BC PUBLIC HEALTH MICROBIOLOGY & REFERENCE LABORATORY

Vancouver, BC

LABORATORY TRENDS

July 16, 2014

In this Issue:

Recent Cluster of Locally-Acquired *Cyclospora*

The single-cell protozoan parasite *Cyclospora cayetanensis* is a rare but well known cause of diarrheal disease. Infections are often foodborne in origin with clusters arising from contaminated fresh produce. Symptoms are non-specific (watery diarrhea, abdominal cramps, and nausea). Cyclosporiasis can be treated effectively. While diagnosis is done by parasitology laboratories, clinicians should highlight suspicious cases to both diagnostic laboratories and to regional public health.

The most common cause of cyclosporaisis occurs due to travel to areas where the parasite is endemic; however, the global distribution of food has changed the probability of acquisition in non-endemic regions due to consumption of imported, contaminated produce such as leafy green vegetables, fresh herbs and berries. In British Columbia (BC), *C. cayetanensis* emerged as a locally-acquired enteric pathogen in 1999 and since then has been responsible for outbreaks in BC linked to imported produce such as Thai basil (2001) and Mexican basil (2007). BC experienced annual spring outbreaks of cyclosporiasis between 2001-2007 and again in 2013. No locally-acquired outbreaks were reported between 2008-2012.

Recently, seven cases of cyclosporiasis have been identified from three different health authorities in BC with no reported travel during their exposure period. Onset of illness has been reported from June 6-25, 2014. The investigation into common sources of contamination for this cluster is ongoing.

In order to assist the current investigation we are asking our laboratory network to submit SAF and unpreserved specimens along with reports and relevant travel history of positive *Cyclospora* species stool specimens to the Parasitology Program at the BC Public Health Microbiology & Reference Laboratory (BCPHMRL).

Requested Laboratory Samples:

1. Stool specimens in SAF; and,
2. Unpreserved specimens; and,
3. Relevant travel history

Clinical illness compatible with *Cyclospora* infection in a person whose onset of symptoms is within a week of a laboratory-confirmed case, who shares food exposures with said laboratory confirmed case and who has not traveled outside Canada and the U.S. within two weeks of onset of symptoms should also be reported to public health.

Identification of additional cases and timely investigation will support the identification of a common source for public health interventions to prevent further cases from occurring.

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Using a Secondary Gene Sequence Target to Identify *Staphylococcus* Species

One of the core functions of the Public Health Advanced Bacteriology/Mycology Program at the BCPHMRL is the reference identification of bacterial isolates submitted by hospital laboratories. The identification algorithm used by the laboratory has evolved over time with the addition in 2003 of partial 16S RNA sequencing to classical biochemical testing as an aid to identify bacteria. Although not definitive for all bacterial species 16S rRNA sequencing has the ability to identify many organisms to species level.

The recent introduction of Matrix-assisted laser desorption/ionization Time of Flight (MALDI-TOF) technology to hospital laboratories as a means of bacterial identification has replaced most if not all other identification systems. Most biomarkers detected in MALDI-TOF spectra are intracellular proteins primarily in the range of 4000 to 15,000 Da. Lysis of organisms with organic solvents in acidic conditions favors extraction of the basic cytoplasmic proteins, specifically ribosomal and mitochondrial proteins, cold shock proteins, heat shock proteins, DNA binding proteins, and RNA chaperone proteins. These highly conserved housekeeping proteins are ideal biomarkers for characterizing species. Characterizing these proteins from bacterial extracts allows for the rapid and accurate identification of most bacterial species giving MALDI-TOF the potential to accurately identify many more species of bacteria than other commercial methods. This enhanced identification capability by clinical laboratories results in the isolates sent to the Public Health Advanced Bacteriology/Mycology Program being more difficult to identify using our current algorithm. Although the 16S rRNA gene is present in all bacteria the heterogeneity within the gene may not be sufficient to identify all isolates to species level. We have found that isolates not well identified by MALDI-TOF usually represent a challenge to identify using 16S rRNA sequencing.

The *Staphylococcus* genus currently consists of 49 species and 26 subspecies, many of which colonize or cause infections in humans and animals. The heterogeneity in the 16S rRNA gene in this genus is not sufficient to identify many of these species and only has very limited capability to identify isolates to subspecies level. This requires the laboratory to sequence an alternate gene to identify some staphylococci. To improve *Staphylococcus* identification the Public Health Advanced Bacteriology/Mycology Program in collaboration with the Molecular Microbiology and Genomic Program explored the use of elongation factor gene (tuf) sequencing as a means for identification.

In preparation for using this gene target as a means of identifying staphylococci a sequence library of the tuf gene was generated for all *Staphylococcus* species not identified by 16S rRNA sequence. Staphylococcal isolates submitted to the laboratory that could not be identified by 16S rRNA sequencing or MALDI-TOF had a 371 to 374 nt region of the tuf gene sequenced to determine their identification. This region was chosen as it was differential for all valid *Staphylococcus* species, could be rapidly sequenced using a single set of primers and was more discriminatory than MALDI-TOF or 16S rRNA sequencing.

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Secondary Gene Sequence Target to Identify *Staphylococcus* Species

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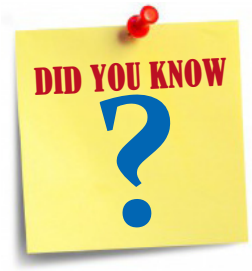
Sequences from archived and clinical isolates were then compared to the sequences in this database. All isolates that were not identified to species level by 16S rRNA or MALDI-TOF were unambiguously identified to species level by tuf gene sequence. Strict criteria for acceptable identifications have been developed with isolates having a ≥99.5% match to the species type strain with at least a 2% sequence separation between the first and second choice matches were considered to be acceptable identifications.

The application of this gene sequence now allows for the identification of all staphylococci submitted to the laboratory for identification. As MALDI-TOF becomes more utilized for bacterial identification by hospitals in British Columbia the need for sequencing of secondary genes to identify isolates will become necessary. The Public Health Advanced Bacteriology/Mycology Program is currently exploring the sequencing of the histone A (hisA) gene for the identification of *Burkholderia* species and the chaperonin 60 (cpn60) gene for the identification of *Streptococcus* species to improve identification of these genera.

For more information please contact Alan McNabb, Section Head, Molecular Microbiology and Genomics Program, alan.mcnabb@bccdc.ca.



Email Orders for Requisitions and Sample Containers

Our newly improved Sample Container Order form is now available for use. In our efforts to reduce the amount of paper used for ordering sample kits and/or requisitions, the updated Sample Container Order form can now be submitted via email to kitorders@hssbc.ca. Ordering via email will also support the maintenance of a client database for orders and assist with more efficient filling of requests. The new form can be found at www.bccdc.ca/PHSALaboratories/OrderForm. (Note that for Google Chrome users, the built-in pdf viewer will need to be disabled to submit the form via email. Simply open Chrome, type in chrome://plugins/ in the address bar, scroll down the list until you see the option "Chrome PDF Viewer" and click Disable).

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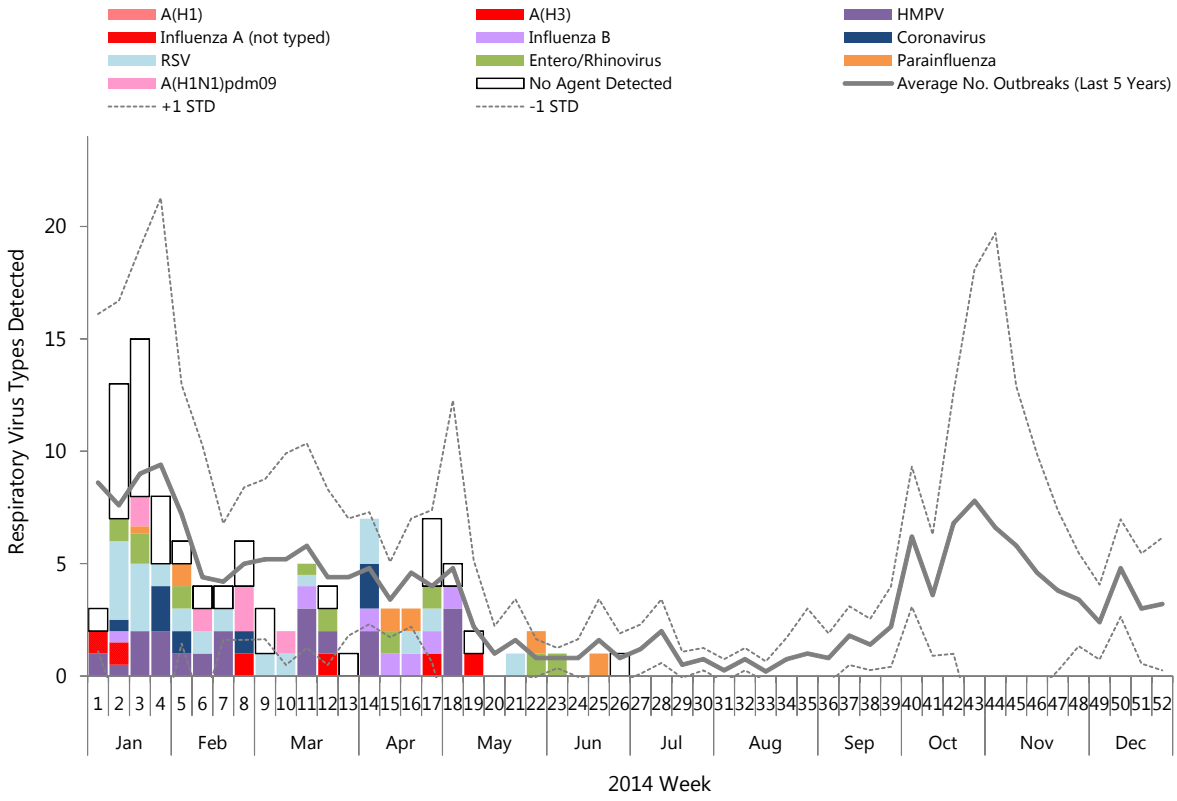
Respiratory Outbreaks

There were 3 respiratory outbreaks investigated for the month of June which is consistent with the historical trend in the summer months (Figure 1). Samples were submitted from longterm care facilities with parainfluenza detected in one facility and entero/rhinovirus detected from samples from another facility.

Figure 1
Respiratory outbreaks investigated* in 2014, Virology Program, BCPHMRL.

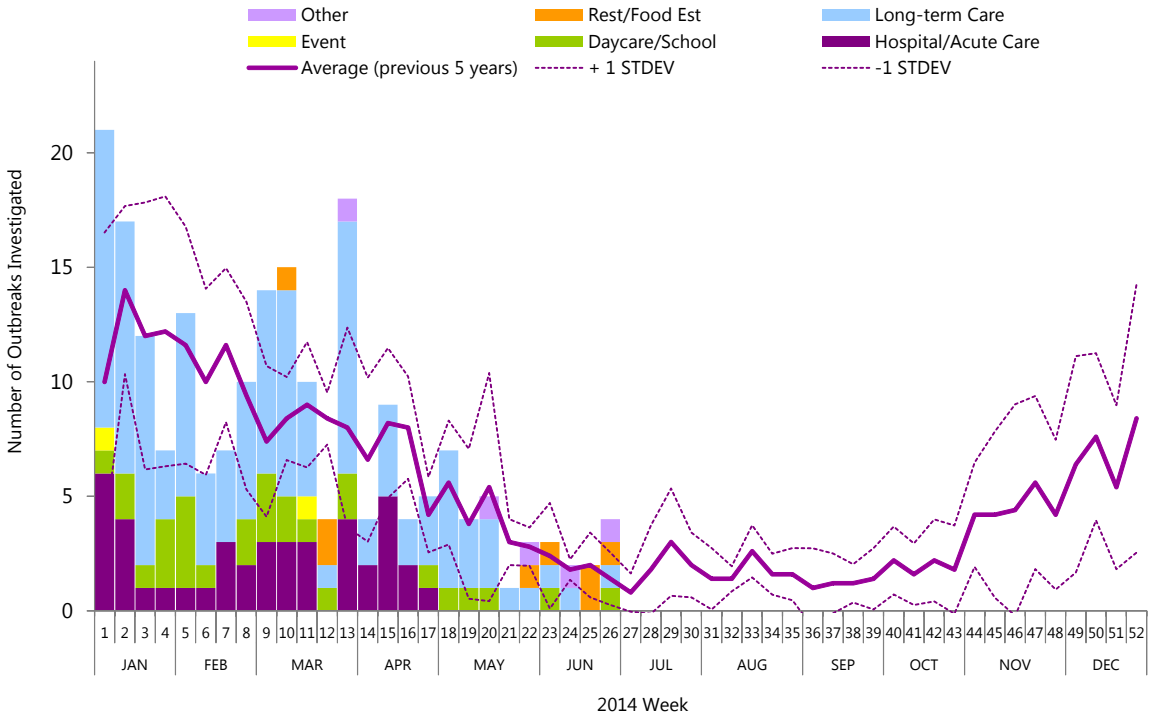


* Figure 1 reflects respiratory sample results submitted for investigation to the BCPHMRL and may not be representative of respiratory outbreaks in the entire BC community.

Gastrointestinal Outbreaks

In June, the BCPHMRL investigated 11 gastrointestinal (GI) outbreaks, consistent with this time in previous years with the exception of the last week of the month (Figure 2). Outbreaks were identified from 4 (36%) food service establishments, 3 (27%) longterm care facilities, 2 (18%) daycares/schools and 2 (18%) other event/facility types (including a cruise ship outbreak). Samples for laboratory testing were submitted for 9 (82%) of these outbreaks with norovirus confirmed in 8 (89%) from 3 (37%) food service establishments, 3 (37%) longterm care facilities, 1 (12%) daycares/school and 1 (12%) other event. *Salmonella* was also identified in 1 (11%) separate outbreak in a food service establishment.

Figure 2
Gastrointestinal outbreaks investigated* in 2014, Environmental Microbiology, Public Health Advanced Bacteriology & Mycology, Parasitology and Virology Programs, BCPHMRL.



* The data available are from outbreaks in which the BCPHMRL has been notified. Some acute care microbiology laboratories are also testing for norovirus in the province and these data may not include outbreaks from all Health Authorities. Given the nature of GI outbreaks, samples are not always available for testing.

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A Report of the BC Public Health Microbiology & Reference Laboratory, Vancouver, BC

The BC Public Health Microbiology Reference Laboratory (BCPHMRL) at the BCCDC site provides consultative, interpretative testing and analyses for clinical and environmental infectious diseases in partnership with other microbiology labs and public health workers across the province and nationally. The PHMRL is the provincial communicable disease detection, fingerprinting and molecular epidemiology centre providing advanced and specialized services along with international defined laboratory core functions province-wide.

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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