Massive SARS-CoV-2 RT-PCR Testing on Rural Communities in Manabi Province (Ecuador) Reveals Severe COVID-19 Outbreaks

Esteban Ortiz-Prado, Aquiles R. Henriquez-Trujillo, Ismar A. Rivera-Olivero, Byron Freire-Paspuel, Alexander Paolo Vallejo-Janeta, Tannya Lozada, and Miguel Angel Garcia-Bereguiain* for the UDLA COVID-19 Team

One Health Research Group, Faculty of Medicine, Universidad de Las Américas, Quito, Ecuador

Abstract. Rural communities from Latin America are particularly susceptible to develop serious outbreaks of infectious diseases. Inadequate diagnosis and poor health infrastructure jeopardize proper contact tracing and other actions to reduce the impact of COVID-19 in the region. We herein describe the preliminary data of our ongoing fieldwork of massive testing among nonhospitalized rural population in Manabi Province of the coastal region of Ecuador. A total of 1,479 people from six different rural communities were tested for SARS-CoV-2 by RT-qPCR following the CDC protocol; 350 individuals tested positive, resulting in an overall attack rate of 23.7% for SARS-CoV-2 infection. This ultrahigh prevalence must urge to the public health authorities from Ecuador to take immediate actions to counteract this dramatic scenario in Manabi Province and to improve SARS-CoV-2 testing countrywide.

Ecuador is one of the most affected countries in terms of COVID-19–related deaths worldwide, ranking third in the world for excess mortality, with more than 166 excess deaths per 100,000 habitants.¹ Recent official data reported at least 116,360 RT-qPCR–confirmed COVID-19 cases and 6,724 officially registered deaths, although these numbers are probably far from the true reality.²

SARS-CoV-2 molecular diagnostic laboratories from the National Institute of Public Health Research (INSPI) are only located at the three biggest urban areas in Ecuador: Guayaquil, Quito, and Cuenca. So far, Ecuador has very limited SARS-CoV-2 RT-PCR testing capacities, ranging 84th in the world in terms of test per million people, processing around 3,000 samples per day with positive testing rates (PTR%) more than 40%, clearly above the WHO's recommendations.

SARS-CoV-2 molecular diagnosis is even less accessible for rural communities far away from the INSPI network of laboratories. For instance, Manabi Province is mainly rural, with 1,562,079 inhabitants and with 19,427 km² of land. This region is the third most populated and fourth in size among the 24 provinces of Ecuador. Moreover, Manabi was the worldwide epicenter of the most lethal earthquake in 2016, killing more than 605 people and causing severe damage in road infrastructure, hospitals, and medical healthcare facilities, still under reconstruction. By the time this study was carried out, there was no SARS-CoV-2 diagnostic laboratory operating in the whole province, and all the samples collected at Ministry of Health (MoH) facilities were sent to the INSPI laboratory at Guayaquil city involving at least a day road trip.

According to official data from the MoH, 17,509 people (1.1% of total population) were tested in Manabi Province since the beginning of the COVID-19 pandemic. A total of 8,414 people were positive for SARS-CoV-2, resulting in an extremely high PTR of 48%. This scenario makes it really difficult to implement a successful COVID-19 surveillance strategy. Moreover, 898 deaths associated to COVID-19 were already confirmed, whereas more than 2,800 excessive

deaths have been reported for this province since the beginning of the COVID-19 pandemic. $^{\rm 3}$

Although MoH SARS-CoV-2 sampling was mainly focused on symptomatic patients attending hospital facilities at urban locations in Manabi, high levels of SARS-CoV-2 community transmission could not be ruled out at undeserved rural communities. In this sense, a massive molecular testing strategy using RT-qPCR for SARS-CoV-2 was deployed by our university in coordination with local community leaders, the MoH, and the regional government at rural communities on the 22 cantons included in the province of Manabi, starting the third week of August 2020.

Nasopharyngeal swabs were collected on 0.5 mL Tris-EDTA (TE) pH 8 buffer for SARS-CoV-2 diagnosis by RT-PCR following an adapted version of the CDC protocol by using PureLink Viral RNA/DNA Mini Kit (Invitrogen, Walthan, MA) as an alternate RNA extraction method and CFX96 BioRad instrument.^{4–12} In brief, the CDC designed RT-qPCR FDA EUA 2019-nCoV CDC kit (IDT, Coralville, IA) is based on N1 and N2 probes to detect SARS-CoV-2 and RNase P as an RNA extraction quality control.^{4,5} Also, negative controls (TE pH 8 buffer) were included as carryover contamination controls, one for each set of RNA extractions, to guarantee that only true positives were reported. Written consent was obtained for all the individuals included on the surveillance. The study was approved by "Comité de Etica para Investigación en Seres Humanos" from "Universidad de Las Américas."

We herein present the preliminary data for the first stage of the ongoing surveillance study including to date six cantons geographically distributed all over Manabi Province: El Carmen (North East), Chone (Mid North), Pedernales (North West), Portoviejo (Mid West), Bolivar (Mid East), and 24 de Mayo (South West). Rural communities with high levels of poverty and with reported cases of COVID-19 were included in the study, one per canton. Within the community, we carried out a convenience sampling where only one family member per household was included on the testing.

We found COVID-19 outbreaks at all communities visited. From the 1,479 people tested, 350 individuals were positive for SARS-CoV-2, resulting in a prevalence of recent or active infection of 23.7%. The attack rates varied from 12.7% at canton 24 de Mayo to 34.9% at canton El Carmen (Table 1). We did not report any deaths during our visits, but community

^{*}Address correspondence to Miguel Angel Garcia-Bereguiain, One Health Research Group, Faculty of Medicine, Universidad de Las Américas, Campus Queri, Calle Jose Queri, 100101, Quito, Ecuador. E-mail: magbereguiain@gmail.com

TABLE 1

SARS-CoV-2 positivity rate by RT-qPCR for the rural communities from the six cantons from Manabi Province included on this study

Canton	Total number of individuals tested	SARS-CoV-2-positive individuals	Positivity rate (%)
El Carmen	146	51	34.9
Portoviejo	378	107	28.3
Pedernales	134	44	32.8
Chone	325	58	17.8
24 de Mayo	251	32	12.7
Bolivar	245	58	23.7

leaders described multiple deaths related to COVID-19, and mild-to-moderate COVID-19 symptoms were also frequently observed by our physicians.

Although we cannot completely rule out some potential bias on our convenience sampling, we believe our data would suggest a potential community transmission of SARS-CoV-2. Further massive SARS-CoV-2 testing at rural communities in Manabi is necessary to confirm this hypothesis and potentially endorse a population lockdown. Although our researchers have been supporting the implementation of the first SARS-CoV-2 in Manabi Province (with funds from MoH, regional government, and Germany Office of International Cooperation for Development) since the end of August 2020, the testing capacity to date, with less than 1,000 samples per week, would not be enough for the entire province demands under the described epidemiological scenario.

During a week of massive testing, our laboratory at Universidad de Las Americas was able to process the equivalent of 8.5% of the total samples collected by the MoH during the whole COVID-19 pandemic period at this province. We urge the MoH to increase SARS-CoV-2 testing capacity at Manabi Province. Moreover, we would potentially expect a similar scenario at other rural communities all over Ecuador. So far, MoH COVID-19 surveillance programs need to be dramatically improved even more considering that, starting on September 14, 2020, the state of national emergency was revoked and restrictions to mobility were lifted.

Received September 16, 2020. Accepted for publication January 12, 2021.

Published online February 8, 2021.

Acknowledgments: We thank the Ministry of Public Health at Manabi Province and Prefectura de Manabi for the logistic support. Publication charges for this article were waived due to the ongoing pandemic of COVID-19.

Financial support: This study was supported by Fundación CRISFE (fondo Sumar Juntos) and Universidad de Las Américas.

Disclosure: All authors contributed to data collection, analysis, and writing of the manuscript.

UDLA COVID-Team: Tatiana Jaramillo, Daniela Santander Gordon, Gabriel Alfredo Iturralde, Julio Alejandro Teran, Karen Marcela Vasquez, Jonathan Dario Rondal, Genoveva Granda, Ana Cecilia Santamaria, Cynthia Lorena Pino, Oscar Lenin Espinosa, Angie Buitron, David Sanchez Grisales, Karina Beatriz Jimenez, Heberson Galvis, Barbara Coronel, Diana Carolina Morales Jadan, Vanessa Bastidas, Dayana Marcela Aguilar, Ines Maria Paredes, Christian David Bilvao, Maria Belen Rodriguez Paredes, Sebastian Rodriguez Pazmiño, Juan Carlos Laglaguano, Henry Herrera, Pablo Marcelo Espinosa, Edison Andres Galarraga, Marlon Steven Zambrano-Mila, Ana Maria Tito, Nelson David Zapata.

Authors' addresses: Esteban Ortiz-Prado, Aquiles R. Henriquez-Trujillo, Ismar A. Rivera-Olivero, Byron Freire-Paspuel, Alexander Paolo Vallejo-Janeta, Tannya Lozada, and Miguel Angel Garcia-Bereguiainon, One Health Research Group, Faculty of Medicine, Universidad de Las Américas, Quito, Ecuador, E-mails: esteban.ortiz.prado@udla.edu.ec, tannya.lozada@udla.edu.ec, ismar.rivera.olivero@udla.edu.ec, byron. freire.paspuel@udla.edu.ec, aquiles.henriquez.trujillo@udla.edu.ec, pavellejoj@gmail.com, and magbereguiain@gmail.com.

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC-BY) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

REFERENCES

- Ortiz-Prado E et al., 2020. Epidemiological, socio-demographic and clinical features of the early phase of the COVID-19 epidemic in Ecuador. *MedRxiv*.
- Ministerio de Salud Pública, 2020. Situación Coronavirus COVID-19. Ecuadorian Ministry of Health.
- Available at: https://www.salud.gob.ec/wp-content/uploads/ 2020/09/Boletin-182_Nacional_MSP.pdf.
- 4. Lu X et al., 2020. US CDC real-time reverse transcription PCR panel for detection of severe acute respiratory syndrome coronavirus 2. *Emerg Infect Dis 26:* 8.
- CDC, 2020. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19). Center for Diseases Control and Prevention. Available at: https://www.cdc.gov/coronavirus/ 2019-ncov/lab/guidelines-clinical-specimens.html. Accessed October 9, 2020.
- Freire-Paspuel B, Vega-Mariño P, Velez A, Castillo P, Cruz M, Garcia-Bereguiain MA, 2020. Evaluation of nCoV-QS (MiCo BioMed) for RT-qPCR detection of SARS-CoV-2 from Nasopharyngeal samples using CDC FDA EUA qPCR kit as a gold Standard: an example of the need of validation studies. *J Clin Virol* 128: 104454.
- Freire-Paspuel B, Vega-Mariño P, Velez A, Castillo P, Gomez-Santos EE, Cruz M, Garcia-Bereguiain MA, 2020. Cottontipped plastic swabs for SARS-CoV-2 RT-qPCR diagnosis to prevent supply shortages. *Front Cell Infect Microbiol 10:* 356.
- Freire-Paspuel B, Vega-Mariño P, Velez A, Castillo P, Cruz M, Garcia-Bereguiain M, 2020. Sample pooling of RNA extracts to speed up SARS-CoV-2 diagnosis using CDC FDA EUA RTqPCR kit. *Virus Res 290:* 198173.
- Freire-Paspuel B, Garcia-Bereguiain MA, 2020. Analytical sensitivity and clinical performance of a triplex RT-qPCR assay using CDC N1, N2 and RP targets for SARS-CoV-2 diagnosis. *Int J Infect Dis.* doi: 10.1016/j.ijid.2020.10.047.
- Freire-Paspuel B, Vega-Mariño P, Velez A, Cruz M, Perez F, Garcia-Bereguiain MA, 2020. Analytical and clinical comparison of Viasure (CerTest Biotec) and 2019-nCoV CDC (IDT) RT-qPCR kits for SARS-CoV2 diagnosis. *Virology* 553: 154–156.
- Freire-Paspuel B, Garcia-Bereguiain MA, 2020. Poor sensitivity of "AccuPower SARS-CoV-2 real time RT-PCR kit (Bioneer, South Korea)". *Virol J 17:* 178.
- Freire-Paspuel B, Vega-Mariño P, Velez A, Castillo P, Masaquiza C, Cedeño-Vega R, Lozada T, Cruz M, Garcia-Bereguiain MA, 2020. "One health" inspired SARS-CoV-2 surveillance: the Galapagos Islands experience. *One Health 11:* 100185.