

# LABORATORY TRENDS



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A Report from the BCCDC Public Health Laboratory



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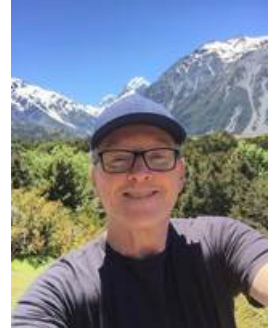
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## New Staff at the BCCDC Public Health Laboratory

In April we welcomed two new staff members to the team here at the BC Centre for Disease Control (BCCDC) Public Health Laboratory (PHL): Dr. Paul Levett and Dr. Chris Fjell.

Dr. Paul Levett is a board-certified clinical microbiologist. He completed his PhD on *Clostridium difficile* in the United Kingdom. After spending many years working in the Caribbean, he began his northward migration, via the Centers for Disease Control and Prevention in Atlanta, where he was the head of the *Leptospira* laboratory and acting head of the Special Bacteriology Reference Laboratory. He was the clinical director at the Saskatchewan Disease Control Laboratory in Regina from 2003, and is an adjunct professor in the School of Graduate Studies and Research at the University of Regina. His research interests include leptospirosis and other vector-borne and zoonotic diseases, and a wide range of viral infections, including HIV. He has authored or co-authored over 200 publications on public health microbiology, including two books on anaerobic bacteria. Dr. Levett will be supporting the Virology and Environmental Microbiology Programs as program head while Dr. Agatha Jassem and Dr. Natalie Prystajecy are on maternity leave.



Dr. Chris Fjell also joined the BCCDC PHL as the public health genomics microbiologist. After initial training and work in engineering, Dr. Fjell switched to academic work with a PhD from the University of British Columbia (UBC) (Experimental Medicine program, computational biology of immunity and infection). He worked in the department of Microbiology & Immunology (Hancock lab) at UBC and in the division of Critical Care Medicine at St Paul's Hospital, Centre for Heart Lung Innovation (Boyd lab). Most recently, Dr. Fjell worked as UBC staff at the Vancouver Coastal Health Research Institute as a health research analyst and at the Centre for Clinical Genomics (BC Cancer Research Centre) on a revamped analytic software pipeline. Dr. Fjell will be supporting our workflows here at the BCCDC PHL, particularly as we move towards implementing routine whole genome sequencing for diagnostics and surveillance.

# Availability of In House Zika Virus IgG and IgM Serology Testing

Infection with Zika virus has been identified to be of special concern, with links to fetal birth defects during pregnancy causing microcephaly and reports of associations with Guillain-Barré syndrome (a cause of paralysis). Diagnosing the infection will determine if increased monitoring of the fetus is required. In addition, determining if partners of pregnant women, or those planning conception, are infected can prevent the spread of infection during pregnancy, as the informed patient can make the decision to delay conception or avoid exposure. To further complicate diagnostic considerations, people who are at risk of Zika virus are also at risk of chikungunya and dengue virus infection.

Since January 2016 the BCCDC PHL Zoonotic Diseases & Emerging Pathogens laboratory has been able to offer direct detection of Zika virus viral RNA through in house PCR testing. However, as no commercial antibody assays were available at the time, all serology testing had been forwarded to the National Microbiology Laboratory (NML) with appropriate history for Zika virus IgM detection. A few commercial Zika virus IgM and IgG tests are now available with Health Canada approval.

## Laboratory Diagnosis

The previously reported and still relevant laboratory testing guidelines are available in the [January 2016](#) issue of *Laboratory Trends*.

A negative serological result does not exclude an infection, particularly in the very early phase of an infection or later when IgM antibodies may have waned and become undetectable. For this reason, testing will be performed with both the InBios ZIKV Detect™ IgM Capture ELISA and the EUROIMMUN Anti-Zika Virus ELISA IgG. These commercial tests have been extensively evaluated and recommended by the NML and further assessed by the BCCDC PHL. A positive serological result means that there has been contact with a *Flavivirus* and further testing will be done to confirm the infection by Plaque Reduction Neutralization Testing (PRNT) at the NML.

## Criteria for Testing

Testing is not recommended without a travel history to an area with Zika virus transmission. Patients with a history of travel to an area with Zika virus transmission and who report two or more symptoms consistent with Zika virus infection (acute onset of fever, maculopapular rash, arthralgia, or conjunctivitis) or pregnant women within 2 weeks of travel, or who have ultrasound findings of fetal microcephaly or intracranial calcifications, should be tested for Zika virus infection.

## Sample collection instructions:

- 1) During acute symptomatic infection collect:
  - a. 5 mL EDTA purple top blood tube
  - b. 5 mL gold top serum separator tube
- 2) If symptoms have resolved collect:
  - a. 5 mL gold top serum separator tube only

EDTA blood and serum is best to diagnose acute infection by PCR and PCR testing can also be performed on urine and other sample types such as nasopharyngeal swabs and semen. Persistence of Zika virus RNA in these sample types are not well understood. For serologic tests serum is the optimal sample.

Please provide both the travel and clinical history, including the date of onset of symptoms. The onset date is extremely important to ensure appropriate testing to determine the stage of the disease. Send samples with relevant history to the BCCDC PHL. If there is a clinical suspicion and a negative test result, clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample is recommended.

The availability of in house serological testing will substantially reduce the long turnaround times previously experienced for Zika virus IgM results. The additional information provided by a patient's Zika virus IgG antibody level will provide a more comprehensive picture of exposure to the virus. **BCCDC PHL has been able to offer in house Zika virus serology testing since April 2018.**



J. Leung performing Zika ELISA, Zoonotic Diseases & Emerging Pathogens Program, BCCDC PHL.

## New *Salmonella* Screening Assay for Food and Environmental Samples

Starting in April, BCCDC PHL Environmental Microbiology Program implemented a molecular screening method for the detection of *Salmonella* species in food and environmental samples. The BAX® System is a polymerase chain reaction (PCR) assay that can detect a variety of *Salmonella* species found in many food types as well as in environmental samples and has been validated for use by the Health Products and Food Branch Division of Health Canada as well as the Environmental Microbiology Program.

Samples undergo an enrichment step of 24-48 hours depending on the matrix with PCR results ready in 3.5 hours. The PCR amplifies three specific genetic sequences unique to *Salmonella* and the system then analyzes the PCR product by fluorescent detection for the presence or absence of the organism. This means negative or presumptive positive results for *Salmonella* may be returned quicker compared to waiting for culture which can take from 2 to 5 days. This method will help improve workflow and increase the capacity for *Salmonella* testing, for outbreak response and for the *Salmonella* Food Quality Check project. There are no changes required from clients for sample submission or test ordering.

On the reports, the results of the *Salmonella* molecular screening will be displayed under the new test name "*Salmonella* NAT" with the following resulting options as follows:

- "*Salmonella* species detected",
- "*Salmonella* species not detected" or
- "*Salmonella* species indeterminate".

Samples that are screened positive or indeterminate for *Salmonella* species will be cultured. All confirmed *Salmonella* isolates will be serotyped and sequenced by Whole Genome Sequencing (WGS).



C. Tchao evaluating samples for *Salmonella* using the BAX® System, Environmental Microbiology Program, BCCDC PHL.

## Case Report: Case of Microfilaria (*Brugia malayi*)

Submitted by: Teresa Lo, Navdeep Chahil and Dr. Muhammad Morshed, Parasitology Program and Dr. Inna Sekirov, TB/Mycobacteriology Program

The BCCDC Public Health Laboratory Parasitology Program recently identified an unusual roundworm (nematode), *Brugia malayi*, in a 20 year old female patient who had extensive travel history in South Asia. *B. malayi* is a causative agent of filariasis, a parasitic disease which may present as lymphatic, subcutaneous or serous cavity filariasis depending on the vector mosquito species. We have not seen this parasite in our laboratory over the last 37 years.

There are three different filarial worm species that can cause lymphatic filariasis in humans: *Wuchereria bancrofti*, *B. malayi* and *B. timori*. *W. bancrofti* is found globally; however, *B. malayi* and *B. timori* are only found in Asia and Southeast Asia. These nematodes spread from person to person through the bites of a variety of mosquitoes. *Culex quinquefasciatus*, *Aedes* and *Mansonia* mosquitoes can transmit the infection in the Pacific and in Asia, whereas the *Anopheles* vector transmits this disease in Africa and the Americas. Transmission of these nematodes is not as common compared to other mosquito-borne diseases such as malaria or arbovirus infection. Bites from many mosquitoes over several months to years are needed to cause lymphatic filariasis. Also to note only a small percentage of people are symptomatic with swelling of the legs being the most common symptom; swelling of the arms, breasts and genitalia can also occur. Men can develop swelling of the scrotum due to infection with *W. bancrofti*.

### Case summary

The patient had acute febrile diarrheal illness early on in her travels, which was treated with albendazole. Despite treatment, the patient continued to experience ongoing gastrointestinal issues such as bloating, lower abdominal pain and alternating diarrhea and constipation. In addition, the patient experienced an episode of right lower leg/ankle swelling without preceding trauma, for which she received a course of antibiotics. On subsequent travel in South Asia she experienced vertigo with dizziness and paresthesias in the hands. The patient did not recall consuming any raw meats or fish but did consume pre-cut fruits during her travels. There were no other pertinent symptoms and the patient did not have prior significant medical history.

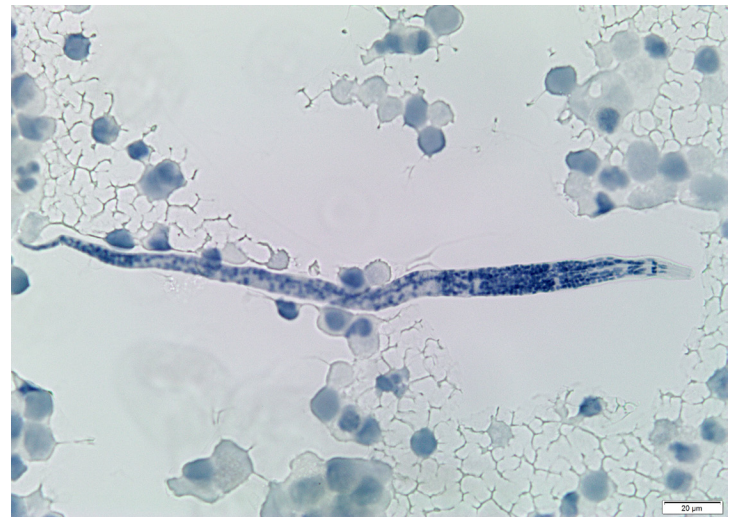
The patient's symptoms prompted a laboratory investigation with the following findings:

- initial filaria serology was equivocal and the *Schistosoma* serology was positive,
- subsequent filaria serology was negative,
- no ova or parasites were found in the stool and urine,
- Strongyloides* serology and *Entamoeba* serology were negative; and,
- malaria smears were negative.

In the laboratory, the patient's EDTA blood sample was concentrated by Knott's concentration method, lysing the red blood cells, and then centrifuging to enhance the recovery of parasites. A microfilaria worm was found in the concentrated blood sediment and subsequently confirmed by iron-hematoxylin stain (Figure 1). The iron-hematoxylin stains the sheath on the worm which aids in identification. Only one microfilaria worm was found from the entire sediment. In light of this finding, it was surprising that the filarial serology was negative, as the assay is based on *B. malayi* antigens, specifically.

Microscopy is still considered a gold stand for diagnosis. Of note, since this parasite circulates in the blood at night (called nocturnal periodicity), blood collection in an EDTA tube should be done at night to coincide with the appearance of the microfilariae. A thick smear should be made and stained with Giemsa or hematoxylin and eosin and concentration techniques can be used for increased sensitivity. Detection of antibodies can also be used for an alternate diagnosis. For antibody testing 5 to 7 ml blood in a serum separator tube (SST) is required. BC residents travelling to filaria-endemic areas are directed to avoid mosquito bites and use appropriate precautions such as insect repellent with DEET and also use mosquito net at night for sleeping.

Figure 1. Microfilaria worm of *B. malayi* stained with iron-hematoxylin, Parasitology Program, BCCDC PHL.



# Raw Poultry Sampling Program Update

Since 1991, the BCCDC Public Health Laboratory (PHL) and the BCCDC Food Protection Services have been working with all BC health authorities to assess sanitation in food premises through the Food Quality Check Program (FQCP). The program enables Environmental Health Officers (EHOs) to submit food or environmental samples for assessment of microbial indicators. These results may provide evidence for the need to correct food handling, food storage and hygienic practices, and ultimately prevent/mitigate of food poisoning incidents.

In 2016, with collaboration from the Ministry of Health and as part of FQCP, a Raw Poultry Sampling Program was initiated with a focus on sampling chicken and egg products. Goals of this special survey are to enhance surveillance for *Salmonella* Enteritidis in the province, to help link food isolates to human cases with epidemiology and laboratory (whole genome sequencing, WGS) data, and to establish WGS as a tool for outbreak investigation. Sampling from restaurants and retail premises are the primary focus for this program. Comprehensive sample information is collected to allow any trace-back activities when needed.

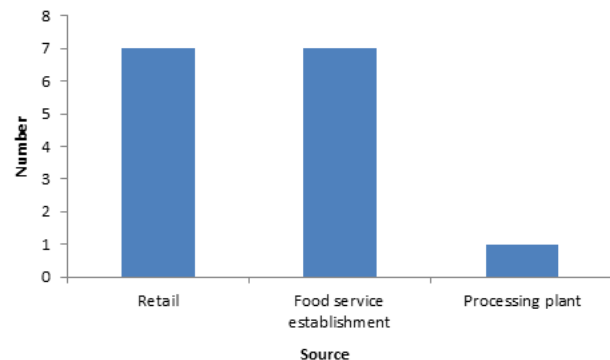
Results from submissions in 2017 are summarized in Table 1. Nearly twenty-five percent of samples submitted were positive for *Salmonella*. Of these, 11% were positive for *S. Enteritidis*. These were mainly raw poultry product samples from either retail or food service establishments (Figure 2), with the exception of one chicken nugget sample. From the 15 samples positive for *S. Enteritidis*, the dominant phage type was 13 (n=11, 73%) (Figure 3). No *Salmonella* was found in the egg samples submitted.

This program will continue into 2018 with strong interest in further understanding *S. Enteritidis* distribution and prevalence in poultry products, especially WGS relevance between food samples and human samples.

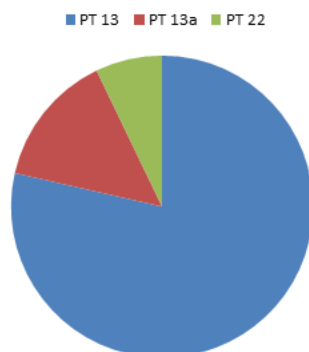
**Table 1.** Results from the Raw Poultry Sampling Program, 2017, Environmental Microbiology, Program, BCCDC PHL.

Product	Number sampled	Number (%) positive for <i>Salmonella</i>	Number (%) positive for <i>S. Enteritidis</i>	Comments
Chicken	139	34 (24.5%)	15 (10.8%)*	Other serotypes detected: <i>S. Anatum</i> (1), <i>S. Reading</i> (1), <i>S. Typhimurium</i> (3), <i>S. Braenderup</i> (1), <i>S. O4512:i:-</i> (1), <i>S. Kentucky</i> (12)
Egg products	6	0	0	3 samples were pooled eggs. Remaining samples were whole eggs.

**Figure 2.** Source of samples positive for *Salmonella* Enteritidis, Environmental Microbiology, Program, BCCDC PHL.



**Figure 3.** Phage types of samples positive for *Salmonella* Enteritidis, Environmental Microbiology, Program, BCCDC PHL.

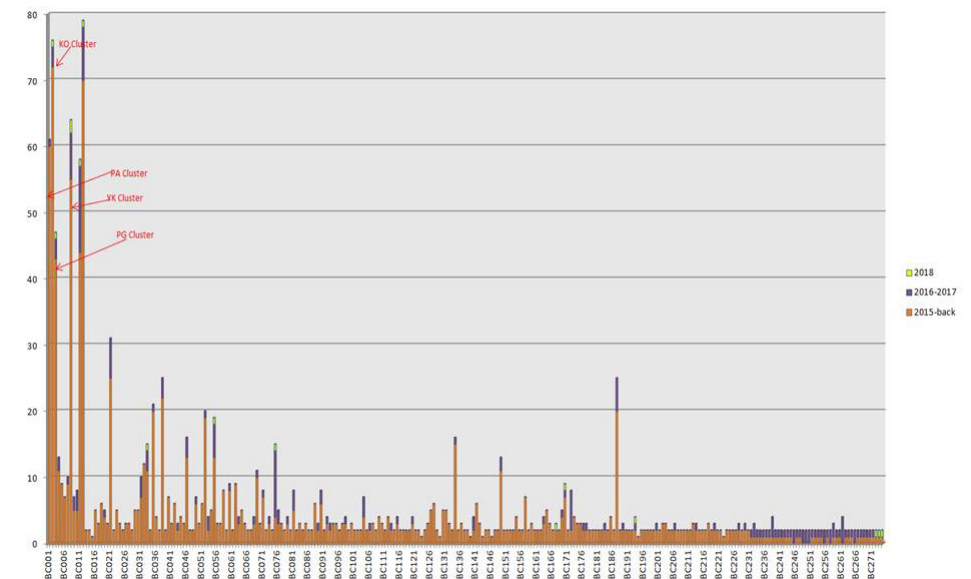


# TB genotyping surveillance

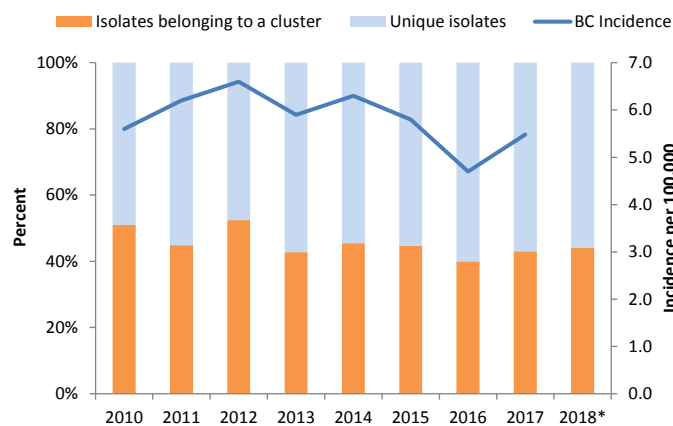
The Tuberculosis (TB)/Mycobacteriology Program at the BCCDC Public Health Laboratory (PHL) has been offering *Mycobacterium tuberculosis* (MTB) genotyping using the Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats (MIRU-VNTR) method since 2009. Where previously select MTB samples from the BC and Yukon were sent to the National Reference Centre for Mycobacteriology at the National Microbiology Laboratory for MIRU-VNTR genotyping, the TB/Mycobacteriology Program began offering routine, prospective in-house MIRU-VNTR genotyping in September, 2015. During the same period, in an effort to improve TB surveillance and epidemiologic investigation capacity, the TB/Mycobacteriology Program began assigning unique MIRU-VNTR -derived designation patterns where formerly, a 24-digit pattern corresponding to the number of repeats at each target was provided. A new cluster code is assigned when at least two isolates (from different patients) have matching MIRU-VNTR patterns.

There are currently 274 designations assigned, representing unique strains in our database. The first 12 clusters assigned included isolates belonging to several outbreaks ongoing at the time and represented concerted effort at understanding relatedness between certain isolates (Figure 4). Although there are several large clusters, the majority of unique patterns belong to clusters of 2-3 individuals likely resulting from limited transmission events within households or other connected networks. In general, from 40-45% of isolates (since 2015) are being clustered each year using MIRU-VNTR genotyping (Figure 5). Active TB incidence rates for BC have been generally decreasing from 2014-2016 with a slight increase in 2017 (Figure 4). Although recent MIRU-VNTR data seems to support the more limited transmission that is being seen in our population, interpretation of these genotype clusters requires correlation with follow-up epidemiological investigations as well as sequencing to provide further resolution.

**Figure 4.** MIRU designations assigned up to the first quarter of 2018 including 23 Yukon isolates from 4 clusters. KO=Kelowna, PA=Port Alberni, YK=Yukon, PG=Prince George, TB/Mycobacteriology Program, BCCDC PHL.



**Figure 5.** MIRU-VNTR genotyping clustering of TB isolates in BC and Yukon from 2010 to 2018\* (first quarter) and TB incidence in BC. Incidence data supplied by BCCDC Clinical Prevention Services.



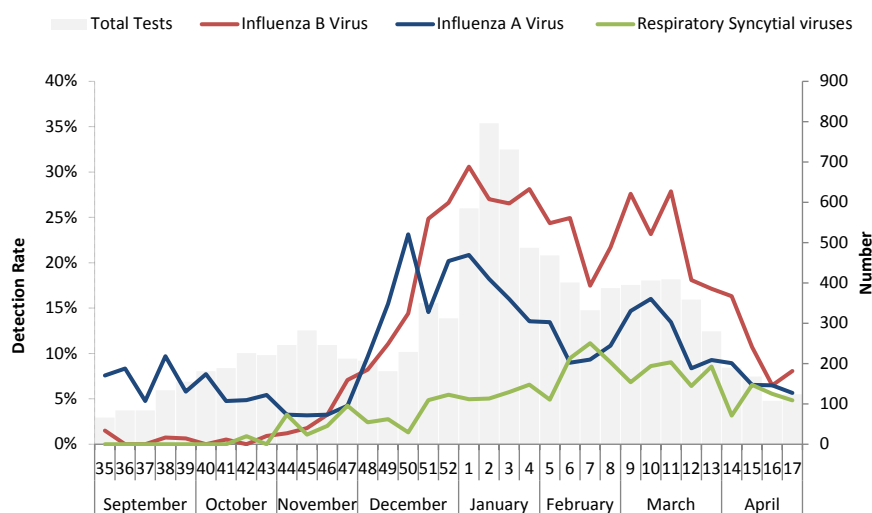
# Respiratory surveillance

The Virology Program at the BCCDC PHL has endured a heavy influenza season with test volumes that surged in January before decreasing but remaining elevated in the months of February and March. In an effort to provide better service and to accommodate the increased respiratory volumes, the program increased the respiratory pathogen panel testing (run after the influenza A/B and RSV NAT) for select populations to four days a week in January. This will continue until volumes do not warrant the increased testing.

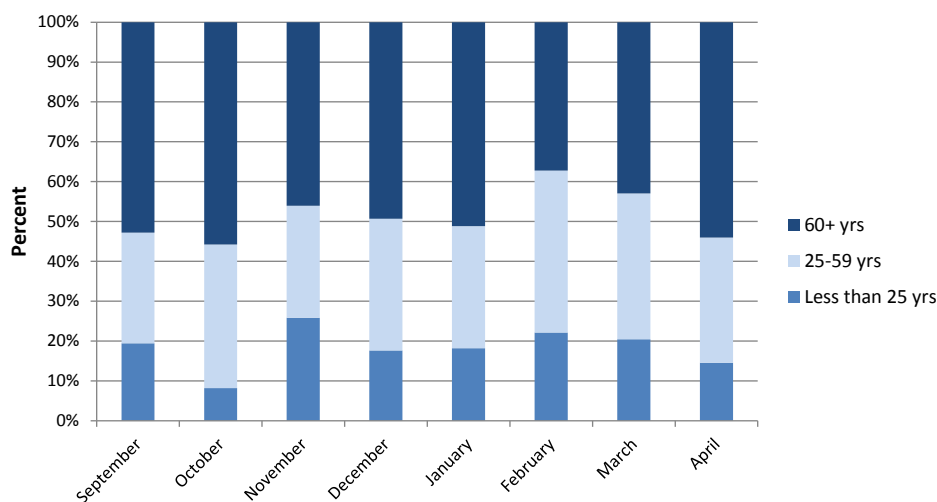
Influenza A began circulating early this season starting in September. In November, influenza B appeared more prominently in the population, co-circulating with influenza A until December before dominating over preceding months (Figure 6). Influenza A rates peaked at 23% in a week at the end of December before decreasing while influenza B rates fluctuated between 17-31% between the last two weeks of December and mid-March. Co-circulation of influenza A/B has been observed for a large proportion if not the majority of the season for Quebec, the Atlantic Provinces, and Ontario while similar to BC, the Prairies have detected influenza B as the dominant influenza virus for a large part of the season (1).

Those 60 years and over were the most affected by influenza this season, representing between 37-56% of all influenza positive specimens (Figure 7). The large number of respiratory outbreaks in longterm care (LTC) facilities are indicative of how this population has been adversely affected this season (see page 9). Adults 25-59 years and those less than 25 years represented between 28-41% and 8-26% of all influenza positive specimens throughout the season (Figure 7).

**Figure 6.** Respiratory virus detection rates of respiratory screen (influenza A/B and RSV NAT) by result week, 2017-18 season, Virology Program, BCCDC PHL.



**Figure 7.** Age groups of individuals with positive influenza specimens from respiratory screen (influenza A/B and RSV NAT) by result month, 2017-18 season, Virology Program, BCCDC PHL.



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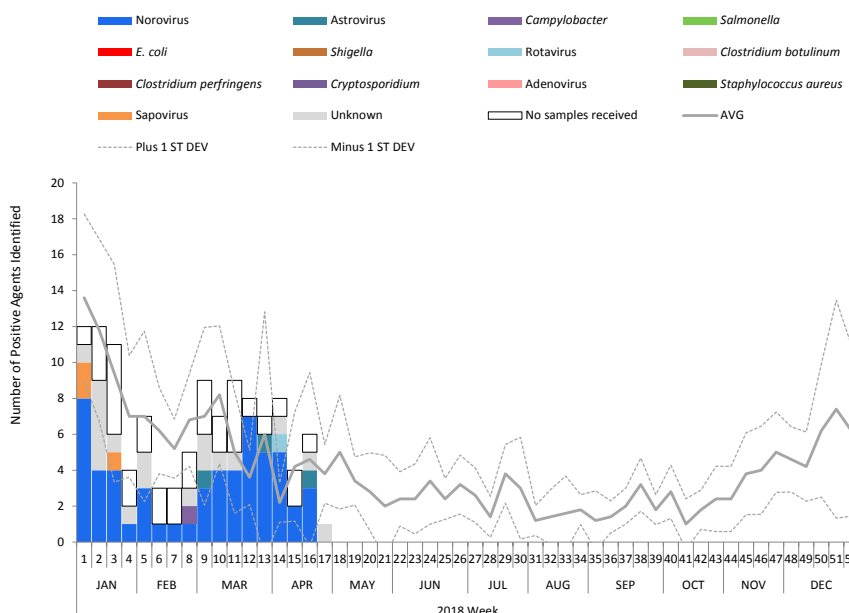
Influenza weekly reports 2017-18 season (Public Health Agency of Canada). Available at: <https://www.canada.ca/en/public-health/services/diseases/flu-influenza/influenza-surveillance/weekly-reports-2017-2018-season.html> (Accessed April 19, 2018)



## Gastrointestinal outbreaks

From January to April there were 116 gastrointestinal (GI) outbreaks investigated by the BCCDC PHL (Figure 8). Outbreaks were investigated from 66 (57%) LTC facilities, 28 (24%) daycares/schools, seven (6%) hospitals, 12 (10%) restaurants, two events (2%) and one (1%) other facility type. Samples were received from 71% of these outbreaks with norovirus detected in 56 (68%) (43 from LTC facilities, seven from restaurants (including six linked to oysters), two from daycares/schools, two from hospitals, from an event where oysters were served and from another facility type. Sapovirus was detected from samples from three wings of a LTC facility, astrovirus was detected from samples from two daycare outbreaks as well as a LTC facility outbreak, *Campylobacter* was detected from a restaurant outbreak, and rotavirus was detected in another LTC facility.

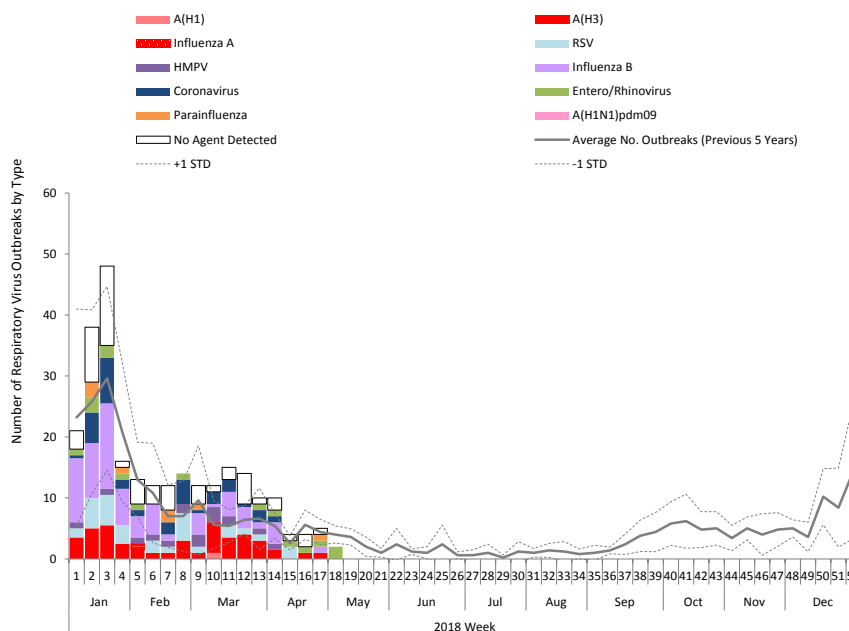
**Figure 8.** Gastrointestinal outbreaks investigated in 2017, Environmental Microbiology, Public Health Advanced Bacteriology & Mycology, Parasitology and Virology Programs, BCCDC PHL. The data available are from outbreaks in which the BCCDC PHL has been notified. Some acute care microbiology laboratories are also testing for norovirus in the province and these data may not include outbreaks from all health authorities.



## Respiratory outbreaks

From January to April there were 252 influenza-like illness (ILI) outbreaks investigated by the Virology Program of BCCDC PHL. Specimens from these outbreaks were submitted from 238 (94%) LTC facilities and 14 (6%) hospitals. The number of outbreaks is at the higher end of average weekly submissions from the past five years during this period (Figure 9). March was particularly busy with elevated numbers when ILI outbreaks are typically on the decline at this time of the year. Influenza B was detected in 59 (23%) outbreaks as well as with other mixed infections in a further 12 (5%) outbreaks. Influenza A(H3) was detected in 43 (17%) outbreaks and with other mixed infections in a further 7 (3%) outbreaks. With two additional outbreaks where influenza was detected (A, unsubtypeable and A(H1N1)pdm09), influenza was responsible for 49% of all outbreaks from January to April this year (compared to 42% of outbreaks in 2017 during this period).

**Figure 9.** Influenza-like illness outbreaks investigated in 2017 to date, Virology Program, BCCDC PHL. Note that some outbreaks are not reflected here if they are awaiting subtyping.



The Public Health Laboratory at the BC Centre for Disease Control (BCCDC) provides consultative, interpretative testing and analyses for clinical and environmental infectious diseases in partnership with other microbiology laboratories and public health workers across the province and nationally. The BCCDC PHL is the provincial communicable disease detection, fingerprinting and molecular epidemiology centre providing advanced and specialized services along with international defined laboratory core functions. The Provincial Toxicology Centre conducts toxicology testing and analysis for clinical patients, including therapeutic drug monitoring, drug screening tests and forensic toxicology analyses for the BC Coroners Service.

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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