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SHORT COMMUNICATION

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The first Italian outbreak of SARS-CoV-2 B.1.1.7 lineage in Corzano, Lombardy

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Abstract

In December 2020, Italy experienced the first case of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) B.1.1.7 lineage. In January 2021, we identified 21 cases of this variant in Corzano, defining the first outbreak of SARS-CoV-2 B.1.1.7 lineage in Italy. The high transmissibility of the B.1.1.7 variant represented an important benefit for the virus, which became rapidly dominant on the territory. Containment measures induced the epidemic curve onto a decreasing trajectory underlining the importance of appropriate control and surveillance for restraint of virus spread.

Highlights The first Italian outbreak of SARS-CoV-2 B.1.1.7 lineage occurred in Lombardy in January 2021. The outbreak originated by a single introduction of the B.1.1.7 lineage. The genomic sequencing revealed, for the first time, the presence of the V551F mutation in the B.1.1.7 lineage in Italy. Surveillance, prompt sequencing and tracing efforts were fundamental to identify and to quickly contain the outbreak.

KEYWORDS

coronavirus, epidemiology, pandemics

1 | INTRODUCTION

Since the first case of coronavirus disease 2019 (COVID-19), the continuous spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) across the world has allowed the virus to generate a large number of mutations leading to distinctive SARS-CoV-2 variants. Identification and tracing of these mutations through whole-genome sequencing have been critical from one side to characterize new variants that could alter the virologic and clinical features of the disease, and from the other side to map outbreaks within the population over time. The first variant that has rapidly expanded across European countries was initially detected in the UK and belongs to the B.1.1.7 lineage (20I/501Y.V1, also called a variant of concern [VOC] 202012/01).[1] In Italy, the first case of the B.1.1.7 variant was identified in December 2020 in a traveler who arrived from the UK.[2] Since then, the variant has been identified in different towns in Italy.[3] Here we describe the first identified outbreak of the B.1.1.7 lineage of SARS-CoV-2 in Italy. At the end of January 2021, the local sanitary authority of Brescia, northern Italy, reported a suspicious increasing number of people who resulted positive for SARS-CoV-2 molecular test in Corzano, a small town near Brescia. In approximately 10 days, the number of reported SARS-CoV-2 cases had reached over 140 (10% of the entire population) with 84 cases (60%) among pupils and teachers of local schools. At that time, to contain the spreading of the virus, the mayor of the town decided to close the schools.

Serena Messali and Giovanni Campisi equally contributed to this study.

2 | MATERIALS AND METHODS

Based on cycle threshold (Ct) values (range: 11.08–24.62) 21 nasopharyngeal swab specimens collected between January 22nd and January 29th 2021 at the Brescia Civic Hospital were selected for genomic analysis. The samples belonged to patients aged from 3 to 59 years (8 males and 13 females). Ct values were obtained using the Allplex™ 2019-nCoV Assay (Seegene Inc.) reagents and automatically evaluated using the 2019-CoV Viewer analysis software (Seegene). Sequencing libraries were prepared using the multiplex PCR Research and Surveillance Panel (Paragon Genomics), according to the manufacturer's protocol. The resulting libraries were normalized, equal amounts of each library were pooled and sequenced on the Illumina MiSeq platform (Illumina). Raw data were checked for quality using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and then analyzed with the specifically designed software SOPHiA GENETICS' SARS-CoV-2 Panel (SOPHiA GENETICS).

Lineage assessment was conducted using Phylogenetic Assignment of Named Global Outbreak LINeages tool (Pangolin), available at https://github.com/hCoV2019/pangolin,[4] to lineages assessment.

For phylogenetic analysis, the 21 whole-genome sequences of SARS-CoV-2, described in this study, were aligned to $n = 3.031$ public SARS-CoV-2 genomes available in the Global Initiative on Sharing All Influenza Data (GISAID) until March 2021 or to $n = 1.047$ public Italian SARS-CoV-2 B.1.1.7 lineage genomes available in GISAID until May 2021. Low-quality genomes and nearly identical sequences (genetic similarity >99.99%) were excluded.

The global datasets of 3.052 and 1.068 whole-genome sequences were aligned by MAFFT (FF-NS-2 algorithm) using default parameters.[5] The alignment was manually curated with Aliview[6] to remove artifacts at the ends and within the alignment. Phylogenetic analysis was performed using IQ-TREE (version 1.6.18) under the best fit model according to Bayesian information criterion indicated by the Model Finder application implemented in IQ-TREE.[7]

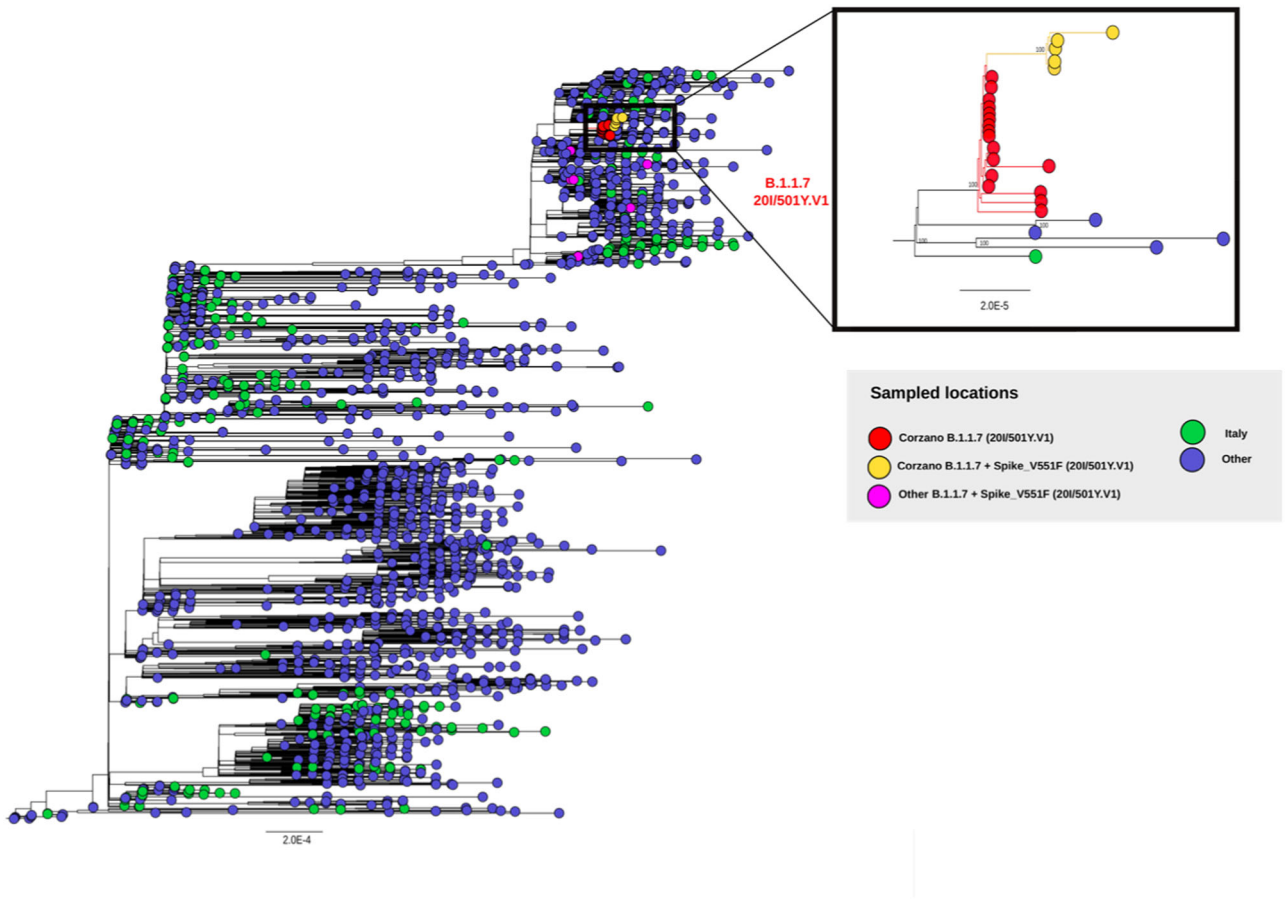

FIGURE 1 Maximum likelihood tree including 21 Corzano genomes plus 3.031 sequences representative of the SARS-CoV-2 lineages globally circulating until March 2021. SARS-CoV-2 B.1.1.7 sequences from Corzano are identified by red circles; SARS-CoV-2 B.1.1.7 sequences from Corzano which carry the Spike mutation V551F are identified by yellow circles; other SARS-CoV-2 Italian sequences are identified by green circles; SARS-CoV-2 sequences from all over the world are identified by violet circles; SARS-CoV-2 B.1.1.7 sequences from all over the world which carry the Spike mutation V551F are identified by pink circles

3 | RESULTS

The first SARS-CoV-2 infection described in this study was identified in Corzano on January 22nd, 2021. The source of infection was unknown. Close contacts from households, students, and teachers were instructed to self-isolate. In the subsequent days, massive COVID-19 testing was conducted. With the increasing number of SARS-CoV-2 infections within 10 days, an outbreak emergence status was declared and schools were closed. None of the infected individuals identified in this outbreak reported severe respiratory, but rather general, unspecific symptoms, such as fatigue, headache, back pain, or exhaustion. Only one of these individuals required nonintensive care unit hospitalization.

To properly define the evolutionary relationships among Corzano SARS-CoV-2 sequences on a global scale, a maximum likelihood (ML) phylogenetic tree was implemented (Figure 1). Thus, we observed a single lineage of virus circulating among the Corzano population, the VOC B.1.1.7. In particular, phylogenetic analysis revealed that all the 21 Corzano B.1.1.7 sequences established an independent cluster with other worldwide sequences belonging to the B.1.1.7 lineage. Interestingly, 5 out of the 21 Corzano sequences were found to carry an additional Spike mutation in the receptor-binding domain at position 551 (V551F), which was not reported elsewhere at the time of sampling. As shown in Figure 1, these sequences (yellow circles) organize a minor cluster within the Corzano clade. Of note, 3 out of the 5 sequences carrying the V551F mutation belonged to pupils (range, 4–10 years) and 2 out of 5 sequences belonged to close contacts (34 and 44 years) of the infected pupils. Up to the end of March 2021, only 7 additional SARS-CoV-2 sequences belonging to B.1.1.7 lineage and carrying the V551F mutation (pink circles) were documented in the GISAID database and they were retrieved in Poland, Germany, Belgium, Denmark, Japan, and the United States.

The SARS-CoV-2 B.1.1.7 variant rapidly spreads worldwide becoming the most prevalent circulating SARS-CoV-2 strain in Italy as well.[3] Thus, to better establish its introduction in Corzano, an ML tree including 1.068 Italian SARS-CoV-2 sequences belonging to B.1.1.7 lineage until May 2021 was built up. As shown in Figure 2, Corzano sequences formed a separate cluster within all the Italian B.1.1.7 circulating strains, suggesting that the epidemic outbreak was caused by a single introduction of B.1.1.7 lineage in the Corzano area. Based on the phylogenetic and epidemiological history, we can exclude any correlation between the V551F mutation and multiple introductions events and assume that the newly identified mutation represents the result of selective pressure and virus-host adaptation. Indeed, the V551F mutation was not found in any other area in Italy, thus its

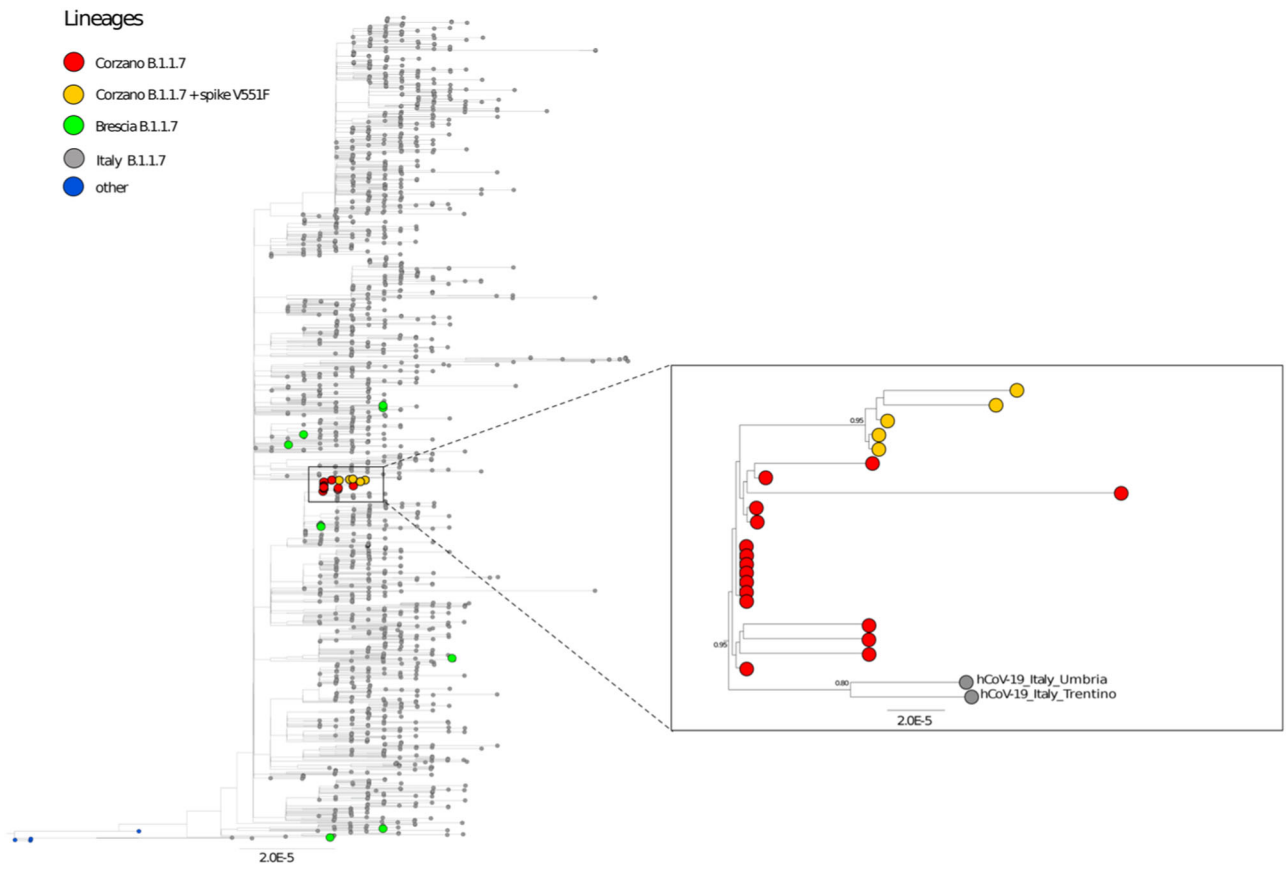

FIGURE 2 Maximum likelihood tree including 21 Corzano genomes plus 1.047 sequences representative of the SARS-CoV-2 B.1.1.7 lineage circulating in Italy until May 2021. SARS-CoV-2 B.1.1.7 sequences from Corzano are identified by red circles; SARS-CoV-2 B.1.1.7 sequences from Corzano which carry the Spike mutation V551F are identified by yellow circles; other SARS-CoV-2 B.1.1.7 sequences circulating in the Brescia area are identified by green circles; other Italian SARS-CoV-2 B.1.1.7 sequences are identified by gray circles

presence in Corzano represents the first evidence on the territory without any history of travel abroad.

4 | DISCUSSION

We report the first Italian outbreak of SARS-CoV-2 B.1.1.7 lineage which occurred in Corzano, a small town in Lombardy, Italy. The phylogenetic and epidemiological analysis demonstrated that the outbreak, which included over 140 cases, originated by a single introduction of the B.1.1.7 lineage in the Corzano area. The first case occurred among pupils of local schools and then the virus rapidly spread among teachers and their close contact. Of particular interest, the genomic sequencing revealed the presence of the V551F mutation in 5 out of 21 sequences, representing the first and only evidence of this mutation in the B.1.1.7 lineage in Italy. Up to the end of March 2021, only 7 additional SARS-CoV-2 sequences belonging to B.1.1.7 lineage and carrying the V551F mutation were documented worldwide in the GISAID database. The presence of this mutation among the Corzano sequences may reflect viral adaptation to the young population's immune response.

To limit the spreading of the infection, strict restriction measures, such as closing of the schools, reduced social contact, and smart working, were adopted. All these procedures resulted to be adequate to control viral transmission. Our data highlight the easy and rapid intergenerational transmission of SARS-CoV-2 B.1.1.7 lineage among children attending primary schools. Nevertheless, asymptomatic individuals among the young population resulted in rapid diffusion of secondary infections even in the adult population. The continuous surveillance in the territory and the prompt sequencing and tracing efforts were fundamental to identify the SARS-CoV-2 B.1.1.7 lineage in Corzano and to quickly contain the outbreak. Indeed, local restriction measures adopted resulted in a rapid decrease in reported cases of SARS-CoV-2 in Corzano and their efficacy is further attested by the lack of V551F mutation diffusion in the surrounding areas.

In conclusion, the presence of the B.1.1.7 variant in Corzano posed critical challenges to epidemic control. Its higher transmissibility represented a strong selective advantage that makes it prone to rapidly become the dominant strain. Our survey analyses show that viral transmission could be effectively and rapidly suppressed by combining the early isolation of infected people with social distancing.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Serena Messali collected data. Serena Messali did the laboratory investigations. Serena Messali, Francesca Caccuri, Marta Giovanetti, and Giovanni Campisi were involved in data analysis and result presentation. Francesca Caccuri and Arnaldo Caruso drafted the manuscript. Arnaldo Caruso and Massimo Ciccozzi critically revised the content. All authors reviewed and approved the final version of the article.

DATA AVAILABILITY STATEMENT

Data have been deposited in Global Initiative on Sharing All Influenza Data (GISAID) database (accession numbers: EPI_ISL_3376580; EPI_ISL_3376678; EPI_ISL_3376679; EPI_ISL_3376731; EPI_ISL_3376841; EPI_ISL_3376843; EPI_ISL_3376844; EPI_ISL_3376846; EPI_ISL_3376848; EPI_ISL_3376852; EPI_ISL_3376885; EPI_ISL_3376950; EPI_ISL_3376986; EPI_ISL_3376988; EPI_ISL_3376990; EPI_ISL_3382429; EPI_ISL_3382431; EPI_ISL_3382432; EPI_ISL_3382475; EPI_ISL_3382688; EPI_ISL_3382923).

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