ORIGINAL RESEARCH

Respiratory tract samples collected from patients in a region of Quebec, Canada, indicate the absence of early circulation of SARS-CoV-2 infection

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BACKGROUND: The first documented case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in Quebec was confirmed on February 27, 2020. Retracing the first cases that occur within a geographical region may provide insight regarding the evolution and spread of SARS-CoV-2 in that region because the spread of undiagnosed cases may facilitate the initial community amplification of the virus. **METHODS:** We performed a retrospective analysis of respiratory tract samples collected for influenza testing in a region of Quebec, Canada, to look for evidence of early circulation of SARS-CoV-2. Frozen nucleic acid extracts initially collected for influenza testing between January 1 and February 20, 2020, were tested for SARS-CoV-2 using a reverse transcription–polymerase chain reaction assay. **RESULTS:** During the study period, 1,440 of 2,121 (67.9%) nucleic acid extracts from individual patients were available for retrospective testing. None of the samples tested positive for SARS-CoV-2. **CONCLUSIONS:** The results suggest that SARS-CoV-2 was not circulating within the region before February 20, 2020, because many samples, representing more than two-thirds of all samples tested for influenza during early 2020, were tested. Further studies using a similar methodology to determine the date of onset of SARS-CoV-2 in different countries and geographic areas could enhance our understanding of the current pandemic.

KEYWORDS: Canada, coronavirus, COVID-19, pandemic, SARS-CoV-2

HISTORIQUE : Le premier cas démontré d'infection par le syndrome respiratoire aigu sévère à coronavirus 2 (SARS-CoV-2) au Québec a été confirmé le 27 février 2020. Le retraçage du premier cas survenu dans une région géographique peut donner un aperçu de l'évolution et de la propagation du virus SARS-CoV-2 dans cette région, car la transmission des cas non diagnostiqués peut favoriser l'amplification initiale du virus dans la communauté. **MÉTHODOLOGIE :** Les chercheurs ont procédé à l'analyse rétrospective des échantillons respiratoires prélevés pour le dépistage de la grippe dans une région du Québec, au Canada, afin de trouver des preuves de circulation précoce du virus SARS-CoV-2D. Les extraits d'acide nucléique congelés entre le 1er janvier et le 20 février 2020 ont été soumis au dépistage du virus SARS-CoV-2 au moyen de l'amplification en chaîne par polymérase après transcriptase inverse. **RÉSULTATS :** Pendant la période de l'étude, 1 440 des 2 121 extraits d'acide nucléique (67,9 %) provenant de patients différents étaient disponibles en vue de tests rétrospectifs. Aucun n'a été positif au virus SARS-CoV-2. **CONCLUSIONS :** D'après les résultats, le virus SARS-CoV-2 n'était pas en circulation dans la région avant le 20 février 2020, car de nombreux échantillons, représentant plus des deux tiers de tous ceux ayant servi au dépistage de la grippe au début de l'année 2020, ont été soumis au dépistage. D'autres études faisant appel à une méthodologie semblable pour déterminer la date d'apparition du virus SARS-CoV-2 dans divers pays et diverses régions géographiques pourraient permettre de mieux comprendre la pandémie en cours.

MOTS-CLÉS : Canada, COVID-19, pandémie, SARS-CoV-2

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INTRODUCTION

The province of Quebec, Canada, has been severely affected by the coronavirus disease 2019 (COVID-19) pandemic, and incidence and death rates in the region are comparable to those in countries such as Spain, the United Kingdom, France, and the United States (1). The first confirmed case of COVID-19 in Quebec was reported on February 27 in a traveller returning from Iran (2). Retracing the earliest detected cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection that occur in a geographical region may provide insight regarding the evolution and spread of SARS-CoV-2 in the region, because the spread of undiagnosed cases may facilitate the initial community amplification of the virus.

SARS-CoV-2 infection shares several clinical features with influenza-like illness, and clinical indications for testing for both types of infections are similar (3). Therefore, we performed a retrospective analysis of respiratory tract samples collected for influenza testing in a region of Quebec, Canada, to look for evidence of early circulation of SARS-CoV-2 before the first case was reported on February 27.

METHODS

Population and design

This study was conducted in the Eastern Townships region of Quebec, Canada, at the Centre intégré universitaire de santé et de services sociaux de l'Estrie-Centre hospitalier universitaire de Sherbrooke (CIUSSSE-CHUS), which provides hospital care to the 170,000 residents of Sherbrooke as well as referral services for the Estrie region (total population 508,000) in southern Quebec. The study population included all patients who underwent testing for influenza between January 1 and February 20, 2020, for whom frozen nucleic acid extracts were available. The CIUSSSE-CHUS institutional review board approved this study. The requirement for informed consent was waived because it was a retrospective study that used deidentified leftover samples.

SARS-CoV-2 testing

Frozen nucleic acid extracts were available from samples tested for influenza virus using our in-house influenza reverse transcription–polymerase chain reaction (RT-PCR) assay. Our laboratory also performed influenza testing using two different commercial PCR assays (Solana Influenza A+B Assay, Quidel, San Diego, CA, and Flu & RSV Molecular Test-Xpert Xpress Flu/RSV GeneXpert Cepheid, Sunnyvale, CA). After use for influenza testing, no residual nucleic acid extracts were available with the commercial systems, and all tests were performed on nucleic extracts used for our in-house influenza RT-PCR assay because primary specimens were not stored.

Total nucleic acids were extracted for influenza testing using an EasyMag automated nucleic acid extraction system (bioMérieux SA, Marcy l'Étoile, France). Surplus nucleic acid extracts were stored at 80°C until they were tested for SARS-CoV-2. SARS-CoV-2 testing was performed between April 27 and April 30. An RT-PCR multiplex assay was used to detect SARS-CoV-2 by amplifying the viral envelope (E gene) and human beta-2-microglobulin mRNA (B2M) genes (4). The B2M primers and the probe developed for this study were as follows: B2M.qref.F3, ACTACACTGAATTCACCCCCACTGA; B2M.qref.R3, GCTGCTTACATGTCTCGATCCCA; and B2M.taqman.3.cy5, Cy5-GCCTGCCGTGTGAACCATGT-BBQ. Reliance One-Step Multiplex quantitative RT-PCR (RT-qPCR) Supermix (BioRad, Hercules, CA) or homemade RT-qPCR Mastermix were used to amplify target genes using a BioRad CFX384 Touch Real-Time PCR Detection System. Commercial SARS-CoV-2 positive and negative controls were used (Exact Diagnostics, Fort Worth, TX).

RESULTS

During the study period, nucleic acid extracts from 1,440 of 2,121 (67.9%) samples collected from individual patients for influenza virus testing were available for retrospective SARS-CoV-2 testing. Of these samples, 339 (23.5%) were from outpatients, 600 (41.7%) were from the emergency room, and 501 (34.8%) were from inpatients. Patients' median age was 45 years (interquartile range 7–74). The initial in-house influenza PCR detected influenza virus in 529 samples (36.7%)—232 influenza A and 296 influenza B. One sample was positive for both influenza A and influenza B. The samples for which there were no extracts available had been tested for influenza using Solana Influenza A+B (n = 675) and Flu & RSV Molecular Test-Xpert Xpress Flu/RSV (n = 6) tests. SARS-CoV-2 RT-PCR testing was performed on all 1,440 available samples. No positive case of SARS-CoV-2 was identified from among the specimens assessed.

DISCUSSION

In this study, we found no evidence of circulation of SARS-CoV-2 infection in the Eastern Townships region of Quebec, Canada, between January 1 and February 20, 2020. These findings indicate that it is unlikely that SARS-CoV-2 was present in the region during this period because many samples, which represented more than two-thirds of all

samples tested for influenza in the region during the study period, were tested for SARS-CoV-2. It is therefore unlikely that the initial spread of COVID-19 in the Eastern Townships region can be attributed to undetected circulation of SARS-CoV-2. Our results are consistent with other reported indicators in the province of Quebec, such as the decrease in influenza-like illness-related consultations and influenza or pneumonia hospitalizations by the end of February and an absence of an increase in all-cause mortality attributable to COVID-19 until the end of March (5, 6). These results suggest that important circulation of COVID-19 before February 27, 2020, was unlikely; however, limited circulation in isolated regions may not have been detected by means of aggregated provincial data.

Current mathematical models of the COVID-19 pandemic integrate data and originate with the earliest detected cases of COVID-19 (7). The retrospective identification of cases that occurred before those currently identified may improve the performance of the models. To our knowledge, a similar retrospective testing approach has not been broadly implemented in many countries, despite its potential utility. Similar approaches in Scotland and Western Switzerland did not retrospectively identify cases before the first reported cases in these regions (8, 9). In the United States, the first documented case of community transmission was identified retrospectively by the Seattle Flu Study, and a patient with SARS-CoV-2 infection 1 month before the first cases of COVID-19 were reported was identified retrospectively in France (10, 11).

The principal limitation of our study is its retrospective nature. RNA extracts degrade over time, but we used purified RNA extracts that were stored at 80°C, and we performed SARS-CoV-2 RNA testing 3–4 months after the RNA was extracted. In this environment, RNA can be stable for more than 50 months (12). Our PCR assay included an internal control to confirm the stability of frozen extracts. The analysis reported here is restricted to a single geographic region, and no international airport exists in the territory. It would be informative to perform a similar study in an urban area such as Montreal, to assess whether there was early spread of the infection in an internationally connected city that faced a particularly great disease burden, or Toronto, where the first known case in Canada was reported (13). Further studies using a similar methodology to determine the date of introduction of SARS-CoV-2 to different countries or territories could enhance our understanding of the pandemic and may facilitate the refinement of mathematical models used to describe the genesis of the pandemic.

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