Confirmed Severe Acute Respiratory Syndrome Coronavirus 2 Reinfections After a Second Wave With Predominance of Lambda in Lima and Callao, Peru

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Background. Coronavirus disease 2019 (COVID-19) infection is a major public health problem in the world and reinfections are becoming more frequent. Our main objective was to describe the epidemiological, clinical, and genomic characteristics of the confirmed cases of reinfection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the capital of Lima and Callao, Peru.

Methods. We searched in the Peruvian laboratory information system from April 2020 up to May 2021, looking for cases having 2 positive molecular tests for SARS-CoV-2 with more than 90 days between them. We performed genomic sequencing to the available pairs of samples and described the clinical characteristics, epidemiological impact, and genomic analysis of the confirmed reinfections.

Results. There were 1 694 164 people with a positive diagnostic test for SARS-CoV-2 in Lima/Callao during the study period. Of these, 1695 had 2 positive molecular tests with more than 90 days between them. Two hundred eleven had both samples available for genomic analysis according to our selection criteria, and these were retrieved and submitted to sequencing. Thirty cases were confirmed to be SARS-CoV-2 reinfections with 2 different lineages in the 2 episodes. The variant Lambda (C.37) was the most common during the second infection and accounted for 19 (63.3%) of the 30 cases.

Conclusions. We report 30 cases of confirmed SARS-CoV-2 reinfections. The Lambda variant was the most common cause of the second infections, in concordance with its predominant circulation during Peru's second wave. This report describes the largest series of confirmed reinfections by SARS-CoV-2 in Latin America.

Keywords. SARS-CoV-2; Peru; SARS-CoV-2 C.37 variant; reinfection; coronavirus.

More than 220 million cases of COVID-19 have been reported worldwide [1]. In Peru, 2 017 968 cases had been reported by the end of May 2021, approaching the end of a devastating second contagion wave [2]. The main characteristic of this period was its high lethality at approximately 9.4% [3]. This huge epidemiological impact had not been foreseen by the health authorities or the community itself, and one of the reasons was the reliance on the protection acquired by the immunity built in the community during the first wave of the epidemic. Some seroprevalence studies in the country showed that during this first wave, 20%–70% of the population had already been exposed to the virus [4, 5], and there were many studies that showed that protection conferred by previous infection was highly effective [6].

In this context, areas with high seroprevalence of COVID-19 were observed to be "protected," and few cases of confirmed SARS-CoV-2 reinfection were reported with genomic sequencing, with little impact on public health at the end of the first pandemic wave [7]. At a global level, the first confirmed case of reinfection was published in Hong Kong [8], and since then, more cases have been confirmed through genomic analyses at the global level [7], 1 of them from Peru [9]. However, this varied when populations that were highly exposed at the end of the first wave, such as Manaus in Brazil, presented high rates of infection during their "second wave". These ravaging successive waves showed that reinfection cases were possible with new variants and waning of immunity, indicating that the scarcity of scientific reports did not reflect the real epidemiology of reinfections [10].

To confirm the reinfections, both respiratory samples—the sample from the first and second infection—must undergo genomic sequencing, and if they present different clades, SARS-CoV-2 reinfection is confirmed [11]. This means having both samples to be able to sequence them, and not all countries retain their positive samples; therefore, in most cases, this confirmation is not possible [12]. Another important constraint is the viral degradation of the samples over time, even when they were kept according to standardized procedures, which makes their genomic sequencing difficult. These bottlenecks for confirmation of reinfections make it difficult to track the real magnitude of the problem, most of all in settings that were barely hit by the pandemic [9]. The objective of this study was to describe the epidemiological, clinical, and genomic characteristics of the confirmed cases of reinfection by SARS-CoV-2 in Lima and Callao, Peru.

METHODS

Study Population

We included subjects with 2 episodes of laboratory-confirmed SARS-CoV-2 infection by reverse-transcriptase polymerase chain reaction (RT-PCR) with 90 or more days between both episodes and presence of 2 different genetic clades of the virus between the first and second samples, according to the PANGOLIN case classification system registered in Nextstrain and GISAID [10].

To identify cases, we use a national database (from the Information System of the National Network of Public Health Laboratories of Peru [NetLabv2]) for the following: (1) positive cases; (2) possible reinfections, which were defined as patients with a positive test (serological [if not vaccinated], antigenic, or molecular) and a second positive test (antigenic or molecular) after >90 days; and (3) probable reinfections, defined as patients with 2 tests positive molecular tests after >90 days (as suggested above) for resource-limited settings [10]. The definitions of possible reinfections and probable reinfections were used as a search strategy until the cases were prioritized for sequencing, and these were also based on current Centers for Disease Control and Prevention definitions [13].

The search for cases was carried out from March 2020 (beginning of the pandemic) to May 2021 (end of the second wave in Perú of lambda predominance) and was limited to Lima (capital city) and Callao because, in addition to the availability for sequencing, they represent approximately 70% of molecular tests performed during the pandemic.

We verified the availability of the pair of samples and the fulfillment of the laboratory requirement of having a cycle threshold (Ct) (RT-PCR Ct) ≤33 [11]. If both samples were available, we proceeded with the genomic sequencing and reviewed their clinical registers, patients were called by phone when necessary, prior consent.

Genomic Analysis

The selected samples were sequenced in an Illumina NextSeq 550 system, and previous preparation of genomic libraries was done with the Illumina COVIDSeq Test Kit. The resulting fastq files were mapped with the Wuhan-Hu-1 reference genome (GenBank accession number NC_045512.2) with the BWA program [14]. The base call was made with a coverage depth of 100× followed by manual editing with the Integrative Genomic Viewer (IGV) program. The acceptance limit of unread nucleotides (N) per genome was 15%, and each base was supported by a read quality of 30. This operation was carried out with the sam-tools and ivar program. The identification of lineages was carried out with the Pangolin program (https://cov-lineages.org/). The resulting genomes were aligned with the MAFFTV.7 program with genomes downloaded from the GISAID database (https://www.gisaid.org/) belonging to the same lineages. A phylogenetic tree was obtained with the RAxML v8.2.10 program using the maximum likelihood methodology with a node support based on 1000 bootstrap repetitions. The resulting phylogenetic tree was edited in FigTree [15] (Supplemental Table 2).

Statistical Analysis and Ethical Considerations

All personal identifiers were removed and encrypted. Categorical variables were reported as absolute and relative frequencies. Numerical variables were reported through measures of central tendency and dispersion according to normality. Stata software version 17.0 (StataCorp, College Station, TX) was used for statistical analyses.

Ethical Considerations

The study respects the ethical principles in research according to the Helsinki Standards. The study received authorization from the Institutional Research Ethics Committee of the National Institute of Health (Registry No. 25618-20) and was carried out under the context of surveillance of reinfections. Informed consent was obtained from the subjects. The protocol of this article has been entered in the Registry of Health Research Projects of Peru (PRISA) through the code EI00000002038.

RESULTS

From March 2020 to May 2021, there were 1 694 164 people with a positive test for SARS-CoV-2 in the provinces of Lima and Callao. A total of 21 614 (1.27%) were cases of possible reinfection and 1695 (0.1%) cases of probable reinfection. The prevalence of possible and probable COVID-19 reinfections in the provinces studied by date can be found in Supplementary Material Figure S1.

Of this latter population, 211 had paired samples available with Cts ≤30 and were sequenced. We identified 30 cases with confirmed SARS-CoV-2 reinfection (Figure 1). Most of the cases were between 30 and 49 years old and were men. No
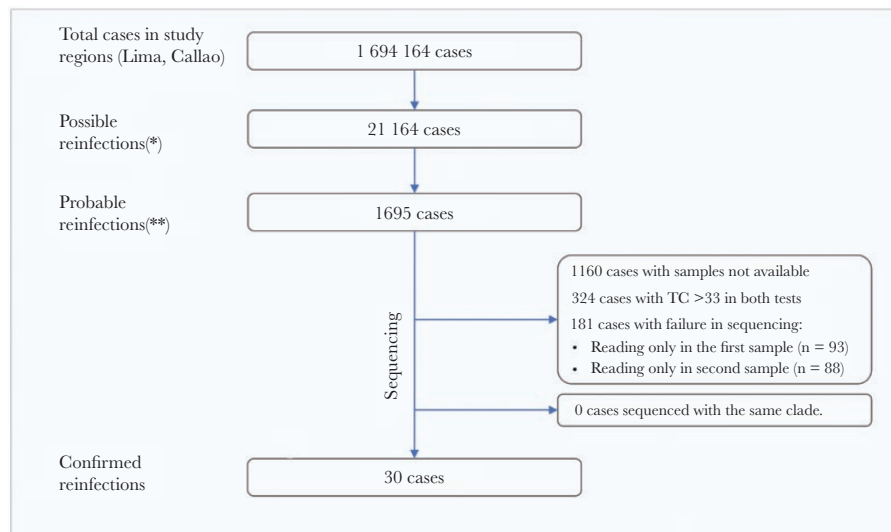
Figure 1. Inclusion of cases of reinfection due to severe acute respiratory syndrome coronavirus 2 in the cities of Lima and Callao.

deaths were reported. A total of 66.6% were people whose occupation was health personnel or police or military personnel, and most of the cases presented comorbidities (73.3%), mainly overweight. The sequenced variants that caused the first episode were mainly B1.1 (30%) and B.1.1.1 (16.7%), and variants that caused the second episode were mainly C.37 (63%), C.37.1 (10%), and P1 (10%) (Table 1).

Table 2 describes the clinical characteristics of both episodes of infection (Supplemental Table 1). In general, the second episodes of infection were mostly mild cases, although we could not report statistical differences between both groups ($P = .30$). The average time to reinfection was more than 6 months (Supplemental Tables 2–4).

The Figure 2B shows the lineages of the first and second episodes of reinfection. Twenty-two of thirty reinfections (73.3%) were due to the Lambda variant of interest (19 corresponding to C.37 and 3 to C.37.1). There were 5 cases of reinfection after 15 days of the second dose of BBIBP-CorV vaccine against COVID-19 ("Sinopharm"); 3 of these were reinfected with the Lambda variant and 2 with the Gamma variant (P.1) (Figure 2, Supplemental Table 3, Supplemental Table 5, Supplemental Table 6). Most reinfections occurred during the devastating second wave of the Peruvian epidemic, which lasted from February to June 2021 (Supplemental Table 5, Figure 3).

DISCUSSION

This constitutes the largest series of SARS-CoV-2 reinfections confirmed through genomic sequencing in Latin America. We have previously addressed the issue of an existing gap in the quantification of the real number of reinfections occurring in the community, due to diagnostic constraints [10]. However, we were able to report 30 cases of reinfection during the second wave of infections in the capital of Peru. This was possible due to more than 1 factor, among which the massive burden of disease due to COVID-19 experienced in Peru was probably the most important [2]. The vast predominance of the lambda variant during the second wave, with its ability to evade humoral response due to previous infections, has also played a relevant role. Finally, the ability of the National Institute of Health of Peru to keep some samples of positive cases all throughout the pandemic, which ensured adequate sampling, transport, and maintenance of the specimens, was an important factor.

Among the main characteristics of our series, we note that most people had mild or asymptomatic COVID-19 in both episodes. Although the presence of milder symptoms cannot be extrapolated outside our 30 cases, this could also be true for the majority of reinfections [15]. Besides our series, this finding was also observed in the report by Abu-Raddad et al [16], who found a 90% lower occurrence of hospitalization or death in reinfections than in primary infections in Qatar [17]. It is noteworthy that the percentage of population accessing vaccination was low during these months [16]; however, we had some cases of vaccinated people who were reinfected. In addition, a high percentage of health personnel and police and/or military personnel was represented in this series, which is probably due to a combination of a higher exposure to infection in these groups [5] and better access to diagnostic testing.

Another important aspect is that we observed a greater genomic diversity during the first SARS-CoV-2 episodes, as evidenced through a greater number of lineages present than in the group of the second infections. This is likely a reflection of the multiple introductions of SARS-CoV-2 to the country [18], and the massive predominance of the Lambda variant during the second wave of infections, despite the parallel circulation of

Table 1. Characteristics of Confirmed SARS-CoV-2 Reinfections After a Second Wave With Predominance of Lambda in Lima and Callao, Peru

Patient's Characteristics	N (%)
Age (years)[a]	35 [32–44]
Age Group	
Less than 29 years	04 (13.3)
30–49 years	22 (73.4)
50 or more	04 (13.3)
Sex	
Male	20 (66.7)
Occupation	
Health worker	10 (33.3)
Police/military	10 (33.3)
Other	10 (33.3)
Comorbidities[b]	
Yes	22 (73.3)
Overweight	18 (62.1)
Obesity	03 (10.0)
High blood pressure	1 (3.3)
Asthma	1 (3.3)
Chronic lung disease	1 (3.3)
Vaccination Status	
No vaccine received	22 (73.3)
1 dose of vaccine	03 (10.0)
2 doses of vaccine	05 (16.7)
Lineages Sequenced During the First Episode	
B.1.1	9 (30.0)
B.1.1.1	5 (16.7)
C.14	5 (16.7)
C.37 (lambda)	3 (10.0)
B.1	2 (6.7)
C.32	2 (6.7)
B.1.1.372	1 (3.3)
B.1.205	1 (3.3)
C.13	1 (3.3)
C.4	1 (3.3)
Lineages Sequenced During the Second Episode	
C.37 (lambda)	19 (63.3)
C.37.1	3 (10.0)
P.1 (gamma)	3 (10.0)
B.1.1.1	2 (6.7)
B.1.1.348	2 (6.7)
B.1	1 (3.3)

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Median [interquartile range].

[b]Comorbidities: asthma, hypertension, tuberculosis, overweight (body mass index 25–30 Kg/m^2), obesity (body mass index >30 Kg/m^2).

other variants of concern [19]. The World Health Organization considered it a variant of interest since June, due to its epidemiological impact in South America and its unique combination of mutations [20] (7 mutations in the spike protein [Δ247-253, G75V, T76I, L452Q, F490S, D614G, T859N]; L452Q is similar to the L452R mutation reported in Delta and Epsilon variants). Acevedo et al [21] reported an increase in the infectivity of the Lambda variant, and immune escape of the neutralizing antibodies [22] and its greater infectivity could be attributed to the T76I and L452Q mutations in the protein S reported by Kimura

et al [21, 23]. It is interesting to note that Lambda (3 cases) and Gamma (2 cases) achieved immune evasion in people who already had hybrid immunity (natural infection plus vaccine) [23]. Continuous genomic surveillance has helped us to better visualize the impact of new variants in our territory, such as delta, which predominated in the second half of 2021, but with little epidemiologic impact [19], unlike omicron, which has produced reinfections massively in many parts of the globe [24].

Differences in immunity generated through vaccines and natural infections have been widely described [25]. Worldwide, before the omicron wave, cases of reinfections had been reported in small amount [7], whereas cases of vaccine breakthroughs were described as percentages of the millions of cases occurring during the third, fourth, and fifth waves of several countries [26]. Three key factors have been widely recognized to drive vaccine breakthroughs: (1) waning of humoral immunity, as evidenced by decreasing serologic titers over the months after vaccination, (2) overall decrease of vaccine effectiveness after 4–6 months, and (3) the rise of variants of concern that have a significant antigenic drift and immune escape [25, 27]. In this context, hybrid immunity or "superimmunity" deserves a closer look in order to better understand its clinical and epidemiological implications, most of all for severe disease and death prevention [23]. Moreover, new vaccines against COVID-19 must be effective against variants with greater transmission and immune evasion capacity, and they must be able to boost not only humoral but cellular immunity [28].

Our study team has been able to access nationwide data of diagnostic testing and genomic surveillance and analyze these together to identify the reinfection cases. Another strength of our study was that we were able to communicate with the cases when epidemiological, clinical, or vaccination status data were missing. However, an important limitation was that we only had paired samples available for genomic analysis in a small fraction of the probable cases of reinfection. Based on previous studies, it is very likely that more cases of SARS-CoV-2 reinfection will appear [29]. We report 30 cases of confirmed SARS-CoV-2 reinfections and Lambda variant was the most common cause of the second infections, in concordance with its predominant circulation during Peru's second wave.

CONCLUSIONS

This report represents the largest description of cases with confirmed SARS-CoV-2 reinfection in Latin America during the second pandemic wave. The relevance of the Lambda variant (C.37) is highlighted during the second Peruvian wave. Reinfections are not an uncommon phenomenon, and health systems need to strengthen their monitoring and analysis to better understand their epidemiological role in the future dynamics of the COVID-19 pandemic in the context of new COVID-19 variants.

Table 2. Clinical Characteristics in Both Episodes of SARS-CoV-2 Infection

Patient's Characteristics	1st Episode COVID-19	2nd Episode COVID-19	P Value
Severity, n (%)			
Asymptomatic	2 (6.7)	1 (3.3)	.30
Mild case	25 (83.3)	29 (96.7)	
Moderate case	2 (6.7)	0 (0.0)	
Severe case	1 (3.3)	0 (0.0)	
Most Frequent Symptoms, n (%)			
Cough	10 (33.3)	15 (50.0)	
Fever	12 (40.0)	09 (30.0)	-
Odynophagia	17 (56.7)	14 (46.7)	
Malaise	13 (43.3)	20 (66.6)	
Rhinorrhea	10 (33.3)	12 (40.0)	
Dyspnea	05 (16.6)	02 (06.6)	
Headache	11 (36.6)	12 (40.0)	
Other	08 (26.6)	05 (83.3)	
Number of symptoms reported, mean ± SD	3.5 ± 1.9	3.3 ± 1.7	.67
Days from disease onset to test, mean ± SD	4.9 ± 3.5	3.6 ± 2.3	.10
Days between both molecular tests, mean ± SD	235.53 ± 71.0		
90–149 days	04 (13.3)		
150–199 days	04 (13.3)		
200 days or more	22 (73.3)		

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation.
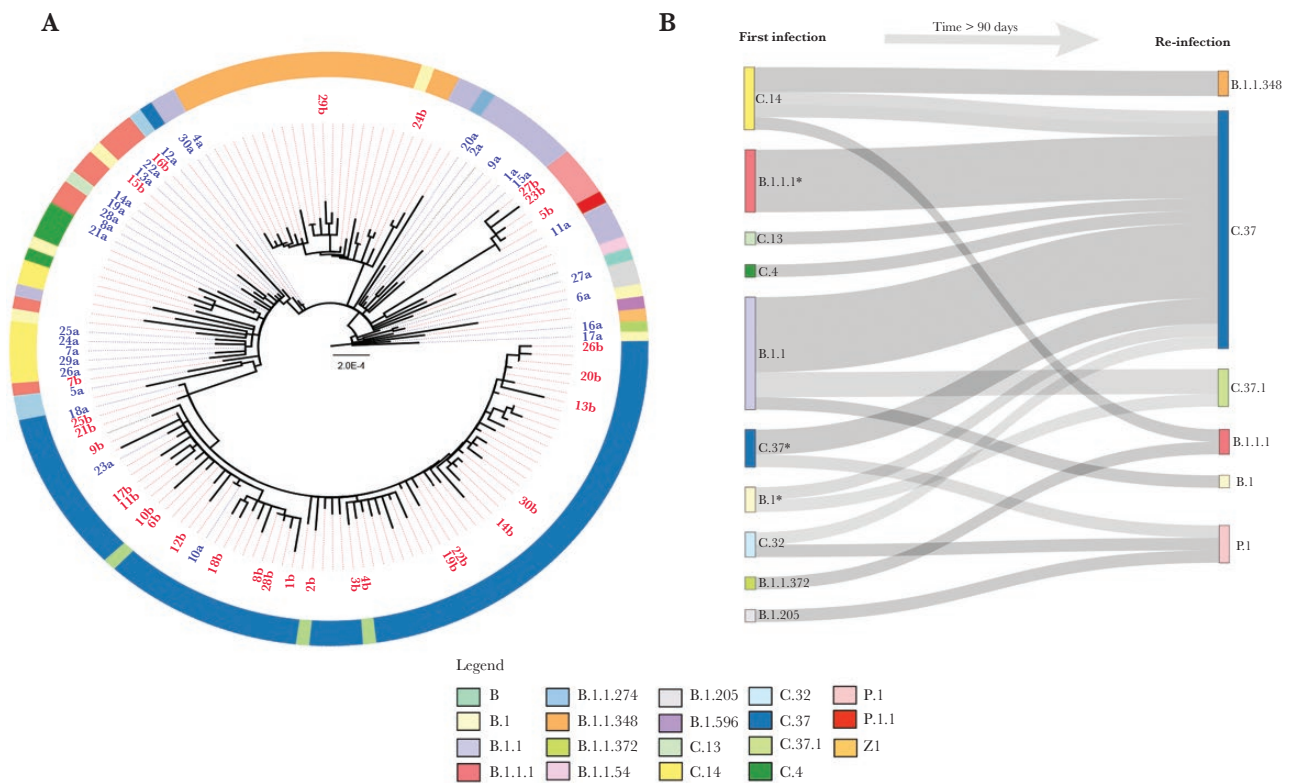

Figure 2. (A) Phylogenetic tree of the sequences obtained from the cases of reinfection. (B) Lineages obtained in the first (left column) episode and the second (right column) episode of severe acute respiratory syndrome coronavirus 2 infection in Lima and Callao, Peru.
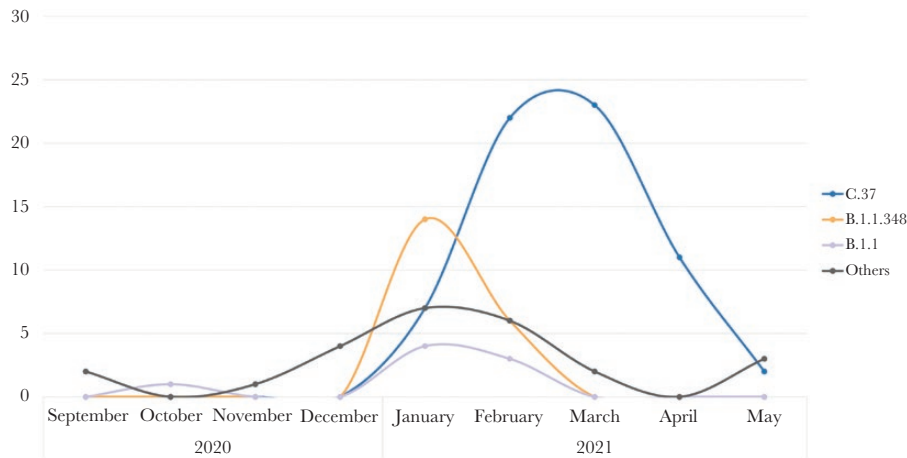
Figure 3. Chronology of the cases of second samples sequenced in Lima and Callao, Peru.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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