PUBLIC HEALTH MICROBIOLOGY & REFERENCE LABORATORY

Vancouver, BC

LABORATORY TRENDS

April 20, 2012

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Laboratory News

Doctor - Your patient has *Mycobacterium stomatepiae*

In January, 2005 following two publications by the Public Health Microbiology & Reference Laboratory (PHMRL) describing the use of partial sequencing of the 65 kilodalton heat shock protein gene (*hsp*65) for the identification of *Mycobacterium* species, the TB/Mycobacteriology Laboratory stopped using traditional classical biochemical tests to identify mycobacteria and initiated identification by *hsp*65 DNA sequencing. The development of this technique involved creating an accurate in-house *hsp*65 DNA sequence database to which *hsp*65 sequences from unknown organisms were BLAST compared. This change in identification process allowed the TB/Mycobacteriology Laboratory to accurately identify a greater number of species than previously possible, allowed for more timely identification, reducing identification times for all species from weeks to days, and saved costs. The technique also allowed identification from positive primary liquid detection media without having to grow the organisms on solid culture media first, further reducing time to microbial identification. This technique has also been validated for use with patient samples authorized by a Medical Microbiologist, allowing identification of mycobacteria directly from patient samples prior to culture.

The accurate identification of mycobacteria using DNA sequencing, like all identification methods, is dependent on the database to which the unknown nucleic acid sequence is compared. Our initial database consisted of all 118 validly described *Mycobacterium* species that cause clinical disease or colonize humans or animals, as well as species described only from the environment or aquatic sources. In addition we also included 47 mycobacteria sequences from patient samples that were unique and could not be assigned to a described species or were related to described species, but not sufficiently close to being assigned to that species. Since the initial development of our database 42 more *Mycobacterium* species have been validly described, as recently as this month (van Ingen *et al.*, 2012), many of which colonize or are associated with disease in humans. The Molecular Microbiology & Genomics and TB/Mycobacteriology Laboratories at the PHMRL are now in the process of incorporating all newly described species into our database and will soon be revising species reporting capabilities.

The recent description of new species does not mean that they are new pathogens, but just previously unrecognized pathogens. The increase in molecular identification capabilities in front line laboratories such as the TB/Mycobacteriology Laboratory at the PHMRL has aided in the detection and description of these new species. For example, *M. yongonense,* a recently detected (2011) but yet to be validly described, species is related to *M. intracellulare*. Analysis of the sequence from this new species against our initial database revealed that this species had a sequence identical to an entry in our database that we detected in 2003 and at that time noted it as *M. intracellulare*-like. We expect that this phenomenon will occur with many of the organisms previously reported only as *Mycobacterium* species by the TB/Mycobacteriology Laboratory as the addition of the new sequence entries reveal that these organisms previously unidentifiable are members of a newly described species.

continued...

As for *M. stomatepiae*, the chance of identifying it from patient samples is very low; the organism was first described from aquatic sources as a possible fish pathogen. However, we have detected *M. hibernia*, a species that was originally described from sphagnum moss in Ireland, from a patient's sputum sample. As we revise our database and incorporate the new species to have world class identification capabilities we are certain that some of these new species will be identified from clinical samples submitted to the PHMRL for detection and identification.

Submitted by: Alan McNabb, Section Head,
Molecular Microbiology & Genomics Program

Reference

van Ingen, J., Rahim, Z., Mulder, A., Boeree, M.J., Simeone, R., Brosch, R., and van Soolingen, D. (2012). Characterization of *Mycobacterium orygis* as *M. tuberculosis* Complex Subspecies. *Emerging Infectious Diseases,* 18(4), 653-655.

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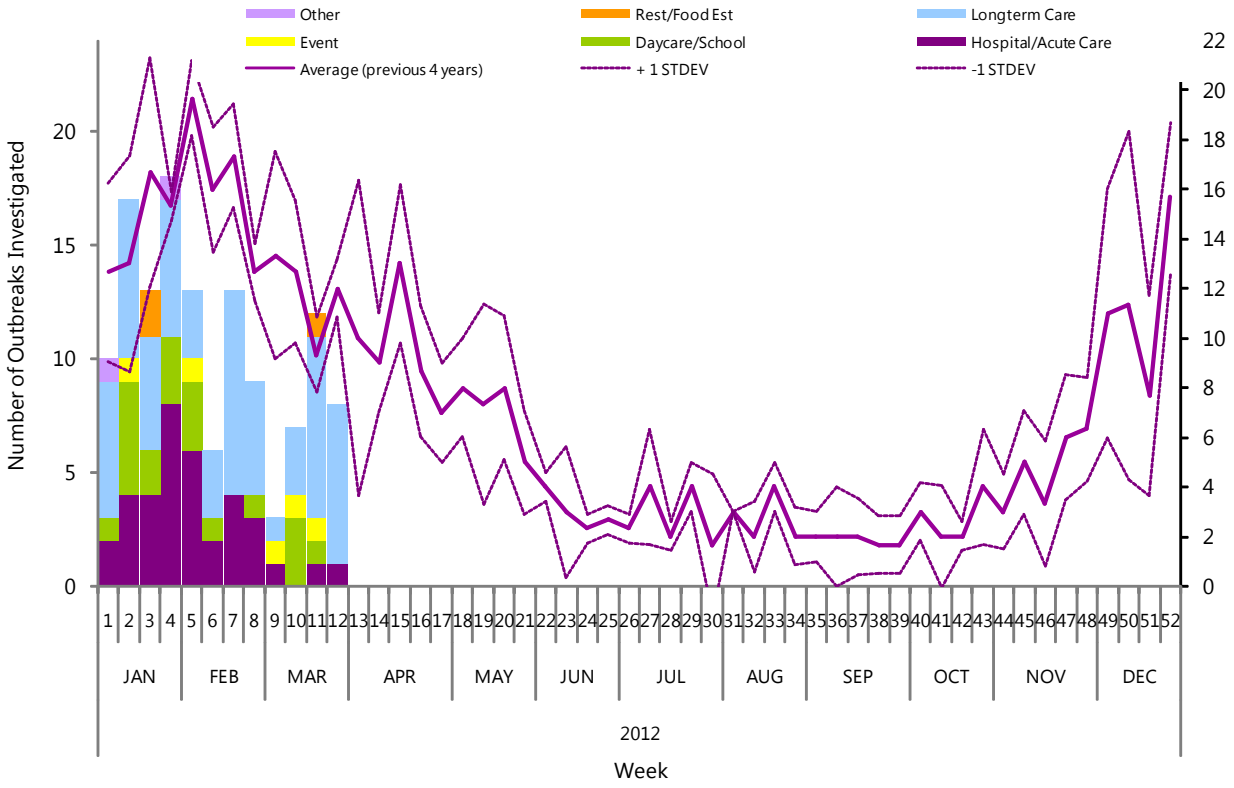
Gastrointestinal Outbreaks

In March, there were 35 gastrointestinal (GI) outbreaks investigated at the PHMRL. There were fewer outbreaks in weeks 9 and 10 compared to this time in previous years (Figure 1). Outbreaks were identified from 25 longterm care facilities, 4 daycares/schools, 3 hospitals, 2 events and 1 restaurant. Samples for laboratory testing were submitted for 23 (66%) of these outbreaks. Of these, norovirus was confirmed in 22 (96%) outbreaks (64% in longterm care facilities, 14% in hospitals, 9% in daycares/schools, 9% at special events and 4% in restaurants).

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 and these data do not include outbreaks from Vancouver Island Health Authority. Given the nature of GI outbreaks, samples are not always available for testing.

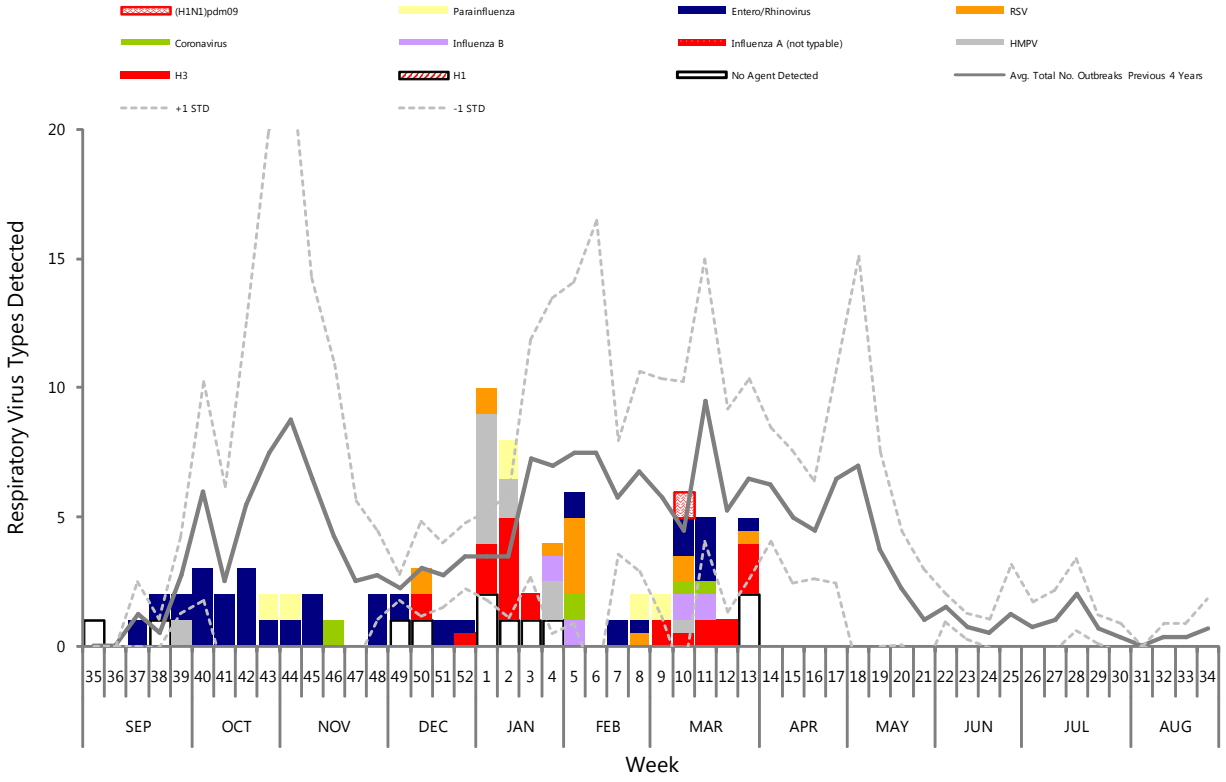
Figure 1
Gastrointestinal outbreaks investigated since January, 2012, Environmental Microbiology, Bacteriology & Mycology, Parasitology and Virology Programs, PHMRL.



Respiratory Outbreaks

In March, samples were submitted to the PHMRL for 17 respiratory outbreak investigations. All were from longterm care facilities except for 1 school-related outbreak. The number of outbreaks investigated was consistent with what has been seen in previous years (Figure 2). Using PCR and Luminex methods, influenza A(H3) was detected in 5 facilities and influenza B detected in 2 facilities. There were 6 outbreaks where two pathogens were detected. Of the other respiratory viruses, entero/rhinovirus was detected in 5 facilities, RSV was detected in 3 facilities, coronavirus detected in 2 facilities, human metapneumovirus detected in 1 facility and influenza A(H1N1)pdm09 detected in 1 facility. There were 2 outbreaks where no agent was detected.

Figure 2
Respiratory outbreaks investigated by respiratory season, Virology Program, PHMRL. This figure reflects respiratory sample results submitted for investigation and is not representative of respiratory outbreaks in the entire BC community.

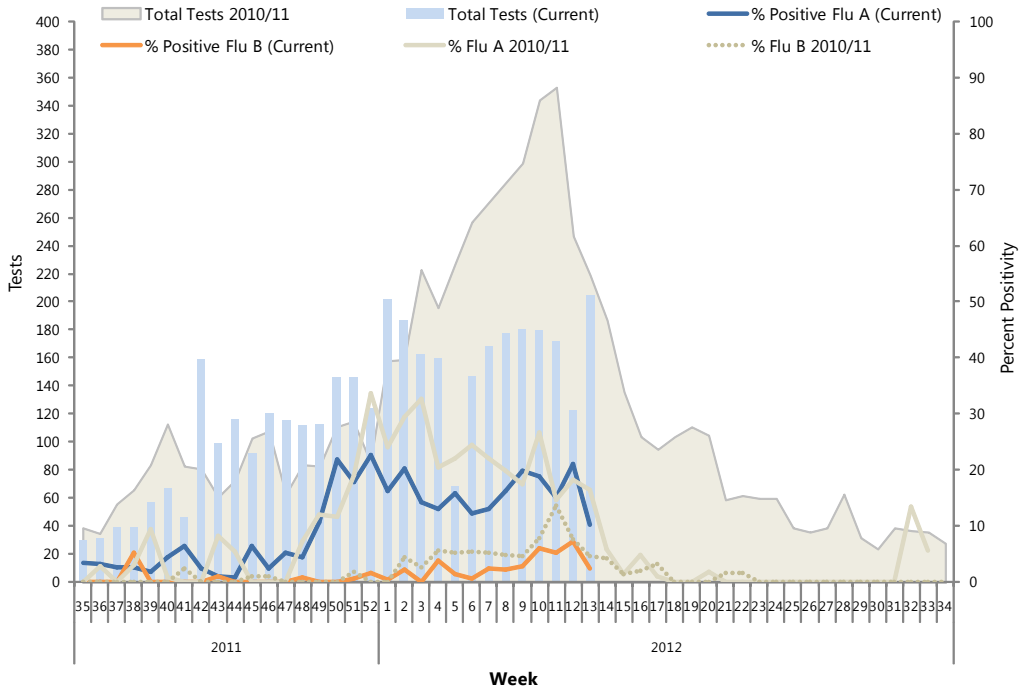


Influenza Surveillance

Volumes for respiratory testing in weeks 10-13 in the 2011/12 season continue to be below that of the same weeks from the 2010/11 season. The positivity rate for influenza A was a little lower in week 10 but was similar for weeks 11-14 compared to this time last seaon. Influenza B positivity followed a similar trend (Figure 3).

In weeks 10-13, influenza positivity rates varied from nearly 12-25% (Table 1). Influenza A (H3N2) was the major virus type detected this period with 69 (9.9%) positive specimens. Influenza B detection increased to 34 (4.9%) positive specimens, followed by 32 (4.6%) detections of (H1N1) pdm09.

Figure 3
Respiratory testing volumes and influenza percent positivity by week, 2011/12, Virology Program, PHMRL.



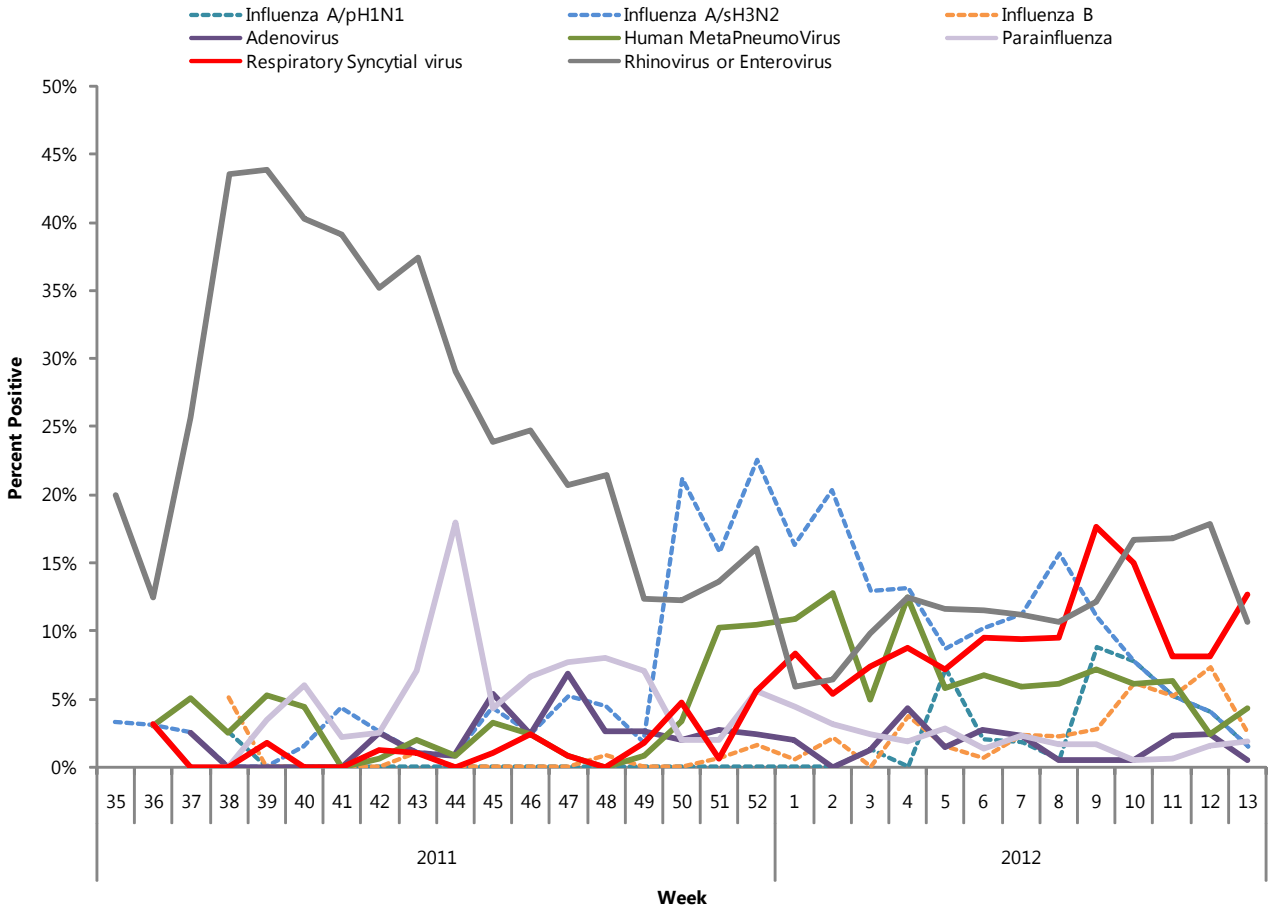
The PHMRL continues to see a mix of respiratory viruses detected from respiratory specimens (Figure 4). Levels of rhino/enterovirus increased in weeks 10-12 while RSV has shown the reverse trend in these weeks. Rates of human metapneumovirus declined slightly in week 12 while rates for adenovirus and parainfluenza virus have been low.

Table 1
Positive influenza A and B detections for weeks 10-13 (March 4 - March 31, 2012, Virology Program, PHMRL. (H1N1)pdm09 refers to the 2009 influenza A(H1N1) pandemic virus.

	Week 10	Week 11	Week 12	Week 13
Number of Specimens Tested	**185**	**176**	**127**	**210**
Number of Positive Specimens	**46 (24.86%)**	**36 (20.45%)**	**32 (25.20%)**	**25 (11.91%)**
Influenza A	35 (18.92%)	26 (14.77%)	24 (18.90%)	18 (8.57%)
(H1N1)pdm09	*15 (8.11%)*	*9 (5.11%)*	*5 (3.94%)*	*3 (1.43%)*
sH3N2	*20 (10.81%)*	*17 (9.66%)*	*19 (14.96%)*	*13 (6.19%)*
Not typeable				*2 (0.95%)*
Influenza B	11 (5.95%)	10 (5.68%)	8 (6.30%)	5 (2.38%)

Influenza Surveillance continued

Figure 4
Percent positivity of respiratory viruses by week, 2011/12, Virology Program, PHMRL.



National influenza trends in March demonstrated low to moderate levels of activity. Rates of influenza A were highest in the Prairies (16-18%) and in BC (9-19%) while the other provinces had less than 10% influenza A positivity. Influenza B rates were highest in Ontario (15-23%) and the Atlantic Provinces (14-22%) and were generally predominant except in Prairies and in BC.

The World Health Organization (WHO) reports that most of the influenza activity in North America, Europe and northern Asia has peaked and is now declining. The most common subtype circulating in Europe and North America continues to be influenza A(H3N2) with increased detection of influenza B. The predominant subtype in Mexico is influenza A(H1N1) pdm09 whereas China is predominantly detecting influenza type B (WHO, 13 Apr 2012 Update).

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