



LABORATORY TRENDS



August 18, 2011

Laboratory News

ViroNet Canada

The Public Health Microbiology & Reference Laboratory (PHMRL) is a member of ViroNet Canada, an initiative of the Canadian Public Health Laboratory Network (CPHLN). The ViroNet network enables the comparison and identification of norovirus outbreak strains and supports a database maintained by the National Microbiology Laboratory, designed with international standards in mind. It is both a repository of genomic sequence information through the BioNumerics platform as well as a web board for communication of molecular subtyping data to partner members. Since norovirus is the leading cause of gastrointestinal illness, a collaborative network is essential in monitoring its genomic diversity and evolution as well as supporting outbreak investigation and source tracking.

Network of Networks Workshop

In March of this year, members of the PHMRL attended a workshop hosted jointly by the Canadian Food Inspection Agency and the Public Health Agency of Canada with the aim of initiating an integrated network linking food, public health and animal health laboratories. This proposed *Network of Networks* integrates partners from multiple sectors to improve food safety through enhanced information sharing, coordinated action and better communication for mitigating risks to food chain threats. Much feedback was generated during the 2-day workshop including identification of success factors for network creation and the implementation of a Network of Networks Steering Committee.

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Global News

Vibrio parahaemolyticus Outbreak in WA State

Illnesses in Washington State has been linked with the consumption of raw oysters containing *Vibrio parahaemolyticus* bacteria. Over 20 cases have been linked to both commercial and recreational harvesting in Puget Sound and on the Washington coast. Prevention of vibriosis by refrigerating or using ice after harvesting and cooking thoroughly is particularly important during summer months when warmer temperatures encourage *V. parahaemolyticus* to thrive. Vibriosis is a mild to moderate illness with symptoms lasting from two to seven days. It can, however, have more serious impact on immunocompromised individuals or on those with chronic liver disease.



Dengue Fever Outbreaks

Dengue is an infectious disease of the tropics, transmitted through the bite of several *Aedes* mosquito species that acquire the dengue virus from the blood of an infected person. It causes flu-like illness and the more serious Dengue haemorrhagic fever and is endemic in over 100 countries with up to 50 million infections estimated annually (www.who.int/en). Since the beginning of 2011, thousands of cases with some fatalities have occurred in Thailand, Viet Nam, India, Sri Lanka and Brazil, who has experienced over 150,000 suspected cases since January. Cases in the Turks and Caicos, Bahamas and St. Lucia have also been recently reported. Tourists to these dengue endemic countries who have contracted the illness may present with symptoms after their return. There are no specific treatments for dengue.





Molecular Detection and Epidemiology of Enteroviral Meningitis in British Columbia, July 2010 to June 2011

Human enteroviruses are responsible for a wide spectrum of diseases from respiratory illness, to hand-foot-mouth disease, conjunctivitis, myocarditis and meningitis. Enterovirus infections demonstrate a seasonal pattern in temperate regions, typically occurring in the summer and fall. They are the most common cause of viral meningitis and can occasionally cause outbreaks. Until recently, virus culture was the most common method for detection, but this method is slow and insensitive. The introduction of reverse transcriptase-polymerase chain reaction (RT-PCR) detection for enteroviruses in our laboratory has revolutionized our diagnosis of enteroviral meningitis by both increasing the detection rate and reducing the time to detection.

In early 2010, the Public Health Microbiology & Reference Laboratory modified and validated a published RT-PCR assay for the detection of enteroviruses. This assay was demonstrated to reliably detect down to 5,000 virus per millilitre of cerebral spinal fluid (CSF). Although our laboratory does not perform all testing for the detection of enterovirus in BC, we have received 820 CSF samples for enterovirus testing in the past 12 months of which 13% were positive for enterovirus RNA. Our detection rate for enterovirus follows the summer-fall seasonal pattern observed in temperate climates (Table 1). The elevated positivity rates in December 2010 and January 2011 are yet to be explained. Due to the quantitative nature of our RT-PCR assay we were able to estimate the viral copy numbers present in the CSF. In most cases, there was less than 500,000 virus copies/mL in CSF, and much higher titres from the stool samples that we examined.

Table 1. Numbers of samples submitted per month and positivity rates

	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Submitted	33	98	106	68	106	21	4	74	72	72	79	95
Positive	16	36	28	7	8	3	1	0	0	0	3	3
% Positive	48.5	36.7	26.4	10.3	7.5	14.3	25.0	0	0	0	3.8	3.2

Enteroviruses have traditionally been identified by serological methods, but now partial sequencing of the Viral Protein 1 (VP1) gene is the preferred method for the identification of enteroviruses. Partial sequencing of the VP1 gene of positive samples by our laboratory indicated that a limited number of enterovirus species are circulating in BC: Coxsackie A9, Echovirus 4-related species, Echovirus 9, Echovirus 14, Echovirus 30 and Echovirus 39. Notably, Coxsackie A9 was the most prevalent enterovirus followed by Enterovirus 30. Even though there are a limited number of species circulating in the province there appears to be significant variation within intra-species sequences, possibly indicating that several strains of the same species are causing disease in our population, but this will have to be investigated further.

Review of the patient demographics of the positive samples to identify the associated Health Authority indicated that enterovirus infections are spread throughout the province, but with some areas of concentration (Table 2). As expected, the majority of positive samples came from the larger population centres.

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Table 2. Numbers of samples submitted per month and positivity rates

Health Authority	Positive Samples	% of Total Positive Samples
VIHA	21	18.7%
IHA	23	20.5%
FIHA	27	24.1%
VCHA	17	15.2%
NHA	4	3.6%
PHSA	None tested	NA
HA unknown	20	17.8%

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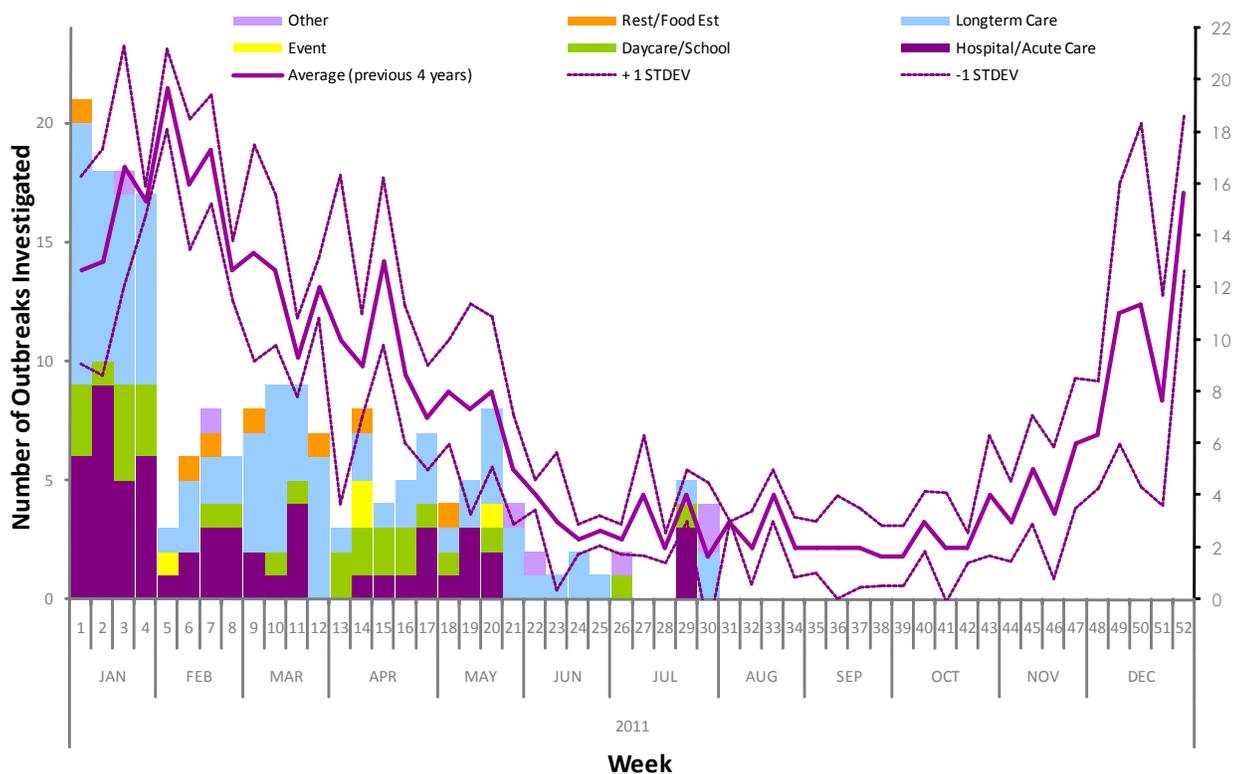
Vancouver, BC



Gastrointestinal Outbreaks

In July, there were 9 gastrointestinal (GI) outbreaks investigated at the PHMRL; 1 was confirmed to be due to norovirus by RT-PCR. Outbreaks were identified from 3 hospitals, 3 longterm care facilities, 1 daycare, and from 2 camps (Figure 1). The data available are from outbreaks in which the PHMRL has been notified. Some acute care microbiology laboratories are also testing for norovirus in the province. Given the nature of GI outbreaks, samples are not always available for testing.

Figure 1
Gastrointestinal outbreaks investigated since January, 2011, Environmental Microbiology, Bacteriology & Mycology, Parasitology and Virology Programs, PHMRL



The BC Centre for Disease Control, the Canadian Food Inspection Agency, Vancouver Coastal Health, Vancouver Island Health Authority and Fraser Health Authority are investigating a foodborne outbreak associated with the consumption of mussels. Several cases of illness have been reported from patrons of restaurants and one retailer who consumed cooked mussels from the end of July to the beginning of August. The product has been distributed within Western Canada as well as Ontario. A voluntary recall has been issued by the harvester and distributors of these mussels that may contain biotoxins causing Diarrhetic Shellfish Poisoning. For more information see www.inspection.gc.ca.

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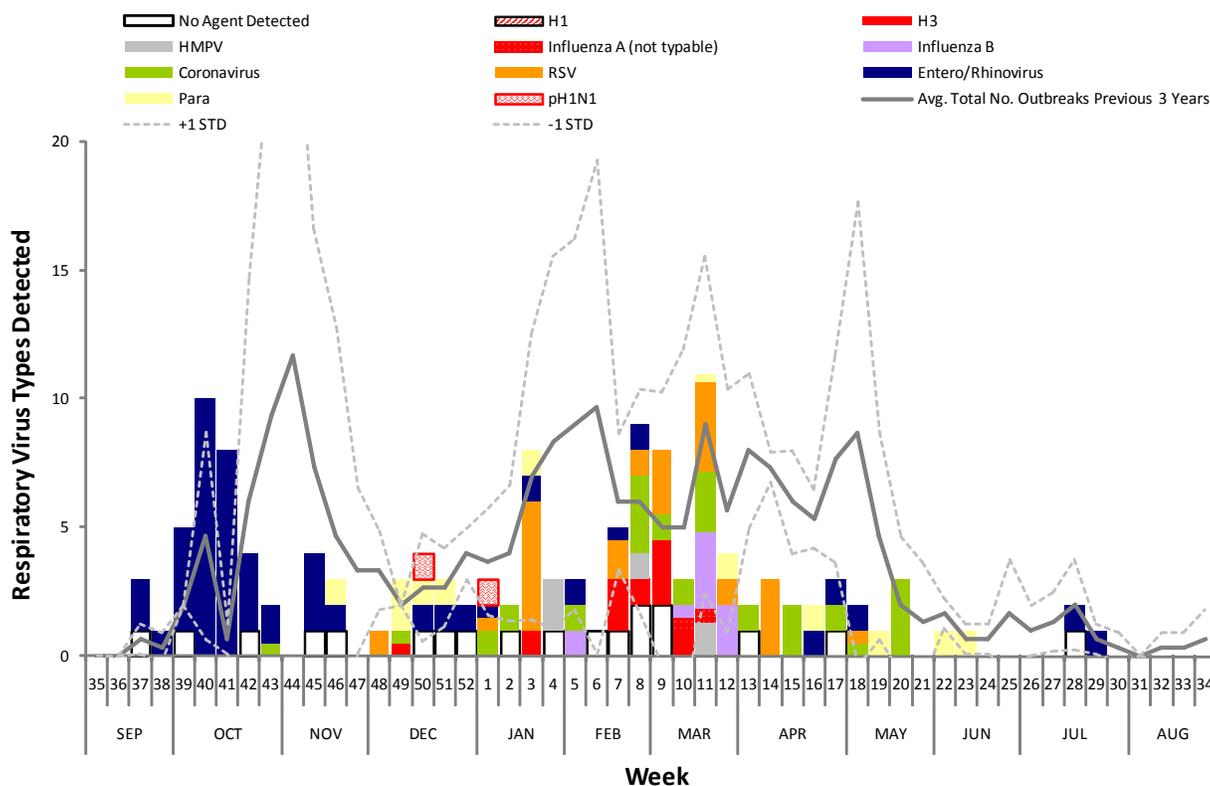


Respiratory Outbreaks

In July, 2011 there were 3 respiratory virus outbreaks investigated at the PHMRL using PCR and Luminex methods (Figure 2). Samples were all submitted from longterm care facilities with enterovirus/rhinovirus detected for two outbreaks and no agent identified for the remaining outbreak.

Figure 2 only reflect respiratory sample results submitted for investigation to the PHMRL and is not representative of respiratory outbreaks in the entire BC community.

Figure 2 Respiratory outbreaks investigated since January, 2011, Virology Program, PHMRL



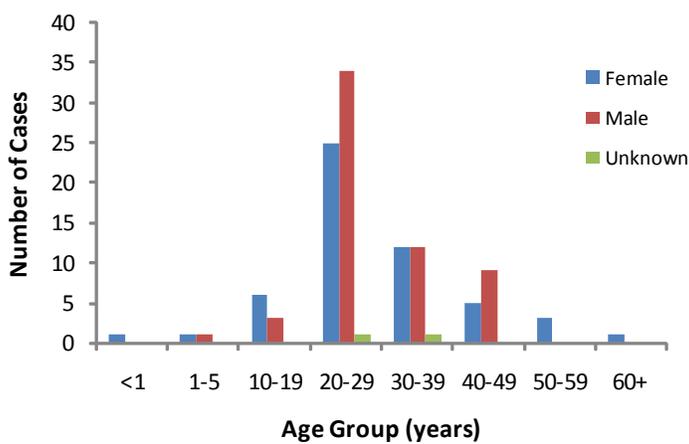


Mumps Outbreak

A mumps outbreak continues in the province. Since January, there have been 115 PHMRL laboratory-confirmed cases of mumps. The outbreak began in the Whistler area but has become more widespread within Vancouver Coastal Health, Fraser Health, Interior Health and Vancouver Island Health Authorities. Over 73% are within the 20-39 age range and 51% are male (Figure 3).

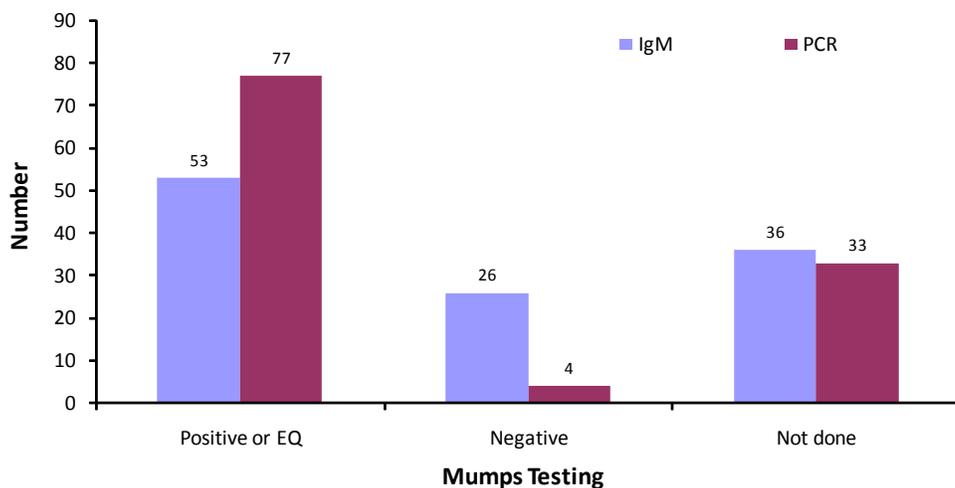
Genotype G has been identified by the NML. As IgM serology may be inconclusive (testing shows that about 30% of IgM non-reactive samples have been PCR positive for mumps virus, Figure 4), both blood for serology as well as urine/buccal swabs for PCR testing are encouraged.

Figure 3 Age and gender of laboratory-confirmed mumps cases, Virology and High Volume Serology Programs, PHMRL.



This outbreak has now spread to Ontario where 11 cases of illness are linked to transmission within Toronto and in surrounding health regions. The index case travelled to Vancouver in early June (see PROMED Mumps - Canada (04): (ON)).

Figure 4 Mumps testing by serology and RT-PCR, Virology and High Volume Serology Programs, PHMRL.





TB Surveillance

Genotyping can be a useful tool in surveillance and outbreak investigation to identify clusters of related cases. Previously, *Mycobacterium tuberculosis* (MTB) genotyping was performed using IS6110-based Restriction Fragment Length Polymorphism (RFLP), but this has now been replaced by the Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats (MIRU-VNTR) method. In BC, select MTB samples are sent to the National Reference Centre for Mycobacteriology at the National Microbiology Laboratory for reference MIRU-VNTR genotyping services. This multiplex PCR-based assay interrogates 24 regions in the MTB genome which contain a variable number of repeat units (Supply et al, 2001). The number of repeat units can be determined through the size of each target region, and the corresponding numerical value is assigned to that region resulting in a 24-digit pattern.

MIRU-VNTR genotyping for BC and Yukon MTB isolates from 2009 to present have identified significant clusters of MTB genotypes from the Yukon, Kelowna, Prince George/Vanderhoof and Port Alberni regions (Table 1). MIRU-VNTR patterns that differ at a single locus represent highly related MTB strains from cases that may have epidemiological linkages. The MIRU-VNTR genotyping suggests that the Prince George and Vanderhoof clusters represent a single outbreak. Interpretation of these genotype clusters requires correlation with follow-up epidemiological investigations and MTB genome sequencing. There are other one-locus differences for the other clusters as well.

Table 1
MIRU-VNTR patterns associated with identified *Mycobacterium tuberculosis* community clusters in BC, 2009 to present, TB/ Mycobacteriology Program, PHMRL.

Region	MIRU-VNTR Pattern	Number
Kelowna	224325153323 444234423373	26
	Kelowna-related pattern	3
Prince George/ Vanderhoof	234325153323 441444223352	11
Vanderhoof	234315153323 441444223352	7
	Vanderhoof-related pattern	1
Yukon Yukon-related	228225113222 343244433483	10
	Yukon-related pattern	1
Port Alberni	125325153224 233234423353	8
	Port Alberni-related pattern	1
Delta	213325351321 242324223452	3
	Delta-related pattern	2
Other Regions	Various patterns	56
Total		129

Supply P, Lesjean S, Savine E, Kremer K, van Soolingen D, Locht C (2001). Automated highthroughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. J Clin Microbiol; 39:3563–71.



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This report may be freely distributed to your colleagues. If you would like more specific information or would like to include any figures for other reporting purposes, please contact us.

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