



Public Health Agence de santé Agency of Canada publique du Canada

The Untold Stories Of The National Microbiology Laboratory:

Innovations to keep ahead in the evolutionary struggle with the microbial world

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Emerging and Remerging Infectious Diseases

- Emerging infections are those that are new or newly discovered in humans – mainly come from animals (H1N1, measles, plague, tuberculosis, influenza, HIV, SARS)
- Infections re-emerge when old microbial foes acquire new weapons through <u>genetic</u> exchange or <u>mutation</u> –resistance to drugs or vaccines, escape from the human immune defences

Emerging and Re-emerging Infectious Disease Over My Career

Bacteria

- Legionairre's disease
- E.coli H7:0157
- Clostridium difficile colitis
- Helicobacter pylori
- Chlamydia trachomatis and C.
 pneumonia
- Staphylococcus toxic shock
- Flesh eating disease
- Methicillin resistant *S. aureus*
- Penicillin resistant gonorrhea and *Hemophilus influenza*
- Extremently drug resistant tuberculosis
- Vancomycin resistant enterococcus

Viruses

- Ebola virus
- HTLV-I
- HIV-1
- HIV-2
- Nipah virus
- Borna virus
- West Nile virus
- SARS
- H5N1 Avian influenza
- H1N1 pandemic influenza
- Hepatitis B & Hepatitis C
- Delta agent
- Prion disease (mad cow)
- NERS

Are Infectious Diseases Threats Increasing?

- Probably "yes"
- 35+ "new" diseases over the past 40 years
- Of all microbial species, we have characterized less than 1% and the unknown 99% represent constant source of new threats.
- Most new infections come from animals
- Organisms mutate in response to human tactics: drugs, vaccines, disinfectants ...

Why Is This Happening?

- Ecologic changes
- Human demographic/behavioural changes
- Globalization
- Rapid growth in technology
- Microbial adaptation and change
- Gaps in public health programs/infrastructure

Anticipating the Puck



The Katrina Lesson

Canada's Response To These Threats – Build The National Microbiology Laboratory In Winnipeg



NML Fast Facts

- Announced 1987
- Construction began 1992
- Official opening 1999
- Only operational Level 4 lab in Canada
- First facility with human and animal CL 4 labs
- 31 metres high
- 29,300 sq. metres



Recent Expansion

\$ 3 story expansion funded
 by the Economic Action Plan
 (\$24 million)

Completed March 31, 2011

Expanded specimen receiving, shipping and receiving, bio-repository and media preparation areas.







Containment Level 2

Most CSCHAH labs are CL2 (60.8%)
Similar to laboratories in hospitals and medical clinics
Work done in a biosafety cabinet with controlled air flow (at right)
Treatment and preventive measures are available

*Examples:*E-coli
Whooping Cough
Hepatitis C

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Containment Level 3

♦ 35.5% of total lab space Can cause serious disease in humans or result in serious economic consequences (animals) Diseases that do not ordinarily spread by casual contact or they are treatable

Staff wear laboratory clothing and shower out (most areas) ♦ Air is filtered; waste is treated

Examples:

- Foot-and-Mouth disease
- West Nile virus









Containment Level 4

3.7% of lab space (1.1% of building)
Dangerous agents that usually produce very serious and untreatable diseases
Staff wear biosafety suits; chemically treated each time

- Each department has one CL4 suite which provides back-up during annual recertification
- Suites are air tight
- All air and waste sterilized

Examples:

- Ebola virus
- Nipah virus
- Marburg Virus

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Canada

Directional Air Flow

- Air pressure is tightly controlled in and around high containment labs
- Doors are interlocking; bioseal (submarine) doors in key locations
- Progressively lower pressure going deeper into lab space dropping by 50 pascals between rooms ensures air flows in toward lab and out through HEPA filters





Some Important Public Health Innovations From The NML

Innovation 1:

Rapid Detection & Alerting of Infectious Disease

New Tools to Increase Speed of Detection and Alerting

- Previously: Countries reported new & emerging diseases to WHO – take weeks
- Now: advanced computer based technologies
 - Canadian Network for Public Health Intelligence
 - Monitors news, internet etc.
- Examples: SARS, H1N1, Listeria

Canadian Network for Public Health Intelligence

Fostering collaboration and consultation through innovation in disease surveillance, intelligence exchange, research, and response to protect, promote and support public health

> Dr Shamir N Mukhi Chief Engineer

Background

•An innovative scientific public health informatics platform developed and managed by PHAC-NML.

•Includes technologies for collaboration, surveillance, alerting, knowledge management, lab systems, and event management supporting large number of F/P/T public health professionals in human, animal and environmental health domains

•Recognized as trusted platform providing key foundational infrastructure for public health surveillance in Canada.

What is CNPHI?

A secure public health intelligence cloud



What is CNPHI?

- CNPHI is an innovative, versatile informatics platform that has been custom built for public health needs
- CNPHI platform is not static; constantly to meet current and future needs of dynamic public health programs
- Respects jurisdictional boundaries
- Helps transform program to structured centralized data collection and analysis
- Innovative current technology
- Six focus areas: Knowledge Management, Collaboration, Alerting, Surveillance, Event Management, Laboratory Systems



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Benefits

•Provides capability to directly link F/P/T epidemiologists and laboratories, enabling bi-directional and close to realtime data and intelligence sharing between partners while respecting jurisdictional boundaries

Innovative reporting and data entry/collection tools
 supporting increased data quality, interactive data analysis

 Provides initiative specific sponsors control to manage access to applications within their platform

- Facy charing with all participants of discussions



CNPHI at a Glance



Event Management

- Command and control
 Real-time data collection and integration
- Intelligence organization, display, and decision making tools
- Integration of organic and external expertise
- Connectivity of external command centres and
- capabilities - Data recovery and long-term analysis

Program Management

- Program/Businesss centres
 Communication and
- coordination - Program driven; configurable and flexible

Alerting

- Targeted alerting
- Role based
- Respect jurisdictional accountabilities
- Program driven; configurable & flexible



- Intelligence presentation using maps, charts, etc.
- Supported by algorithms and other decision support tools
 Program driven; configurable
- and flexible



- Manual and automated
- targeted data collection - Respects privacy issues
- Program driven; configurable and flexible

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*General Alerts will accommodate disease areas not specifically addressed in other modules until program areas define disease specific user requirements

Laboratory Feature of CNPHI

PFGE tiff file

Dendrogram created in Bionumerics



Patterns entered into Bionumerics and analyzed



Each lab analyzes the image and replies to listserv: Any pattern matches? Any known sources of infection? What is known about the pattern?

Clusters identified Is this a new pattern? Is this a common pattern? Patterns designated

How DNA fingerprinting* was used to identify and investigate the 2008 listeriosis outbreak, example:

Source	Listeria DNA Fingerprint	Result
Patient 1 Patient 2 Patient 3 Patient 4 Patient 5 Food Product 1 Food Product 2		not outbreak strain not outbreak strain outbreak strain outbreak strain outbreak strain outbreak strain outbreak strain
Patient 6 Patient 7 Patient 8 Patient 9 Food Product 3 Patient 10		outbreak strainnot outbreak strainnot outbreak strainnot outbreak strainnot outbreak strainnot outbreak strainnot outbreak strain

Listeria monocytogenes DNA fingerprints analyzed by the National Microbiology Laboratory (NML), June – October, 2008

Includes all human, food, and environmental samples from the 2008 national Listeriosis outbreak investigation



Jumber of Listeria I solate

Innovation 2:

Rapid Containment - Sending the Lab to the Specimen!

Microbiologic Emergency Response Teams

- use of portable CL3 units to create safe work environment
- isolator for basic microbiology
- isolator for specialized techniques
- equipment needed to perform testing



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Arrival In Uige



Mobile Laboratory Equipment



Uige Airfield

Operations for Ebola in DRC 2007



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Second team to new location –> breakdown lab and transport, second team sets up again.

High Containment in the Bush









Perfectly Functional Laboratory









Innovation 3:

Using Viruses to Fight Viruses!

Viral Hemorrhagic Fevers

Scary Viruses !

Why Feared?

- Ebola
- Marburg
- Lassa
- CCHF
- Machupo

- Zoonotic
- Mortality as high as 80%
- No treatment, no vaccines
- "A list" of bioterrorism threats

Ebola Vaccine Development

- Uses a Vesicular
 Stomatitis Virus to fool the immune system into thinking it's Ebola.
- Effective in non-human primates, seemingly proved effective for human use in West Africa







Non-human primates





A not so brief history of VSV-EBOV

Ebola vaccine developed in Winnipeg is headed for Africa

Canada is donating 800 to 1,000 doses of the vaccine, which was developed at the National Microbiology Laboratory



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Ebola vaccine efficacy trial ready to launch in Guinea

Joint news release WHO/MSF/NIPH

5 MARCH 2015 I GENEVA - Based on promising data from initial clinical trials in late 2014, WHO with the Health Ministry of Guinea, Médecins Sans Frontières (MSF), Epicentre and The Norwegian Institute of Public Health (NIPH), will launch a Phase III trial in Guinea on 7 March to test the VSV-EBOV vaccine for efficacy and effectiveness to prevent Ebola. The vaccine was developed by the Public Health Agency of Canada. A second vaccine will be tested in a sequential study, as supply becomes available.

A humble history of VSV-EBOV



Ebola virus disease

Learn about Ebola virus disease, its causes, symptoms, risks, treatment and prevention. Also find information on surveillance and guidance for health professionals.





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Emergency vaccination A. Ring vaccination B. Post-exposure vaccinatio





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 Kebela (DRC)

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE









Public Health Agency of Canada

Agence de la santé publique du Canada



National Institute of Allergy and Infectious Diseases Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.



U.S. ARMY MEDICAL DEPARTMENT U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

These Vaccines Could Help Save the Great Apes





Innovation 4:

Monoclonal Treatment For Ebola Virus Infection!

Therapeutic options

- Coagulation regulators (e.g. MAPc2)
- Convalescent plasma
- Monoclonal antibodies (e.g. ZMapp)
- PMOs, siRNA
- Small drug molecules (e.g. BCX4430, T705)
- Interferons

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1H3 Escape mutation





French

- ZMapp in Sierra Leone; Dr. Khan.
- ZMapp in Liberia; Jimmy.
- Zmapp versus Zmab and sleep deprivation.
- Compassionate use in emergencies and the law.
- What do we need to believe.

A COMPARATIVE STUDY OF LYMPH AND SERUM FERMENTS DURING PROTEIN SHOCK REACTIONS. Davis BF, Petersen WF. J Exp Med. 1917 Nov 1;26(5):699-706.

- Russia got the world's first communist government, lead by Lenin.
- US declares war on Germany, Jerusalem falls to Britain
- NHL forms with Montreal Canadiens, Montreal Maroons, Toronto Arenas, Ottawa Senators & Quebec Bulldogs. 1st NHL championship game ever played, Toronto Arenas beats Montreal Canadiens 7-3 in 1st of 2 game set.
- Secret Service extends protection of president to his family

and most effective w

Measles, hepatitis A, ra based bacterial disease well as pneumococcal a

 Antibody therapy wa the discovery of anti



Monoclonal Antibody Therapy

- The introduction of hybridoma technology in 1975 made possible the generation of one antibody or monoclonal antibody (mAb) produced from one B-cell clone, specific for one antigenic epitope.
- The technique was initially developed in mice and later mAbs were partially or fully humanized.
- There are currently around 350 mAbs in clinical development, with most in early developmental stages.
- The majority (~90%) of these mAbs target antigens relevant either to cancer or inflammatory or immunological disorders.

Innovation 5:

Using High Throughput Machines To Understand Genetics

H1N1



April 17 Agence de santé publique de canada April 17 Mexican Health Officials Request Assistance from NML

CC



"celiam" <celiam@servidor.unam.mx> 2009-04-17 07:30 PM

To <frank_plummer@phac-aspc.gc.ca>

Subject Probable Influenza with severe pneumonia cases in México

Dear Dr. Plummer:

I hope this mail will find you very well. I am bothering you to comment a situation regarding some confirmed and probable cases of Influenza with rapid evolution to interstitial pneumonia reaching mechanical ventilation that we are having in México right now. I would appreciate any comment and help for other possible diagnosis.

The attack rate among health workers seems to be higher than normal. We do not have the entire picture because under notification is still a problem in México.

So far I can tell you we have seem a late peak in March and April of probable Influenza and confirmed cases, mostly adults 17-45 years old (we do have vaccination programs for >60 years old and small children). We detected several outbreaks in three different States with no fatalities, in the last two months. One of these outbreaks, in Tlaxcala, central part of México, with almost 60 probable cases and we confirmed 35 of them with both A and B influenza virus. Other outbreak in a small town in Veracruz (gulf coast) with a clinical picture "influenza like" with an attack rate of 30%, almost 400 probable cases, no fatalities, but we only confirmed influenza in 4 (three A and one B, un SRV, 3 Parainfluenza and 2 adenovirus). The most of the sampling is late after 5 days then we lost sensitivity. However since the last 2 weeks one of the biggest National Institutes of Health in México(NIH), National Institute of Respiratory Diseases(INER) reported us (Tuesday almost 50 cases of Probable influenza with 10 cases of severe pneumonia, 2 death in small children and 1 physician (transferred from another hospital), only 6 of these cases were positive for Influenza account and commercial Real time PCR kit at the chinical lab in the hospital. We confirmed 3 of these cases (two of the deaths), unfortunately the other 3 were secondary samples 2 or 3 days later in patients already receiving Oseltamivir and they were negative for us. These virus were 2 Influenza A (H1N1) and 1 B. The physician death was 59 years old and 2 of his daughters had the same clinical manifestation with pneumonia, both of Down syndrome one of the mole shopital iced recovering. In these cases they have a high attack rate within hospital workers, physicians and nurses mainly, not severe pneumonia in them only severe myialgias, arthralgias, fever, and so on. The physician is one of the positive for Influenza A at the hospital lab ut negative for us.

The personnel concern, allowed us to find about this and we started our investigation. Other big NIH Hospital reported 2 cases with rapid evolution with severe pneumonia with the requirement of mechanical ventilation and one is an Anesthesiologist fellow of that hospital. The confirmed influenza in the "outpatient "but the fellow was negative, both of them receive immediately oseltamivir and they are recovering right now. Oaxaca state reported a 37 year old diabetic woman with the same clinical picture with rapid evolution to severe pneumonia and she died after 4 days of hospitalization no more cases are reported, no outbreak of respiratory diseases in the area. I got bronchoaspiration fluid, nasopharyngeal swab, serum, lung and liver biopsy of this death patient. Autopsy was not authorized by the family. So far all the samples including lung tissues are negative for influenza, parainfluenza 1-3, adenovirus SRV, metapneumovirus, coronavirus, Hantavirus in serum, etc. However in a private clinical laboratory in Oaxaca they ran some brochoaspiration swab and the report was positive for coronavirus. We have tested this twice in two different places here in México city and they are negative for coronavirus. Today we detected that there is another fellow hospitalized in a City near Mexico City with severe pneumonia and mechanical ventilation, they never notified and we do not have samples. In the central part of México San Luis Potosí a Private ID physician called us to say he received 10 cases of severe respiratory diseases 3 of them with severe pneumonia previously healthy and one death, we checked in the area and we found one severe pneumonia case in another hospital and the antecedent of another one death all of them are between 19 and 47 years old. They will send me autopsy tissues of one of the deaths and biopsy and samples of the other one by Monday.

All the cases are interstitial pneumonia, no hemorrhagic data, some of them with shock in the beginning, laboratory suggesting viral diseases, some of them with longer hospitalization suggest bacterial infection added. In addition I can tell you that influenza surveillance is improving in México, is limited, but so far in this season we have tested a little bit above 4000 probable cases and we confirmed 316, 37% B (increased amount in comparison to last season and before, less than 15%) and even we have not subtype all the A isolations the most of them are HIN1 and H3N2 including those from Tlaxcala outbreak and the recent 2 A isolations from INER.

After all the analysis we considered we have a late peak of influenza season with more severe presentation because of the confirmation in some of the cases and they are not confined to specific area but there are association among some of these cases. We are concern regarding the health care workers attack rate, but we found no matter vaccination for them is free the vaccination coverage in them is only 20-40%, we are using late vaccination now to improve coverage in health care workers, we reinforced all precaution measurements including high efficiency mask for health care workers in close contact with probable cases in hospitals and health promotion regarding respiratory diseases campaign for public, in general we did a press conference today without panic pointing these issues.

I would really appreciate any comment about this situation and advice for more diagnosis and if it is possible to send some of the tissues or samples to MNL.

Best regards

Celia

Dra. Celia M. Alpuche Aranda Dirección General Adjunta Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE)

MX contacts NML requesting





swine flu cases in California





NML determines outbreak in Mexico is **H1N1 Influenza A** (human swine flu)



North America (A/Mexico/ /2009 [H1N1]), Influenza A virus, April 2009:

- Reassortment H1N1: An unusual mix of genetic sequences
- 4 unique virus sources





April 29 200 clinical samples

Mexico City direct to Winnipeg

ASHING

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Innovation 6:

Using Systems Biology to Understand Infectious Disease

Solutions for the HIV Pandemic

HIV Prevention With The Old Science

Finding	Time Lag to General Acceptance
Women were readily infected with HIV through sex	2 years
Other STD promote HIV transmission	5 years
Breast feeding is a major route of mother to child HIV transmission	5 years
Male circumcision reduces HIV susceptibility	19 years
Hormonal contraception enhances susceptibility to HIV	?
Behavioural interventions targetted at "core groups" reduced HIV in the general population	?

Resistance to HIV-1 Infection

Highly-exposed persistently seronegative.

3 years follow, HIV-1 serology and PCR negative, Active in sex work.

10% of highly exposed sex workers resistant to HIV-1.



Nature of HIV Resistance



Gene expression profiling in HIV Resistant CD4 T cells Women Whole Blood







Conclusion

- Microbial threats are growing and ever changing
- The National Microbiology Laboratory has become a key global institution in innovation for response to emerging infectious diseases.
- The importance and achievements of the National Microbiology Laboratory are relatively unknown by Canadians.

Next Steps

- In general existing bioinformatics has yielded limited insights into the basis for resistance to HIV infection and other complex data problems.
- In collaboration with a machine learning company we are exploring the utility of artificial intelligence for understanding these kinds of complex data.