

Wastewater Monitoring of Incoming Aircraft at Canadian Ports of Entry Policy Brief

Introduction

In a globalized world with increased air passenger traffic over the last several decades (Tay et al. 2024), emerging pathogens can be spread across the world within a few days. The rapid, worldwide dissemination of SARS-CoV-2 in recent years exemplifies this phenomenon, as international air travel played a key role in introducing new variants to previously unaffected regions (Jones et al. 2023).

Wastewater surveillance (WWS) is a rapid, sensitive, non-invasive, and cost-effective tool for monitoring various pathogens. It serves as an early detection system for outbreaks and can provide vital information on spatial and temporal trends of infectious diseases on a community level (Karthikeyan et al. 2022). Additionally, it can be applied to detection of novel pathogens, their variants, or re-emerging pathogens (Tisza et al. 2023). First implemented in the 1930s to combat a polio outbreak, WWS saw a resurgence in interest from researchers during the SARS-CoV-2 pandemic, allowing public health officials to better understand and respond to public health threats (Diamond et al. 2022). Currently, the Public Health Agency of Canada (PHAC) has ongoing efforts in conducting regular WWS in several cities (Halifax, Montreal, Toronto, Edmonton, and Vancouver) for select pathogens (Government of Canada, Wastewater monitoring, 2024). Sewage samples from the municipal water treatment stations are tested for COVID-19, influenza A and B, respiratory syncytial virus, mpox and polio. While the data obtained from this surveillance serves to monitor the general level of these diseases over time on the population level, it cannot trace the origin of the specific pathogens. In addition, municipal wastewater is combined with sewage from the airports, which may provide challenges in interpreting the results.

Studies have shown that the detection of new variants of SARS-CoV-2 in airport wastewater preceded their detection in the local water treatment plant station by approximately two weeks (Tay et al. 2024, Wongprommoon et al. 2024). Testing wastewater from airplanes and airport terminals would serve as the earliest alert system, provide complementary data on dynamics of introduced pathogens, and potentially determine the origin of an outbreak, which ultimately can be used to inform public health response and policy.

This surveillance program requires routinely collecting aircraft wastewater samples for microbiological analysis and sequencing and linking the resulting data with associated international air traffic information. We suggest a policy that provides unparalleled strengths, such as variability between individual plane wastewater testing and upscale testing as a response to immediate public health threats and implementing precautions for incoming flights from areas of particular concern to protect citizens (Image 1).

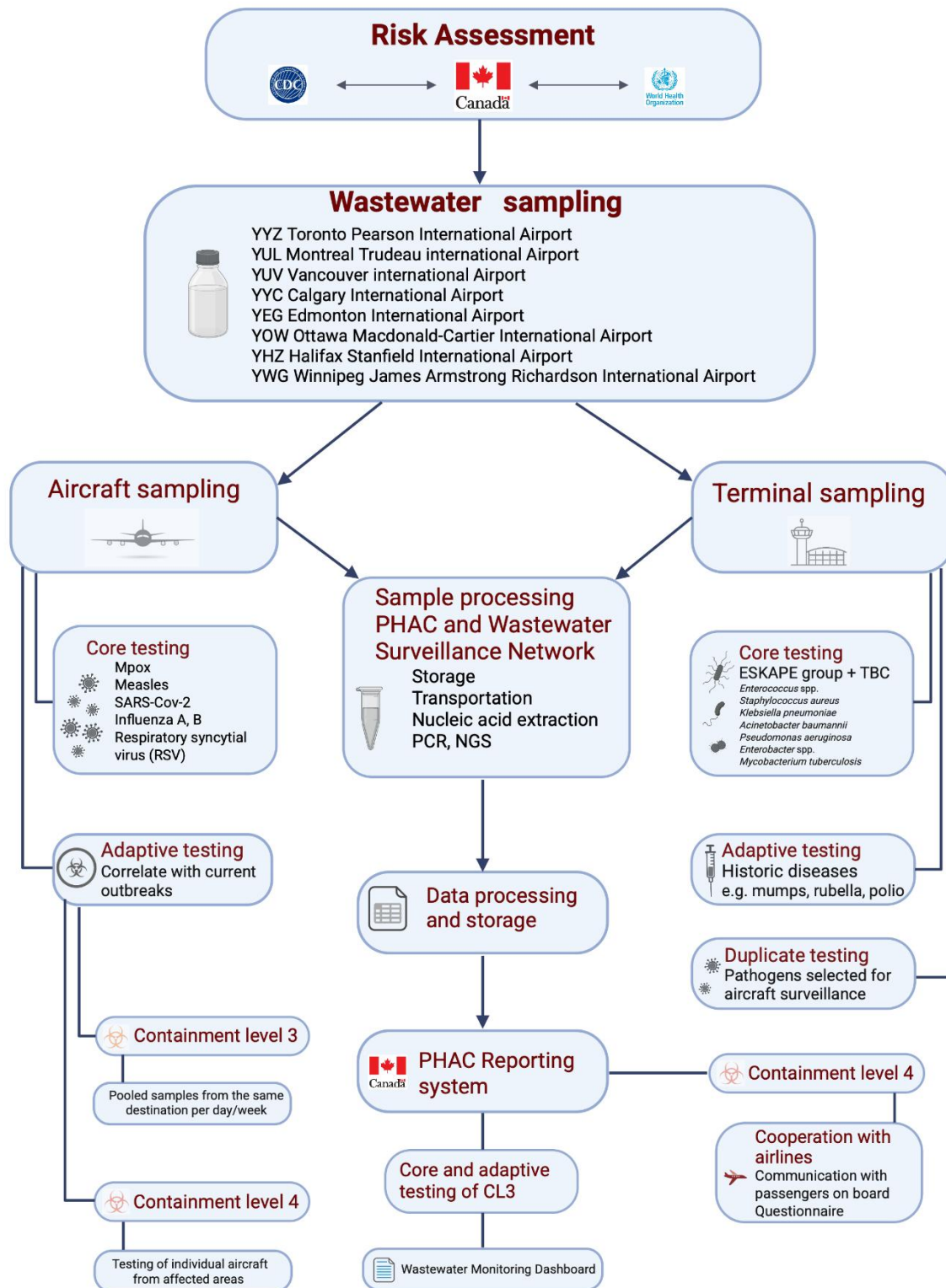


Image 1. Visual representation of the policy for the WWS testing of the airports

Pathogens of interest

Our program comprises a core list of pathogens and an adaptive list of pathogens suitable for aircraft wastewater monitoring. Core pathogens of concern include measles, Mpox, SARS-Cov-2, Influenza A and B, and respiratory syncytial virus (RSV). While the suggested pathogens overlap with the PHAC list for routine wastewater monitoring, this approach may be a great supportive tool for epidemiological surveillance with its focus on imported health threats. Aircraft WWS provides a clear relationship between incoming pathogens from international flights and their transmission within municipalities that already have routine testing. On the other side, for the municipalities that are not a part of the Canadian Waster Survey, this approach will serve as the primary tool for wastewater surveillance of public health threats.

In addition to our suggested core list is surveillance of antimicrobial resistant (AMR) bacteria that pose a significant threat to global public health, specifically pathogens from ESKAPE group: *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (Patil et al. 2021). In addition, rifampicin-resistant *Mycobacterium tuberculosis* will be routinely monitored. The World Health Organization (WHO) classified these bacteria as critical and high-priority pathogens, due to high transmissibility and disease burden, increasing AMR trends, and few to no potential treatment options (WHO Bacterial Priority Pathogens List, 2024). Monitoring entry of AMR organisms would provide essential data to the Canadian Antimicrobial Resistance Surveillance System (CARSS), facilitate prediction, and inform preparedness to mitigate existing and raising AMR burdens in Canada. This routine surveillance will also help to determine the impact of international transport on the global spread of AMR (Jones et al. 2023, Nguyen et al. 2021).

Beyond the core list for routine surveillance, PHAC will maintain an adaptive list of pathogens depending on national and global public health trends. Pathogens suitable for wastewater monitoring must match several criteria such as ability to be shed in excreta, characterization as having a high-community impact level, and causing an ongoing epidemic or pandemic (Tiwari et al. 2024, Singh et al. 2024). In brief, a research group responsible for aircraft WWS would coordinate with various public health organizations such as WHO and CDC to determine which pathogens are suitable for surveillance on flights coming from affected countries or regions. Once an outbreak is reported with a causative agent presenting a public health risk to Canada, a temporary focused surveillance should be implemented. All planes arriving from the region or country of interest will undergo a sewage collection procedure and be tested to determine the risk of introducing this pathogen into the country.

Our adaptive policy also maintains a list of “historic” diseases such as polio, rubella, and mumps. While in Canada the number of clinical cases of these diseases have significantly declined and rarely reported over the past several decades due to vaccination programs, these viruses are still circulating in many regions and could be re-introduced to Canada.

Despite being one of the most successful public health interventions, immunization coverage has drastically dropped worldwide due to the COVID-19 pandemic and its associated impact on the healthcare system. For instance, in 2024 WHO reported that only 84% of children received three full doses of the Measles-Mumps-Rubella vaccine, whereas the coverage rate sufficient to provide herd immunity against these diseases must be approximately 95% (WHO, 2024). In addition, these numbers are highly variable worldwide in countries such as Canada, where vaccination coverage has declined and an increased trend in public hesitancy toward immunization, surveillance measures must be considered. Surveillance of these vaccine-preventable diseases should be implemented when the immunization coverage declines to a critical level. Monitoring these pathogens at ports of entry can act as an essential risk-assessment tool to predict and prevent potential re-emergence of these pathogens due to international transit from endemic regions. In addition, this approach will facilitate an implementation of corresponding policies such as promotion of vaccination campaign and prepare the healthcare system for potential upcoming challenges.

Sampling strategy

Collected samples will be maintained at 4°C in a laboratory or stored in the airport before being tested to prevent potential degradation of the genetic material targeted in the detection procedure. Depending on the risk of an outbreak starting in Canada, and the volume of flights arriving from the region of concern, samples may be pooled together (i.e., samples from incoming flights from the same destination may be collected throughout the week, combined and tested once to reduce workload). Airport terminal sewage samples will be collected through an automated pump and processed on a weekly basis. The testing frequency for an outbreak may be subject to change throughout its course to reflect changes in threat level. For example, if the number of cases in the affected country increases, then samples can be tested 3 times a week instead of once a week.

However, during an outbreak caused by pathogens of containment level 4, wastewater samples will not be pooled, and aircraft will be tested individually to ensure traceability. As the risk associated with the outbreak reduces, testing will be scaled down until the outbreak is over, or it is deemed to no longer present as a threat for international travel. Additionally, pathogens selected for aircraft testing will also be monitored in the sewage from the terminal.

Airports of interest

Canada's major airports with high volumes of international flight traffic will be selected for wastewater monitoring. These airports include Toronto Pearson International Airport (YYZ, 27.6 million international passengers), Montreal Trudeau international Airport (YUL, 14.1 million international passengers), Vancouver international Airport (YVR, 11.7 million international passengers), Calgary International Airport (YYC, 5.8 million international passengers), Edmonton International Airport (YEG, 1.21 million international passengers), Ottawa Macdonald-Cartier International Airport (YOW, 0.85 million international passengers), Halifax Stanfield International Airport (YHZ, 0.49 international passengers), and Winnipeg James Armstrong Richardson International Airport (YWG, 0.48 million international passengers) (Statistics Canada, 2024). Additionally, these airports are situated in relatively large cities that have access to the necessary facilities to conduct detection procedures. Currently, PHAC has ongoing efforts in conducting regular wastewater monitoring in select cities (Halifax, Montreal, Toronto, Edmonton, and Vancouver).

Table 1. Canadian airports of interest for wastewater monitoring

No	Airport Name	Airport Code	Number of direct international destinations	Number of international passengers, million/year
1	Toronto Pearson International Airport	YYZ	199	27.6
2	Montreal Trudeau international Airport	YUL	145	14.1
3	Vancouver international Airport	YUV	79	11.7
4	Calgary International Airport	YYC	64	5.8
5	Edmonton International Airport	YEG	28	1.21
6	Ottawa Macdonald-Cartier International Airport	YOW	27	0.85
7	Halifax Stanfield International Airport	YHZ	29	0.49

8	Winnipeg James Armstrong Richardson International Airport	YWG	16	0.48
---	-----------------------------------------------------------	-----	----	------

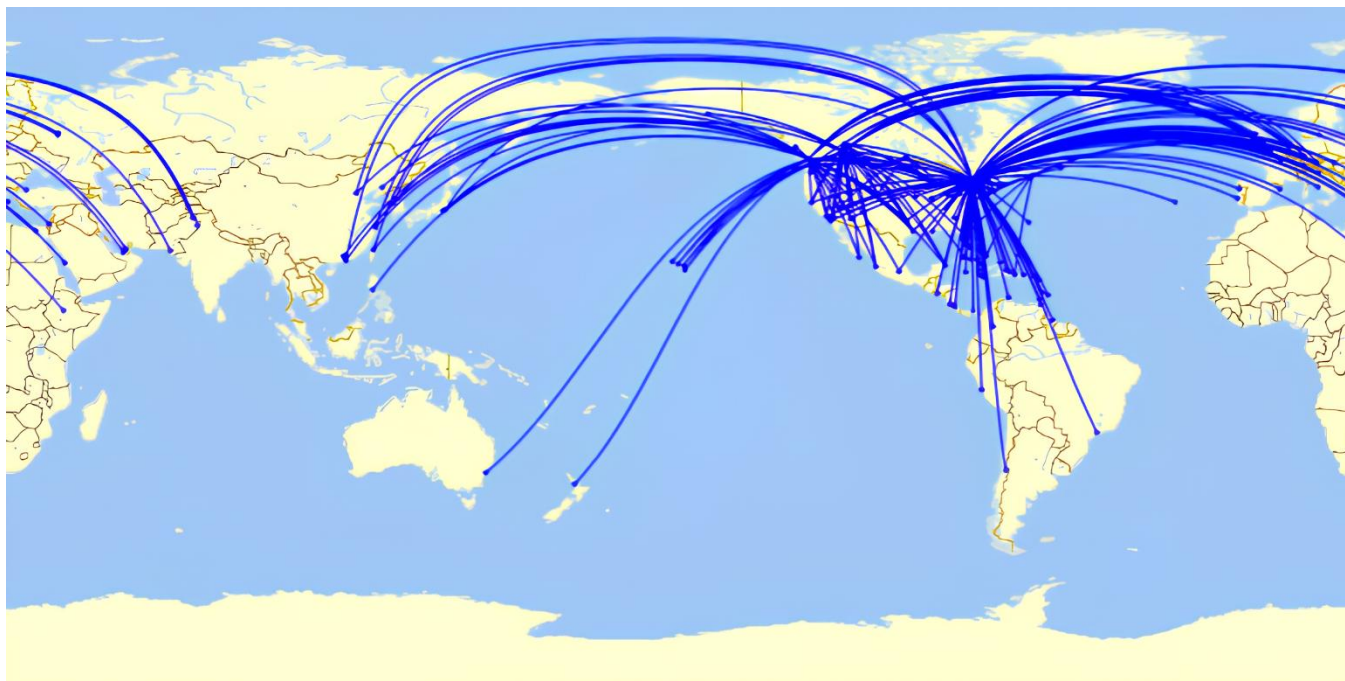


Image 2. Map of international air routes to the major Canadian airports (Data obtained from OpenFlights.org, Flightsfrom.com, and Flightconnections.com)

WWS Reporting System

The data collected from the surveillance program (both core and adaptive surveillance) will be submitted to PHAC for further analysis and integration into the Canadian Wastewater Survey program and other local surveillance networks. We suggest that PHAC publishes the core surveillance reports through the existing WWS dashboard either as a separate section for incoming flights or integrates it into pre-existing sections. Additionally, PHAC will be reporting data on AMR bacteria to the Canadian Antimicrobial Resistant Surveillance System (CARSS) to boost the country's AMR monitoring program. For the adaptive surveillance program, a separate section on the WWS dashboard would be created to inform the public of potential threats. For containment level 3 (CL3) pathogens, the website will indicate if the targeted pathogen was detected in incoming planes and an estimate of when it would have entered. For containment level 4 (CL4) pathogens, PHAC would be working directly with the airlines to contact passengers on positive flights. They will be sent a questionnaire to obtain more information on their travel history. Passengers would be recommended to get tested and begin self-isolation as soon as possible to reduce the risk of spreading an infection. The announcement on the website will indicate the exact

flight where the pathogen was detected. Ensuring airplane samples for CL-4 pathogens are tested for each individual plane and not pooled is essential to ensure the proper public health response. Passengers will be provided with a guidance on quarantine and recommendations on follow-up check-ups. The raw data obtained throughout the surveillance process by PHAC may be available to provincial and municipal public health authorities upon request. These data may also be available to Canadian researchers to help identify trends and help improve the surveillance network.

Policy strengths

The strength of this policy is inextricably linked to its flexibility. Costs will be minimized during times when no outbreaks of concern are identified, and testing is restricted only to core pathogen surveillance. Our policy will also have minimal to no impact on airline and airport functions when there are no pathogens of concern and even when wastewater is being tested from individual airplanes, the impact on regular function would be extremely minimal. Sampling procedures are not time-consuming and can be readily integrated into standard waste disposal procedures. Given the standardized detection protocols for wastewater, the data obtained from our surveillance will be useful for international cooperation across different surveillance frameworks.

Another advantage of this policy is that the reliability of the sampling process will not be affected by weather events and climate throughout the year. For example, municipal wastewater samples can be diluted by heavy rainfall and spring runoff (Dhiyebi 2023). These can lead to underestimation of pathogens in the population or bring concentrations below the limit of detection of assays. Additionally, ambient temperature may affect the quality of wastewater samples collected during conventional WWS. In our program, samples collected from airplanes may be stored and transported in refrigerated conditions before arriving at the lab. This would greatly improve the reliability of our policy in comparison to traditional WWS.

The final strength of this policy is its inherent adaptability to varying incoming flight volumes. For core pathogen surveillance, our program will aim to test a constant proportion of all incoming flights (for example, 5% of all incoming flights will have their wastewater sampled). Thus, the rate of testing will always reflect the volume of flights coming into Canada. For example, the number of planes being tested will increase to reflect the increase in incoming flights during the summer and winter holidays. The number of planes tested will likewise be reduced in the fall and spring months where air travel is lowest. This is also the case for the flexible pathogen surveillance program where all airplanes from the targeted region will be sampled, so the number of tests will depend on the number of incoming flights.

Value to Government

PHAC would immediately be made aware if a pathogen of concern has been introduced into Canada and where the pathogen entered. With that knowledge, they can send notifications to passengers and airport workers, implementing temporarily restrictive measures for flights coming from affected regions, such as individual testing of passengers prior to departure or upon arrival. This would greatly increase detection of introduced pathogens into Canada from abroad, preparing the public health system for threats. Unlike traditional WWS, which cannot determine the origin of the pathogen, this policy can be utilized to track travelers that have potentially been infected and quickly prevent the spread of infection. Moreover, this policy would complement the routine WWS currently being conducted in the cities that have international airports (Halifax, Montreal, Toronto, Edmonton, and Vancouver) to determine the impact of pathogens introduced through travel on local populations.

Value to Citizens

The public can feel confident in the government's efforts to address and assess potential threats early and effectively. This proactive approach will help protect citizens far more than reactive policies. Returning public health confidence through pro-active measures is essential for public buy-in and confidence of public health at large. In addition, the information presented on the WWS dashboard will be easy to understand and access for the public.

Public Health Significance

Identifying flights through which pathogens enter the country through is an important first step in prevention and protection of Canadian citizens against pathogens of concern arriving from other countries. Having specific information on what pathogens were introduced on a single flight from a specific area provides vital information for preventing the spread of the pathogen into the community at large and can prevent future introductions. For the routine surveillance aspect of our proposed plan, the information gained over time will help experts understand the movements of different pathogens and help guide future public health policy.

Expense Estimate and Return on Investment

This program will have limited overhead during times without pathogens of concern but will have the ability to ramp up testing intensity in times of need. This flexibility will reduce the cost of the program unless there are imminent global threats. Disease outbreaks, epidemics, and pandemics have significant costs for governments globally, and the sooner a pathogen of concern can be detected, the sooner preventative measures can be implemented to prevent or limit their impact. This will save a significant amount of money in addition to saving human lives. However, before the program can be implemented, financial

investments will be necessary to train ground operators on how to conduct proper sampling. Once the training program is developed, current employees of the target airports will be trained. This training will be offered to new ground operators who start working after the policy is implemented.

Policy Limitations

Although our policy has the potential to trace the origin of introduced pathogens through individual or destination-based testing of aircraft, it has a limited capability of tracking pathogens that may be brought through connections. To overcome this limitation, we suggest all passengers who were on board the aircraft tested positive through WWS to fill up a questionnaire with their latest travel information. Establishing an international network with a similar monitoring approach at foreign international hubs for comprehensive tracking of health threats is ideal but outside our scope.

The effective detection of pathogens in wastewater depends significantly on their shedding potential in human excreta and stability. Naturally, respiratory pathogens that may be found in sewage are less detectable than enteric pathogens, which may impact effectiveness of our suggested WWS (Tay et al. 2024). In addition, it is critical to collect and process samples at a proper temperature condition of 4°C to prevent nucleic acid degradation (Williams et al. 2024).

Another key consideration is passenger habits and their probability of using an aircraft toilet on short-, medium-, and long-haul flights. Some studies demonstrate that the aircraft WWS testing for SARS-CoV-2 can capture only a small fraction of infected passengers on board, and the number of samples tested positive was greater on the long-haul flights (Jones et al. 2023). To address this limitation, we suggest that the pathogens primarily selected for aircraft testing should also be monitored in the sewage from the terminal.

While surveillance of pathogens entering the country through airports may help reduce the impact of foreign pathogens on Canada, it is worth noting that cross-border ground transportation and maritime traffic, such as cruise ships, may also pose a risk of introducing pathogens. Thus, a similar policy should be applied to these modes of transport to maximize the detection capacity of pathogens among international travelers.

Communication Strategy

To communicate this wastewater surveillance policy with the public, a webpage will be designed in collaboration with PHAC to introduce the policy, explain the methodology, and publish data on surveyed pathogens. The webpage will be composed of 3 pages: introduction page, pathogen activity dashboard, which is split between routine and emerging pathogens, and methods. A questionnaire will be available for passengers aboard

flights that tested positive for a pathogen for contact tracing, provide information to public health officials and advice to the passengers.

Introduction Page

Statement of Purpose and Interest. This page provides a statement of purpose and importance for aviation wastewater surveillance. PHAC will outline why sampling wastewater from air travel is critical for the early detection of routine and emerging pathogens, and how this early detection will support public health preparedness.

Wastewater Sampling and Analysis. Here the Wastewater Surveillance Network will provide a brief description of the sampling process of aircraft and airports, outlining that collection of samples from aircraft wastewater tanks and terminals. A brief overview of laboratory methods used will also be provided, explaining DNA extraction, quantification methods, and identification of target pathogens. The turnaround time from sample collection to data reporting will also be published.

Early Warning System. Should a target pathogen be detected above its alert threshold, a warning will be issued on the webpage. These same alerts will also be communicated with public health authorities. Based on the alerted pathogen, resources for recommended public health actions. These resources include links to preventative safety measures, recommended courses of action if one believes they may be infected, and other information regarding the illness.

Frequently Asked Questions. Common questions regarding the dashboard's purpose, data, interpretation, privacy, and various other concerns will be posted. Explanations of scientific terms and methodologies will also be posted.

Contact Information. Contact information will also be included for dashboard management and technical support.

Pathogen activity dashboard

Routine Pathogens. This page will contain a dashboard to present detection data for core pathogens and will be updated weekly. This page will include national and regional detection data, an interactive map, instructions for interpreting the data, and information on additional resources.

There will be a national overview that summarizes pathogen activity levels across airports in Canada. Activity levels will be displayed using a population-weighted average index for each pathogen, ranging from low, moderate, to severe.

For further resolution, pathogen activity levels will also be presented per airport. This section will contain an interactive map displaying pathogen activity across different airports in Canada. Each airport will also have time-series graphs displaying pathogen detection

from aircraft and airports levels over time. This map will also have a heat-map overlay to better visualize high risk airports or terminals. The map can be filtered by airport, airline, origin of departure, and time period. Additionally, the frequency of positive cases for each given pathogen from subcontinental regions (i.e. Western Europe, West Africa, Southeast Asia) will be listed. In collaboration with PHAC, this map can also be compared with other relevant public health metrics such as the number of reported clinical cases and travel patterns. This will help index the detection of pathogens to relevant clinical information.

This page will also contain graphs displaying weekly population-weighted averages of pathogen levels. However, a disclaimer will be posted explaining that these averages are likely to be underreported, as not all passengers will use the restroom on flights, integrity nucleic acids in samples can be affected during transport, and shedding capacity of various pathogens in wastewater is variable. Weekly minimum and maximum signals will be highlighted. Hovering over the graphs will reveal daily values and historical minimum and maximum values. The page will also advise caution when interpreting short term changes by explaining that ongoing trends are a more reliable indicator. Consistent with the interactive map, graphs of other relevant public health metrics such as number of reported clinical cases, municipal WWS, and travel patterns will also be displayed. All data used to generate the interactive map and graphs will be available to download in comma-separated value format (CSV).

Emerging Pathogens. Emerging pathogens present distinct threats and challenges to public health. PHAC will explain the importance of monitoring pathogen variants and strains at airports as a major transportation hub. It will be outlined that monitoring emerging strains allows us to detect new variants before they spread and survey population dynamics of variants over time. The geographic scope of the surveillance system across major international airports in Canada will be highlighted.

On this page the Wastewater Surveillance Network will provide a brief explanation of the testing methods distinct to identifying emerging strains from aircraft and airport wastewater. Specifically, it will be outlined how genomic sequencing and bioinformatic strategies differ for identifying emerging as opposed to routine pathogens. Wastewater data will also be integrated with other municipal surveillance data sources for a more complete overview.

Interactive maps and graphs for emerging strains will be implemented in the same format as for routine pathogens. However, there is no pre-set list of emerging strains, therefore maps and graphs would need to not only track pathogen activity, but also clearly identify when a new strain has emerged for a given pathogen.

Methods and additional notes

The goal of this section of the dashboard will be to provide transparency regarding not only the methodology the Wastewater Surveillance Network will be using to monitor aircraft and airport wastewater, but also the limitations and challenges this monitoring technique faces when it comes to protecting public health. This tab of the webpage will be broken down into four main sections, namely methodology, limitations, definitions, and data changes or updates

In the methodology section, PHAC will focus on providing more detailed information relating to protocols surrounding sample collection and analysis. This will include descriptions of sampling strategy protocols for wastewater collection from both aircraft lavatories and airport sewage systems, as well as explanations for the frequency of sample collection both during routine surveillance and the heightened monitoring proposed for emerging pathogens. Detailed explanations of techniques such as polymerase chain reactions and sequencing used to quantify and identify pathogens within wastewater samples will also be provided in this section. Equipment, software, and additional techniques required to complete the wastewater analysis will be clearly identified and described as well.

The limitations described in the next section of this tab of the website will focus primarily on confounding factors. This will focus on confounding factors such as variations in passenger and crew counts, or the location of sampling points along airport wastewater pipelines. The influence these factors may have on data collection and analysis will be explained in this section as well, as will concerns related to sensitivity and detection limits. The sensitivity and reliability of proposed testing methods will be explored, especially when it comes to establishing acceptable detection thresholds to observe and identify low levels of pathogens within a sample. Also to be discussed in this section is the potential for higher pathogen concentrations in airport and/or aircraft wastewater relative to municipal concentrations, and how this adds an additional layer of complexity to the proposed analyses.

This section's third tab will focus on definitions of scientific terminology to increase the accessibility of the methodology and the data it creates. These definitions will focus on terms surrounding wastewater sampling and analysis, initially covering terms such as wastewater signal, sensitivity, and detection threshold, and expanding to cover any additional terms as needed. This definition list will be expanded to include any terms which begin to feature on the FAQ tab as well.

The final tab in this section of the dashboard is meant to display data changes and updates. As such, this page will contain a changelog of all data presented to the public on

the website. This will include information on the change itself, but also the date the adjustment was made and the reason(s) an update to the data was considered necessary.

Data Privacy

In this tab, PHAC will address which, if any, personal information is collected, and at which steps of the protocol they are collected. It will be highlighted that personal information is not shared with the public and is not published on this dashboard. It is important for the adherence to personal privacy policies that microbial genomic data collected from wastewater collection samples cannot be indexed back to a single individual.

Questionnaire

A questionnaire will be designed to collect information from passengers that boarded a flight that tested positive for a pathogen. The primary function of the form is to help public health authorities trace the origin of the infection, monitor at-risk individuals, and determine appropriate follow-up steps to ensure passenger safety and contain further spread. The questionnaire is to be organized into several sections: (1) Flight Information, which requests comprehensive details for all legs of the trip (including connecting flights), including booking references, airline, flight numbers, departure and arrival dates, and airport codes; (2) Contact Information, which gathers names, dates of birth, phone numbers, email, and a Canadian address or connecting flight details; (3) Additional Passengers, where contact information for all individuals in the same booking is provided; and (4) Symptom Assessment, where respondents indicate whether they have experienced any symptoms associated with the identified pathogen. The form will conclude with instructions on where to seek medical advice if necessary.

References

- Government of Canada, Wastewater monitoring (2024). Open access
<https://www.canada.ca/en/public-health/services/emergency-preparedness-response/wastewater-monitoring.html>.
- Dhiyebi, H. A., Abu Farah, J., Ikert, H., Srikanthan, N., Hayat, S., Bragg, L. M., Qasim, A., Payne, M., Kaleis, L., Paget, C., Celmer-Repin, D., Folkema, A., Drew, S., Delatolla, R., Giesy, J. P., & Servos, M. R. (2023). Assessment of seasonality and normalization techniques for wastewater-based surveillance in Ontario, Canada. *Front Public Health*, 11, 1186525. <https://doi.org/10.3389/fpubh.2023.1186525>
- Diamond, M. B., Keshaviah, A., Bento, A. I., Conroy-Ben, O., Driver, E. M., Ensor, K. B., Halden, R. U., Hopkins, L. P., Kuhn, K. G., Moe, C. L., Rouchka, E. C., Smith, T., Stevenson, B. S., Susswein, Z., Vogel, J. R., Wolfe, M. K., Stadler, L. B., & Scarpino, S. V. (2022). Wastewater surveillance of pathogens can inform public health responses. *Nat Med*, 28(10), 1992-1995. <https://doi.org/10.1038/s41591-022-01940-x>
- Jones, D. L., Rhymes, J. M., Wade, M. J., Kevill, J. L., Malham, S. K., Grimsley, J. M. S., Rimmer, C., Weightman, A. J., & Farkas, K. (2023). Suitability of aircraft wastewater for pathogen detection and public health surveillance. *Science of The Total Environment*, 856, 159162. <https://doi.org/10.1016/j.scitotenv.2022.159162>
- Karthikeyan, S., Levy, J. I., De Hoff, P., Humphrey, G., Birmingham, A., Jepsen, K., Farmer, S., Tubb, H. M., Valles, T., Tribelhorn, C. E., Tsai, R., Aigner, S., Sathe, S., Moshiri, N., Henson, B., Mark, A. M., Hakim, A., Baer, N. A., Barber, T.,...Knight, R. (2022). Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. *Nature*, 609(7925), 101-108. <https://doi.org/10.1038/s41586-022-05049-6>
- Karthikeyan, S., Levy, J. I., De Hoff, P., Humphrey, G., Birmingham, A., Jepsen, K., Farmer, S., Tubb, H. M., Valles, T., Tribelhorn, C. E., Tsai, R., Aigner, S., Sathe, S., Moshiri, N., Henson, B., Mark, A. M., Hakim, A., Baer, N. A., Barber, T.,...Knight, R. (2022). Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. *Nature*, 609(7925), 101-108. <https://doi.org/10.1038/s41586-022-05049-6>
- Nguyen, A. Q., Vu, H. P., Nguyen, L. N., Wang, Q., Djordjevic, S. P., Donner, E., Yin, H., & Nghiem, L. D. (2021). Monitoring antibiotic resistance genes in wastewater treatment: Current strategies and future challenges. *Sci Total Environ*, 783, 146964. <https://doi.org/10.1016/j.scitotenv.2021.146964>
- Patil, A., Banerji, R., Kanojiya, P., & Saroj, S. D. (2021). Foodborne ESKAPE Biofilms and Antimicrobial Resistance: lessons Learned from Clinical Isolates. *Pathog Glob Health*, 115(6), 339-356. <https://doi.org/10.1080/20477724.2021.1916158>

- Singh, S., Ahmed, A. I., Almansoori, S., Alameri, S., Adlan, A., Odivilas, G., Chattaway, M. A., Salem, S. B., Brudecki, G., & Elamin, W. (2024). A narrative review of wastewater surveillance: pathogens of concern, applications, detection methods, and challenges [Review]. *Frontiers in Public Health*, Volume 12 - 2024. <https://doi.org/10.3389/fpubh.2024.1445961>
- Statistics Canada. Air passenger traffic at Canadian airports, annual (2024). Open access <https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=2310025301>.
- Tay, M., Lee, B., Ismail, M. H., Yam, J., Maliki, D., Gin, K. Y., Chae, S. R., Ho, Z. J. M., Teoh, Y. L., Ng, L. C., & Wong, J. C. C. (2024). Usefulness of aircraft and airport wastewater for monitoring multiple pathogens including SARS-CoV-2 variants. *J Travel Med*, 31(5). <https://doi.org/10.1093/jtm/taae074>
- Tisza, M., Javornik Cregeen, S., Avadhanula, V., Zhang, P., Ayvaz, T., Feliz, K., Hoffman, K. L., Clark, J. R., Terwilliger, A., Ross, M. C., Cormier, J., Moreno, H., Wang, L., Payne, K., Henke, D., Troisi, C., Wu, F., Rios, J., Deegan, J.,...Maresso, A. W. (2023). Wastewater sequencing reveals community and variant dynamics of the collective human virome. *Nat Commun*, 14(1), 6878. <https://doi.org/10.1038/s41467-023-42064-1>
- Tiwari, A., Radu, E., Kreuzinger, N., Ahmed, W., & Pitkänen, T. (2024). Key considerations for pathogen surveillance in wastewater. *Sci Total Environ*, 945, 173862. <https://doi.org/10.1016/j.scitotenv.2024.173862>
- World Health Organization [WHO]. Immunization Coverage (2024). Open access <https://www.who.int/news-room/fact-sheets/detail/immunization-coverage>.
- World Health Organization [WHO]. Bacterial Priority Pathogens List (2024). Open access <https://iris.who.int/bitstream/handle/10665/376776/9789240093461-eng.pdf?sequence=1>.
- Williams, R. C., Perry, W. B., Lambert-Slosarska, K., Fitcher, B., Pellett, C., Richardson-O'Neill, I., Paterson, S., Grimsley, J. M. S., Wade, M. J., Weightman, A. J., Farkas, K., & Jones, D. L. (2024). Examining the stability of viral RNA and DNA in wastewater: Effects of storage time, temperature, and freeze-thaw cycles. *Water Res*, 259, 121879. <https://doi.org/10.1016/j.watres.2024.121879>
- Wongprommoon, A., Chomkatekaw, C., & Chewapreecha, C. (2024). Monitoring pathogens in wastewater. *Nature Reviews Microbiology*, 22(5), 261-261. <https://doi.org/10.1038/s41579-024-01033>