Journal of Travel Medicine

International Society of Travel Medicine
Established 1991
Promoting healthy travel worldwide

Journal of Travel Medicine, 2021, 1–4
https://doi.org/10.1093/jtm/taab180
Research Letter

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High SARS-CoV-2 viral load in travellers arriving in Spain with a negative COVID-19 test prior to departure

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Submitted 26 August 2021; Accepted 12 November 2021

Key words: SARS-CoV-2, COVID-19, travellers, high viral load

International air travel has been a key factor in the global spread of SARS-CoV-2. In the early stages of the pandemic, borders were closed to international travellers, while at later stages, a number of different control strategies have been implemented. Measures aimed at minimizing the importation of COVID-19 cases include symptom identification in ill travellers, quarantining on arrival and finally, travel restrictions allowing only those showing a negative pre-departure SARS-CoV-2 test to travel.

The aim of our study was to assess the robustness of the current control measures based on negative pre-departure tests by evaluating whether, despite the requirement for a negative test on departure, it is still possible to identify travellers testing positive for SARS-CoV2 by RT-PCR on arrival. The study was approved by the ethical research committee of Gregorio Marañón Hospital (Reference MICRO.HGUGM.2021-009).

Our study ran over 2 months, between 22 April and 26 June 2021, when it was mandatory for any person taking a flight to Spain to have a negative pre-departure SARS-CoV test. Tests were required to be taken within 72 hours or 48 hours before departure for nucleic acid and rapid antigen tests (RAT), respectively; considering the potential intermediate stops for some international flights, this time window before arrival might extend up to 96 hours before arrival for certain countries of departure.

The screening protocol followed at Madrid international airport (https://www.mscbs.gob.es/profesionales/saludPublica/sanidadExterior/ControlHS.htm) was based on a two-stage procedure. Firstly, a primary control based on (i) temperature measure (>37.5°C), (ii) visual examination on the passenger status and/or symptoms and (iii) data compiled in the Health Control Form (SpTH), which is obligatory to enter to Spain. Then, a secondary control for all cases with suspicion of suffering COVID-19 in the primary control; it included a clinical and epidemiological study, including a RAT test (Panbio COVID-19 Ag Rapid Test Device (nasopharyngeal); Abbott, Chicago, IL). In addition, RATs were performed at arrival on a random selection of asymptomatic travellers. The random selection was done by using the SpTH (Spain Travel Health) programme from the Ministry of Health, which uses the QR codes obtained by each traveller after having fulfilled the SpTH form. The percentage of travellers randomly selected to be tested was determined according to common criteria determined for entering in any country from the EU (SND orders for the quarantine).

We selected all the 196 positive cases identified with a SARS-CoV-2 RAT test on arrival at Madrid international airport and, later, laboratory-confirmed by a positive RT-PCR test (TaqPath, Thermo Fisher, MA, USA) from a new specimen taken at our hospital using the same materials, reagents and procedures, which were used for COVID-19 diagnosis on the community setting.

The 196 travellers with a positive SARS-CoV-2 test on arrival represented 0.43% of the 45.211 travellers tested. Most of these came from Colombia (114), followed by the Dominican Republic (30) and Peru (12). The 40 remaining cases were distributed

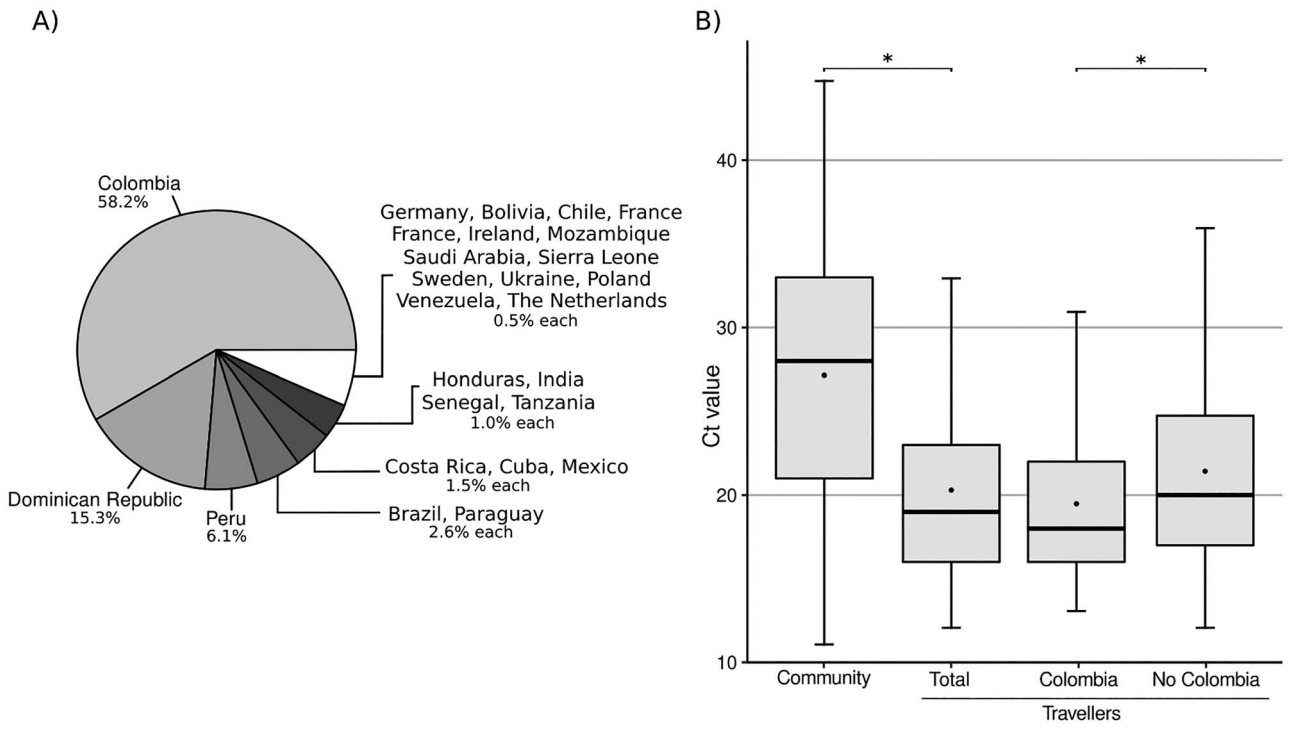

Figure 1. (A) Distribution of the COVID-19 cases in travellers according to their country of origin; (B) differences in the Ct values (asterisks indicate statistically significant differences, black thick lines in each box correspond to the median values, dots within boxes correspond to mean values) for cases from the community, travellers in total, travellers from Colombia and travellers from countries other than Colombia

among 22 different countries of origin (Supplementary Table 1, available as Supplementary data at *JTM* online, Figure 1A). Most of them (175/196) had not been vaccinated; for the remaining 21 travellers, 13 and 8 had received two or one vaccine doses, respectively.

Different studies have also identified SARS-CoV-2 patients in travellers on arrival, although most were performed in 2020 before pre-departure testing was generally mandated for international travel. One study in Alaska identified 951 asymptomatic travellers with positive SARS-CoV-2 test results and another two studies from Canada identified around 1–2% of asymptomatic travellers as being positive on arrival.[1] Other studies have focused on the analysis of SARS-CoV-2 infection prevalence in repatriation flights,[2–4] offering figures ranging from 0.44 to 6.3%. These figures led to some of the authors to recommend the implementation of pre-departure testing.

The unexpectedly common finding of travellers with SARS-CoV-2 on arrival in our study, despite the compulsory negative test result prior to departure, was even more alarming when we analysed SARS-CoV-2 viral loads. Based on RT-PCR Ct values (N gene), the mean viral load of those travellers diagnosed on arrival was inferred to be high (Ct: 20.3). During the same period of time, 2543 COVID-19 cases were diagnosed at our institution from community cases, excluding travellers, and their mean Ct value was significantly higher (Ct: 27.1, $P < 0.001$, Figure 1B) when compared with the average Ct value of the travellers group. After analysing the mean Ct values of cases from the three best represented countries of origin, namely, Colombia (Ct: 19.5), the Dominican Republic (Ct: 22) and Peru (Ct: 19.9), those from Colombia showed the lowest values (P: 0.0198, Wilcoxon rank-sum test, Figure 1B) of all positive travellers. Our data contrast with those from another study in Canada in which the mean viral loads of travellers diagnosed on arrival were much lower (average Ct: 32) and only 22% of positive travellers showed high viral loads ($Ct < 25$).[5]

Our data revealed clear weaknesses in the current international control regulations based on pre-departure testing for international flights. Testing within 24 hours before the flight's departure[6] has been proposed to minimize the proportion of cases that may be missed with the current 72-hour time window. However, the high viral load values among the travellers in our study suggest that they should have been detected within the 72 hours prior to departure. The low efficiency in identification of these highly infective asymptomatic COVID-19 cases would appear to be due to the low sensitivity or lack of validity of the tests applied in the country of origin and even due to the possibility of fake test certificates.

WGS analysis was performed on 171 (87%) of positive travellers. The most frequently identified SARS-CoV-2 variant was B.1.621 (72, 42.1%), as was expected for a sample in which most cases came from Colombia (Table 1). Some VOCs were also identified in 39.2% of the cases, among them B.1.1.7 (Alpha: 14, 8.2%), B.1.351 (Beta: 3, 1.8%), P.1 (Gamma: 45, 26.3%) and B.1.617.2 (Delta: 5, 2.9%) (Table 1). The variant frequency distribution differed from the one in our population in the same time period (based on a random sample of 571 community cases sequenced; 22.5% of total diagnosed cases), in which the dominant variants were the Alpha, Gamma and Delta VOCs (89% altogether, Alpha 68%, 17% Delta and 4.2% Gamma), while the majority B.1.621 variant in the travellers accounted for only 3.5% of our population.

The impact of in-flight exposure to positive cases has been evaluated.[7,8] In our case, we were unable to determine the secondary cases caused by in-flight exposure since we did not

Table 1. SARS-CoV variants identified in travellers with WGS data available

Country of origin	*N*	*N* (%) sequenced	SARS-CoV-2 variant (*N*)
Colombia	114	109 (95.6)	B.1.621 (58), P.1 (25), B.1.623 (13), B.1.526 (2), P.1.1 (1), C.37 (3), B.1.621.1 (2), B.1.1.7 (4), B.1.617.2 (1)
Brazil	5	5 (100)	P.1 (5)
Peru	12	10 (83.3)	P.1 (3), C.37 (6), P.1.7 (1)
Dominican Republic	30	26 (86.7)	B.1.621.1 (11), B.1.630 (3), P.1 (7), B.1.1.7 (4), P.1.1 (1)
Costa Rica	3	2 (66.7)	P.1 (1), B.1.1.7 (1)
Cuba	3	3 (100)	B.1.351 (3)
Mexico	3	2 (66.7)	B.1 (1), B.1.621.1 (1), P.1 (1)
Paraguay	5	3 (60.0)	P.1 (2), B.1.526 (1)
∗countries with ≤ 2 travellers: Germany (1), Bolivia (1), Chile (1), France (1), Netherlands (1), Honduras (2), India (2), Ireland (1), Mozambique (1), Saudi Arabia (1), Senegal (1), Sierra Leone (1), Sweden (1), Tanzania (2), Ukraine (1), Poland (1), Venezuela (1)	20	11 (55.0)	B.1.1.7 (5), P.1 (1), B.1.631 (1), B.1.617.2 (4)
	196	171 (87.0)	

have access to those cases developing symptoms after arrival and therefore sequencing data could not be obtained for comparisons. We compared our sequencing data with sequences obtained from 572 cases from our population (community cases) diagnosed in the same time period and no transmission clusters were identified involving travellers and community cases.

Conclusions

Our findings indicate clear weaknesses in the control measures based on pre-departure screening for SARS-CoV-2 and raise concerns about the frequent presence of undiagnosed asymptomatic carriers with high viral loads on intercontinental flights. There is a real need to reinforce travel regulations to rule out international movements of highly infective COVID-19 cases.

Author's contributions

Data analysis was done by Andrea Molero-Salinas, Carla Rico-Luna, Sergio Buenestado-Serrano, Pilar Catalán and Laura Pérez-Lago. Data compilation was by Andrea Molero-Salinas, Carla Rico-Luna and Pilar Catalán. MS revision was done by Andrea Molero-Salinas, Laura Pérez-Lago, Sergio Buenestado-Serrano, Pilar Catalán and Patricia Muñoz. Technical procedures were performed by Víctor Manuel de la Cueva García, José Egido and Javier Adán-Jiménez. Experimental tasks were carried out by Víctor Manuel de la Cueva García and Javier Adán-Jiménez. Andrea Molero-Salinas was in charge of the experimental analysis. Carla Rico-Luna and Carmen Losada took care of the diagnostics. Sergio Buenestado-Serrano performed bioinformatic analysis. Laura Pérez-Lago performed genomic analysis. Darío García de Viedma took care of the conceptualization, design, supervision, analysis and MS writing. Gregorio Marañón Microbiology-ID COVID-19 Study Group: Adán-Jiménez (Javier), Alcalá (Luis), Aldámiz (Teresa), Alonso (Roberto), Álvarez (Beatriz), Álvarez-Uría (Ana), Berenguer (Juan), Bermúdez (Elena), Bouza (Emilio), Buenestado-Serrano (Sergio), Burillo (Almudena), Candela (Ana), Carrillo (Raquel), Catalán (Pilar), Cercenado (Emilia), Cobos (Alejandro), de la Cueva (Víctor Manuel), Díez (Cristina), Egido-Balzategui (Jose), Escribano (Pilar), Estévez (Agustín), Fanciulli (Chiara), Galar (Alicia), García (María Dolores), García de Viedma (Darío), Gijón (Paloma), González (Adolfo), Guillén (Helmuth), Guinea (Jesús), Haces (Laura Vanessa), Herranz (Marta), Kestler (Martha), López (Juan Carlos), Losada (Carmen Narcisa), Machado (Marina), Marín (Mercedes), Martín (Pablo), Martín-Escolano (Javier), Molero-Salinas (Andrea), Montilla (Pedro), Muñoz (Patricia), Olmedo (María), Otero-Sobrino (Álvaro), Padilla (Belén), Palomo (María), Parras (Francisco), Pérez-Granda (María Jesús), Pérez-Lago (Laura), Pérez (Leire), R Maus (Sandra), Reigadas (Elena), Rico-Luna (Carla Margarita), Rincón (Cristina), Rodríguez (Belén), Rodríguez (Sara), Rodríguez-Grande (Cristina), Rojas (Adriana), Ruiz-Serrano (María Jesús), Sánchez (Carlos), Sánchez (Mar), Serrano (Julia), Sola-Campoy (Pedro J), Tejerina (Francisco), Valerio (Maricela), Veintimilla (María Cristina), Vesperinas (Lara), Vicente (Teresa) and de la Villa (Sofía).

Supplementary data

Supplementary data are available at *JTM* online.

Acknowledgements

We are grateful to Janet Dawson for her editing and proofreading assistance. We are indebted to Ana Clara Zoni from Subdirección General de Información Sanitaria, Secretaria General de Salud Digital, Información e Innovación del Sistema Nacional de salud, Ministerio de Sanidad, Spain and personnel from Área de Control Sanitario, Subdirección General de Sanidad Exterior, Dirección General de Salud Pública, Secretaría de Estado de Sanidad and Ministerio de Sanidad for their invaluable help to obtain data from the travellers tested at the airport and information about the diagnosis schemes applied.

Funding

L.P.-L. is receptor of a Miguel Servet Research contract (CPII20/00001) from Instituto de Salud Carlos III.

Conflict of interest

None declared.

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