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Research Letter

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Cluster characterization of SARS-CoV-2 in military personnel deployed to Egypt and subsequent introduction of B.1.1.7 and C.36 lineages to Colombia

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineage B.1.1.7, also known as the Alpha variant, and catalogued a variant of concern (VOC), was first identified in the UK in September 2020,[1] and is now rapidly spreading with 812 731 deposited genomes from 135 different countries, including Colombia[2] as of 9 June 2021. Sequence data have shown a rapid expansion of this lineage, suggesting a likely selective advantage, most probably mediated by accumulation of critical mutations associated with increased virulence and transmission.[3,4] Evidence suggests that B.1.1.7 transmits more efficiently than other variants, displaying increased infectivity and risk of hospitalization.[5]

B.1.1.7 was first detected in Colombia on 16 April 2021, and is steadily increasing with a total of 75 deposited sequences (by 9 June 2021).[2] Here we conducted a screening study to assess the status of SARS-CoV-2 infection among Colo mbian military personnel deployed to Sinai, Egypt, on 4 November 2019. A total 126 active-duty service members returned to Colombia on 6 April 2021. All of them were screened by RT-qPCR (Reverse Transcription-quantitative Polymerase Chain Reaction) on 2 April 2021, and resulted negative before returning to the country (Figure 1A). Upon arrival (10 April 2021), a repeat RT-qPCR was performed on the 126 individuals, with 42 of them testing positive for SARS-CoV-2, of which only two were symptomatic (cough, headache and odynophagia). Subsequently, due to onset of symptoms in other previously negative-tested individuals, a third RT-qPCR was run on 13 April 2021, with 71 servicemen testing positive. Of these, 67 were symptomatic mainly with odynophagia. On 16 April 2021, a fourth RT-qPCR was performed on those who had remained negative, with seven subjects testing positive. Of these, all were asymptomatic except one, who presented cough. The six who remained negative received a fifth test on April 22, which was positive on all six individuals, four of them whom later developed odynophagia (Figure 1A). It is important to note that the group of soldiers who returned to Colombia had not been previously vaccinated against SARS-CoV-2 upon their return or at the time of their arrival in the country. These soldiers were included in the national vaccination plan only after the event described in this report. Short after diagnosing the first case, all servicemen were placed under supervised and strict quarantine in Fusagasuga, Colombia. This quarantine consisted of the confinement of all these military

personnel in quarters adapted for this purpose. Personnel arrived on April 6 and the last individuals were released on 8 May 2021 (around 1 month of quarantine and release after testing negative for RT-qPCR). After arrival at the country, periodic medical follow-up was conducted, and tests were administered as soon as possible, and the onset of signs and symptoms related to the infection were identified (Figure 1). Individuals during their stay were required to use all basic biosecurity measures such as the use of a face mask, hand washing and social distancing. However, due to space conditions, rooms had to be shared at certain times when distancing from staff could not be fully ensured, particularly during rest hours. The minimum isolation time after the positive test was 15 days. It is important to emphasize that to proceed to the quarantine lifting of any individual from the group, a clinical evaluation and an RT-qPCR test were performed to ensure a negative test.

We sequenced and analysed epidemiological variables on a subgroup of the above-described military personnel. Thirty subjects were selected, for whom clinical, epidemiological and cycle-threshold (Ct) data are summarized in Supplementary Table 1, available as Supplementary data at *JTM* online.

Long-Read Oxford Nanopore MinION-sequencing and bioinformatics analysis were conducted as previously reported.[6] Most genome sequences ($n = 28$) belonged to B.1.1.7 lineage, with one classified as C.36 lineage, reported mainly from Egypt, and now here for the first time in Colombia. Finally, the remaining samples could not be resolved due to insufficient quality reads.

A maximum-likelihood tree was built to evaluate the phylogenetic relationships of the genomes included. Three groups of VOC, B.1.1.7, were built including: (i) All genomes reported for Colombia and available until date-of-analysis (9 June 2021; $N = 24$), (ii) All available genomes from Egypt ($N = 16$) and (iii) A selected group of British genomes within the same timeframe ($N = 23$). In addition to the B.1.1.7 groups, a collection of 1902 high-quality reference genomes embracing different lineages used in Nextclade (https://clades.nextstrain.org/) for phylogenetic inferences was included and built from GISAID (Global Initiative on Sharing All Influenza Data) database (downloaded on 19 May 2021). The complete dataset with 1965 sequences was used to perform phylogenetic inference using Nextstrain.[7] A monophyletic cluster, which showed to be divergent from the comparative genomes, was identified during phylogenetic inferences (Figure 1B). This cluster included all the B.1.1.7 genomes from the different analysis groups. Five monophyletic sub-clusters with four or more genomes (C1–C5) were found within this divergent cluster that included most of the genomes analysed (Figure 1C). Two clusters belonged to genomes from Egypt (C2 and C4), whereas the UK and Colombian each denoted a cluster (C1 and C3, respectively). All the genomes analysed from the military in this study were found within the fifth cluster (C5, Sinai-Col). Interestingly, Sinai-Col genomes were phylogenetically closer to Egyptian genomes than to those from Colombia (Figure 1D).

In addition, SNPs analysis was performed to assess differences between B.1.1.7 groups. Remarkably, all the Sinai-Col genomes featured a SNPs repertoire containing 15 SNPs previously described for the lineage[8] along with additional ones. Of these, some were observed in the Egyptian and UK groups, but two added mutations were only present in the Sinai group (C5944T and T15096C), specifically located at ORF1a and ORF1b (Figure 1E). Furthermore, the Sinai-Col genomes, such as the UK ones, presented three lineage-specific signature deletions[8] and an additional one in position 28 274 (Figure 1F). Based on SNPs-profiling, our phylogenetic analysis revealed a closer relationship of Sinai-Col genomes with Egyptian compared with Colombian genomes, with the latter exhibiting few mutations of interest (9/15; Figure 1E).

Phylogenetic network (SplitsTree 5 v5.2.26) was constructed from the 29 Sinai-Col genomes. Two groups were identified, with 19 (Group-1) and 10 (Group-2) sequences, respectively (Supplementary Figure 1, available as Supplementary data at *JTM* online). No significant statistical differences were found between groups pertaining age and Ct-values for the target-genes. All symptomatic cases were identified within Group-1 (74%, $n = 14$), whereas those in Group 2, remained asymptomatic (0%, $n = 0$; Fisher's exact test, $P = 0.0002$). Odynophagia was the prevailing symptom in Group 1 (58%, $n = 11$; Fisher's exact test, $P = 0.003$) and no statistical differences were observed for cough or headache amongst groups (Supplementary Table 1, available as Supplementary data at *JTM* online).

Herein, we document a new introduction event of the VOC Alpha and the first report on the occurrence of lineage C.36 in Colombia by returning military personnel from Sinai to Colombia on April 2021. Considering that the first report of the Alpha variant in Colombia was given on 16 April 2021, and that the military population evaluated here entered the country on 6 April 2021, we can suggest that this was one of the first introductions of this variant in Colombia, in this case, occurring in a group coming from Sinai, Egypt. Likewise, it should be noted that in these military personnel it was also identified the C.36 variant reported mainly in Egypt, which had not previously been identified in the country.

Despite inherent differences in disease dynamics between countries, travel-related control measures remain a key element to contain the spread of coronavirus disease of 2019 (COVID-19).[9,10] However, in this case, prevention measures aimed to mitigate travel-related risk of transmission, taken prior to returning to the country, as well as those implemented during the isolation in Colombia, appear to have been insufficient, highlighting the need for homogenization of international standard testing and the supervision of the traveler's isolation to prevent new introductions of the virus. This highlights the importance of genomic surveillance of SARS-CoV-2 and monitoring for emerging variants, specifically, VOCs associated to enhanced-transmissibility, to halt further spread across the country.

Finally, our findings reinforce and highlight how travel is evidently a high-vulnerability event, particularly for fragile public health systems like Colombia, and emphasize the need for coordinated efforts with the national and local governments, as well as health and academic institutions to detect and track emerging SARS-CoV-2 cases and variants in context of the reopening of national/international flights to the country. Since the beginning of the pandemic in Colombia, the country has faced a series of limitations related to insufficient testing capacity, poor hospital care infrastructure, delayed government decision-making, among others, that have hindered the management

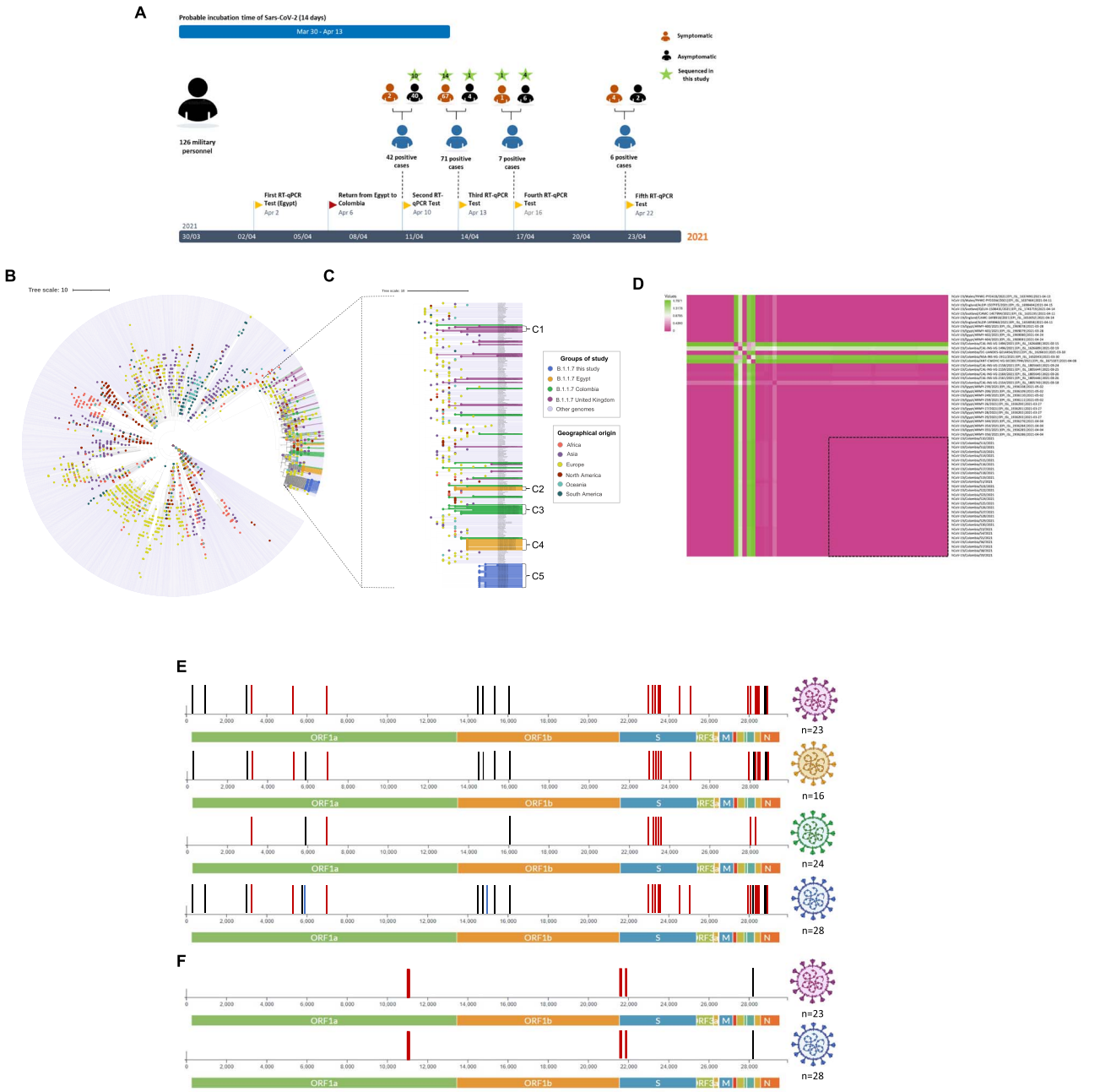

Figure 1. (A) Graphical representation of the epidemiological dynamic of the Colombian militaries with travel history in Sinai, Egypt. SARS-CoV-2 clusters of B.1.1.7 genomes from Colombia, Egypt and UK. (B) Maximum likelihood tree built of 1995 genomes used in this study and a worldwide representation. (C). Magnification of the cluster that includes all the B.1.1.7 genomes, grouped in five clusters (C1–C5) colored by region. (D). Heatmap visualization of the distance matrix of the B.1.1.7 genomes included in the analysis, highlighting the genomes from this study by dotted lines. SNPs representation of the four groups of the B.1.1.7 genomes analysed in this study, substitutions of the genomes from UK, Egypt, Colombia and Sinai-Col (in order from top to bottom) (E), and deletions of the genomes from the UK and this study (in order from top to bottom) (F). The red lines represent the mutations and deletions of interest previously described for the lineage, and the blue lines represent the unique substitutions observed for the Sinai-Col group.

and control of this event of global epidemiological concern. Although improvements have been implemented in some health care processes and epidemiological and genomic surveillance, greater investments and efforts are required in order to have an effective control of the pandemic based on data and information. Therefore, it is essential to carry out efficient, large-scale genomic surveillance to monitor spread, prevent onward introductions of variants and ultimately contain SARS-CoV-2 transmission.

Data availability

The data here described is available in the public database, GISAID (https://www.gisaid.org) under the accession IDs EPI_ISL_1 824 179–1 824 188 and EPI_ISL_2 339 776–2 339 794.

Supplementary data

Supplementary data are available at *JTM* online.

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Authors' contributions

N.B., S.C., M.M., L.H.P. and J.D.R contributed to the study design, data collection, data analysis, interpretation, writing and review. C.M., C.O., J.P., L.A., E.K.M., M.T.A., F.S.O., Y.R., C.A.C., M.C.D. and S.G. contributed to the data collection and review. A.P. contributed to the review of the manuscript.

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