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Application of genomic surveillance in Cantabria to keep the COVID-19 pandemic under control

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Abstract:

A number of variants of the SARS-CoV-2 virus are now causing special concern all around the world due to its purported ability to evade the immune response in previously immunized people. Therefore, many governments, including that of Spain, have implemented non-pharmaceutical interventions (NPI) in an attempt to prevent variants of concern (VOCs) from being imported and, if they are, facilitate their timely detection and control their expansion throughout new regions.
In this report, the real efficacy of the current NPI is subjected to examination, using the first COVID-19 outbreak of P.1 lineage (Gamma variant) arisen in Cantabria as a practical example. Likewise, the usefulness of genomic surveillance in keeping a pandemic situation under control is also assessed. Finally, the issue of whether there really exists in Spain, as well as in every Autonomous Community, the capacity to perform sufficiently broad genetic analyses, in a sufficiently short time and in a sufficient amount of samples, as to have real-time data on the evolution of the variants is addressed here.
The COVID-19 outbreak reported in this work was kept under control and its expansion was avoided. However, analysing the facts, this success seems to be largely due to a

KEYWORDS

Genomic surveillance.
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COVID-19.
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P.1 lineage.
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E484K.
N501Y.
Immune escape.
Spreading containment.

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Non-pharmaceutical interventions (NPI).

surprisingly low transmission capacity of this strain of the virus, while not so much to a high efficiency of the existing NPI. Indeed, the attack rate of this outbreak has been as low as 3.5%.

PALABRAS CLAVE

Vigilancia genómica.
Vigilancia epidemiológica.
COVID-19.
Variante de preocupación (VOC).
Brote.
Linaje P.1.
Variante Gamma.
Mutación E484K.
Mutación N501Y.
Escape inmune.
Control de la expansión.
Intervenciones no farmacológicas (NPI).

Aplicación de la vigilancia genómica en Cantabria para mantener bajo control la pandemia de COVID-19

Resumen:

Algunas variantes del virus SARS-CoV-2 están causando, actualmente, especial preocupación en todo el mundo por su supuesta capacidad de evadir la respuesta inmune en personas previamente inmunizadas. Debido a esto, muchos gobiernos, incluido el de España, han implementado intervenciones no farmacológicas (NPI) en un intento de evitar que las variantes de preocupación (VOCs) sean importadas y, en caso de que lo sean, facilitar su detección temprana controlar su expansión en nuevas regiones.
En este trabajo, la eficacia real de las actuales NPIs es sometida a examen utilizando, como ejemplo real y práctico, el primer brote de COVID-19 debido a la variante P.1 (variante Gamma) surgido en Cantabria. Asimismo, se evalúa la utilidad de la vigilancia genómica de cara a mantener una situación pandémica bajo control. Por último, se plantea la cuestión de si realmente existe en España, y en cada Comunidad Autónoma, la capacidad de realizar análisis genéticos suficientemente amplios, en un tiempo suficientemente corto y a un número de muestras suficientemente elevado, como para tener datos en tiempo real sobre la evolución de las variantes.
El brote de COVID-19 que se reporta aquí se pudo mantener bajo control, evitando su expansión. Sin embargo, tras el correspondiente análisis, este éxito parece haberse debido más a una capacidad de transmisión sorprendentemente baja de este linaje que a una gran eficiencia de las medidas no farmacológicas existentes. De hecho, la tasa de ataque de este brote fue solo del 3,5%.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the virus which causes the Coronavirus Disease 2019 (COVID-19). Since its first identification, on December 2019 in Wuhan (China)[1], its genomic sequence has been evolving, giving rise to different lineages and variants, some of which are considered of special concern. P.1 lineage is one of the variants of concern (VOC-202101/02) of this virus. This variant reportedly arose on December 2020 in Manaus[2, 3], a region located in the Northern Brazil where very high attack rates had been noticed short time before, despite the existence of an elevated previous immunization rate[4, 5]. This new lineage was descendent of B.1.1.28 and contained a unique constellation of defining mutations[6]. Some of the substitutions carried by this VOC are of known biological importance, such as E484K, K417T, and N501Y (table I), the three of them affecting the receptor-binding domain of the spike protein that the virus uses to bind the human ACE2 receptor[3]. The single-nucleotide variant (SNV) G23012A causes the E484K change in the protein, which may affect the antigenic profile of SARS-CoV-2, and thus, the ability of antibodies –generated either through a previous natural infection or through vaccination– to recognize and neutralize the virus[7, 8]. Therefore, it has been associated with a viral ability to escape from neutralizing antibodies. For its part, the SNV A23063T causes the N501Y amino acid change, which seems to be related with increased binding specificity, higher viral loads and easier transmission[9, 10]. This substitution –N501Y– is shared between P.1 and B.1.351 lineage (VOC-202012/02), first described in South Africa[11], in samples dating back to the beginning of October 2020.

The rapid emergence and expansion of variants harbouring risk genetic markers is rising concerns of a potential increase in hospitalization rates, together with a potential decrease in the effectiveness of immunization. A variant is considered of concern (VOC) when, in addition to the possible attributes of a variant of interest (VOI), there is evidence of interference with testing, treatments and vaccines; evidence of increased transmissibility; and/or evidence of increased disease severity (tables I and II)[12]. Regarding this, P.1 lineage has been associated with a resurgence of COVID-19 in some areas, independently of seroprevalence level among population, as well as with an exponential increasing in hospitalisations[4, 13]. Accordingly, it seems crucial for any region in the world to develop the capacity to detect the entrance of this VOC and promptly deploy resources to avoid its spreading.

Table I
SARS-CoV-2 variants of concern (VOC)

Pango lineage	VOC Code	Defining mutations in Spike protein	First detected	Known attributes
B.1.1.7	202012/01	Δ69/70, Δ144Y, (S494P*), N501Y, A570D, D614G, P681H.	United Kingdom, October 2020	▪ 50% increased transmission. ▪ Likely increased severity based on hospitalizations and case fatality rates. ▪ Minimal impact on neutralization by monoclonal antibody therapeutics. ▪ Minimal impact on neutralization by convalescent and post-vaccination sera.
B.1.1.7 cluster with E484K	202102/02	All the above plus E484K	United Kingdom, February 2021	▪ Likely similar to B.1.1.7 but with greater immune escape capacity.
B.1.351	202012/02	K417N, E484K, N501Y, D614G.	South Africa, October 2020 (reported on December)	▪ 50% increased transmission. ▪ Moderate impact on neutralization by monoclonal antibody therapeutics. ▪ Moderate reduction on neutralization by convalescent and post-vaccination sera.
P.1 (B.1.1.248)	202101/02	K417N/T, E484K, N501Y, D614G.	Japan ex Brazil, January 2021	▪ Moderate impact on neutralization by monoclonal antibody therapeutics. ▪ Reduced neutralization by convalescent and post-vaccination sera.
B.1.617	TBA	L452R, D614G, P681R.	India, October 2020	▪ Increased transmission ▪ Moderate impact on neutralization by monoclonal antibody therapeutics.

Lineages with scientific evidence of an increase in transmissibility, more severe disease (increased hospitalizations or deaths), significant reduction in neutralization by antibodies, reduced effectiveness of treatments or vaccines and/or diagnostic detection failures[12, 36, 37]. Only mutations affecting the Spike protein are listed in the table, but each of the variants detailed here carries other mutations in different areas of the genome.
TBA: to be assigned

Table II
SARS-CoV-2 variants of interest (VOI)

Pango lineage	VOI code	Defining mutations in Spike protein	First Detected	Ascribed attributes
P.2	202101/01	E484K, D614G, V1176F.	Brazil, April 2020	▪ Potential reduction in neutralization by monoclonal antibody treatments. ▪ Potential reduction in neutralization by convalescent and post-vaccination sera.
A.23.1 cluster with E484K	202102/01	R102I, F157L, V367F, E484K, Q613H, P681R.	United Kingdom, February 2021	
B.1.525	202102/03	A67V, Δ69/70, Δ144, E484K, D614G, Q677H, F888L.	New York, December 2020	
B.1.1.318	202102/04	T95I, Δ144, E484K, P681H, D796H.	TBC	
B.1.324.1 cluster with E484K	202103/01	E484K, S494P, N501Y, D614G, P681H, E1111K.	TBC	
B.1.526	TBA	(L5F*), T95I, D253G, (S477N*), (E484K*), D614G, (A701V*).	New York, November 2020	
B.1.427*	TBA	L452R, D614G.	US-California, June 2020	All the above plus: ▪ 20% increased transmissibility
B.1.429*	TBA	S13I, W152C, L452R, D614G.	US-California, June 2020	

They harbour specific genetic markers that have been associated with changes in receptor binding, reduced neutralization by antibodies, reduced efficacy of treatments, potential diag-nostic impact, increase in transmissibility or increase in disease severity[12, 36, 37]. Only mutations affecting the Spike protein are listed in the table, but each of the variants detailed here carries other mutations in different areas of the genome.
TBC: to be confirmed
TBA: to be assigned
(*) B.1.427 and B.1.429 have been classified by the CDC (USA) as VOC, however, at a global level and by the WHO, they have, for now, been classified as VOI[37].

Context

At the beginning of the year 2021, the B.1.177 lineage of SARS-CoV-2 was the most commonly found among the samples sequenced in Cantabria[2]. This was to be expected, given that this lineage was the most prevalent in Spain since shortly after its emergence in this country, during the summer of 2020[14]. However, since the first detection of B.1.1.7 variant in this region–on a specimen dated from December 23st 2020– this new VOC, which had allegedly arisen in the United Kingdom short time before[15], begun to progressively replace the previously majority variant. Thus, at the beginning of March 2021, 95% of samples analysed in this region of Spain produce Spike gene target failure (SGTF) with TaqPath COVID-19 RT-PCR assay (ThermoFisher)[16] and, most of them, were subsequently confirmed as B.1.1.7 by whole genome sequencing (WGS)[2].

In this background, health authorities in Cantabria devised and launched an active search system aimed at achieving the early detection of new SARS-CoV-2 variants. As part of this system, new technical approaches were incorporated into routine genetic testing at the Microbiology Service of the main hospital, which allow the detection of specific mutations of concern, such as E484K and N501Y, in a much shorter time than next generation sequencing (NGS) (see methods).

These techniques started to be applied in order to buy time, not to replace the WGS, which was in any case carried out at the same time, although providing results later. In a parallel effort, epidemiological surveillance duties in the Public Health Department were focused on identifying every situation that could mean a noteworthy probability of having a new SARS-CoV-2 variant circulating, following the next criteria:

- Imported cases. All cases with a confirmed active infection that had arrived from any other country within the previous 10 days would be rapidly identified, carefully followed (both cases and close contacts) and their samples immediately analysed (by PCR specific to detect mutations of concern and by WGS).
- Reinfected cases. All cases with two positive PDIAs separated by, at least, 90 days, and with a cured status between them, would have the two positive RNA specimens completely sequenced.
- Cases of possible escape to immunity. Although there is no evidence that any of the current mRNA COVID-19 vaccines can completely prevent people from being infected, all cases in Cantabria with a positive PDIA after a complete vaccination schedule would be considered as possible immunization failures their samples would be sequenced. Among them, those cases that presented symptoms would be prioritized, under the highest suspicion of being actual immunization failures.

Arrival of the P.1 variant to Cantabria

The PRIMARY CASE (see figure 1) was a 40-year-old woman who caught a plane in Cuiabá (Brazil) on 27/02/2021 bound for Sao Paulo (Brazil). That same day, she took a second flight from Sao Paulo (Brazil) to Madrid (Spain). She presented a negative PCR result at origin and, upon arrival at the Adolfo Suárez Barajas airport (Madrid), on 28/02/2021, she was subjected to an antigenic test, which also gave a negative result. She then took a third flight, Madrid-Bilbao (in the North of Spain, next to Cantabria). The journey from Bilbao to El Astillero (Cantabria) was done in a private vehicle driven by another person (later considered close contact). She arrived in Cantabria on the same day 28th February 2021.

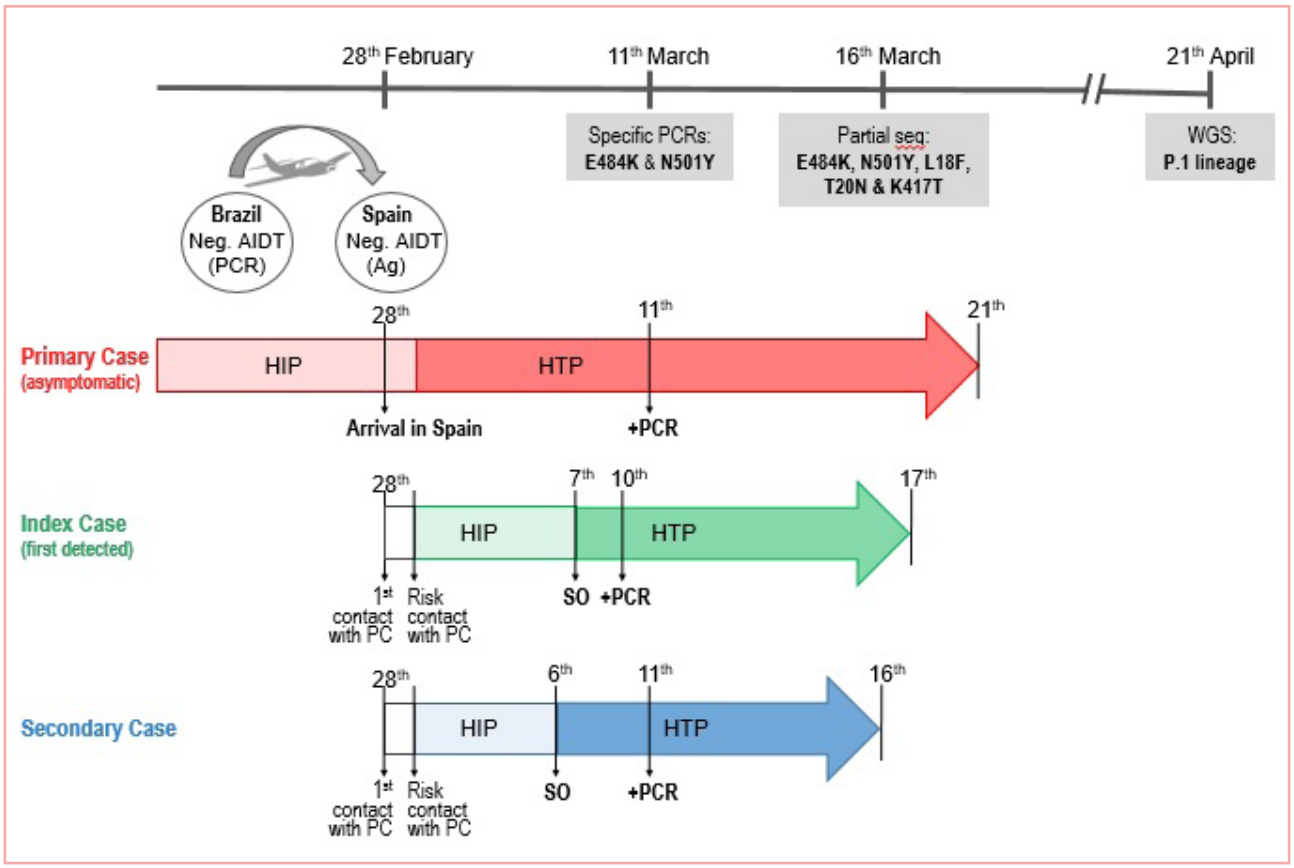

Figure 1. Chronology of the first cases of the P.1 variant detected in Cantabria.

Upon arrival, she started the mandatory quarantine, so she hardly has close contacts, except for two cohabitants (index and secondary cases, respectively) and the person who drove her from Bilbao. The contact tracing unit (from Cantabria's Government) found that she had only left home to go to a pharmacy, whose dependents were treated as casual contacts, subjecting them to a RT-PCR tests (with a negative result), without quarantine.

The *index case*, a 14-year-old woman, was the daughter and cohabitant of the primary case. She started feeling COVID-19 symptoms on 07/03/2021 and a positive PDIA came out on 10/03/2021 (figure 1). Up to that moment, she had not been obliged to quarantine, so she had close contacts in different scopes: educational, social (including an academy) and familiar.

During the contact-tracing interview, the (indirect) link of this case with Brazil was discovered and registered in databases. This was the signal which triggered a contingency plan. The primary case (described above) was considered, for the moment, a close contact of this one. She was still completely asymptomatic.

As the contingency plan had been activated, while all contacts were sampled by nasopharyngeal smear, and subsequent habitual RT-PCR tests for COVID-19 diagnosis were performed, addition-al laboratory tests were started over the positive RNA sample (the one from the index case), trying to find a clue as to which variant of the virus it was. The presence of the E484K mutation in the viral RNA was tested by means of a specific PCR (see methods). If the substitution were found, a specific PCR to detect N501Y mutation plus a partial sequencing of the whole S gene (a technique which is faster than WGS and more complete than the mere presence of a single ge-netic marker to elucidate the specific variant) would be performed. Sequencing of the complete viral genome was urgently requested, as well.

The *secondary case* (figure 1), a 25-year-old woman, was the niece of the primary case, cousin of the index case, and cohabitant of both. She was studied (like the primary case) as a close con-tact of the index case, despite the fact that she had started symptoms the day before, on 06/03/2021. However, she had not associated these symptoms with COVID-19 and had led a normal life. For instance, she had attended an educational center (different from that of the primary case) between 08/03/2021 and 10/03/2021. In addition, she had made two bus trips, within Cantabria (El Astillero-Santander, round trip), each one lasting about 30 minutes. She had also visited several supermarkets on 10/03/2021, as well as a pharmacy on the same day. Em-ployees of these establishments were subjected to PCR without quarantine, as casual contacts.

Genetic results and triggering of outbreak control mechanism

On 11/03/2021 several laboratory results came out (see figure 1), showing that:

1. The viral genetic material obtained from the index case did carry the E484K mutation.
2. The two cohabitants of the index case (primary and secondary cases, respectively) test-ed positive for COVID-19.
3. Immediately after this result, the RNA specimens from the primary and secondary cases had been tested for the E484K and N501Y substitutions, which had been detected in both.

The E484K mutation is a G>A transition at 23012 position of the viral genome that entails an ami-no acid change in the S protein of the SARS-CoV-2 virus. It has been reported to be present in, at least, 9 different SARS-CoV-2 variants[17] (tables 1 and 2). Indeed, the fact that this substitution is being incorporated by different lineages, apparently without connection, in different areas of the world, seems to indicate that it likely confers an adaptive advantage to the virus. Therefore, the mere presence of this genetic marker does not allow a differential discrimination of any spe-cific variant. However, in that situation, given the epidemiological link of the cases with Brazil, it was considered highly probable that it was P.1 or P.2. The second one (P.2) is considered –as the rest of lineages harbouring the E484K mutation– variant of interest (VOI) (table 1). However, among this two options, the presence of N501Y mutation suggested that it was P.1 variant, whose genetic profile has led to term it variant of concern (VOC)[12] (table 2). Consequently, a maximum containment protocol was activated in Cantabria, while molecular studies on the sam-ples continued, in order to obtain, as soon as possible, deeper information.

As part of this containment protocol, the usual definition of close contact was expanded to in-clude casual contacts, which would not be quarantined but would be subjected to PDIA. In addi-tion, trace-back investigations were initiated with the primary case, to trace all contacts since her arrival to Cantabria, taking into account that she was asymptomatic. In this way, a total of 92 con-tacts were studied, mainly belonging to the index and secondary cases, given that the primary one had remained quarantined, so had a small number of contacts. In addition, an intensive sur-veillance system was initiated to closely follow cases and contacts, with a dual purpose: identify any quarantine breach (and, when appropriate, identify the possible cause and solve it) and de-tect any onset of symptoms. When a symptom compatible with COVID-19 was identified in a contact, it would be subjected to a new nasopharyngeal smear followed by RT-PCR test.

On 16/03/2021 new laboratory results came out, this time from targeted partial sequencing of the S gene (see figure 1), which made it possible to dismiss that the variant was P.2, since the 3 samples (coming from index, primary and secondary cases, respectively) carried E484K, N501Y, L18F, T20N and K417T mutations, among others. The whole nucleotide sequence of all analysed regions was homologous to that described for the P.1 variant. Thus, the fence tightened

around P.1, although the definitive confirmation will come with the results of the sequencing of the complete genome (something that may take several more days).

On 21/04/2021 whole genome sequencing results were received (see figure 1). Index case was confirmed as P.1 lineage carrier (GISAID accession ID: EPI_ISL_1908182), although inconclusive results were obtained for the other two cases, probably due to low viral load.

Outbreak Evolution

The three cases with a COVID-19 diagnosis remained in isolation at home until the end of the transmissibility period. Two of them had mild symptoms (without requiring hospitalization) and one of them (the imported case) always asymptomatic.

All close contacts were followed up by public health technicians until the end of each incubation period (10 days after the last risk contact). On day 9, they underwent a second nasopharyngeal smear and, once the second negative PCR result was obtained, all of them were epidemiologically discharged.

The outbreak was considered non-active since 22/03/2021 and closed on 05/04/2021. P.1 variant remained in a single dwelling with three cases.

Prevention and control actions. Public health response

A total of 92 contacts was identified and carefully monitored. The distribution of these contacts was as follows:

- The imported (and primary) case had:
  - 1 close contact: the person who drove her from the airport (Bilbao) to her home, a trip lasting about an hour.
  - 1 casual contact: the clerk who treated her at a pharmacy, with all the necessary security measures.
- The index case had:
  - 56 close contacts, which were distributed as follows:
    = 34 with an exposure in the educational field (forma education)
    = 10 with an exposure in a different educational aria: no-formal academy
    = 12 with a social exposure.
  - 4 casual contacts:
    = 2 with an exposure in the no-formal academy, but with an aloof and brief contact.
    = 1 with a social exposure, also aloof and brief (a friend's mother).
    = 1 with occupational exposure, with all the necessary security measures (the teacher)
- The secondary case had:
  - 25 close contacts:
    = 24 with an exposure in the educational field (formal education)
    = 1 due to a social interaction.
  - 5 casual contacts, all of them employees of pharmacies and supermarkets, with an aloof contact and all security measures.

Close contacts were informed and supported in self-quarantine needs. All of them received pe-riodic phone calls, in order to detect any possible sign of quarantine breach, as well as any onset of symptoms. In case of the latter, they were COVID-19 PDIA. Conversely, casual contacts were only quarantined only until having the COVID-19 PDIA result. As all of then tested negative, re-ceived the epidemiological discharge.

Weak points in outbreak control actions

The secondary case made two bus trips (same route, round trip) within the infectivity period, with a duration of 30 minutes each journey, which could not be traced, following the guidelines of the Spanish Government regarding contact tracing in means of transport. Taking into account the time slots in which the trips took place, it is expected that the bus was crowded. For this rea-son, the possibility that transmission of the P.1 lineage has occurred in this medium cannot be rejected, while it is true that, for the moment, this variant has not been detected in Cantabria in non-imported cases, despite the local active searching efforts.

Methods

The personal and epidemiological data of each positive case and each contact were collected by the Epidemiological Surveillance Unit. These data were recorded and anonymized in the Go.Data application, a tool designed by the WHO for outbreaks investigation and contact tracing during public health emergencies.

Mutations E484K and N501Y were detected through an assay based on "*allele-specific PCR*" technology, but applied, in this case, to identify a viral single nucleotide polymorphism (SNP). Briefly, two DNA self-designed probes were used, together with a primer pair, in a PCR reaction. In E484K assay, one of the probes hybridizes with the wild type sequence (G nucleotide) and the other, with the mutated one (A nucleotide). Similarly, in N501Y assay, one of the probes hybrid-izes with the wild type sequence (A nucleotide) and the other, with the mutated one (T nucleo-tide). Each probe of each specific assay incorporates a reporter fluorophore that emits radiation with a different wavelength.

Targeted sequencing of the S gene was done using specific RT-PCRs for marker regions of the Spike gene, following a protocol described by Geneva University [18]. Products of RT-PCR were sequenced by Eurofins (Hamburg, Germany) with Sanger technology.

Whole genome amplification for sequencing was performed by Spanish National Center for Mi-crobiology (CNM, National Health Institute Carlos III). The RNA preparations were amplified by means of reverse-transcriptase PCR (RT-PCR) with the use of random primers[19, 20], followed by the amplification of the cDNA products, according to ARTIC network's PCR protocol, based on the use of the primer pool "*ARTIC nCoV-2019 v3*"[21]. In this case, libraries were performed ac-cording to Nextera DNA library preparation kit instructions

(Illumina, California, USA). Libraries were barcoded with unique dual indexes, pooled and sequenced using a NextSeq Reagent kit 300 cycles (Illumina, California, USA) in a NextSeq sequencer (Illumina, California, USA). The se-quences are available in GISAID[2].

Viral consensus genomes were obtained using a mapping against viral reference genome ap-proach, followed by variant calling and consensus genome generation. This pipeline, called Vi-ralrecon[22], was written using Nextflow framework[23] in collaboration with the nf-core team[24]. FastQC files containing raw reads were first analyzed for quality using FastQC v0.11.9[25]. Raw reads were trimmed for low-quality 3′ ends and adapter sequence removal using fastp v.0.20.1[26]. Trimmed reads were mapped against the reference SARS-Cov2 genome (NC_045512.2) with bowtie2 v.2.3.5.1[27], and the mapping files coming from amplicon sequenc-ing were trimmed with iVar v.1.2.2[28] to remove amplicon primers. We used Picard v.2.22.8 (https://github.com/broadinstitute/picard) and SAMtools v.1.9[29] to generate viral mapping stats. To obtain statistics about the host genome content, we performed kmer-based mapping of the trimmed reads against the GRCh38 NCBI human genome reference with Kraken2 v.2.0.9beta[30].

Discussion

Since last February 17th 2021, travellers arriving in Spain from some specific countries, among which Brazil is included, are bound, by ministerial order[31, 32], to quarantine for 10 days. The outbreak presented here shows that this measure is necessary, given that the rest of the non-pharmaceutical measures aimed at preventing the import of risk variants appear to be failing in their purpose.

Spanish Government requires, since November 23rd 2020[33], a negative PCR result to all pas-sengers who enter the country by air or sea from countries considered at risk for COVID-19 dis-ease. The analysed biological sample must have been taken within the previous 72 hours before the arrival in Spain. A list of countries considered at risk is available on official websites[34, 35] and updated every fifteen days. Furthermore, at the arrival airport –once in Spain– random anti-gen tests are carried out. The primary case of this outbreak passed these controls: she had a negative PCR result from Brazil and, as soon as she landed in Spain, she was subjected to an anti-gen test, which was, likewise, negative. In addition, she never felt symptoms of illness, fact that delayed the diagnosis. Not until a secondary case developed the disease was the primary one detected. This fact has two drawbacks: First, it is more difficult to find the link to a country at risk interviewing a case that has not traveled. On this occasion, the contact tracing system was effec-tive and made possible to identify the indirect link with Brazil which triggered the alarms. Second, whilst the primary case is not diagnosed, their co-habitants are not quarantined, increasing the risk of spreading the disease and generating tertiary cases. With this in mind, perhaps the possi-bility of extending the quarantine to traveller's co-habitants should be considered.

In another vein, the necessity of finding laboratory methods that allow the differential identifica-tion of SARS-CoV-2 variants in a reasonable lapse of time has become evident. Develop the ca-pacity to obtain genomic information as complete as possible in the shortest possible time seems now essential. The detection of a small number of specific mutations can be done quickly in al-most any molecular biology laboratory, but the information is often insufficient. In the study of this outbreak, the delay of weeks to obtain WGS results has been faced, and relatively solved acting from the precautionary principle, that was, assuming that the variant was of concern ra-ther than waiting for a conclusive confirmation.

Finally, taking into account all data presented here, it might seem surprising the extremely low capacity of transmission that this strain of the SARS-CoV-2 virus has shown to have. Leaving aside the casual contacts, the total size of this outbreak has been 3 cases and 82 close contacts, which gives a secondary attack rate of only 3,5%. This can be easily noticed in figure 2 (although all con-tacts are represented, including casual ones). From the moment that P.1. lineage was described, several works[2-8] have suggested an ability to evade the immune response, but not much has been said about its transmission capacity, which could be, actually, limited.

Justification of the number of authors

The number of authors who sign this paper is higher than 6 because it has been a multidisciplinary work, which has encompassed epidemiological surveillance, public health actions, as well as microbiological, genetic and genomic analyses. A large number of specialists in different fields have had to work coordinately. The contribution of all of them has been essential. None of the authors has conflicts of interest to disclose.

Contributions

SCO managed the procedure followed with imported cases, the activation of a containment protocol, compiled the genomic and epidemiologic data, supervised the actions taken, followed the outbreak evolution, conceived the work and wrote the manuscript. JRL, SV and MG performed some of the genetic analyses and raised the alarm as soon as the E484K mutation was identified in the index case. AHA supported methodologically the epidemiological investigation, the exploiting the Go.Data tool as well as the ethical committee approval. He also conceived the work. MIC performed the Whole Genome Sequencing and the lineage assignation. GB performed the contact tracing of the index case, discovered the link with Brazil and gave immediate notice to SCO to initiate the appropriate actions. Later, she followed up contacts in the non-educational scope. JP and ED tracked and followed up contacts in education scope and managed actions in this area.

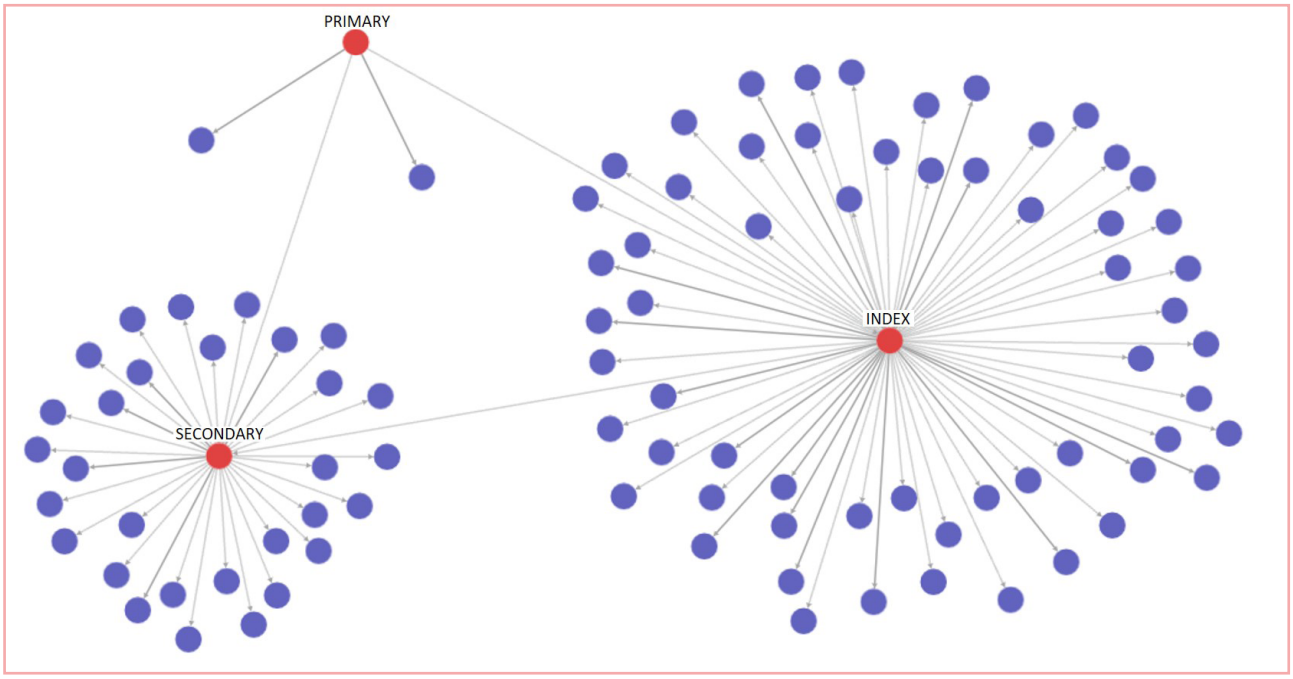

Figure 2. Graphic representation of the hierarchical relationship between cases (red) and close contacts (blue).

MLG and AF solved incidents and gave support in the elaboration of the figure 1. TL gave technical support with Go.Data tool and obtained the figure 2. JCM and RW devised and launched the Genomic Surveillance Plan in Cantabria, conceived the work, supervised the whole process and revised the final version of the manuscript. RW gave the order to activate a maximum containment protocol. All authors critically revised the manuscript, providing important intellectual contributions, and approved its final version.

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Conflicts of interest
None.

References

1. WHO - World Health Organization., "WHO | Pneumonia of unknown cause – China." [Online]. Available: https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/. [Accessed: 02-Apr-2021].
2. "GISAID - Initiative." [Online]. Available: https://www.gisaid.org/. [Accessed: 26-Mar-2021].
3. N. R. Faria et al., "Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings - SARS-CoV-2 coronavirus," Virological.org, vol. January 12, 2021.
4. A. F. Martins et al., "Detection of SARS-CoV-2 lineage P.1 in patients from a region with exponentially increasing hospitalisation rate, February 2021, Rio Grande do Sul, Southern Brazil," Eurosurveillance, vol. 26, no. 12, p. 2100276, Mar. 2021, doi: 10.2807/1560-7917.ES.2021.26.12.2100276.
5. L. F. Buss et al., "Three-quarters attack rate of SARS-CoV-2 in the Brazilian Amazon during a largely unmitigated epidemic," Science (80-.)., vol. 371, no. 6526, pp. 288–292, Jan. 2021, doi: 10.1126/science.abe9728.
6. C. M. Voloch et al., "Genomic characterization of a novel SARS-CoV-2 lineage from Rio de Janeiro, Brazil," medRxiv. medRxiv, p. 2020.12.23.20248598, 26-Dec-2020, doi: 10.1101/2020.12.23.20248598.
7. J. M. Bursky, R. E. Chen, and X. Zhang, "Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies," doi: 10.1038/s41591-021-01294-w.
8. A. J. Greaney et al., "Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition," Cell Host Microbe, vol. 29, no. 1, pp. 44-57.e9, Jan. 2021, doi: 10.1016/j.chom.2020.11.007.
9. ECDC - Eurpoean Centre for Disease Prevention and Control;, "Risk related to spread of new SARS-CoV-2 variants of concern in the EU/EEA," 2020.

10. T. Golubchik and K. A. Lythgoe, "Early analysis of a potential link between viral load and the N501Y mutation in the SARS-COV-2 spike protein," medRxiv, vol. 1, no. 2, p. 2021.01.12.20249080, Jan. 2021, doi: 10.1101/2021.01.12.20249080.
11. H. Tegally et al., "Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa," medRxiv, vol. 10. medRxiv, p. 2020.12.21.20248640, 22-Dec-2020, doi: 10.1101/2020.12.21.20248640.
12. CDC - Centers for Disease Control and Prevention;, "SARS-CoV-2 Variant Classifications and Definitions." [Online]. Available: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#Interest. [Accessed: 24-Mar-2021].
13. E. C. Sabino et al., "Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence," The Lancet, vol. 397, no. 10273. Elsevier B.V., pp. 452–455, 06-Feb-2021, doi: 10.1016/S0140-6736(21)00183-5.
14. J. L. Guthrie et al., "Detection of the novel SARS-CoV-2 European lineage B.1.177 in Ontario, Canada," J. Clin. Virol. Plus, vol. 1, no. 1–2, p. 100010, Jun. 2021, doi: 10.1016/j.jcvp.2021.100010.
15. A. Rambaut et al., "Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations - SARS-CoV-2 coronavirus / nCoV-2019 Genomic Epidemiology - Virological," Virological.org, 2020. [Online]. Available: https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563. [Accessed: 26-Mar-2021].
16. V. Borges et al., "Tracking SARS-CoV-2 VOC 202012/01 (lineage B.1.1.7) dissemination in Portugal: insights from nationwide RT-PCR Spike gene drop out data - SARS-CoV-2 coronavirus - Virological," Virological.org, 2021. [Online]. Available: https://virological.org/t/tracking-sars-cov-2-voc-202012-01-lineage-b-1-1-7-dissemination-in-portugal-insights-from-nationwide-rt-pcr-spike-gene-drop-out-data/600. [Accessed: 02-Apr-2021].
17. The Economist, "It's a family affair - The same covid-19 mutations are appearing in different places | Graphic detail | The Economist," 2021. [Online]. Available: https://www.economist.com/graphic-detail/2021/02/27/the-same-covid-19-mutations-are-appearing-in-different-places. [Accessed: 24-Mar-2021].
18. Université.de.Genève, "Protocol for specific RT-PCRs for marker regions of the Spike region indicative of the UK SARS-CoV-2 variant B.1.1.7 and the South African variant 501Y.V2," 2020.
19. G. Palacios et al., "Panmicrobial Oligonucleotide Array for Diagnosis of Infectious Diseases," Emerg. Infect. Dis., vol. 13, no. 1, pp. 73–81, 2007, doi: 10.3201/eid1301.060837.
20. U. Pérez-Sautu et al., "Target-independent high-throughput sequencing methods provide evidence that already known human viral pathogens play a main role in respiratory infections with unexplained etiology," Emerg. Microbes Infect., vol. 8, no. 1, pp. 1054–1065, Jan. 2019, doi: 10.1080/22221751.2019.1640587.
21. J. Quick, "ARTIC Coronavirus Method Development Community 1 more workspace Illumina Nextera DNA Flex library construction and sequencing for SARS-CoV-2: Adapting COVID-19 ARTIC protocol NEBnext library construction and sequencing for SARS-CoV-2: Adapting COVID-19 ARTIC protocol," Apr. 2020, doi: 10.17504/protocols.io.bdp7i5rn.
22. "GitHub - nf-core/viralrecon: Assembly and intrahost/low-frequency variant calling for viral samples." [Online]. Available: https://github.com/nf-core/viralrecon. [Accessed: 20-May-2021].
23. "Nextflow - A DSL for parallel and scalable computational pipelines." [Online]. Available: https://www.nextflow.io/. [Accessed: 20-May-2021].
24. P. A. Ewels et al., "The nf-core framework for community-curated bioinformatics pipelines," Nature Biotechnology, vol. 38, no. 3. Nature Research, pp. 276–278, 01-Mar-2020, doi: 10.1038/s41587-020-0439-x.
25. "Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data." [Online]. Available: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. [Accessed: 20-May-2021].
26. S. Chen, Y. Zhou, Y. Chen, and J. Gu, "Fastp: An ultra-fast all-in-one FASTQ preprocessor," in Bioinformatics, 2018, vol. 34, no. 17, pp. i884–i890, doi: 10.1093/bioinformatics/bty560.
27. B. Langmead and S. L. Salzberg, "Fast gapped-read alignment with Bowtie 2," Nat. Methods, vol. 9, no. 4, pp. 357–359, Apr. 2012, doi: 10.1038/nmeth.1923.
28. N. D. Grubaugh et al., "An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar," Genome Biol., vol. 20, no. 1, Jan. 2019, doi: 10.1186/s13059-018-1618-7.
29. H. Li et al., "The Sequence Alignment/Map format and SAMtools," Bioinformatics, vol. 25, no. 16, pp. 2078–2079, Aug. 2009, doi: 10.1093/bioinformatics/btp352.
30. D. E. Wood, J. Lu, and B. Langmead, "Improved metagenomic analysis with Kraken 2," Genome Biol., vol. 20, no. 1, pp. 1–13, Nov. 2019, doi: 10.1186/s13059-019-1891-0.

31. Gobierno.de.España - Ministerio.de.Sanidad, "BOE.es - BOE-A-2021-2458 Orden SND/133/2021, de 17 de febrero, sobre las condiciones de cuarentena a las que deben someterse las personas procedentes de la República Federativa de Brasil y la República de Sudáfrica a su llegada a España, durante la situac," 2021. [Online]. Available: https://www.boe.es/buscar/doc.php?id=BOE-A-2021-2458. [Accessed: 08-Apr-2021].
32. Gobierno.de.España-Ministerio.de.Sanidad, "BOE.es - BOE-A-2021-3310 Orden SND/181/2021, de 2 de marzo, sobre las condiciones de cuarentena a las que deben someterse las personas procedentes de países de riesgo, durante la situación de crisis sanitaria ocasionada por el COVID-19." [Online]. Available: https://www.boe.es/diario_boe/txt.php?id=BOE-A-2021-3310. [Accessed: 08-Apr-2021].
33. Gobierno.de.España-Ministerio.de.Sanidad, "BOE.es - BOE-A-2020-14821 Resolución de 23 de noviembre de 2020, de la Subsecretaría, por la que se publica la Adenda al Convenio entre la Dirección General de Salud Pública y AENA, S.M.E, SA, para el control sanitario de la entrada de pasajeros de vuelos," 2021. [Online]. Available: https://www.boe.es/diario_boe/txt.php?id=BOE-A-2020-14821. [Accessed: 08-Apr-2021].
34. "Spain Travel Health." [Online]. Available: https://www.spth.gob.es/. [Accessed: 08-Apr-2021].
35. "Ministerio de Sanidad, Consumo y Bienestar Social." [Online]. Available: https://www.mscbs.gob.es/. [Accessed: 08-Apr-2021].
36. PHE-Public.Heath.England, "SARS-CoV-2 variants of concern and variants under investigation in England," 2021.
37. WHO - World Health Organization., "COVID-19 Weekly Epidemiological Update Global overview."