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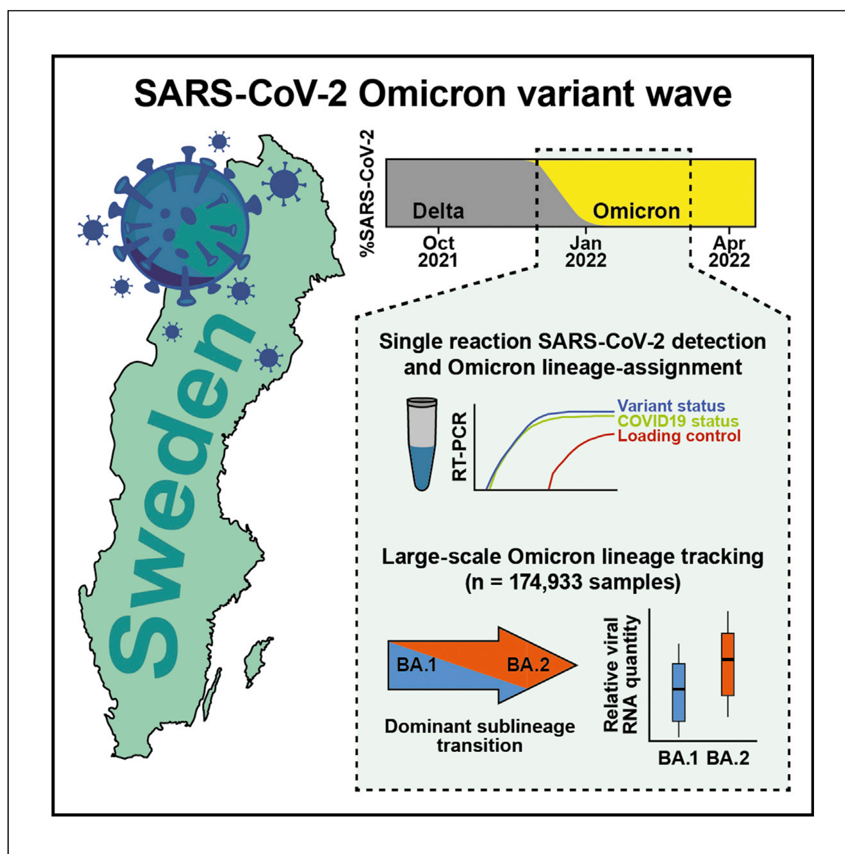
Clinical and Translational Report

Monitoring of the SARS-CoV-2 Omicron BA.1/BA.2 lineage transition in the Swedish population reveals increased viral RNA levels in BA.2 cases



The SARS-CoV-2 pandemic has been characterized by waves of novel mutated virus variants. Lentini et al. tracked the ongoing Omicron emergence in the Swedish population in real time, showing how population-scale lineage tracking can be achieved with a single laboratory test and revealing the rapid shift in dominant subvariant.

Translation to Population Health

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Highlights

An RT-PCR assay directly classifying COVID-19 and Omicron BA.1 sublineage status

Monitoring of the Omicron BA.1 to BA.2 sublineage dominance transition in Sweden

Day-by-day resolved analysis encompassing 174,933 clinical upper airway samples

2-fold higher viral RNA levels in upper airway of BA.2 relative to BA.1 cases

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Clinical and Translational Report

Monitoring of the SARS-CoV-2 Omicron BA.1/BA.2 lineage transition in the Swedish population reveals increased viral RNA levels in BA.2 cases

Antonio Lentini,[1] Antonio Pereira,[2] Ola Winqvist,[2] and Björn Reinius[1,3,*]

SUMMARY

Background: Throughout the SARS-CoV-2 pandemic, multiple waves of variants of concern have swept across populations, leading to a chain of new and yet more contagious variants dominating COVID-19 cases. Here, we tracked the remarkably rapid shift from Omicron BA.1 to BA.2 sublineage dominance in the Swedish population in early 2022 at a day-by-day basis.
Methods: Using a custom SARS-CoV-2 Omicron BA.1 lineage-typing RT-PCR assay, we analyzed 174,933 clinical upper airway samples collected during January to March 2022.
Findings: Our study demonstrates the feasibility and reliability of parallel lineage assignment of select variants at population scale, tracking the dominant sublineage transition from BA.1 to BA.2 at day-to-day resolution and uncovering nearly 2-fold higher levels of viral RNA in cases infected with Omicron BA.2 relative to BA.1.
Conclusions: Our data provide unique insights into the Omicron BA.1 to BA.2 transition that occurred in Sweden during early 2022, and later, across the world. This may help to understand the increased transmissibility of the BA.2 variant.

CONTEXT AND SIGNIFICANCE

Early population tracking is critical for informed decision-making and handling of the SARS-CoV-2 pandemic during outbreaks of variant strains of concern. Omicron variant infections emerged during late 2021, but at the time, lineage assignment relied on sequencing or separate genotyping assays in addition to the clinical SARS-CoV-2 RT-PCR, burdening laboratories and restricting the scope of lineage monitoring. Here, researchers at the Karolinska Institute and ABC Labs developed a single RT-PCR assay to simultaneously classify COVID-19 and Omicron BA.1 lineage status and demonstrated the feasibility of parallel lineage assignment of select variants at population scale. The authors show, at day-to-day resolution, the rapid transition from Omicron BA.1 to BA.2 sublineage dominance in the Swedish population, exposing factors that could influence this transition.

INTRODUCTION

The SARS-CoV-2 pandemic has been characterized by the emergence and subsequent dominance of new variants of concern, such as Alpha B.1.1.7 during late 2020, Delta B.1.617.2 in mid-2021, and the Omicron variant B.1.1.529.1 (BA.1) in late 2021 and early 2022.[1] It is estimated that a peak of nearly 50 million new infections occurred daily worldwide during the Omicron wave of January 2022, far exceeding the peak of 14 million daily Delta infections during April 2021[2] and signifying an unprecedented level of transmission of Omicron. In late December 2021, during the midst of the Omicron BA.1 wave in Sweden, the Omicron sublineage BA.2 (B.1.1.529.2) arrived and rapidly spread through the population. At this time, we had in place an RT-PCR assay capable of genotyping Omicron BA.1 cases directly in primary SARS-CoV-2 RT-PCR testing, enabling day-by-day tracking of the BA.1 frequency at massive scale. Here, we provide details of this assay and present data from 174,933 clinical upper airway samples analyzed with the method in the Swedish population January to March 2022, demonstrating how the Omicron BA.1 wave was consistently outcompeted by BA.2. Importantly, we report approximately twice as high viral RNA levels detected in upper airway samples from Omicron BA.2 cases, suggesting that a higher viral load might contribute to the increased transmissibility of the BA.2 lineage.

RESULTS

As we observed an acceleration of Omicron BA.1 cases across several European countries in early December of 2021, we rapidly developed a modified version of

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the CDC SARS-CoV-2 RT-PCR assay,[3] simultaneously detecting general SARS-CoV-2 infection status (*N1-FAM*), human RNaseP sample integrity (*RP-HEX*), and Omicron BA.1-lineage status (S_{BA1}-*Cy5*) leveraging BA.1-specific indels in the spike (*S*) gene (Figure 1A). To attain a multiplex assay of high sensitivity and specificity, suitable for RNA-extraction-free RT-PCR on heat-inactivated samples employed in mass testing,[4] we designed 144 combinatorial S_{BA1}-primer-probe sets, matching CDC *N1* properties and with minimized amplicon length (Figure S1A and Table S1). We obtained the first *in vitro* expanded Omicron BA.1 inoculate in Sweden (GISAID: EPI_ISL_7452247) that was used to identify a BA.1-specific primer-probe set for large-scale oligonucleotide production at an early phase of the Omicron wave (Figures 1B and S1B). By serial dilution of clinical BA.1 specimens, we found that the detection sensitivity and log-linear cycle thresholds (C_T) range were similar for both the *N1* and S_{BA1} sets in the assay conditions (Figure 1C). Using 185 clinical upper airway samples (combined throat swab, nose swab, and saliva; see STAR Methods) of known COVID19-infection status, we validated that addition of S_{BA1}-*Cy5* probes did not affect *N1* C_T values and the sensitivity to detect general SARS-CoV-2 infection in primary RT-PCR (Figure 1D and Table S2). Parallel genotyping of 133 SARS-CoV-2-positive samples using Thermo Fisher TaqMan SARS-CoV-2 Mutation Panel Assay as well as whole-genome sequencing (WGS, n = 103 samples) provided Omicron BA.1-case classification that was 100% consistent with our direct RT-PCR assay (Figures 1E and 1F). Omicron (both the BA.1 and BA.2 lineage) carries a C28311T substitution at the third base position of the *N1* probe, which we confirmed had negligible effect on C_T values by tests using CDC *N1* and custom Omicron *N1* probes (Figure S1C).

Having optimized and validated the assay, it was deployed to monitor the Omicron wave across central Sweden day by day, totaling 174,933 clinical samples from nine healthcare regions analyzed between January 26 and March 8, 2022, using a standard operation procedure and self-sampling kit (Table S3, see STAR Methods and Data S1 for details of specimen collection). While BA.1 was the dominant lineage among COVID-19-positive cases during January, the BA.1 fraction steadily decreased to as low as 11% by March (Figure 2A). This trend was observed in all monitored healthcare regions across Sweden (Figure 2B), indicating that Omicron BA.1 was being outcompeted by another lineage. To trace this lineage switch, we whole-genome sequenced and lineage-assigned 698 samples picked among SARS-CoV-2-positive cases. Strikingly, 100% of clinical samples typed as Omicron BA.1 negative in our direct RT-PCR assay were found to be of the Omicron BA.2 lineage by WGS (Figure 2C), lacking the BA.1-specific "EPE" insertion in the *S* gene targeted by our S_{BA1}-probe (Figure S1A), demonstrating that Omicron BA.2 was the lineage outcompeting BA.1 in the Swedish population. Combining these sequenced cases with the validation set of 103 samples mentioned in a previous section, we had classified a total of 801 clinical samples by WGS (Table S4), demonstrating >99% agreement with the BA.1 calls obtained directly in the RT-PCR (796/801 samples) (Figure 2D). In summary, our RT-PCR data demonstrated in real time that two Omicron waves co-occurred in the Swedish population and that BA.2 outcompeted BA.1. Why Omicron BA.2 is more contagious and replaced BA.1 is not well understood, and multiple non-exclusive mechanisms such as increased binding affinity of the spike protein to host angiotensin-converting enzyme two receptor, evasion of neutralizing antibodies, and increased viral load are plausible. Our massive RT-PCR dataset allowed us to compare viral RNA levels detected in BA.1 and BA.2 cases with high statistical power. Intriguingly, samples genotyped as BA.1-negative in RT-PCR contained 1.9-fold higher levels of viral RNA than BA.1-positive samples (median *N1* C_T

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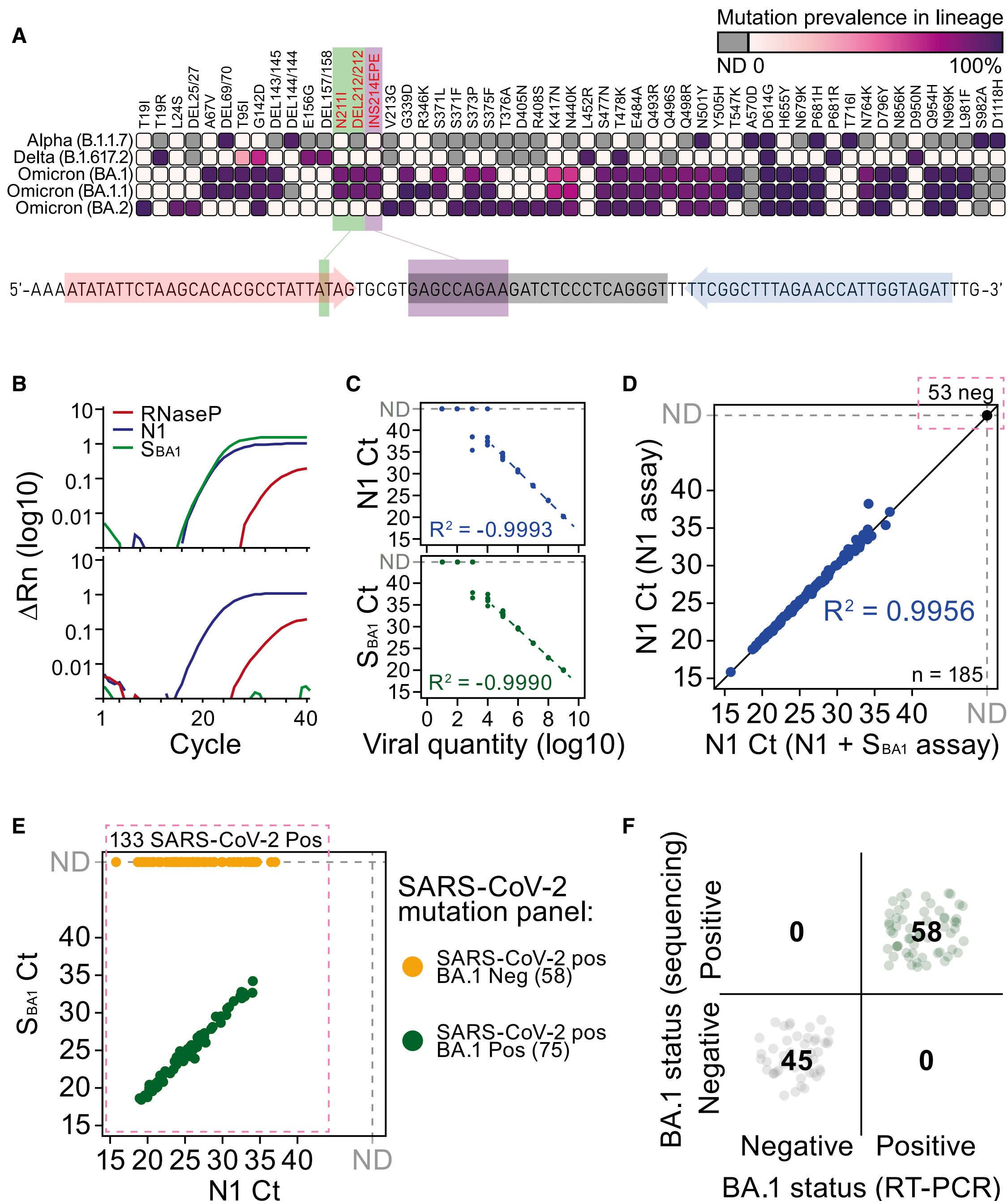

Figure 1. An RT-PCR assay providing general SARS-CoV-2 infection status and Omicron BA.1 classification in parallel

(A) Mutational spectrum of the S protein among different SARS-CoV-2 lineages and location of Omicron BA.1-specific primer-probes used in the assay. Mutation prevalence data adapted from outbreak.info/compare-lineages, accessed 03-23-2022, where prevalence is based on total GISAID sequence data.

(B) RT-PCR amplification curves for N1, S_{BA1}, and RNaseP targets in representative Omicron BA.1-positive (top) and BA.1-negative (bottom) samples.

(C) RT-PCR sensitivity of N1 (top) and S_{BA1} targets in dilution series of a BA.1-positive sample. Each dilution point represented by n = 8 replicates.

(D) Linear relationship between N1 RT-PCR Ct between the N1 assay and N1+S_{BA1} assay for n = 185 clinical specimens.

(E) Agreement between lineage assignment of N1+S_{BA1} RT-PCR assay and Thermo Fisher TaqMan SARS-CoV-2 Mutation Panel (colors) for n = 133 COVID-19 positive samples (See also Table S2).

(F) Agreement between N1+S_{BA1} RT-PCR assay and WGS lineage calls for n = 103 clinical specimens.

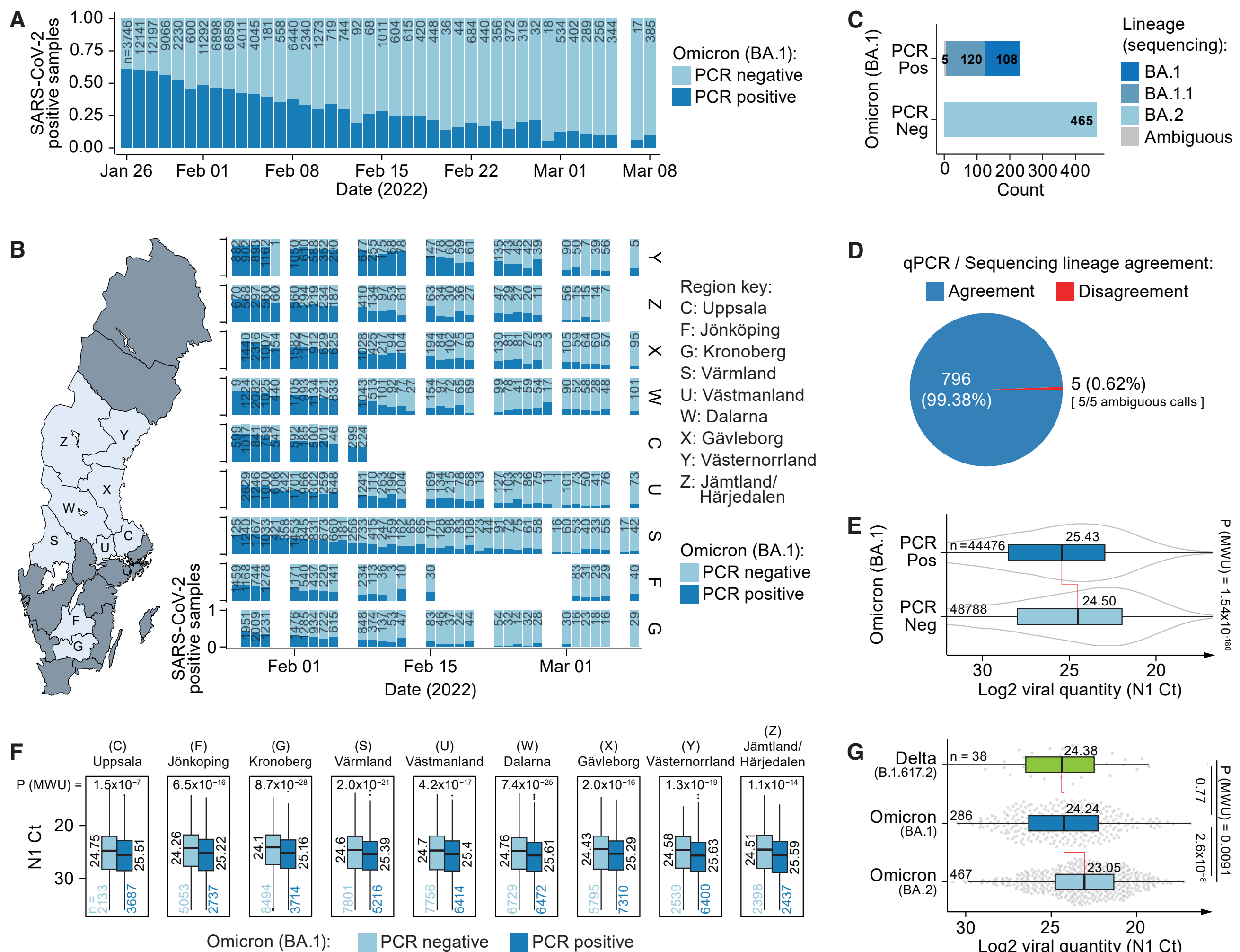

Figure 2. Omicron BA.1/BA.2 lineage transition and viral load in Swedish COVID-19 cases

(A) Omicron BA.1 lineage assignment by RT-PCR over time in n = 93,126 SARS-CoV-2-positive cases (174,933 tests performed including negative cases).

(B) Same as (A) but separated by originating Swedish healthcare regions.

(C) SARS-CoV-2 Lineage assignment by WGS based on qPCR lineage call (x axis) for n = 698 biological replicates.

(D) Agreement between lineage calls for RT-PCR and WGS for n = 801 clinical specimens.

(E) Difference in viral RNA load (N1 RT-PCR Ct) for BA.1-positive and -negative, SARS-CoV-2-positive, samples based on RT-PCR calls for n = 93,264 COVID-19-positive cases. Data are shown as median, first, and third quartiles, and 1.5x inter-quartile range. p values calculated using two-tailed Mann-Whitney U-tests.

(F) Same as (E) but with samples separated based on originating health-care region.

(G) Same as (E) but with classification based on WGS lineage calls for n = 791 sequenced clinical specimens.

$BA.1_{Neg}$ = 24.50, $CI_{95\%}$: 24.45–24.55 versus $BA.1_{Pos}$ = 25.43, $CI_{95\%}$: 25.37–25.47; p = 1.54 x 10^{-180}, Mann-Whitney U-test) (Figure 2E). Importantly, higher viral RNA levels in BA.2 were not due to differences in sample material amounts (Figure S2B) and were consistently detected across day-by-day timepoints spanning the length of the study, with sampling date having negligible effects (β_{Date} = −0.00016, P_{Date} = 0.944, $\beta_{BA.1}$ = 0.928, $P_{BA.1}$ < 2 x 10^{-308}, median quantile regression) (Figure S2C), as well as across originating healthcare regions (average BA.1-BA.2 difference = 0.9 ± 0.14 C_T ± 1 SD, p < 1.5 x 10^{-7}, Mann-Whitney U-test) (Figure 2F). As the *N1* target sequences utilized for RNA quantification in our assay are identical in the BA.1 and BA.2 genomes, the same RT-PCR efficiency (*E*) is expected and should not influence their relative C_T values, which we experimentally

confirmed by serial dilution of BA.1 and BA.2 positive clinical samples (mean *N1* $E_{BA.1}$ = 94%, SEM = 1.7%; mean *N1* $E_{BA.2}$ = 94%, SEM = 2.3%; Figure S2D). A similar efficiency was recorded for the $S_{BA.1}$ primer-probe set (mean *S* $E_{BA.1}$ = 94%, SEM = 4.1%). The difference in viral RNA load between the BA.1 and BA.2 cases remained when N1 C_T values were normalized to sample-specific RNaseP amounts as well as after correcting for plate variation using a standardized calibrator sample that was included in each RT-PCR run (Figures S2E–S2G). Furthermore, the viral RNA level for Omicron BA.1 was similar to that of Delta cases sampled in mid-December (p = 0.77, Mann-Whitney U-test) (Figure 2G). Similar viral RNA levels between Delta and BA.1 have previously been reported,[5,6] whereas the increased load in BA.2 cases was surprising. To ensure that the difference in viral RNA quantity detected in samples of Omicron BA.1 and BA.2 cases was not attributed to our specific RT-PCR assay, nor due to potential differences in amplification of the 3′-end of the SARS-CoV-2 genome (where the *N* gene is located) due to unequal discontinuous transcription found in coronaviruses,[7] we subjected 3,392 samples in parallel to both our *N1/RP/*S_{BA1} assay and an extraction-based assay (SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N Genes, IVD, NZYTech; Table S5) targeting *N* as well as *RdRp*, located further 5′ inside *ORF1ab*. Our RT-PCR assay for the *N* gene showed a strong linear correlation with both *N* and *RdRp* in the extraction-based assay (R^2 > 0.949) (Figure S2H), and we indeed confirmed increased copy numbers in BA.1-negative COVID-19 samples in both assays and genes probed (Figure S2I). Furthermore, lineage classification by WGS for 118 of these samples confirmed that BA.1-negative samples were indeed of BA.2 lineage (81/81 samples tested) (Figure S2J). Between March and May 2022, additional BA.2 sublineages were annotated, and we therefore retrospectively repeated lineage classification of our sequenced samples, which showed good agreement with our initial lineage assignments (Figure S3A). Interestingly, a considerable fraction of BA.2 samples could now be assigned to the sublineage BA.2.9, demonstrating its presence in the Swedish population at the time of our study. Notably, the viral RNA levels of BA.2.9 samples were on par with BA.2 samples (p = 0.74, Mann-Whitney U-test) (Figure S3B), and BA.2.9 sublineage was primarily found in the southern-most healthcare region in our data (Figure S3C). Finally, Delta-Omicron recombination events have previously been reported,[8] but such cases were not detected in our sequenced data (Figures S3D and S3E and Table S6).

DISCUSSION

Together, we devised an effective RT-PCR assay to call general COVID-19 status and Omicron BA.1 lineage status simultaneously and robustly in a single RT-PCR reaction, which enabled day-by-day monitoring of the Omicron BA.1/BA.2 transition in Sweden at immense scale. As such, our work demonstrates the feasibility of lineage typing of selected relevant lineages or variants for population monitoring directly in the primary SARS-CoV-2 RT-PCR, without straining the clinical laboratory with separate genotyping RT-PCR assays and the logistics and time lag of WGS. We observed in real time that the predominant Omicron BA.1 lineage was gradually and consistently outcompeted by Omicron BA.2 throughout nine Swedish health-care regions (Figures 2A and 2B). Retrospective analysis of lagging WGS data from the same time period indeed confirmed this trend at the national level (Figure S3F), visualizing the co-occurrence of the BA.1/BA.2 waves in Sweden. Importantly, our data showed that cases of BA.2 infection presented nearly 2-fold higher levels of viral RNA in the upper airways compared with BA.1 infection. Given droplet and aerosol transmission of the virus, this result is consistent with the notion of an elevated viral load contributing to

increased transmissibility of BA.2, as immune evasion does not seem to be increased compared to BA.1.[9–11] However, our results do not exclude or question the possibility of other mechanisms that might add to the BA.2 transmissibility. Our direct Omicron BA.1-typing assay is a directly compatible addition to the well-established CDC *N1/RNaseP* probe sets and extraction-free SARS-CoV-2 RT-qPCR[4] and can thus easily be deployed for instant monitoring of the Omicron BA.1/BA.2 transition where needed, avoiding the time lag and sample number bottleneck of genotyping by WGS. Importantly, lineage assignment in the primary RT-PCR enabled an inclusive quantification of viral RNA levels in all samples of a large population. This is in contrast to backtracking RNA levels after obtained WGS results, which biases sample inclusion toward strongly positive samples from which successful WGS libraries can be obtained (Figure S2A). The S_{BA1}-*Cy5* primer-probe set that we developed could readily be replaced to target and monitor other SARS-CoV-2 variants, and our approach can be adapted to other viruses of interest in the future.

Limitations of the study

Here, we have measured viral RNA levels by quantitative RT-PCR, which although being the gold standard for clinical testing, may not necessarily scale with amounts of active viral particles.[6] Coronaviruses generate subgenomic RNAs that are more abundant than genomic RNA copies,[7] which could affect quantification of SARS-CoV-2 copy numbers by RT-PCR. Quantification of infectious viral levels requires active virus isolation in cell culture, which is not practically feasible to achieve from clinical samples at a large scale, such as in this study. We present our RT-PCR data in qualitative (e.g., COVID-19 and BA.1 status) or relative terms (e.g., BA.1 versus BA.2 C_T values/fold changes), which we thoroughly show to be robust within our experimental settings. However, exact C_T values, while correlative, will differ depending on sample preparation procedures and experimental protocols (e.g., see Figures S2E and S2F).

ETHICS STATEMENT

Ethical oversight and approval were obtained by the Swedish Ethical Review Authority (Dnr 2020-01945 and 2022-01139-02, Etikprövningsmyndigheten).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- METHOD DETAILS
  - SARS-CoV-2 RT-PCR
  - SARS-CoV-2 WGS
  - Sequencing data analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2022.07.007.

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AUTHOR CONTRIBUTIONS

A.L. performed WGS data processing, data analysis and visualization, statistical analyses, prepared figures, wrote the initial draft, and edited the manuscript. A.P. performed Thermo Fisher TaqMan SARS-CoV-2 Mutation Panel Assay. O.W. provided resources, coordinated the project, and edited the manuscript. B.R. provided resources, coordinated the project, designed the BA.1 assay, performed RT-PCR, analyzed the data, prepared figures, wrote the initial draft, and edited the manuscript. All authors had unrestricted access to all data, read and approved the final version of the manuscript, and take responsibility for its content.

DECLARATION OF INTERESTS

Björn Reinius is a founder and a shareholder of GeneBeats and has worked as a consultant on SARS-CoV-2 RT-PCR diagnostics. Ola Winqvist is a founder and a shareholder of ABC Labs.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
SARS-CoV-2 Omicron BA.1 *in vitro* expanded	Public Health Agency of Sweden	N/A
SARS-CoV-2 Alpha strain *in vitro* expanded	Public Health Agency of Sweden	N/A
Biological samples		
SARS-CoV-2 upper airway samples (throat, saliva, and bilateral nostril sampling)	Public Health Agency of Sweden	N/A
Critical commercial assays		
TaqPath 1-Step RT-qPCR Master Mix, CG	Thermo Fisher	Cat#A15300
Thermo Fisher TaqMan SARS-CoV-2 Mutation Panel Assay	Thermo Fisher	Cat#A51814 Cat#A51808 Cat#A51813 Cat#A51819
SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N Genes, IVD	NZYTech	Cat#MD0483
Illumina COVIDSeq Test	Illumina	Cat#20043675
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	Thermo Fisher	Cat#A48383
Deposited data		
Raw sequencing data	ArrayExpress	E-MTAB-11650
Assembled SARS-CoV-2 genomes	GISAID	EPI_ISL_11414777 - 11415389 and EPI_ISL_11450500 - 11450677
RT-PCR C_T values	This paper	Table S2, S3, and S5
Tables S1, S2, S3, S4, S5, and S6	Mendeley Data	https://doi.org/10.17632/2z3t52wdy8.1
Oligonucleotides		
SARS-CoV-2 primers and probes	This paper	Table S1
Software and algorithms		
QuantStudio Design & Analysis Software	Applied Biosystems	v1.5.2
fastp	github.com/OpenGene/fastp	v0.20.0
MEGAHIT	github.com/voutcn/megahit	v1.2.9
RagTag	github.com/malonge/RagTag	v2.1.0
Pangolin	pangolin.cog-uk.io	v3.1.20 / v4.0.6
Minimap2	github.com/lh3/minimap2	v2.24-r1122
Nextclade	clades.nextstrain.org	v1.14.0
R	r-project.org	v4.2.0
data.table	CRAN	v1.14.2
ggplot2	CRAN	v3.3.6
quantreg	CRAN	v5.93
Other		
Computational code	This paper	https://doi.org/10.5281/zenodo.6866026

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Björn Reinius (bjorn.reinius@ki.se).

Materials availability

- This study used custom RT-PCR primer-probe sets. Their sequences and oligonucleotide modifications of these are provided in Table S1.

Data and code availability

- Raw sequencing data have been deposited at ArrayExpress and are publicly available as of the date of publication. Assembled SARS-CoV-2 genomes have been deposited at GISAID and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.
- All original code has been deposited at https://github.com/reiniuslab/OmicronWaves/ and is publicly available as of the date of publication. Release version is listed in the key resources table.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

In vitro expanded SARS-CoV-2 strains

The *in vitro* expanded Omicron BA.1 reference RNA sample used for the evaluation of S_{BA1} primer-probe sets (Figures S1A and S2B) was obtained from the Public Health Agency of Sweden (sample isolated Nov 30, 2021; GISAID Accession ID: EPI_ISL_7452247), and the non-Omicron BA.1 reference sample (lacking "S:N211IN212-" and "S:214:EPE") was an Alpha B.1.1.7 (sample isolated Feb 1, 2021[12]).

Human SARS-CoV-2 samples

Following the initial selection of the S_{BA1} primer-probe set, which was performed on *in vitro* specimens to allow timely large-scale oligonucleotide synthesis needed for the population monitoring, all further evaluation was performed on anonymized clinical specimens, as described in the results. All samples were collected by self-sampling using the same kit and a standard operating procedure for throat, saliva, and bilateral nostril sampling, established by the Public Health Agency of Sweden (See instructions in Data S1). 174,933 upper pharynx samples were analysed January 26th – March 8th 2022 (Table S3). The specimens were collected in a fixed volume of 1 mL physiological saline (0.9% NaCl). Upon arrival to the laboratory, the tubes were inactivated by heating at 70°C for 50 min in a hot air oven. All samples included in the study were analysed for presences of SARS-CoV-2 as part of clinical diagnostics performed at ABC Labs, Stockholm, by demand of the Public Health Agency of Sweden. The self-testing program as stated by the Public Health Agency of Sweden was open during the whole period, and sampling was open on Monday through Saturday every week. Indication for testing was self-assessed symptoms up until the 9th of February 2022, at which the Swedish national COVID-19 testing policy changed. From the 9th of February and onwards, testing was restricted to persons with medical indication, patients and care givers in elderly care, tracing, and regional screening of patients. This policy shift is reflected as a drop in overall sample numbers after February 9th (Figure 2A). Importantly, we registered the difference in SARS-CoV-2 viral RNA load between BA.1 and non-BA.1 SARS-CoV-2 positive cases consistently before as well as after the change in testing policy (P_{Before} = $7.35\text{x}10^{-157}$, N_{Before} = 80,264 SARS-CoV-2 positive samples, P_{After} = $5.78\text{x}10^{-14}$, N_{After} = 12,862 SARS-CoV-2 positive samples, Mann-Whitney U-test) (Figure S2C). Testing was provided free of charge. The use of the Omicron BA.1 screening protocol was validated at ABC Labs and approved by the Public Health Agency of Sweden. For the purpose and analyses of the current study, sample identities were anonymised, and IDs were replaced by a randomized code (i.e., those listed in supplementary tables). The vaccination status of the individual specimens included in this study was not accessible, but the overall vaccination rate in Sweden was: 86.4% (1 dose); 83.5% (≥2 doses) at the start of the study January 26th, and 86.8% (1 dose); 84.5% (≥2 doses) at the end of the study March 8th (data obtained from the Public Health

Agency of Sweden). Informed consent for the use of anonymized C_T values or samples obtained in routine clinical diagnostics was not obtained and not required, in accordance with the study permit obtained by the Swedish Ethical Review Authority (Dnr 2020-01945 and 2022-01139-02, Etikprövningsmyndigheten).

METHOD DETAILS

SARS-CoV-2 RT-PCR

The SARS-CoV-2 RT-PCR assay used in this study represents an improved, multiplex-version of our previously described RNA-extraction-free protocol[4] with increased sample and reaction volume and increased sensitivity. For each reaction, 24 uL RT-PCR master mix was prepared, containing 7.5 μL TaqPath 1-Step RT-qPCR Master Mix, CG (Thermo Fisher, containing ROX as passive reference), 0.9 μL 10% Tween20 (Sigma), N1 primer-probe mix (IDT 2019-nCov CDC; FAM/BHQ1, Integrated DNA Technologies), S_{BA1} primer-probe mix (Cy-5/BHQ2; Merck), RNaseP primer-probe mix (HEX/BHQ1; Merck), and nuclease free water up to 24 uL. Primer/Probe concentrations in the final reactions were 246/62 nM (N1), 491/125 nM (S_{BA1}), and 122/37 nM (RNaseP). Primer and probe sequences of the final assay are provided in Table S1B. For RT-PCR testing, 6 μL heat-inactivated nasopharyngeal swab sample (in 0.9% NaCl) was added to optical 96-well PCR plates (EnduraPlate, Applied Biosystems) containing 24 μL master mix, using a liquid handling robot with automatic sample barcode scanner (Fluent 480, Fluent 780, or Fluent 1080; Tecan). Each PCR plate contained a negative control (water) and an Omicron BA.1 positive control sample of low viral load (median *N1* C_T = 30.4), utilized as calibrator sample (Figures S2F and S2G). Plates were sealed and centrifuged for 30 s at 1500 *g*. RT-PCR was performed on QuantStudio real-time PCR machines (Applied Biosystems) using the QuantStudio Design & Analysis Software v1.5.2 and temperature cycles: 25 °C for 2 min, 50 °C for 10 min, 95 °C for 2 min, and 40 cycles of 95 °C for 3 s and 56 °C for 30 s. To test log-linear C_T range for the *N1* and S_{BA1} primer-probe sets in the assay conditions, a strongly positive Omicron BA.1 clinical sample was serial-diluted 1:10 in steps and RT-PCR was performed in 8 replicates per concentration. To test whether addition of the S_{BA1} primer-probe affected N1 C_Ts, we performed SARS-CoV-2 RT-PCR with and without presence of the S_{BA1} primer-probe set in parallel on 185 clinical samples (133 known positives, 52 negatives) and compared N1 C_Ts (Figure 1D). Plate-normalized C_T values (Figure S2F) were calculated $C_T\,N1_{sample\ i,\ plate\ j} - (median(C_T\,N1_{calibrator,\ plate\ 1\text{-}1952}) - C_T\,N1_{calibrator,\ plate\ j})$. In the parallel genotyping of 133 SARS-CoV-2-positive samples (Figure 1E), we used the Thermo Fisher TaqMan SARS-CoV-2 Mutation Panel Assay (according to the manufacturer's instructions) and our SARS-CoV-2 $N1/RP/S_{BA1}$ assay. Extraction-based SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N Genes, IVD (NZYTech) (Figures S2H–SJ) was performed according to the manufacturer's instructions (Version 13/2021, December 2021).

SARS-CoV-2 WGS

For sequencing of SARS-CoV-2 genomes, we used the Illumina COVIDSeq Test kit (Illumina) and sequencing was performed on the Illumina NextSeq 550 Sequencing System. RNA extraction prior to WGS library preparation was performed using the KingFisher Flex System and the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. A48383).

Sequencing data analysis

Raw data was quality trimmed using fastp[13] v0.20.0 and *de novo* assembled using MEGAHIT[14] v1.2.9 [–min-contig-len 5000]. Disjoint contigs were scaffolded against the SARS-CoV-2 reference genome [ASM985889v3] using RagTag[15] v2.1.0 and

SARS-CoV-2 lineage was assigned using pangolin[16] v3.1.20 [lineages version 2022-02-28] and repeated using pangolin v4.0.6 [pangolin-data v1.8] for updated sublineage classification. Alignment statistics was obtained using Minimap2[17] v2.24-r1122 [-ax sr Sars_cov_2.ASM985889v3.dna_sm.toplevel.fa.gz] and samtools[18] v1.10 [stats] [coverage]. Identification of SARS-CoV-2 mutations was performed using Nextclade[19] v1.14.0. Fraction of total sequenced genomes falling into defined variant or lineage groups at national level was obtained for Swedish samples from Covariants.org, accessed 2022-05-24. Computational code is available at https://github.com/reiniuslab/OmicronWaves/ and https://doi.org/10.5281/zenodo.6866026.