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Integrated analyses of the transmission history of SARS-CoV-2 and its association with molecular evolution of the virus underlining the pandemic outbreaks in Italy, 2019-2023

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ABSTRACT

Background: Italy was significantly affected by the COVID-19 pandemic, experiencing multiple waves of infection following the sequential emergence of new variants. Understanding the transmission patterns and evolution of SARS-CoV-2 is vital for future preparedness.
Methods: We conducted an analysis of viral genome sequences, integrating epidemiological and phylodynamic approaches, to characterize how SARS-CoV-2 variants have spread within the country.
Results: Our findings indicate bidirectional international transmission, with Italy transitioning between importing and exporting the virus. Italy experienced four distinct epidemic waves, each associated with a significant reduction in fatalities from 2021 to 2023. These waves were primarily driven by the emergence of VOCs such as Alpha, Delta, and Omicron, which were reflected in observed transmission dynamics and effectiveness of public health measures.

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Conclusions: The changing patterns of viral spread and variant prevalence throughout Italy's pandemic response underscore the continued importance of flexible public health strategies and genomic surveillance, both of which are crucial for tracking the evolution of variants and adapting control measures effectively to ensure preparedness for future outbreaks.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a single-stranded RNA virus, emerged in late 2019 and quickly spread worldwide, overwhelming healthcare systems [1]. This ongoing transmission led to new variants with increased ability to spread and infect people [2]. A key mutation in the spike protein, D614G, appeared early in the pandemic; this variant quickly became dominant, replacing the original strain by June 2020 [3]. Between October and December 2020, WHO has identified five concerning variants (VOC)—Alpha, Beta, Delta, Gamma, and Omicron that spread easily and evade immunity [4-7], hindering pandemic control efforts.

Italy was among the first countries outside China to be severely affected by the COVID-19 pandemic, experiencing a high number of cases and fatalities during the initial circulation of nonvariants of concern. With the first case reported in Italy on January 31, 2020, the epidemic quickly escalated in March 2020, prompting the Italian government to implement strict restrictions to mitigate the viral spread across the country and within its regions [8,9]. By February 15, 2024, Italy had reported more than 26.7 million cases of COVID-19 and over 196,200 associated deaths. To date, approximately 150.32 million vaccine doses have been administered, with 85% of the total population vaccinated with at least one dose (https://covid19.who.int/region/euro/country/it). Despite the early establishment of sustained SARS-CoV-2 transmission in Italy, significant gaps in our understanding of the virus's transmission dynamics at a national scale persist. Unraveling such dynamics and potential determinants that may have driven the progression of the Italian epidemic is crucial for both immediate outbreak response and long-term epidemic/pandemic preparedness [1,10,11]. By examining the expansion and displacement of viral lineages, the study seeks to comprehensively understand the dynamic interaction between viral evolution, epidemic waves and public health responses.

Materials and methods

Sequence data collection

To conduct a thorough analysis of the genomic epidemiology of SARS-CoV-2 in Italy, we retrieved all full-length Italian viral genomes available on GISAID (https://www.gisaid.org/) up to December 31, 2023. We rigorously assessed sequence quality using NextClade to exclude incomplete, ambiguous, or problematic data, ensuring data accuracy for subsequent analysis [12]. To address the disparate sequencing efforts across Italian regions and accurately represent the prevalence of VOC, we implemented both an Italy-specific and a global subsampling strategy. We employed the subsampler method [13], which subsamples sequences per country based on case counts throughout the study period. This facilitates a sample that is geographically, temporally, and epidemiologically representative. The resulting datasets included 19,483 sequences of Alpha (including 8,312 Italian genomes), 21,478 sequences of Delta (including 8,601 Italian genomes), 20,871 sequences of Omicron BA.1 (including 8,547 Italian genomes), 20,673 sequences of Omicron BA.2 (including 8,196 viral genomes from Italy), 20,580 sequences of Omicron BA.5 (including 8,160 Italian genomes), and 15,261 sequences of BQ.1 (including 3,474 genomes from Italy). Due to a scarcity of genomes from Italy, sequences of Omicron BA.4 were not included.

Phylogenetic analysis

Sequences were aligned using ViralMSA with default parameters [14] and manually edited with Aliview [15] to remove artifacts from the terminal regions and within the alignment itself. Phylogenetic analysis of these sequences was performed using the maximum likelihood (ML) method implemented in IQ-TREE v.2 [16] and trees was calibrated using a molecular clock in TreeTime [17] (see additional methods [1]). Outlier sequences that deviated from the strict molecular clock assumption, as flagged by TreeTime until a robust time-scaled phylogeny was obtained. For each VOC, time-scaled tree topologies were generated, and discrete ancestral state reconstruction (of locations) was performed to infer the global dissemination of each variant using the mugration package extension of TreeTime under a GTR model. Using a custom Python script, we counted the number of state changes by iterating over each phylogeny from the root to the external tips. We recorded state changes whenever an internal node transitioned from one country to a different country in the resulting child node or tip(s). The timing of these transition events was then documented, serving as the estimated import or export events.

Epidemiology data assembly

We analyzed COVID-19 case counts in Italy using publicly released data up to December 31, 2022, sourced from the Italian Civil Protection Department repository (https://github.com/pcm-dpc/COVID-19). This repository provides daily updates on confirmed cases, deaths, vaccine administration, and recoveries, broken down by region. For analytical convenience, we grouped geographical locations into Italian macro-regions: Northeast, Northwest, Central, South, and Insular, aligning with regional policy applications. Additionally, metadata including the date of sampling, macro-regional location, and viral lineage were compiled from the GISAID database.

We estimated the reproductive number (Re) of cases [18]. The first step involves estimating the probability that case j (infected at time tj) infected case i, compared to the probability that any other case in the data infected case i.

$$p_{ij} = \frac{w\left(t_i - t_j\right)}{\sum_{k \neq j} w\left(t_i - t_k\right)}$$

where:

p_{ij} is the probability that case j infected case i;

$w(t_i - t_j)$ represents a function that assigns a time weight to the contribution of case j to the infection of case i, based on the time difference between the two events; and

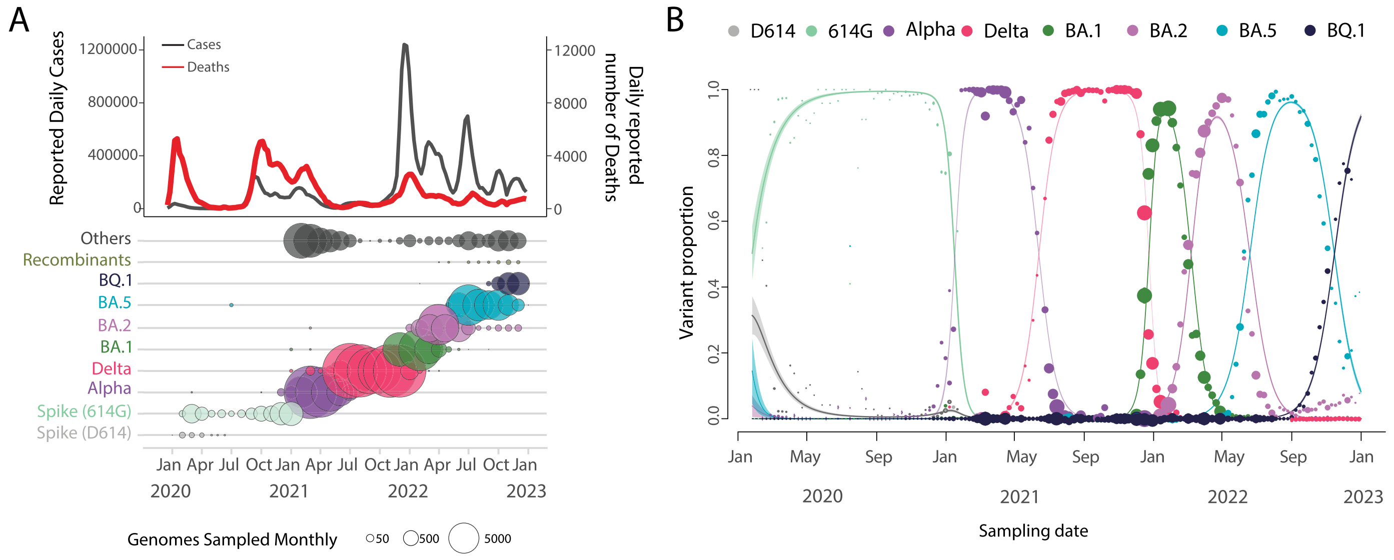

Figure 1. Epidemiological progression of the COVID-19 pandemic in Italy. (a) Total reported new case counts (dark gray) and the daily reported number of deaths (red) in Italy (Data Source: Our World in Data [OWID]) along with the distribution of Variant of Concern VOCs, and other lineages through time (size of circles proportional to the number of genomes sampled per month for each lineage). (b) Modelled proportion of SARS-CoV-2 variants over time in Italy presented in a linear scale. Model fits are based on a multinomial logistic regression. The size of the dots corresponds to the weekly sample size of SARS-CoV-2 genomes (independently of variants, such that each week will have the same point size across all variant curves).

$\sum_{i\neq j} w(t_i - t_j)$ represents the sum of the time weights for all cases except j.

Next, the individual reproductive number for case j, denoted as R_j, is defined as the sum of the probabilities that case j infected all other cases i:

$$R_j = \sum_i p_{ij}$$

Variant prevalence in each age group was calculated using the formula:

$$PV = \frac{CVX}{CS} x100$$

Here, “number of variant X cases in the age group” refers to the count of confirmed variant X cases, and “total number of cases in the age group” refers to the count of all confirmed cases, variant-agnostic. These percentage calculations help standardize the relative frequency of variants by age group, aiding in the demographic analysis of variant spread.

Results

The COVID-19 epidemic in Italy was characterized by four main waves, with a notable reduction in total deaths per year from 2021 to 2022. Although the specific drivers of this reduction are unclear, it was likely a consequence of significant herd immunity (both natural and vaccine-related) developed since the introduction of SARS-CoV-2, as suggested by other studies [19,20]. The first wave was marked by the circulation of ancestral SARS-CoV-2 lineages between January and March 2020 (Figures 1a, 2b-f).

In early February 2020, viral strains carrying the glycine residue at position 614 replaced the ancestral strains (Figure 1b), becoming the dominant form in the country, mostly in northern Italy first, with all G614 samples having shifted before March 1, 2020. The Spike 614G mutation led to a steady rise in the COVID-19 death toll in Italy (Figures 1a, 2b-f). In March 2020, the main hospital in Bergamo, Lombardy (Northwest Italy), became the European epicenter of the unfolding pandemic, as evidenced by the peak in deaths (Figure 2c). During this period, the country reached a daily total of 919 deaths, the highest number of daily fatalities from COVID-19 recorded in the country (Figures 1a, 2b-f). To mitigate viral spread and reduce the death toll, Italy implemented the largest lockdown in European history on March 9, 2020, lasting 70 days. This was followed by a decrease in the number of daily cases and deaths by April 2020. After a seemingly stable epidemic recession, with very few new cases detected between June and July 2020, a second wave began at the end of 2020, marked by a dramatic resurgence in cases and deaths. This wave was driven by the emergence and circulation of several VOCs, including Alpha and later Delta (Figures 1, 2b-f). The Alpha variant was widely detected in the South, followed by the Northeast macro-region (Figure 2b, e). In early May 2021, Delta displaced Alpha, which had dominated infections for about three months, becoming the predominant strain nationwide (Figures 1, 2) and leading the viral population to remain uniform across each Italian macro-region for nearly nine consecutive months.

Following the detection of Delta in Italy, the period from March to October 2021 reported reduced levels of transmission. This was evidenced by the low incidence of reported COVID-19 cases and deaths (Figures 1, 2). Identifying the precise cause of this trend remains challenging; however, it is plausible that immunity acquired from prior infections with earlier variants (e.g., D614G and Alpha), combined with effective vaccination coverage (exceeding 80%) that began on December 31, 2020, contributed to the decrease in reported cases and deaths in the country (Figures 1, 2). It is also possible that during the low transmission period the virus kept circulating in hidden reservoirs of asymptomatic patients [21].

Delta, which predominantly drove the second epidemic wave, began to be replaced by the Omicron BA.1 VOC in late November 2021 (Figures 1, 2). The initial cases of Omicron were detected in early November 2021 in Milan, involving a traveler returning from Mozambique [22]. Subsequently, a notable increase in its prevalence was recorded, leading to sustained transmission and a clear transition from Delta to Omicron BA.1 as the predominant circulating lineage at the national level (Figures 1b, 2). This period saw the highest peak of cases across all macro-regions, particularly in the Central, South, and Insular macro-regions, where several small epidemic peaks were observed. Additionally, a simultaneous peak in daily reported deaths was noted in the Insular macro-region (Figure 2). Subsequently, several Omicron family

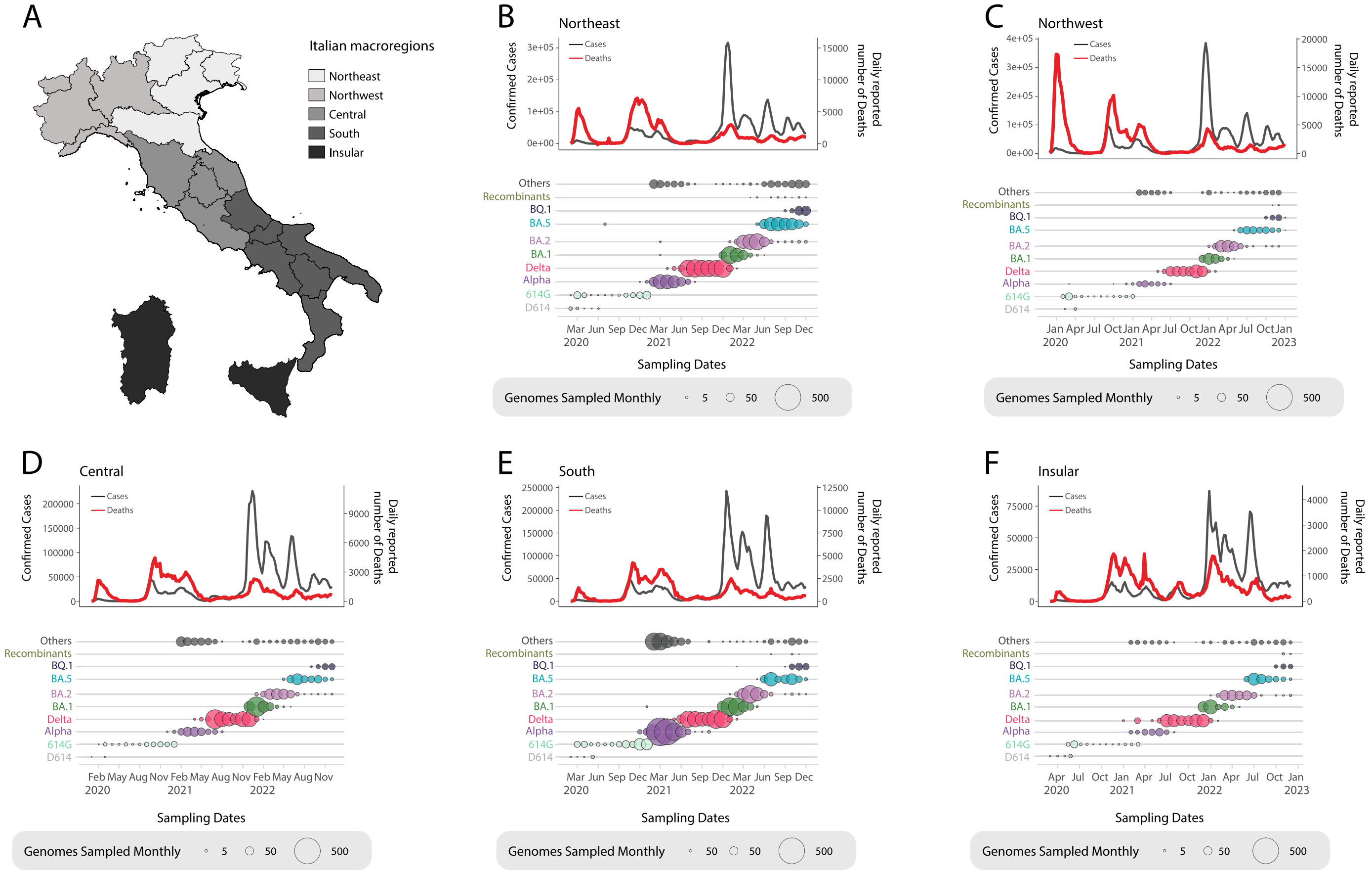

Figure 2. Epidemiological progression of the COVID-19 pandemic within Italian macro-regions. (a) Map of Italy highlighting the five different Italian macro-regions under investigation: Northeast, Northwest, Central, South, and Insular; (b-f) The total reported new case counts (dark gray) and the daily reported number of deaths (red) in each macro-region, with data sourced from the Italian Civil Protection Department (http://www.protezionecivile.gov.it/), are presented alongside the distribution of variants of concern (VOCs) and other lineages over time. The size of the circles is proportional to the number of genomes sampled per month for each lineage.

lineages were identified, including BA.1, BA.2, and BA.5, BA.1, and BA.2 were responsible for the third peak of infections in Italy, with BA.2 demonstrating a significant growth advantage over BA.1, thus becoming one of the most common Omicron sublineages in Italy (Figures 1b, 2). This was associated with a concerning ability to reinfect patients who had initially been infected with Omicron BA.1. However, as the BA.2 wave subsided in May 2022, BA.5 emerged as the dominant lineage in Italy (Figure 1b), driving the fourth national epidemic peak. Interestingly, the emergence and subsequent dominance of BA.5 did not coincide with an increase in reported COVID-19 cases, hospitalizations, or deaths. Instead, there was a sharp peak followed by a swift decline, again likely reflecting the effective impact of the vaccination program and long history of infection of the population.

In early September 2022, a novel and rapid shift from the BA.5 variant to its descendant lineage BQ.1 was reported, also without an increase in deaths or cases (Figures 1, 2). BQ.1 and its sublineages, previously classified as Variants of Interest (VOI), carry mutations that could challenge existing immune responses, although no associated large epidemic peak has been reported to date. This situation indicated a shift towards an endemic phase of SARS-CoV-2, characterized by seasonal patterns and generally causing mild respiratory disease in a population with high immunity levels due to vaccination and/or prior infections. Notably, while BA.2 was displaced by BA.5 in May 2022, it remained minimally detectable, leading to the independent evolution of multiple second-generation BA.2 variants. This phenomenon, observed globally, is likely due to reinfections with this variant, as immunity from previous non-Omicron infections showed limited effectiveness [23].

The efficacy of genomic surveillance programs is critically dependent on their capacity to accurately represent the epidemic under investigation. This is typically assessed by the proportion of reported cases subjected to sequencing and the turnaround time from sample collection to the availability of results. Notably, early in the pandemic, Italy experienced substantial delays in this process [24]. In our analysis of Italian macro-regions, we noted a relatively consistent sequencing rate across the country, averaging 0.03% of total cases. The South macro-region, which was significantly impacted, accounted for about 34.5% of all sequenced viral genomes from Italy (Table S1). Conversely, the Northwest region, initially the epicenter of the pandemic, had a lower sequencing rate of just 0.02% of total cases but still contributed 12.5% of the total number of Italian strains in public databases.

To explore why Italy was the most impacted country during the circulation of non-VOCs and why the Spike 614G mutation led to a steady rise in the COVID-19 death toll, we estimated the variant-specific effective reproductive numbers (Re) at the national level. This analysis investigated the transmission dynamics of the variants predominantly influencing the SARS-CoV-2 epidemic in Italy (Table S2 and Figure 3a).

The D614G variant had a slightly higher Re value than the Alpha variant and was notably more transmissible than the Delta variant (Figure 3a). The higher Re of the D614G variant was likely attributed by a high level of susceptibility in the population at the beginning of the epidemic. Analysis of the Delta variant's Re

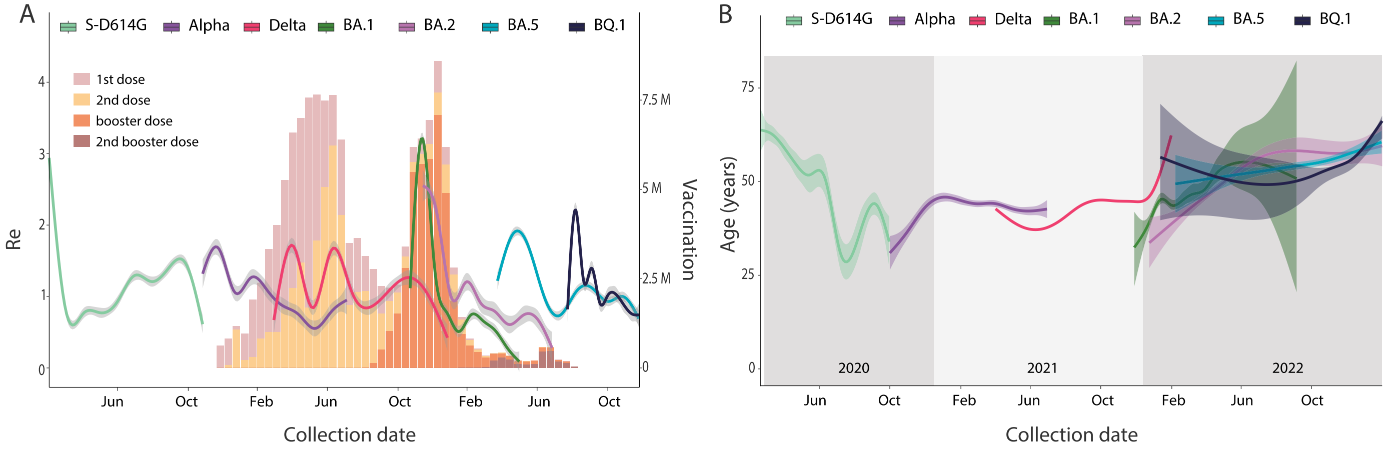

Figure 3. Epidemiological distribution of variants across different age groups and variant-specific effective reproductive numbers in Italy. (a) Epidemiological distribution of variants and vaccination impact across Italian macro-regions. Estimated effective reproductive number (Re) against the backdrop of vaccination rates over time. Different colors highlight each vaccine dose. The national Re average across all regions is represented by colored lines, according to the legend in the top left. The left y-axis indicates the Re values while the right y axis indicates the vaccination coverage; (b) Temporal progression of age distribution among individuals infected with SARS-CoV-2 variants across Italy. The y-axis indicates age in years. The lines depict the average age of collected cases, while the shaded areas represent the confidence intervals.

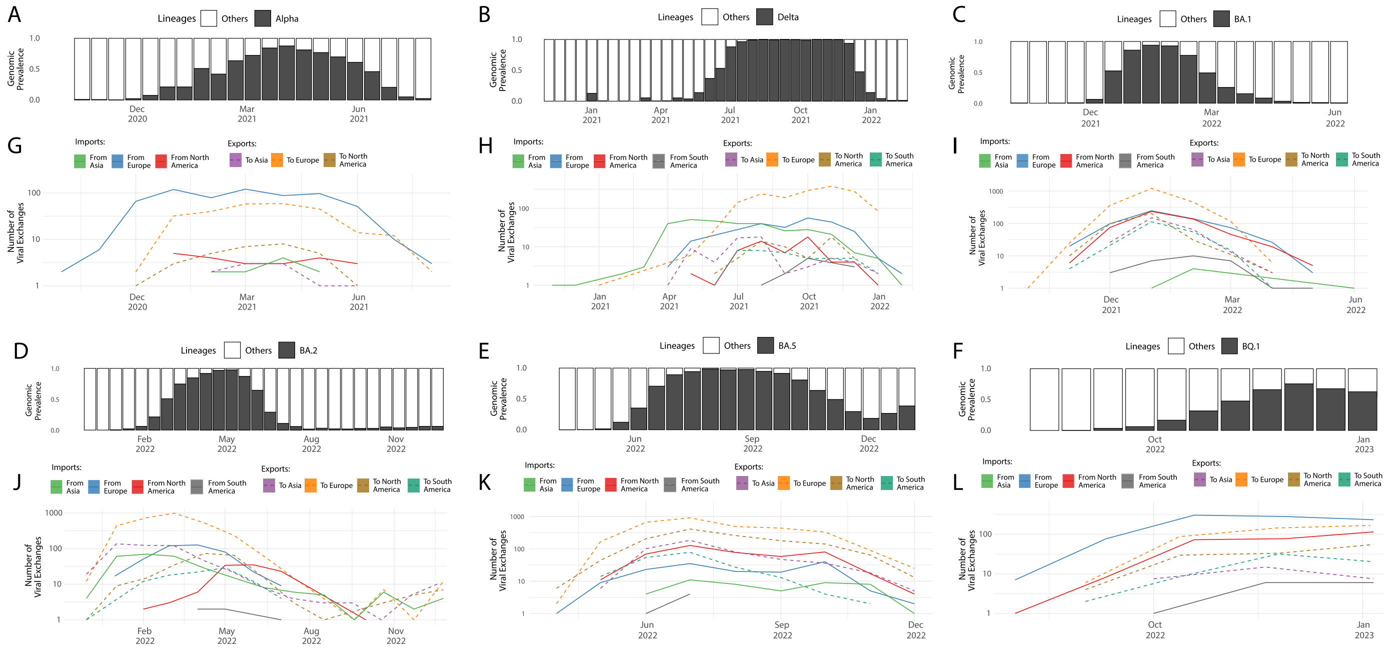

Figure 4. Inferred viral dissemination patterns of variant of concern VOCs in Italy. (a-f) Genomic prevalence of VOCs Alpha, Delta, Omicron BA.1, Omicron BA.2, Omicron BA.5, and BQ.1 in Italy over time. (g-n) Inferred viral exchange patterns to and from Italy for the five VOCs. Introductions in Italy are shown in solid lines and exports from Italy are shown in dotted lines and these are colored by continent.

showed fluctuations indicating phases of rapid growth, stabilization, and decline, likely influenced by the quick and synchronized vaccination rollout, control measures, and seasonal effects. Despite this, an initial increase in Re was observed during the circulation of Omicron-like variants, they yielded comparable median Re values, indicating a similar propensity for transmission. Specifically, BA.1 exhibited rapid expansion followed by stabilization, with decreasing Re values suggesting the effectiveness of control measures or accumulation of herd-immunity, as well as for BA.2. BA.5 demonstrated variable transmissibility dynamics, with temporary peaks followed by stabilization, while BQ.1 initially displayed high transmissibility, gradually stabilizing at lower levels. Omicron's mutations likely enabled it to bypass immune defenses, allowing for faster and wider spread compared to previous variants.

Figure 3b shows how different age groups were affected by various COVID-19 variants. The D614G variant initially affected older individuals but later spread to younger adults. The Alpha variant primarily affected young adults but later spread across a wider age range. The Delta variant mainly affected people under 50, while Omicron variants (BA.1, BA.2, BA.5, BQ.1) had a more varied impact across different age groups. D614G, Delta, and Omicron BA.1 affected a wider range of ages, including the youngest and oldest.

We combined epidemiological data with phylogenetic analysis to study how the virus spread in Italy, focusing on VOCs (Figures 4, 5).

Employing ancestral location state reconstruction on the dated phylogeny enabled us to infer the number of viral import and export events involving Italy (Figures 4, 5). Most of the imported introductions, estimated at approximately 677 for Alpha, 857 for Delta, 1,187 for Omicron BA.1, 838 for BA.2, 697 for BA.5, and 1,071 for BQ.1, predominantly originated from Europe, Asia, and North America, respectively (Figures 4, 5). These introductions occurred

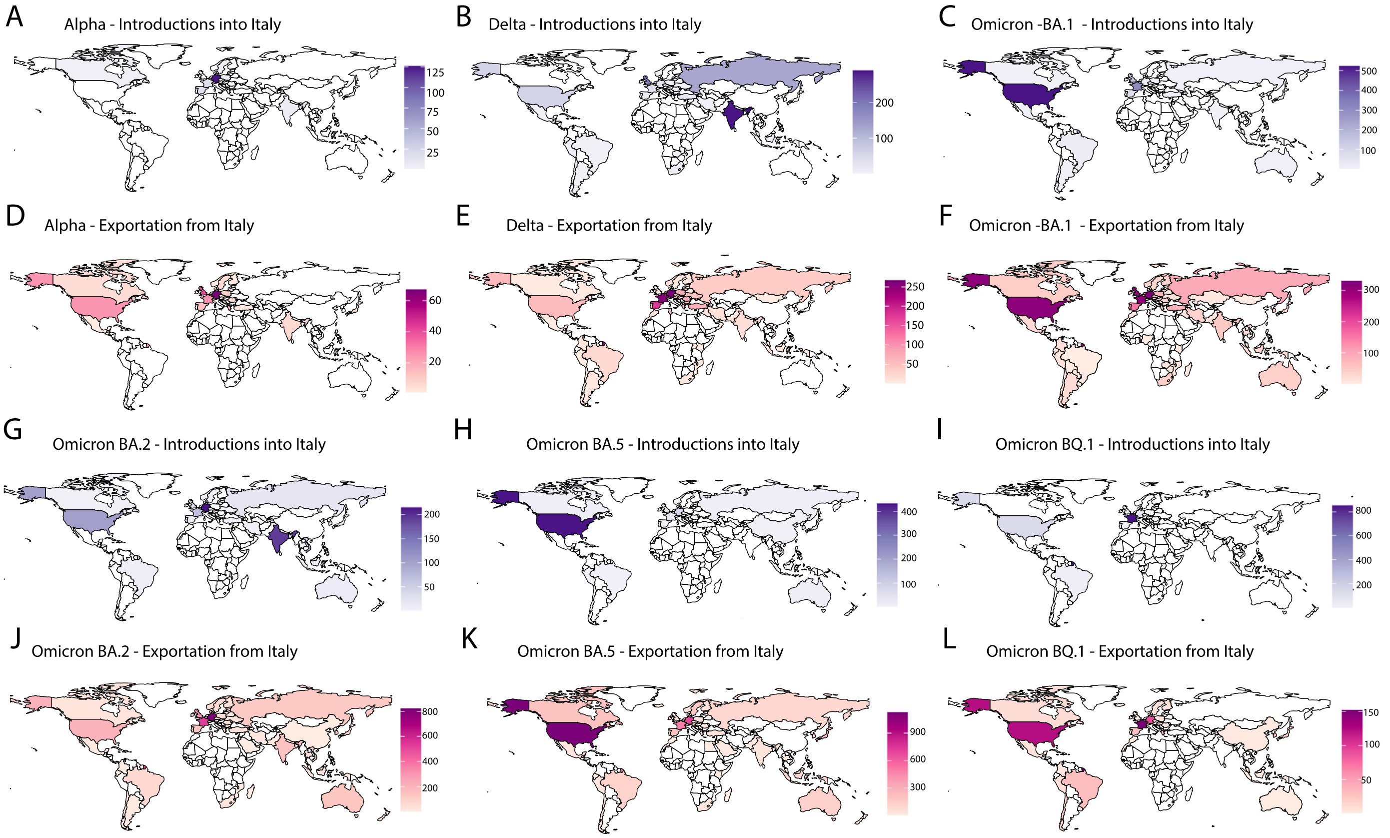

Figure 5. Transmission dynamics of the VOCs that drove the Italian epidemic over time. (a-c and g-i) Inferred locations of importations of the VOCs that drove the Italian epidemic over time -Alpha, Delta, Omicron BA.1, Omicron BA.2, Omicron BA.5, and BQ.1 into Italy. (d-f and j-l) Inferred locations of exportation of the VOCs that drove the Italian epidemic over time -Alpha, Delta, Omicron BA.1, Omicron BA.2, Omicron BA.5, and BQ.1 into Italy.

during periods when travel restrictions were relaxed, and the epidemic was rapidly progressing (Figures 4, 5).

In Italy, Alpha was detected across all macro-regions by December 2020 (Figure 4a). The discrete state maximum likelihood reconstruction, based on globally case-sensitive genomic subsampling, suggested that approximately 65% of Alpha's importation could be attributed to the UK, increasing to 97% when considering Europe as a whole, indicating significant dispersal within the continent (Figures 4g, 5a). Delta, initially reported in India in April 2021, was swiftly detected worldwide, prompting new epidemic waves. It appeared in Italy in early July 2021 (Figure 4b). While a moderate number of introductions were traced to Asia (36.5%, with 33.8% from India alone), a substantial portion was attributed to European countries, accounting for 31.5% (Figures 4h, 5b). Omicron, the third VOC detected in Italy, was first characterized in November 2021 in Southern Africa. Its spread within Italy unfolded in several stages as various Omicron sublineages emerged. BA.1 and BA.2 were some of the initial sublineages identified, with BA.1 leading from the end of 2021 to March 2022, and BA.2 becoming predominant in late February 2022 (Figure 4c, d). At least 44.3% of BA.1 introductions stemmed from North America (Figures 4i, 5c), whereas BA.2 imports were primarily from European countries (50%), followed by Asia (32.5%) and North America (12%) (Figures 4j, 5g). The BA.5 and BQ.1 Omicron sublineages, first noted in January 2022, became dominant in Italy during mid-June and mid-October 2022, respectively (Figure 4e, f). The introduction pattern of BA.5 was similar to that of BA.1, with 63% linked to North America (Figures 4k, 5h), while BQ.1 was mostly associated with European countries (84%) (Figures 4l, 5i).

Italy's role as a significant virus exporter, particularly to North American and European countries, is also noteworthy (Figures 4, 5). Data suggest a dynamic bidirectional transmission and dissemination pattern, with total exportation events to Europe ranging from 51.4% to 86%, compared to 3% to 21.8% for North America. Additionally, there were distinct exportation patterns for each VOC. For the Alpha and BQ.1 variants, exportation events were fewer than the number of introductions (approximately 31.2% and 40.0% of the total events, respectively). Conversely, for the Delta, Omicron BA.1, BA.2, and BA.5 variants, the estimated number of exportation events exceeded that of introductions, with roughly 69.9%, 73.0%, 83.5%, and 89.6% of the total events, respectively (Figures 4, 5).

Discussion

Italy has experienced one of the most severe SARS-CoV-2 scenarios globally, also having been the first European nation to face the pandemic's surge after emergence in China [21]. This study has provided insights into the historical transmission and evolution of SARS-CoV-2 from (2019-2022). Such insights were generated from analyzing a large dataset of reported cases and viral genome sequences, demonstrating Italy's surveillance efforts during the epidemic, despite a slow increase in available sequences throughout 2020. Like elsewhere globally, the Italian SARS-CoV-2 pandemic was characterized by successive waves of infections and variant displacements [21]. Italy initially reported cases with the Spike D614 mutation, but the large outbreak with autochthonous transmission was associated with the 614G mutation, likely imported from Germany. This mutation is transmitted as part of a conserved haplotype marked by four mutations that commonly track together. This rapid change was driven by strong selective pressure [25], with 614G associated with higher viral nucleic acid levels in the upper respiratory tract, indicative of increased viral loads and infectivity, reflected in higher estimated Re. This strain became prevalent across almost every age group, initially affecting the oldest and eventually spreading to the youngest age groups (Figure 3). During this phase, which recorded the highest mortality rates, the emergence of the Alpha variant was mirrored by a decreasing daily death rate (Figures 1, 2). The Delta wave persisted the longest, sustaining the pandemic in Italy for nearly nine months (Figure 1b) and was characterized by reciprocal viral exchange with European countries (Figures 4, 5). Daily case and death rates significantly diminished throughout this period, likely due to acquired immunity through natural infection or vaccination. However, displacement is not solely contingent on immunity; it also depends on a complex interplay of local interventions, infrastructure, and variant phenotype/fitness [25]. The highest case peak coincided with the spread of Omicron lineages across all Italian macro-regions, accompanied by an increased daily death rate nationwide (Figures 4, 5), especially in the Central, South, and Insular regions (Figure 2d-f). A significant level of viral transmission continued globally, with Italy playing a central role (Figures 4, 5). Moreover, Omicron BA.1 showed a similar behavior of D614G in terms of Re and wider age prevalence but with opposite trend, first the youngest and then eventually the oldest (Figure 3).

With air travel volumes nearing prepandemic levels, the spread of variants such as Delta and Omicron was primarily from secondary hubs rather than the initial countries of detection [26]. Targeted genomic surveillance of travelers was and remains pivotal in curbing the global dissemination of SARS-CoV-2 variants [27,28]. Early detection of new variants can facilitate their investigation through specialized studies and integrate with routine community-based surveillance.

Throughout the pandemic, the global movement of the SARS-CoV-2 virus was closely aligned with patterns of human mobility [26,27]. Multiple independent viral importations were estimated into Italy, mainly from Europe, Asia, and North America, coinciding with the relaxation of travel restrictions and during periods of rapid epidemic growth. Italy evolved from a viral importer to a viral exporter, primarily to European and North American countries, suggesting bidirectional transmission dynamics. Notably, Italy's role as a virus exporter coincided with the detection of VOCs and VOIs. While we did not measure passenger traffic, the viral migration patterns we observed are consistent with those from other studies that synthesized viral genetic, epidemiological, and travel data [28]. Our analysis of the Italian SARS-CoV-2 pandemic over almost three years highlights the transition of viral patterns from a pandemic to an endemic state, marked more recently by seasonality in epidemic peaks. In the endemic phase, the periodicity of SARS-CoV-2 outbreaks will be influenced by various factors, including viral immune escape, the influence of population turnover (susceptibility), and social behavior [29]. The variance in infection rates across different variants and age groups emphasizes the need for demographic-specific, surveillance, and vaccination-centric strategies. Continual surveillance is essential for understanding variant evolution and informing effective public health interventions. Our findings suggest a transition towards active surveillance to keep pace with the future emergence of new variants [29]. This approach should be extended to all emergent or reemergent pathogens, given its demonstrated potential during the pandemic in public health monitoring and informing response strategies.

Limitations

Our study analyzed 163,124 SARS-CoV-2 sequences from Italy, accounting for uneven regional data collection. We adjusted our data to better represent the national epidemic. While this improved our analysis, it's important to note that the varying levels of sequencing across regions might affect our results. Despite this, our study provides valuable insights into the virus's spread within Italy.

Declarations of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

Not applicable.

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Author contributions

Conception and design: MG. Investigations: EC; VF; FB; ST; KM; GS; SA; SNS; FS; LAS; SK; EW; HT; CM; AB; FC; MS; TdO; TA; AMBdF; LCJA; GC; AC; VC; AM; JL; MC; and MG. Data Analysis: EC; VF; FB; and MG. Visualization: EC, VF; FB; and MG. Writing – Original: EC; and MG. Revision: EC; VF; FB; ST; KM; GS; SA; SNS; FS; LAS; SK; EW; HT; CM; AB; FC; MS; TdO; TA; AMBdF; LCJA; GC; AC; VC; AM; JL; MC; and MG.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107262.

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