector Borne Disease Expansion: Emerging Arboviruses in the Pacific – Dengue, Chikungunya, and Zika in 2014

Humans are amplifying hosts in outbreaks! Similarities in symptoms and test results (flaviviruses - Zika Den etc.) may confound initial diagnosis.
ZIKA Virus Introduction & History

Zika virus (ZIKV):

- Family Flaviviridae, genus Flavivirus
  - Related to Dengue, West Nile, Yellow fever, Japanese encephalitis viruses
- Enveloped virus with +ssRNA genome
- Originally isolated in Uganda from sentinel monkeys in Zika forest in 1947 & from Aedes africanus mosquitoes in 1948
- First human cases in early 50’s, only sporadic small outbreaks of mild disease
- Primates including humans likely reservoir
- ZIKV from sylvatic to urban settings in Africa and Asia – Aedes aegypti strongly suspected as key vector
- Previously assumed that clinical cases -- mild disease, 80% asymptomatic
- Sexual transmission (cases in 2008 US resident, 2013, French Polynesia patient)
• First report of ZIKV outside of Asia/Africa
• Attack rates: 3.6 – 21.5 / 1,000
• ~ 73% of population developed antibodies
• Only 18% reported symptoms
• Clinical features were mild and no hospital admissions or other complications reported
Clinical Features

Clinical illness is usually mild with acute onset. Symptoms include:

- Fever
- Conjunctivitis (no purulent)
- Arthralgia
- Myalgia
- Headache
- Asthenia
- Maculopapular rash
- Swelling in lower limbs
- Pruritus (itch)

- Incubation period is 3-12 days
- Approximately 80% of infections are asymptomatic
- Among those that are symptomatic, symptoms are mild, self limited and last 2 – 7 days

Pacific Islands Outbreaks: 2013-2015

- Islands of French Polynesia
- 383 confirmed cases of ZIKV
- 32,000 estimated cases (~12%)
- **First reports of Guillain-Barré syndrome** in ZIKV patients
- One case of perinatal transmission
- 2014 Cook Islands, Pascua, New Caledonia
- 2015 New Caledonia, Solomon Islands, Fiji, Samoa, Vanuatu
- January 2014 – First report of ZIKV in Americas when reported on Easter Island (Chile)
Significant Burden of ZIKA Disease: 2015-2016-

- But additional outbreaks of Dengue, Chikungunya, and recently Yellow Fever!!

<table>
<thead>
<tr>
<th>Country</th>
<th>Cases</th>
<th>Total Exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>309,000</td>
<td>(~ 1.5 million Non-reported cases / exposures + CHIK and YF)</td>
</tr>
<tr>
<td>Texas &amp; Florida</td>
<td>21,038</td>
<td>Zika tests performed on 13,966 samples from 12551 patients from Canada</td>
</tr>
<tr>
<td>Mexico</td>
<td>6657</td>
<td></td>
</tr>
<tr>
<td>El Salvador</td>
<td>11,404</td>
<td></td>
</tr>
<tr>
<td>Guatemala</td>
<td>32,161</td>
<td></td>
</tr>
<tr>
<td>Costa Rica</td>
<td>104,372</td>
<td></td>
</tr>
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<td>Honduras</td>
<td>2,900</td>
<td></td>
</tr>
<tr>
<td>Panama</td>
<td>2,835</td>
<td></td>
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<tr>
<td>Venezuela (Bolivarian Republic of)</td>
<td>61,527</td>
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<tr>
<td>Bolivia (Plurinational State of)</td>
<td>874</td>
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NML - End of 2016: 21,038 Zika tests performed on 13,966 samples from 12551 patients from Canada!

Febrile, Microcephaly, GBS
Other Modes of Transmission
Confirmed or Suspected

- Sexual transmission (all possible combinations; persistence in sperm)
- Blood products/transfusion
- Virus/RNA detected in urine, saliva & breast milk
- Intrauterine and perinatal infections
Spectrum of ZIKV Disease Appears to Change in Brazil

- Neurological complications including: Guillain-Barré Syndrome in adults and microcephaly in newborns reported at heightened frequency
- Association with microcephaly prompts WHO to declare ZIKV “Public Health Emergency of International Concern” (February 1 2016)

Microcephaly now observed in other countries, other developmental effects, New Study: infected babies with no apparent microcephaly at birth, head growth deceleration after birth observed, other neuro issues (MMWR,CDC)

Did virus mutate or were “novel” clinical aspects of ZIKV previously not detected? Perhaps Both are Factors?

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ZIKV Phylogenetics

American strains constitute a “Western Hemisphere Group”

Epidemic strain emerged via genetic changes in Asian lineage virus in Yap State and French Polynesia (increased virulence ?)

Genetic recomb & amino acid changes identified, significance ?

Canadian Imported Case, Alberta Patient

Lanciotti et al., EID 2016:
http://wwwnc.cdc.gov/eid/article/22/5/160065_article
Initial & Ongoing Canadian Public Health Responses

1. Rapid risk assessment for Canadian travelers (sexual partners) including travel advice

2. Development of case definitions and guidelines for diagnostic testing (Canadian Public Health Laboratory Network – CPHLN, CATMAT, PHAC)

3. Diagnostic testing for ZIKV infection and evaluation of new assays (with provincial labs) and ZIKV research
Ongoing Impact of a Non-Endemic Virus With Severe Clinical Implications (ZIKV) on Canada:

• Travel related infections

• Estimated the 4+ million Canadians travel annually to affected regions

• Additional travel to Florida (ZIKV endemic?) significantly increases the population of travelling Canadians

• 1% of those pregnant or conceived?

• Possibility of sexual transmission upon return home

• Vector-borne transmission in Canada (endemic, establishment)?
Who should get tested:

<table>
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<th>Recommended:</th>
<th>Not currently recommended:</th>
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<td>• Any symptomatic traveller returning from an affected region</td>
<td>• Asymptomatic men with travel history to an affected region</td>
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<tr>
<td>• Asymptomatic pregnant women with travel history</td>
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<tr>
<td>• Sexual contacts of a confirmed Zika case</td>
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<tr>
<td>• Asymp. men or women with travel history who cannot delay pregnancy for medical reasons (IVF)</td>
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There are some caveats!
Current Zika Transmission
Declining activity, but not in all areas and resurgence possible.
However, the NML continues to receive large numbers of samples for testing!

NML - End of 2016: **21,038 Zika tests** performed on **13,966 samples** from **12551 patients from Canada**!

Currently ~ 500 samples a week received for testing!
Summary:

2016: 21,038 Zika tests performed on 13,966 samples from 12551 patients

Cases to date 2015-2017:

- 486 travel related cases
- 3 cases associated with sexual transmission
- 28 pregnant women tested positive
- 2 newborns with anomalies, 2 without
- Positivity Rates Have Decreased (Seasonal Factors - Mosquito Abundance, Viral Ecology - Decreased Virus Circulation, Increased Immunity, Preventative Measures, etc, new mosquito season looming !)
Laboratory Case Definitions for Confirmation of ZIKV Infections

1. **Detection of ZIKV** by **PCR**, antigen presence (IHC), or viral isolation,
   Specimens for PCR & Isolation include: serum, urine, CSF, semen, etc.,

   OR

2. **Detection of ZIKV-specific Antibodies**
   **IgM positive** (e.g., ELISA) and presence of **ZIKV-specific neutralizing antibody** using plaque reduction neutralization tests (PRNTs)

In a PRNT assay Zika virus is mixed with a patient’s serum sample and added to a cell culture monolayer to see if the patient has antibodies that will neutralize the ability of the virus to infect and kill the cells.
Patient’s sera is also mixed with dengue virus to compare neutralization titres (dilution of sera)

(PRNT assays, ZIKV vs Dengue titres, see below) in acute and/or convalescent samples

Eg. PRNTs -- Zika 640  Dengue 20/40  (observed in PCR positive patients)
Diagnostic Testing

**Molecular**
- Two target **real-time RT-PCR** specific for ZIKV
- Works best on **acute specimens**
  - **Serum**: 10-14 days onset (peak 3-5 days post-onset)
  - **Urine**: 14 days onset
- **Viral Isolation is difficult**

**Serological**
- **IgM capture ELISA**
  - Screening assay
  - Sensitive, not specific
  - Fast, 2 days
- **ZIKV PRNT**
  - Confirmatory assay to detect and titer virus-specific antibodies
  - Cross PRNTs performed (ZIKV and DENV)
  - Laborious & time consuming (7 days)
- Significant serological cross-reactivity amongst flavi’s can make interpretations difficult even when PRNTs are used!

Different Cell Lines need to be evaluated for test refinements
Zika virus is a “flavivirus” closely related to West Nile, Dengue, Yellow Fever and other members of this virus genus.

Both are transmitted by the same mosquito: *Aedes aegypti* – the “yellow fever mosquito”

In exposed individuals both can induce a set of antibodies that can bind both viruses.

Because of this ab cross reactivity a patient’s serum from a dengue infection can give a positive result in a standard Zika ELISA test.
**Original Antigenic Sin**, immune system preferentially utilizes immunological memory based on a previous infection when a second slightly different version of that foreign entity (e.g., a virus or bacterium) is encountered.

I.e. **Acute exposure to a pathogen** initially generates specific Abs / “**immune boost**” to a distinct but related pathogen (antigen) that the individual was previously exposed to in the past, the immune response to the current infection may be significantly decreased / sub optimal (until later in infection).

Associated with Influenza, HIV, dengue infections, etc.  \[\text{Den}_1 \rightarrow \text{Den}_2, \uparrow \text{abs to Den}_1\]

--- **suboptimal immune response** during secondary infection, **decreased IgM** (implications for vaccination, immunopathology, & diagnostics)
Antibody Testing Caveats: Significant cross-reaction problems especially for IgM ELISA tests but can also confound interpretation of PRNTs when Secondary Exposures occur.

e.g., Classic “original antigenic sin” issues caused by other flavivirus infections (travellers) followed by ZIKV exposure, or previous flavivirus vaccinations (YFV, JEV). Paired samples may help resolve the identity of infecting virus on a neutralization assay (PRNT).

But Not Always !!!! As well flavivirus secondary infections may lead to “Impaired IgM Responses” so screening IgM ELISAs need to be sensitive ! Commercial assays so far lack appropriate sensitivity !

Secondary Flavivirus Exposure Case Example:

Previous Dengue Exposure

Initial Serum PRNT (neutralization titres, Dengue 20, Zika 0

Second Serum Sample (2 weeks later) PRNT titres, Dengue 80, Zika ???

Recent Zika Exposure

D + Z

D 25

Z

D

Z

160 !!!

Continuing Co-circulation of Dengue, Zika, Yellow Fever, - Diagnostic Challenges !!
ZIKA Virus IgM ELISA Kit Formats and Evaluations:

Eg. Euroimmun (NS1), NovaTec (NS1), Diasorin (NS1), In Bios (Env)

*Initial* assessment of NS1 based assays indicate increased **specificity** for distinguishing between antibodies to related flaviviruses such as ZIKV and DENV.

However, **the sensitivity** for detecting the presence of IgM in acute samples is decreased as compared to the CDC – NML “in house” IgM ELISAs

“*Whole virus*” / E based antigens in ELISAs appear to provide required **sensitivity** but may have decreased **specificity** characteristics

Combining NS1 IgM & IgG ELISAs increase overall sens, however, some issues with distinguishing current from past infections, cost

Multiplex E, NS 1, 5 platforms (MIAs) & avidity measurement “promising” (See Friday Session D 05)
Combining New Technology with Old!
Utilizing Various DIAGNOSTIC FORMATS

Conventional Isolation, Serological, Molecular Assays + Next Generation Diagnostic Approaches
“Multiplex” Microsphere / Luminix Platform - Detection of antibodies to Zika proteins (Wong S et al. 2017)
Flow Cell

Quantitative Measure of Extrinsic Fluorescence Bead Identification (E, NS1-5)

Laser 1
Measurement of Antibody / Antigen Binding on Tagged Beads

Laser 2

Microsphere / Luminix Based Assays

Bead Identification (E, NS1-5)

Quantitative Measure of Extrinsic Fluorescence
Application of New and Higher Throughput Neutralization Assays for Serological Testing

Conventional PRNTs versus neutralization assays employing ZIKV Luciferase Platform

(Shan et al. 2017)
Novel **Point of Care** Molecular Detection Platforms for ZIKA, etc.:

Multi-Plex **RT – LAMP** based procedures being developed with Smartphone monitoring / recording capabilities

Tubes, Reaction Wells and Microfluidic Cassette Variations (Saliva, Blood and Urine matrices).

Priye A et al 2017
Song J et al 2016
Additional Zika Research

Commercial ELISA, etc. kit evaluations, ---

Models for Pathogenesis, Therapeutics – Vaccines, Studies on Vector Competence, Mosquito Surveillance.
Zika Virus Tissue Tropism and Vaccine Considerations: Research Models and Experimentation

From Diamond, 2017
Animal Models Are Essential For Determining Factors Associated With ZIKV Virulence (Fetal, GB, etc.), Mechanisms of Virus-Host Interaction, and Provide Framework For Design & Efficacy Testing of Therapeutics and Vaccines

**MICE** --- Various types of immunocompromised mice available including those lacking interferon genes or receptors -- ZIKV strains

**Non-Human Primates** --- eg. Rhesus – Cynomolgus Macaques Infected with Asian – lineage / outbreak ZIKV strains

**Pig Models** --- viremia, organ infection immune responses, placental piglet transmission, potential reservoir ? Darbellay et al. 2016
Lazear et al 2016 --- Infected Ifnr1 mice had high viral loads in brain, spinal cord and testes.

Govero et al 2016 --- used mouse adapted ZIKV to compare infection and pathology with Dengue

- Persistence in testes with ZIKV not Dengue
- Diminished testosterone and oligospermia, cell death and destruction of seminiferous tubules

Sapparapu et al 2016 --- mAbs against ZIKV E protein reduced tissue, plaental and fetal infection & mortality
NHP Models

**Dudley et al 2016** --- rhesus macaques susceptible to Asian Lineage ZIKV present in saliva, urine, and CSF. Non-pregnant animals remain viremic 21 days, **Pregnant 57 days**! Rechallenge – no viral replication

**Osuna et al 2016** --- rhesus and cynomolgus macaques infected with similar results but also detected in brain, semen and vaginal secretions.
Vaccines Being Developed / Initial Phase 1, 2 Trials

1. Two DNA vaccine candidates (Inovio, NIH) - Phase 1 trials, Phase 2 initiated

2. Inactivated virus vaccines (Walter Reed Army Research Institute)

3. Live attenuated vaccine platforms using chimeric flavivirus formats (pre M-E)
   -- Combined Dengue and Zika LAV formulations may provide utility
   (Laboratory of Infectious Diseases, NIAID)

4. Attenuated weakened live virus (NIH)

Possible Factors/Concerns Effecting Vaccine Development and Application:

---- "Antigenic Sin"— Pre-existing flaviviral antibody decreases initial ZIKV immune response

--- Antibody Dependent Enhancement
   Zika antibody enhances flaviviral infection
DNA Vaccine Approach

Gene encoding surface protein from Zika virus

Inject DNA containing Zika gene

Body’s cells produce virus-like particles, the basis of the vaccine

DNA Vaccine Utilized To Protect Against ZIKV Testes Damage in Mice (Griffin BD et al 2017)
**Antibody and Monoclonal / Polyclonal Based Therapeutics:**

--- Neutralizing human antibodies to ZIKV replication and maternal-fetal transmission & disease in mice (Sapparapu et al 2016, Nature)

Broadly reactive mAb panel from subjects previously infected, cloned hybridomas, possible therapeutic and vaccine design insights

**Genetic Engineering of Bovines For Generating Therapeutic Antibodies**

Tranchromosomal Cows:
- > 30 litres of conc ab
- SAB Biotherapeutics
Trans-Chromosomal Bovine Antibodies

• Ideal for rapid large scale production
• SAB Biotherapeutics has developed humanized cattle
• The bovine immunoglobulin genes have been knocked out and replace with fully human germ line antibody sequences
• Allows for hyper-immunization, and production of fully human polyclonal antibody to emerging pathogens.

Zika Neutralizing Polyclonal Antibody
Transchrom Bovine Ab Results in Mouse Model, Stein, Safronetz et al 2017,

100% Protection 1 Day Post Infection from lethal Zika infection in IFN^-/- mice, reduced Viremia, No virus detected in brain, testes etc. after bovine ZIKA polyclonal Ab dose

Significant weight loss in Isotype control treated mice
Persistence of ZIKV in Seminal Fluid

- **CDC preliminary studies** (CDC-PR, 2017) –

- Significant but **limited** lingering of virus, **majority of infections** result in 1-3 month semen persistence.

- However there is evidence of 6 month persistence in rare cases which has guided current public health recommendations.

- **UK Study (EID publication) with similar results*** ---
Arbovirus Mutation / Evolution Can Lead To Increased Transmission, Expansion, etc.

**West Nile Virus 1999 – 2002**
Genotypic Changes Led to Increased Transmission & Adaption

Chikungunya E protein mutation (s)
Increased viral transmission by *Aedes. Albopictus*

(*Albopictus* is found in more northerly regions than *Aedes aegypti* (Eg. Europe, North East US, Canada ?))

Non-structural proteins
Structural proteins

CAP

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Distribution of Primary ZIKV Vectors in USA – Expansion to Canada?

Possible adaption of vectors to different climates, and/or viral adaption to “new” mosquitoes?
Surveillance for *Aedes Aegypti* and *Aedes Albopictus* In Canada

Mosquitoes found in Windsor, Ontario in 2016!

Risk for Establishment is low, however, continual monitoring is warranted.
Vector Competence Studies on Canadian Mosquitos (Aedes vexans, etc) Lindsay et al.

Oral infection

Infection through needle inoculation

Collection of saliva to assess transmission ?!
Orally Infected Mosquitoes with Two Strains of ZIKV, Winnipeg, MB, Summer 2016

<table>
<thead>
<tr>
<th>Species</th>
<th>Number tested</th>
<th>No. infected (%) infected</th>
<th>No. disseminated (% dissem.)</th>
<th>Min. No Saliva Pos. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ae. vexans</em></td>
<td>131</td>
<td>4 (3.2)</td>
<td>2 (1.5)</td>
<td>2* (1.5)</td>
</tr>
<tr>
<td><em>Oc. euedes</em></td>
<td>7</td>
<td>1 (14.3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Oc. fitchii</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Oc. sticticus</em></td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Cx. tarsalis</em></td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Cq. perturbans</em></td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Live ZIKV isolated from these saliva samples
Conclusions on Canadian Vector Competence For ZIKV, Lindsay R et al

• To-date, mosquitoes from southern Manitoba have demonstrated poor competence as vectors for ZIKV

• ZIKV multiplied in the bodies of many of the species that were inoculated

• Small numbers of Ae. vexans successfully transmitted ZIKV under laboratory conditions

• There was no significant difference in rates of transmission between the two strains of ZIKV

• Further studies are required to determine whether climatic conditions in Canada are permissive for local transmission of ZIKV
Vector control strategies for Zika virus and other arboviruses

Main components include:

1. Entomological surveillance

2. Vector control options
   - Measures targeting aquatic stages
   - Measures targeting adult mosquitoes

3. Personal protective measures
Other Mosquito Pathogens

IT'S O.K. GUS!
IT TESTED NEGATIVE
FOR THE WEST
NILE VIRUS!
## Mosquito Transmitted Arboviruses Isolated in Canada

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<th>Antigenic group</th>
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<td>+ humans, + animals</td>
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</tr>
<tr>
<td>St. Louis encephalitis</td>
<td>Flavivirus</td>
<td>+ humans, - animals</td>
</tr>
<tr>
<td>West Nile</td>
<td>Flavivirus</td>
<td>+ humans, + animals</td>
</tr>
<tr>
<td>California encephalitis</td>
<td>California-Bunya</td>
<td>+ humans, - animals</td>
</tr>
<tr>
<td>Snowshoe hare (SSH)</td>
<td>California-Bunya</td>
<td>+ humans, + animals</td>
</tr>
<tr>
<td>Jamestown Canyon</td>
<td>California-Bunya</td>
<td>+ humans, + animals</td>
</tr>
<tr>
<td>Trivittatus</td>
<td>California-Bunya</td>
<td>- humans, - animals</td>
</tr>
</tbody>
</table>

**Seasonal trends** are key: Most infections occur late in the summer when virus levels are peaking. However, some arbos such as California serogroup cause infections in late spring – should be part of differential when mosquitoes present.
Bunyaviruses -- California Serogroup (Jamestown Canyon and Snowshoe Hare virus Transmission Cycles (Canada wide!))

Incidental hosts
Febrile and Neurological Disease

Transovarial Transmission

Snowshoe hare virus (SSHV), Jamestown Canyon Virus (JCV)
Case History (Webster D et al 2017):

On July 23, 2015 a 73 year old male from Grand Manan Island, New Brunswick developed symptoms of fatigue, nausea, fever.

Several days later febrile illness progressed to delirium and increasing confusion- encephalitis. Condition further declined – post encephalitic dementia in Jan

Herpes, Bartonella, Borrelia. Anaplasma, Coxiella etc. negative (frequent outdoor activities)
Case for “VZ Swap Team, & Dr. Duncan Webster”
Mosquito-borne disease threats: Atlantic Canada

• Sporadic activity limited to enzootic cycles of transmission of
  – **West Nile virus** (infected birds, horses in early 2000’s, no humans)
  – **Eastern equine encephalitis virus** (horses, no human cases)

• Greater seasonal exposure to other arboviruses like **Snowshoe hare virus (SSH)**, **Jamestown canyon virus (JCV)** and other California serogroup viruses
  – Wide range of vectors, high infection prevalence and typically high rates of human exposure, most infections asymptomatic or only mild course of disease but neuroinvasive cases do occur
JCV Serosurvey of NS residents and deer (Patriquin G, Drebot M, .. Hatchette T et al. 2017)

JCV Human Seroprevalence
DHA 1     - 48.2%
DHA 2-8   - 22.6%
DHA 9     - 15.2%
Acute Serum Sample Tested For CSG (JCV & SSH) IgM: ELISA negative - equiv

However PRNT – IgG positive!

2nd serum sample IgM equivocal

Diagnostic 4 fold rise in neut IgG

4 weeks PO IgM pos!, neut IgG +

Impaired IgM induction, high neut / IgG titres to 2 CSGs

Secondary CSG Exposures & Antigenic Sin !?
IgM Dynamics of CSG Case NB Native, Sepsis Case in MN, WNV case

IgM Ab Positivity

9
8
7
6
5
4
3
2
1

IgM Neg

3
6
9
12
15
18
21
24

Days Post Onset of Symptoms

“Typical”
JCV IgM

NB Case

Pos Equivocal

JCV IgG / Neut titres
SSH IgG / Neut titres

40!
160
320!
20!
320
320!

NB Case

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IgM production delay and Original Antigenic Sin as seen in Dengue & Zika flaviviruses.

When similar virus co circulate at high levels previous exposures or co infections can occur that may confound serological test Interpretations (Previous Canadian serosurveys indicated 9-10% previous Den exposures)

May also cause issues involving Increased Disease Severity and reduced Vaccine efficacy!
**Mosquito Transmitted Arboviruses Isolated in Canada**

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<td>+ humans, + animals</td>
</tr>
<tr>
<td>Trivittatus</td>
<td>California-Bunya</td>
<td>- humans, - animals</td>
</tr>
<tr>
<td><strong>Cache Valley</strong></td>
<td>Bunyamwera</td>
<td>+ humans, + animals</td>
</tr>
<tr>
<td>Northway</td>
<td>Bunyamwera</td>
<td>- humans, - animals</td>
</tr>
<tr>
<td>Turlock</td>
<td></td>
<td>- humans, - animals</td>
</tr>
</tbody>
</table>
West Nile Virus continues to be our most important domestic arbovirus in Canada, North America

Average Case Density Value per 100,000 population of West Nile virus disease, by provinces and territories in Canada: 2002-2013
West Nile virus continues to have a significant public health impact, future outbreaks are always a possibility!

US cases ~ 1600 in 2016
Canada -- 100 cases in 2016
--- 6 positive blood donors
Culiseta melanura distribution and Eastern Equine Encephalitis Virus

First Non-Imported Case in Canada September, 2016 !!

13 year old, Encephalitis case, Ont

Prior to 2016 livestock cases (horses, emu) but no human cases in Canada

Collection localities for Culiseta melanura in Canada: • specimens
Vector-borne / mosquito borne zoonotic diseases continue to be of importance as public health issues for both travelling and non-travelling Canadians.

Mosquito associated pathogens continue to emerge and increase in frequency both in Canada and Internationally and need to part of physicians / health care provider’s differential.

National and Global Partnerships Using a “One Health” Approach Are Key For Identifying, Monitoring and Characterizing These Pathogens and Assessing The Risk for Both Preparedness & Response
Questions ?