Comparison of SARS-CoV-2 Viral Loads in the Nasal Mucosa of Patients Infected With BA.1, BA.2, or BA.5 Omicron Lineages

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Lower viral loads were observed in the upper respiratory tract of patients infected with BA.1, whereas patients infected with BA.2 and BA.5 had comparable viral loads to those seen with Alpha or Delta. This suggests that viral loads are likely not responsible for the increased transmission of the Omicron lineages.

Keywords. SARS-CoV-2; Omicron lineage; viral infection; BA5 variant; viral load.

The global coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been punctuated by the emergence of viral lineages with increased transmissibility and/or virulence designated as variants of concern (VOCs). These VOCs include the Alpha (B.1.1.7), Delta (B.1.617.2), and most recently Omicron (B.1.1.529) variants and their respective sublineages. The emergence of both BA.1 and BA.2 sublineages resulted in a worldwide surge of cases and hospitalizations [1]. More recently, BA.5 has emerged and has resulted in surging cases in many regions, even among vaccinated individuals [2].

The Alpha and Delta variants have been associated with increased viral load in the upper respiratory tract, which likely accounted for their increased transmission [3]. It remains unclear if increased transmission of the sublineage variants of Omicron is due to higher viral loads in the upper respiratory tract, immune evasion, or potentially a combination of factors. Data from hamster and mouse studies showed lower levels of viral replication compared with infection with other VOCs [4]. By contrast, viral kinetics studies in ex vivo lung and bronchus tissues suggested that Omicron replicated better in the upper compared with the lower respiratory tract [5]. Recent studies have shown differing viral load dynamics associated with the Omicron sublineages, potentially due to small sample sizes or different viral targets being compared [6–8]. Additionally, recent studies have not compared BA.5 with other sublineages [9].

Whole-genome sequencing allows for specific identification of viral lineages [10, 11], which is not possible through other molecular tests [12], allowing for better association of emerging variants with observed phenotypes. Here we evaluate SARS-CoV-2 viral load at diagnosis using upper respiratory tract samples from patients during the multiple COVID-19 waves that occurred in Ontario to determine if it correlates with the observed increased transmission efficiency.

METHODS

Patient Samples
Patient diagnostic samples and data were collected from 4 clinical laboratories in Ontario between February 2021 and July 2022. These laboratories provide service to 27 academic/community hospitals and community assessment centers. Swabs, either nasopharyngeal, nasal, and/or oral, were tested using laboratory-developed dual-target (E-gene) polymerase chain reaction (PCR) [13].

Whole-Genome Sequencing and Bioinformatics
Whole-genome sequencing (WGS) was done using the COVIDSeq Test library preparation kit (Illumina) and ARTIC V4.1 primers. Libraries were loaded at 9pM for 2×150-bp sequencing on the MiSeq instrument (Illumina). Sequencing files were de-multiplexed using the native instrument software for downstream analytics. The analysis of the WGS data, including dehosting, generation of a consensus sequence, and variant calling with freebayes, was performed using a Nextflow pipeline for running the ARTIC field bioinformatics tools (https://github.com/jts/ncov2019-articnf). Viral lineages were assigned using pangolin, version 4.06, Scorpio, version 0.3.17, and constellations, version 0.1.9. Data were analyzed using PRISM 9.0 and R Studio.
Statistical Analysis
Patient data were anonymized and sent for analysis by each institution. All data were then compiled with institution location included. Data were analyzed using PRISM 9.0 and MS Excel. Sequence data are available on GISAID.

RESULTS
In total, 21,383 patient specimens positive for SARS-CoV-2 were included in the study from multiple regions in Ontario with a catchment area that includes 5 million people, representing 34% of the population and covering multiple geographical areas. Moreover, these areas included both rural and urban centers (Figure 1). Sex data were available for 83% of the patients in the cohort (n = 17,814) and showed approximately equal numbers of males (n = 8,905) and females (n = 8,909). Age distribution was similar for males and females and included <19 (female: 311/5258; male: 385/5252), 19–64 (female: 3,717/5258; male 3,556/5252), and ≥65 years (female: 1,229/5258; male 1,311/5252). Samples were from both ambulatory and hospitalized patients. Whole-genome sequencing indicated that samples collected in this study included 3 VOCs: Alpha (n = 1,625), Delta (n = 6,930), and Omicron (n = 12,828).

To investigate viral load in the upper respiratory tract, we used the E-gene cycle threshold (Ct) value as a surrogate marker. Median viral loads were equivalent in patients infected with the Alpha and Delta variants (Alpha 19.49 vs Delta 19.45; P = .9). However, despite the increased rate of transmission and the faster doubling time for case numbers [14] associated with the Omicron BA.1/BA.1.1 variant, patients infected with the BA.1 lineage demonstrated a lower VL (higher Ct) when compared with patients infected with either the Delta (median, 20.81 vs 19.45; P = .0001; mean, 21.68 vs 20.70) or Alpha variant (median, 20.81 vs 19.45; P = .0001; mean, 20.81 vs 20.04).

Figure 1. Sampling area included Ottawa to Hamilton including testing sites North to Sudbury.
In contrast, the BA.2 lineage was associated with higher viral loads compared with BA.1/BA.1.1 \((P = .0001)\). Furthermore, as shown in Figure 2A, infection with the BA.5 lineage was associated with higher viral loads compared with BA.1/BA.1.1 \((20.81 \text{ vs } 19.94; \ P = .0001)\) but was comparable to BA.2. Data from each of the sites evaluated individually also demonstrated the same phenomenon seen in the aggregate data. There was no evidence to suggest that biological sex impacted viral load \((P = .316)\). Additionally, analysis of the primer binding sites was performed and demonstrated no significant mutations in the primer or probe binding regions to any of the lineages that could affect assay efficacy and Ct value (Figure 2B).

**DISCUSSION**

The emergence of new Omicron sublineages has been associated with increased numbers of infections and greater transmission, often replacing previously circulating variants [14]. It is often unclear if this displacement of 1 variant by another is due solely to evasion of neutralizing antibodies, higher viral replication, other virological factors, or a combination of elements.
We compared the viral loads of samples from patients infected with either the Alpha, Delta, or Omicron lineage. Our findings indicated that individuals infected with the Omicron BA.1 lineage had lower viral loads compared with the Alpha and Delta lineages, as well as the BA.2 and BA.5 sublineages. Interestingly, the currently circulating variants, BA.2 and BA.5, have higher viral loads than BA.1, which could be contributing to their ability to displace BA.1.

This large multisite study addresses differences that may be attributed to sample collection, extraction chemistries, or instrumentation. In silico analysis of primer and probe binding sites revealed no mutations that would interfere with PCR efficiency. Although changes in anatomical sampling sites have been suggested as a potential confounding factor when evaluating viral loads for SARS-CoV-2, there have been no changes in sampling following the initiation of the Omicron wave in Canada. The significant difference in viral load in the upper respiratory tract between patients with the BA.1 vs BA.2 and BA.5 variants suggests that sampling site does not play a role in this phenomenon. Our study does have several limitations, as we were unable to determine reinfection or vaccination status of the participants or assess the impact these may have on viral load, although data suggest that vaccination may have limited impact on viral load and duration of shedding [15]. Additionally, the time between symptom onset and collection and underlying comorbidities was not available. However, recent studies have highlighted that there is no difference in viral loads between symptomatic and asymptomatic patients [3, 16]. Notably, a large study performed by Jones and colleagues found little difference in viral load among >25000 positive patients in Germany regardless of symptoms or hospitalization [3].

Our data are similar to what was reported by a recent US study that reported no differences in Ct value between these lineages, and this group was able to examine this among both vaccinated and unvaccinated individuals [1]. Another study also noted no differences in Ct values between the Omicron and Delta variants in symptomatic or asymptomatic patients [8]. Moreover, infection studies in mice and hamsters comparing Omicron and Delta have also shown lower viral loads associated with Omicron BA.1 [4]. Interestingly, recent work by Migueles et al. highlighted that similar nasopharyngeal viral loads of BA.1 and BA.2 indicate that the advantage of the BA.2 variant was not due to higher replication [9]. However, their initial categorization was performed using a VOC-specific RT-PCR, which is less accurate than the gold-standard WGS and can misclassify sublineages, and they only fully sequenced a subset of samples. Chan et al. identified that BA.2 shows an increased replication efficiency in vivo within the nasal cavity due to a lowered dependence on transmembrane protease serine 2 (TMPRSS2) interaction compared with BA.1 [17]. This was further corroborated by Shuai et al., who identified poor efficiency of TMPRSS2 use in Omicron in a lineage-dependant manor [18]. Additionally, our data further suggest that the selective advantage of BA.5 over BA.2 is due to immune evasion, as viral loads were similar.

CONCLUSIONS

Our data indicate that increased viral loads, often seen as a viral mechanism for increasing transmissibility, are not likely responsible for the increased transmission efficiency of the BA.1/BA.1.1 lineages compared with Delta. Moreover, viral load in the upper respiratory tract is also not likely contributing to the subsequent displacement by BA.2 and BA.5. This supports recent studies highlighting that evasion of the host antibody response is likely contributing to the transmissibility of the Omicron variant, rather than increased amounts of the virus being shed [2]. Further studies are needed to investigate transmission of the BA.2 and BA.5 lineages.

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Patient consent. This work was conducted on de-identified specimens submitted as part of routine clinical testing; hence, evaluation determined ethics approval was not required.

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