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Director's Update

Laboratory Networks

Many of you will have received a copy of BCCDC Laboratory Service's Annual Report. If you haven't seen it, we would be pleased to send you a copy (call 604.660.6030). Alternatively it can be viewed on our website (<http://www.bccdc.org/content.php?item=33>). The theme of this last year's report was Networking. We are building a laboratory system together - our links are national (such as the CPHLN, Canadian Public Health Laboratory Network), provincial (such as PLCO, the Provincial Laboratory Coordinating Office) and regional (such as LMIC, Laboratory Medicine Infection Control Services).



Network Impacts

One of the population health benefits of improved information management includes linking for better surveillance. Over the last number of months, the BCCDC has helped identify and manage many outbreaks of infectious disease such as:

- Norovirus (cruise lines, hospitals and Long Term Care facilities impacted). Thanks to **Lorraine McIntyre**, the Environmental Health Team and **Alan McNabb**, Molecular Services for their leadership on understanding the molecular epidemiology of this emerging pathogen, previously named Norwalk Virus.
- *Clostridium difficile* is of current interest. Mr. Bruce Gamage is working on a survey to gather BC information. Alan McNabb is working with a large team (Ana Paccagnella, Dr. Martin Petric, Carol Shaw, Dr. Swee Han Goh and others) on molecular fingerprinting.
- **Dr. Linda Hoang** and **Dr. Corinne Ong** have been, with your help in the BC Lab Network, studying the third year of a cluster of locally acquired *Cyclospora* parasitic infection. In addition, experts from Southeast Asia are working with Drs Ong and Hoang (funded by a CIHR grant) to improve our understanding of this protozoan.
- Avian influenza was a major concern for public health. While there were only two human infections (ocular) from direct contact from this outbreak in birds, the BCCDC has been working, (thanks to Dr. Martin Petric and the Virology team) on new molecular methods for identifying and fingerprinting influenza.

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From the Enterics Team's perspective, the use of pulsed field gel electrophoresis (PFGE), helped public health separate out different clusters and their sources of *E. coli* O157 infections this past summer.

- One of the best networks was the WNV Team, led in Laboratory Services by **Dr. Morshed**. He and **Dr. Murray Fyfe** worked with many regional partners to prepare for the arrival of this emerging pathogen.

Hepatitis B Testing

It is widely accepted in clinical and public health practice that a level of 10 mIU/ml anti-HBs is protective for hepatitis B infection. However, there is little information about the accuracy and consistency of test results; both between repeat runs on the same instrument and between different assay platforms. In July 2003, Abbott Diagnostics notified Canadian laboratories of inaccuracies in standard calibrator lots for anti-HBs tests, which may have led to readings up to 24% above the 'true' readings.

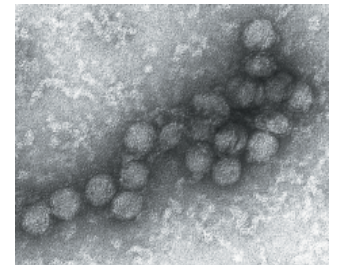
This prompted BC Centre for Disease Control virology laboratory to evaluate different assays. 155 sera previously determined by an accurate standard calibrator to have anti-HBs levels between 10 and 20 were retested using the same (Abbott AxSYM) and three different instruments (Abbott Architect, Bayer ADVIA Centaur and Ortho VITROS anti-HBs). On retesting by the same assay, two specimens changed from immune to non-immune; and non-immune status determined in 17, 39 (and 44 equivocal) and 27 (and 42 equivocal) specimens respectively using different assays. Thus, we can see there is substantial variability in anti-HBs levels between different tests. Unfortunately there is no gold standard and vaccination history must be considered in interpreting borderline test results.



West Nile Virus : Not Here Yet But On the Move

It may not be here in British Columbia yet (no positives in our bird or mosquito surveillance program) but it is only a matter of time. There has been much written about this emerging pathogen but we thought it would be useful for information with a clear laboratory diagnostic focus.

The West Nile Virus (WNV) was first introduced into the northern hemisphere in 1999 in New York. WNV is maintained in nature in a mosquito-bird-mosquito transmission cycle primarily involving *Culex* mosquitoes. Humans, mammals, and reptiles are incidental hosts. During 2003, there were 1335 cases reported in Canada.



West Nile Virus

Description of the Agent

WNV is a spherical enveloped RNA virus serologically classified within the Japanese Encephalitis (JE) virus antigen complex. Morphologically it is about 50nm in diameter and consists of a host-derived lipid bilayer membrane surrounded by a nucleocapsid core containing single stranded RNA.

Clinical Presentations

While most WNV infections in humans are asymptomatic, approximately 20 percent of infected patients have a fever. The majority of reported illnesses have been designated as West Nile Fever, a dengue-like illness presenting with fever, headache, fatigue, myalgia, arthralgia, lymphadenopathy, and maculopapular rash. The virus can also infect the nervous system (West Nile neurological syndrome or WNNS), with clinical presentations including meningitis, encephalitis, polio-like paralysis, movement disorders, and Parkinsonism. During 2003, 160 (12%) of reported cases in Canada and 2863 (29%) of cases in the USA were WNNS. The presence of WNV in breast milk, transmission of WNV from human to human via organ transplants, blood transfusion, and the intrauterine route as well as laboratory-acquired infection have been reported.

Laboratory Diagnosis

The accurate and timely diagnosis of WNV infection requires good communication with laboratories. Information needed by the BCCDC (sole testing laboratory) includes the patient's travel history and symptoms, onset date. Consideration of other agents, (such as herpes simplex), in the differential diagnosis for encephalitis should also prompt the order of other appropriate diagnostic tests.

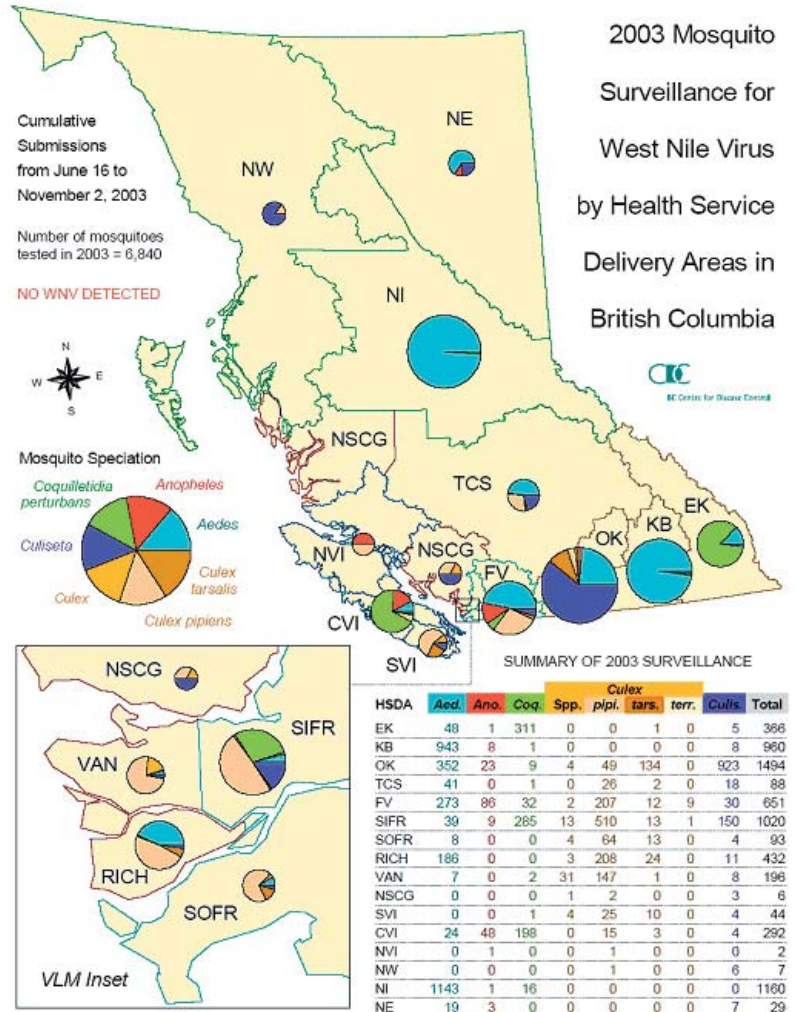
Diagnostic tests should be ordered on patients with clinical presentations consistent with WNV infection (WNV fever or WNS) during the period when WNV could be transmitted by mosquitoes in BC (spring until the first hard frost in autumn).

One or more appropriate specimens include:

- Acute serum collected on presentation and convalescent serum collected 14 - 21 days later. Serum (7-10 mls) should be collected in a pink-top tube and sent to the BCCDC Laboratory Services (Zoonotic and Emerging Pathogens Section).
- Acute plasma collected on presentation. Plasma (7 - 10 mls) should be collected in a purple-top tube and sent to BCCDC Laboratory Services, Zoonotic and Emerging Pathogens Section.
- Cerebrospinal fluid (CSF): 1 - 2 mls should be collected in each of two tubes (no preservatives) and should be kept at 4°C or frozen for transport to the BCCDC Zoonotic and Emerging Pathogens Section.

Serum samples will be used for detecting antibodies against WNV. A screening test (EIA) will be used first along with a second serological test (HI test) when appropriate. Confirmatory of serologically positive tests are done by the BCCDC's reference laboratory. CSF and plasma specimens will be used for detecting WNV antigen (RNA) by a polymerase chain reaction (PCR) test.

PCR testing services will be provided by the BCCDC Laboratory Services on all Cell, Tissue and Organ specimens for WNV as recommended.



BCCDC Laboratory Services ZEP Laboratory Services (serology - 604.660.6054), Parasitology (PCR mosquito surveillance - 604.660.6055), and Virology Services (PCR human specimens - 604.660.4672).

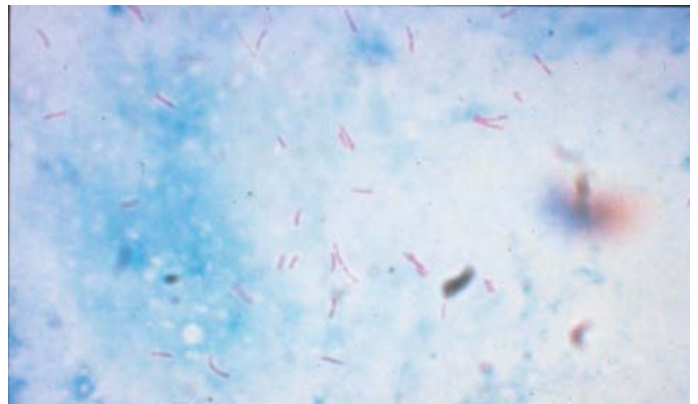
Malaria Reference Services

BCCDC Parasitology Services provides public health diagnostic, contact follow ups, outbreak investigation, surveillance and reference testing for parasitic infections. One parasitic infection, malaria, is potentially fatal. Infections due to *P. falciparum* must be diagnosed rapidly and accurately. Thick and Thin Giemsa stained smears, repeated as appropriate, are recommended for optimal diagnoses. Besides, Quality Assurance studies using PCR (to identify and speciate malaria parasites), technical experts from this section are On Call (24/7) for malaria emergencies. Requests for this emergency service (after hours Monday-Friday, weekends and holidays) must be made through the BCCDC Medical Microbiologist On Call (604.661.7033).

Improved Testing for TB: Identification of Mycobacterium Species by hsp65 Gene Sequencing

The **Molecular Services** and the **Mycobacteriology (TB) Laboratory** initiated a four phase study to determine the feasibility of using partial sequencing of the 65 kiloDalton Heat Shock Protein Gene (hsp65) for the routine identification of mycobacteria from clinical specimens.

Phase One encompassed the creation of a comprehensive and validated hsp65 database which contained all valid mycobacterial species type strains as well strains from putative species and groups and resulted in a world class database.



AFB Smear Positive

Phase Two dealt with the identification of over 600 isolates that were held in the Mycobacteriology Laboratory stock culture collection or clinical isolates encountered during the study. The outcome of this work was peer reviewed (J. Clin. Microbiol. 2004; 42:3000-3011) and represents the largest study of the use of hsp65 sequencing for the identification of mycobacteria. From this work several points were apparent, biochemical testing, although considered by some sources to be the gold standard for the identification of mycobacteria, is an inadequate method for mycobacterial identification, being both inaccurate and more costly than hsp65 sequencing as well as more time consuming. In fact, isolates that took four or five weeks to identify by biochemical tests could be identified in two days by hsp65 sequencing.

Phase Three was the investigation of identifying mycobacterial isolates directly from our primary liquid detection media (Bac T Alert 3D as well as MGIT bottles). This phase has been successfully completed and the data of isolates from over 600 bottles indicates a 99.6% correlation between hsp65 sequencing and all other means of identification. This work discovered several undefined groups of mycobacteria found from clinical sources and is being prepared for publication. The benefit accorded to this technique is that all organisms detected in liquid media can be rapidly identified to species, whereas previously, some organisms once detected in liquid detection media would have to be subcultured from the detection bottle and their growth could require several weeks to occur. Direct sequencing from primary bottles allows the mycobacteriology laboratory to identify 99.6% of all isolates within two days of the primary detection bottle being indicated as containing acid fast bacilli. This provides not only a faster identification for the patient's isolate, but has allowed us to recognize cost savings.

Phase Four is an attempt to apply the technique developed for the identification of mycobacteria from both solid and liquid growth media directly to samples from patients. To date we have analyzed a small number of clinical samples with some success, but it is evident that further development and experience is required to determine the efficiency of applying this test directly to clinical samples.

The BCCDC is working with the CPHLN (Canadian Public Health Laboratory Network) towards understanding the molecular epidemiology of TB across Canada. New methods also used by USA CDC are considered for use in a national algorithm.