ORIGINAL ARTICLE

Retrospective testing of respiratory specimens for COVID-19 to assess for earlier SARS-CoV-2 infections in Alberta, Canada

Jamil N Kanji MD, DTM&H, FRCPC[1,2], Mathew Diggle PhD, MSc, DLSH&TM, FRCPath[2,3], Dennis E Bulman PhD[5,6], Stacey Hume PhD[5,6], Sherry Taylor PhD[5,6], Rhonda Kelln PhD[5], Shelagh Haase BSc[5], Robert Tomaszewski BSc[5], Christine Walker BSc[5], Kanti Pabbaraju MSc[4], Vincent Li MSc[2], Matthew Croxen PhD[2,3], Nathan Zelyas MD, MSc, FRCPC[2,3], Deena Hinshaw MD, MPH, FRCPC[7,8,9], Graham Tipples PhD[2,10,11]

BACKGROUND: The first case of coronavirus disease 2019 (COVID-19) in Alberta, Canada, was confirmed on March 5, 2020. Because the virus testing criteria had changed significantly over this time period, we wanted to ascertain whether previous cases of COVID-19 had been missed in the province. **METHODS:** Our aim was to retrospectively evaluate specimens submitted for respiratory virus testing from December 1, 2019, through March 7, 2020, for undetected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections before the first confirmed case. **RESULTS:** Testing of 23,517 samples (representing 23,394 patients) identified 1 patient positive for SARS-CoV-2. This specimen was collected on February 24, 2020, from a patient with symptoms consistent with COVID-19 who had recently returned from the western United States. Phylogenetic analysis confirmed this viral isolate belonged to lineage B.1. The epidemiology of this case is consistent with those of other early cases before sustained community transmission, which included a travel history outside of Canada. **CONCLUSION:** This exercise provides support that local public health pandemic planning was satisfactory and timely.

KEYWORDS: COVID-19, rtRT-PCR, SARS-CoV-2, travel-related

HISTORIQUE : Le premier cas de maladie à coronavirus 2019 (COVID-19) en Alberta, au Canada, a été confirmé le 15 mars 2020. Puisque les critères de dépistage ont beaucoup évolué pendant cette période, les chercheurs voulaient vérifier si des cas antérieurs de COVID-19 avaient été omis dans la province. **MÉTHODOLOGIE :** Les chercheurs ont procédé à l'évaluation rétrospective d'échantillons soumis en vue du dépistage d'un virus respiratoire entre le 1er décembre 2019 et le 7 mars 2020, afin de retracer les infections par le coronavirus 2 du syndrome respiratoire aigu sévère (SARS-CoV-2) non décelées avant le premier cas confirmé. **RÉSULTATS :** Le dépistage de 23 517 échantillons (représentant 23 394 patients) a fait ressortir un patient positif au SARS-CoV-2. Le prélèvement avait été effectué le 24 février 2020 chez un patient éprouvant des symptômes correspondant à la COVID-19 revenu récemment de l'ouest des États-Unis. L'analyse phylogénétique a confirmé que l'isolat viral appartenait à la lignée B.1. L'épidémiologie de ce cas est compatible avec celle des autres premiers cas précédant une transmission communautaire soutenue, qui incluait un voyage à l'extérieur du Canada. **CONCLUSION :** Cet exercice appuie la pertinence et la rapidité de la planification sanitaire locale de la pandémie.

MOTS-CLÉS : COVID-19, rtRT-PCR, SARS-CoV-2, voyages

[1]Division of Infectious Diseases, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Albert, Canada; [2]Public Health Laboratory, Alberta Precision Laboratories, University of Alberta Hospital, Edmonton, Alberta, Canada; [3]Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada; [4]Public Health Laboratory, Alberta Precision Laboratories, Foothills Medical Centre, Calgary, Alberta, Canada; [5]Genetics and Genomics, Alberta Precision Laboratories, University of Alberta Hospital, Edmonton, Alberta, Canada; [6]Department of Medical Genetics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada; [7]Chief Medical Officer of Health, Alberta Health, Edmonton, Alberta, Canada; [8]Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada; [9]Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada; [10]Department of Medical

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Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada; [11]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada

Correspondence: Mathew Diggle, Public Health Laboratory, Alberta Precision Laboratories, University of Alberta Hospital, 8440 112 Street NW, Edmonton, Alberta T6G 2B7, Canada. Telephone: 780-407-3068. Fax: 780-407-3864. E-mail: mathew.diggle@aplabs.ca

INTRODUCTION

Since the identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as the causative agent of coronavirus disease 2019 (COVID-19) on January 9, 2020, the virus has spread to every inhabited continent (1). The timing of subsequent global spread of SARS-CoV-2 since its identification is not clearly known. Time to most recent common ancestor analysis from more than 7,600 global SARS-CoV-2 genomes estimates the emergence of the virus as ranging from October 6 to December 11, 2019 (2). This suggests that travelers from the area of the virus's origin may have imported it to other countries earlier than first thought.

As a consequence, several countries have found in retrospective studies that SARS-CoV-2 was circulating earlier than their first detected cases. These reports describe SARS-CoV-2 virus detection during time periods before governments implemented strict screening and quarantine measures for travelers arriving in their countries. One example, from a retrospective study reported in April 2020, is that of a SARS-CoV-2–positive man hospitalized in France in December 2019, much earlier than the initial first routine testing case in that country from January 24, 2020 (3). More recently, a rapid communication from the Public Health Agency of Canada (PHAC) confirmed that SARS-CoV-2 was circulating not only internationally but also in Canada more broadly than was initially detected by routine surveillance (4).

In Canada, the first case of COVID-19 was confirmed on January 25, 2020, in Toronto, Ontario, in a traveller returning from Wuhan, China (5). COVID-19 testing in the Western Canadian province of Alberta began on January 21, 2020, with the first case detected on March 5, 2020 (6). Alberta is Canada's fourth most populous province, with a population of 4.43 million (7). During the initial testing period, testing was restricted to those with epidemiologic history in terms of travel to high-risk areas, as defined by PHAC. Thus, it is plausible that cases of locally acquired SARS-CoV-2 or cases not meeting the initial travel criteria for testing may have been missed. We undertook a retrospective evaluation of all samples submitted for testing for influenza and other respiratory viruses from across Alberta from December 1, 2019, through March 7, 2020, to assess for the presence of undiagnosed SARS-CoV-2 in the province before the first detected case.

METHODS

Setting

The vast majority of respiratory virus testing in Alberta is conducted at the Public Health Laboratory (ProvLab), which has locations in two cities (Edmonton and Calgary). Samples were collected and submitted to the laboratory at the clinician's discretion. Therefore, testing for respiratory viruses was typically performed for patients with influenza-like illness, respiratory distress, or any clinical encounter in which a clinician felt that testing was warranted. Specimens accepted include nasopharyngeal swabs or aspirates in universal transport media, bronchoalveolar lavage specimens, and endotracheal aspirate samples. Community (outpatient) specimens are routinely tested for influenza A and B using a multiplex real-time reverse-transcriptase polymerase chain reaction (rtRT-PCR) developed by the US Centers for Disease Control and Prevention to detect seasonal influenza A and B (8). For the purposes of health care delivery, Alberta is divided into five health care zones: North, Edmonton, Central, Calgary, and South zones (9). In most of the province (North, Edmonton, and Calgary zones), samples from hospital and acute care settings (urgent care centres and emergency departments) are subjected to a multiplex-PCR panel of respiratory viruses including influenza A (H1-pdm09 and H3 subtypes) and B viruses, human enterovirus or rhinovirus, human metapneumovirus, respiratory syncytial virus (RSV), adenovirus, human coronaviruses (NL63, OC43, HKU1, 229E), and parainfluenza viruses (types 1–4) (NxTAG® Respiratory Pathogen Panel [RPP], Luminex Corporation, Austin, Texas, USA). In some areas of the province (Central and South zones), hospital and acute care respiratory virus samples are initially tested at nearby community hospital laboratories for influenza A or B and RSV (Simplexa® Flu A/B and RSV Kit, Diasorin, Saluggia, Italy) and forwarded to ProvLab for further RPP testing if requested by the clinician.

All samples submitted for respiratory virus testing throughout the province of Alberta are forwarded to the ProvLab for long-term storage (regardless of the testing results and location of collection in the province) for up to 6 months.

SARS-CoV-2 testing

A pooling methodology was used to facilitate screening of large numbers of samples for the presence of SARS-CoV-2 (10). All sample pooling and viral RNA extractions were

performed using a Tecan Liquid Handler (Tecan, Männedorf, Switzerland), Chemagic MSM1 Magnetic Bead Separator (Perkin Elmer, Waltham, Massachusetts, USA), and Chemagic Viral DNA/RNA (H96) extraction kit within a Big Neat Biohazard Level 2 Containment System (BioSero, San Diego, California, USA). To pool samples, 50 µL aliquots from each of four individual specimens were combined in a single well of a 96-well plate (final volume of 200 µL in each well).

SARS-CoV-2 detection in extracts of pooled samples was done using a rtRT-PCR targeting the SARS-CoV-2 envelope (*E*) gene (and bacteriophage MS2 as an internal control) adapted from the assay described by Corman et al (11). The assay combined 5 µL of pooled extract with 2.5 µL Thermo Fisher Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and primer–probe mix. Testing was conducted using an Applied Biosystems 7500 FAST Real Time PCR system (Thermo Fisher Scientific). Pooled samples with cycle threshold (Ct) values of less than 45 cycles were subject to repeat rtRT-PCR in duplicate from the original extract. Those with repeat positive results were confirmed using an alternate rtRT-PCR assay targeting the N1 and N2 portions of the SARS-CoV-2 nucleoprotein gene (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) (12).

SARS-CoV-2 genomic sequencing

The SARS-CoV-2 genome was amplified from RNA extracts following the ARTIC V3 protocol (13), except the PCR amplification was done at 64°C. Sequencing was performed on the Oxford Nanopore Technologies MinION. Guppy_basecaller (version 3.6.1; Oxford Nanopore Technologies, Oxford, United Kingdom) and guppy_barcoder (version 3.6.1; Oxford Nanopore Technologies) were used to basecall and demultiplex the sequencing data, respectively. A consensus genome was generated using the ARTIC bioinformatics pipeline (14) with nanopolish calling the consensus sequence. Lineage designation was assigned using pangolin (15,16).

Patient identification and contact tracing

In consultation with provincial public health officials during the design phase of the study protocol, it was determined that any retrospectively identified cases of SARS-CoV-2 infection from this study would not be identified or reported to public health or contacted, nor would contact tracing be conducted, given that this study occurred many months after the start of the pandemic. The main aim of this study was to inform and assess the pandemic preparedness of our laboratory and public health team.

Table 1: Demographics of patients with respiratory specimens submitted from December 1, 2019–March 7, 2020 (*N* = 23,517 samples from 23,394 individual patients)

Variable	No. (%)*
Age, y	
Median (mean)	38 (39.8)
Range	0–106
Sex	
Female	12,129 (51.8)
Month of collection	
December	6,603 (28.1)
January	8,376 (35.6)
February	6,254 (26.6)
March	2,284 (9.7)
Specimen type	
Bronchial wash	495 (2.1)
Nasopharyngeal swab or aspirate	21,870 (93.0)
Throat swab	344 (1.5)
Endotracheal suction sample	793 (3.4)
Non-validated samples†	15 (0.1)
Patient type	
Inpatient (hospitalized)	15,062 (64.4)
Emergency or urgent care	4,170 (17.8)
Outpatient (ambulatory non-emergency)	3,334 (14.3)
Long-term, continuing, or congregate care facility	828 (3.5)

** Unless otherwise stated*
† Validated specimens include nasopharyngeal swabs and aspirates, auger suction, throat swabs, endotracheal suction samples, bronchial wash samples, and lung tissue

RESULTS

A total of 23,517 samples collected during the specified time period were retrieved, representing 23,394 individual patient specimens (Table 1). The patients' median age was 38 years, and 51.8% of them were female. The majority of specimens were nasopharyngeal swabs or aspirates (93.0%), with inpatients (64.4%) making up the most common patient type. Because the ProvLab stores all respiratory specimens submitted for evaluation for up to 6 months, these represented all of the samples submitted for influenza and other respiratory virus testing collected across Alberta during the specified time period.

Testing of 5,880 pools identified 1 pool with a Ct value of 19.2 cycles. Individual testing of each of the four samples

in the pool identified one sample (representative of one patient) which was positive on the *E*-gene rtRT-PCR (Ct of 18.2 cycles). This specimen was also confirmed on the N1 and N2 target rtRT-PCR assays (Ct values of 18.5 and 17.6 cycles, respectively).

This specimen, originally collected on February 24, 2020, which tested negative for influenza A and B, was from a patient who had presented to their family physician with symptoms of fever, cough, myalgia, and pleuritic chest pain 2 days after return from the Western region of the United States. No chest radiography or bloodwork was conducted at that encounter, and the patient never required hospitalization. Analysis of the genome sequence assigned this patient's viral isolate to lineage B.1.

INTERPRETATION

To date, all of the first-detected COVID-19 cases across Canada's provinces and territories have been travel related (17). Introduction of SARS-CoV-2 across the Canadian landscape occurred between January 25 and March 22, 2020 (although local transmission within many jurisdictions was established between these dates).

Our results indicate the presence of SARS-CoV-2 infection in Alberta, Canada, on February 24, 2020, 9 days earlier than the first confirmed case. Similar to other first-detected cases, it is presumed to be related to travel. Although tested for influenza, this patient did not meet the recommended criteria at that time for COVID-19 testing, which from January 17 to February 25, 2020, included only travel through Wuhan, China (18). Eligibility expanded on February 26, 2020, by PHAC guidance to include travellers returning from mainland China, Hong Kong, Italy, Iran, Singapore, Japan, and South Korea (19). This case also preceded the implementation of mandatory 14-day quarantine periods for all returning travelers to Canada on March 25, 2020 (20).

The SARS-CoV-2 isolate amplified from this patient's respiratory sample genotyped as lineage B.1, representing one of the predominant initial strains found in Europe, as well as one of the most predominant lineages in Europe, North America, and South America (16, 21), and hence was consistent with this patient's travel to the United States. Overall, introduction of SARS-CoV-2 in Canada was from numerous independent importations, as evidenced by multiple genetic lineages (A.1, B.1, B.3, B.4, and others) that have been found to date (22). The first seven confirmed cases of COVID-19 in Alberta were all travel related, including passengers on multiple cruise ships and also travelers returning at that time from Ukraine, Netherlands, and Turkey (23).

The major strength of this retrospective testing study lies in the large number of samples evaluated (>23,000) to ensure undiagnosed cases were not missed and that the start of the time period from which these specimens were collected was before the first case of COVID-19 was reported outside of China (in Thailand on January 13, 2020) (4). The main limitation of this study lies in the use of a pooling strategy to test the large number of samples. Pooling has been widely evaluated as a reasonable approach to large-scale screening of specimens for SARS-CoV-2 molecular testing in low-prevalence settings (10, 24). On the basis of an internal validation study of pooling conducted at ProvLab, we found that for specimens with low viral levels (Ct value ≥ 35), pooling in a 1:4 fashion was approximately 96% as sensitive as non-pooled testing on our SARS-CoV-2 *E*-gene rtRT-PCR assay (unpublished data) compared with lower sensitivities of pooling in a 1:6 or 1:8 fashion. Similar results were found in a validation study that evaluated a similar pool ratio (1:2, 1:4, or 1:8) conducted in eastern Canada during the first wave of the COVID-19 pandemic, with sensitivities ranging from 92.6% to 98.3% (25). On the basis of this information, we felt that the increased throughput using a 1:4 pool ratio was reasonable in light of the fact that the majority were likely collected from symptomatic patients and because we expected that an extremely high number of samples would need to be tested to find any positive results (the prevalence of infection would be low because SARS-CoV-2 had not established local transmission patterns in the time period evaluated).

Finding only one case in this extensive retrospective testing highlights the strength of the pandemic preparedness and response on behalf of our Public Health Laboratory in collaboration with local public health officials, which included expansion of testing criteria to any symptomatic individuals as of March 7, 2020, and widespread closures of schools, universities, libraries, and public recreational facilities in the days that followed.

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REFERENCES

1. World Health Organization. WHO statement regarding cluster of pneumonia cases in Wuhan, China[Internet]. Geneva: The Organization; 2020. https://www.who.int/china/news/detail/09-01-2020-who-statement-regarding-cluster-of-pneumonia-cases-in-wuhan-china (Accessed January 9, 2020).
2. van Dorp L, Acman M, Richard D, et al. Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. Infect Genet Evol. 2020;83:104351. https://doi.org/10.1016/j.meegid.2020.104351. Medline: 32387564
3. Deslandes A, Berti V, Tandjaoui-Lambotte Y, et al. SARS-CoV-2 was already spreading in France in late December 2019. Int J Antimicrob Agents. 2020;55(6):106006. https://doi.org/10.1016/j.ijantimicag.2020.106006. Medline: 32371096
4. Public Health Agency of Canada COVID-19 Surveillance and Epidemiology Team. A retrospective analysis of the start of the COVID-19 epidemic in Canada, January 15–March 12, 2020. Can Communic Dis Rep. 2020;46(7/8):236–41. https://doi.org/10.14745/ccdr.v46i78a04.
5. Government of Canada. Coronavirus disease (COVID-19): 0utbreak update [Internet]. Ottawa: Government of Canada; 2020. https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection.html#a4 (Accessed August 25, 2020).
6. Pabbaraju K, Wong AA, Douesnard M, et al. A Public Health Laboratory response to the pandemic. J Clin Microbiol. 2020;58(8):e01110-20. https://doi.org/10.1128/JCM.01110-20. Medline: 32513860
7. Government of Alberta. Population statistics [Internet]. Edmonton: Government of Alberta; 2020. https://www.alberta.ca/population-statistics.aspx (Accessed December 17, 2020).
8. Centers for Disease Control and Prevention. CDC realtime RT-PCR (rRTPCR) protocol for detection and characterization of influenza (version 2007). CDC ref. no. I-007–05. Atlanta: The Centers; 2007.
9. Alberta Health Services. AHS in my zone [Internet]. Edmonton: Alberta Health Services. https://www.albertahealthservices.ca/zones/zones.aspx (Accessed October 12, 2020).
10. Petrucca A, Borro M, Lionetto L, et al. Validation of a small-size pooling approach targeting hospital surveillance of SARS-CoV-2 infection. Infect Control Hosp Epidemiol. 2020:1–2. https://doi.org/10.1017/ice.2020.380. Medline: 32734852
11. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045
12. Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention. Research use only 2019-novel coronavirus (2019-nCoV) real-time RT-PCR primers and probes [Internet]. Atlanta: The Centers; 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html (Accessed August 6, 2020).
13. Quick J. nCoV-2019 sequencing protocol v2 [Internet]. 2020. https://www.protocols.io/view/ncov-2019-sequencing-protocol-v2-bdp7i5rn.html (Accessed August 7, 2020).
14. ARTIC bioinformatics pipeline [Internet]. Version 1.0.0. 2020. https://github.com/connor-lab/ncov2019-artic-nf (Accessed month day, year).
15. Pangolin [Internet]. Version 1.0. 2020. https://github.com/cov-lineages/pangolin (Accessed month day, year).
16. Rambaut A, Holmes EC, O'Toole A, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages

to assist genomic epidemiology. Nat Microbiol. 2020;5:1403–7. https://doi.org/10.1038/s41564-020-0770-5. Medline: 32669681

17. Zhao N, Liu Y, Smargiassi A, Bernatsky S. Tracking the origin of early COVID-19 cases in Canada. Int J Infect Dis. 2020;96:P506–8. https://doi.org/10.1016/j.ijid.2020.05.046. Medline: 32425633
18. Alberta Precision Laboratories. Laboratory bulletin: novel coronavirus detected in Wuhan, China [Internet]. Calgary: The Laboratories; 2020. https://www.albertahealthservices.ca/assets/wf/lab/wf-lab-bulletin-novel-coronavirus-detected-in-wuhan-china.pdf (Accessed August 8, 2020).
19. Alberta Health Services. Update to COVID-19 specimen collection processes [Internet]. Alberta: Alberta Health Services; 2020. https://www.albertahealthservices.ca/assets/info/hp/phys/if-hp-phys-moh-covid-19-specimen-collection-processes.pdf (Accessed August 8, 2020).
20. Allin S, Grignon M, Karsenti N, et al. COVID-19 Health System Response Monitor: Canada [Internet]. World Health Organization Regional Office for Europe, European Observatory on Health Systems and Policies; 2020. https://www.covid19healthsystem.org/countries/canada/countrypage.aspx (Accessed August 9, 2020).
21. Meredith LW, Hamilton WL, Warne B, et al. Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study. Lancet Infect Dis. 2020;20(11):P1263–71 https://doi.org/10.1016/S1473-3099(20)30562-4.
22. British Columbia Centres for Disease Control. COVID-19 and going forward [Internet]. Vancouver: The Centres; 2020. https://news.gov.bc.ca/files/June4_Covid19_PPP_V8.pdf (Accessed August 9, 2020).
23. Government of Alberta. Chief medical officer of health COVID-19 update—March 9, 2020. Edmonton: Government of Alberta; 2020. https://www.alberta.ca/release.cfm?xID=6984359BABB75-F775-450B-31D517787EB15A39 (Accessed August 9, 2020).
24. de Salazar A, Aguilera A, Trastoy R, et al. Sample pooling for SARS-CoV-2 RT-PCR screening. Clin Microbiol Infect. 2020;26(12):P1687.E1–E5. https://doi.org/10.1016/j.cmi.2020.09.008. Medline: 32919074
25. LeBlanc JJ, Patriquin G, Pettipas J, et al. Look before diving into pooling of SARS-CoV-2 samples on high throughput analyzers. medRxiv. 2020. https://doi.org/10.1101/2020.08.17.20176982.