TESIS DOCTORAL

Optimización de metodologías para la detección de SARS-CoV-2 y aplicación a la vigilancia epidemiológica en Ecuador

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PUBLICACIÓN 1:

- ARTÍCULO
- PUBLICADO
- Ramírez-Córdova, C*.; **Morales-Jadán, D***.; Alarcón-Salem, S.; Sarmiento-Alvarado, A.; Proaño, M.B.; Camposano, I.; Sarmiento-Alvarado, B.; Coello, D.; Hidalgo-Jiménez, J.F.; Rodríguez, Á.S.; et al.
- 2023.
- Fast, cheap, and sensitive: Homogenizer-based RNA extraction free method for SARS- CoV-2 detection by RT-qPCR. Frontiers in Cellular and Infection Microbiology 2023, 13, 1–5. D.O.I: https://doi.org/10.3389/fcimb.2023.1074953
- Factor de impacto: 6.073 (2022). Q1 en la categoría Microbiology.
- Diseño experimental, trabajo de laboratorio y escritura del primer borrador del manuscrito (co-primera autora).

PUBLICACIÓN 2:

- ARTÍCULO
- PUBLICADO
- **Morales-Jadan, D**., Viteri, C., Castro-Rodríguez, B., & García Bereguiain, M. A.
- 2023.
- The quality of commercial SARS-CoV-2 nucleic acid tests in Ecuador: lessons from COVID-19 pandemic for advancing social equity through microbiology. Frontiers in

Cellular and Infection Microbiology, 13, 536. D.O.I: https://doi.org/10.3389/FCIMB.2023.1179786

- Factor de impacto: 6.073 (2022). Q1 en la categoría Microbiology.
- Recogida de datos, trabajo de laboratorio y escritura de artículo.

PUBLICACIÓN 3:

- ARTÍCULO
- PUBLICADO
- Freire-Paspuel, B.; **Morales-Jadan, D**.; Zambrano-Mila, M.; Pérez, F.; García-Bereguiain, M.A.
- 2022.
- Analytical sensitivity and clinical performance of "COVID-19 RT-PCR Real TM FAST (CY5) (ATGen, Uruguay) and 'ECUGEN SARS-CoV-2 RT-qPCR' (UDLA-STARNEWCORP, Ecuador)": High quality-low-cost local SARS-CoV-2 tests for South America. PLoS Neglected Tropical Diseases 2022, 16. D.O.I: https://doi.org/10.1371/JOURNAL.PNTD.0010082
- Factor de impacto: 4.781 (2022). Q1 en la categoría Public Health, Environmental and Occupational Health.
- Trabajo de laboratorio, colección de datos y escritura de artículo.

PUBLICACIÓN 4:

- ARTÍCULO
- PUBLICADO
- **Morales-Jadán, D**.; Viteri-Dávila, C.; Castro-Rodriguez, B.; Vallejo-Janeta, A.P.; Rivera-Olivero, I.A.; Perez, F.; Garcia-Bereguiain, M.A.
- 2022
- Clinical Performance of Three Commercial SARS-CoV-2 Rapid Antigen Tests for Community-Dwelling Individuals in a Tropical Setting. Frontiers in cellular and infection microbiology 2022, *12*. D.O.I: https://doi.org/10.3389/FCIMB.2022.832235
- Factor de impacto: 6.073 (2022). Q1 en la categoría Microbiology.
- Toma de muestra, diseño experimental, trabajo de laboratorio y escritura del primer borrador del manuscrito.

PUBLICACIÓN 5:

- ARTÍCULO
- PUBLICADO
- Rodriguez-Paredes, M.B.; Vallejo-Janeta, P.A.; **Morales-Jadan, D**.; Freire-Paspuel, B.; Ortiz-Prado, E.; Henriquez-Trujillo, A.R.; Rivera-Olivero, I.A.; Jaramillo, T.; Lozada, T.; Garcia-Bereguiain, M.A.; et al.
- 2022.
- COVID-19 Community Transmission and Super Spreaders in Rural Villages from Manabi Province in the Coastal Region of Ecuador Assessed by Massive Testing of Community-Dwelling Population. *The American Journal of Tropical Medicine and Hygiene* **2022**, *106*, 121. D.O.I: https://doi.org/10.4269/AJTMH.21-0582

- Factor de impacto: 3.707 (2022). Q2 en la categoría Infectious Diseases.
- Trabajo de laboratorio, recogida de datos y revisión del manuscrito final.

PUBLICACIÓN 6:

- ARTÍCULO
- PUBLICADO
- Vallejo-Janeta, A. P., **Morales-Jadan, D.,** Paredes-Espinosa, M. B., Coronel, B., Galvis, H., Bone-Guano, H. R., Amador Rodriguez, B., Gomez Abeledo, G., Freire-Paspuel, B., Ortiz-Prado, E., Rivera-Olivero, I., Henriquez-Trujillo, A. R., Lozada, T., & Bereguiain, M. A. G.
- 2022
- Sustained COVID-19 community transmission and potential super spreading events at neglected afro-ecuadorian communities assessed by massive RT-qPCR and serological testing of community dwelling population. Frontiers in Medicine, 9, 2326. D.O.I: https://doi.org/10.3389/fmed.2022.933260
- Factor de impacto: 5.058 (2022). Q1 en la categoria Medicine.
- Trabajo de laboratorio, recogida de datos y revisión del manuscrito.

PUBLICACIÓN 7:

- ARTÍCULO
- PUBLICADO
- **Morales-Jadán, D**.; Vallejo-Janeta, A.P.; Bastidas, V.; Paredes-Espinosa, M.B.; Freire-Paspuel, B.; Rivera-Olivero, I.; Ortiz-Prado, E.; Henriquez-Trujillo, A.R.; Lozada, T.; Garcia-Bereguiain, M.A.
- 2023.
- High SARS-CoV-2 infection rates and viral loads in community-dwelling individuals from rural indigenous and mestizo communities from the Andes during the first wave of the COVID-19 pandemic in Ecuador. Frontiers in Medicine 2023, 10. D.O.I: https://doi.org/10.3389/FMED.2023.1001679.
- Factor de impacto: 5.058 (2022). Q1 en la categoría Medicine.
- Toma de muestra, diseño experimental, trabajo de laboratorio y escritura del primer borrador del manuscrito.

PUBLICACIÓN 8:

- ARTÍCULO
- PUBLICADO
- Vallejo-Janeta, A. P., **Morales-Jadan, D**., Velez, A., Vega-Marino, P., Freire-Paspuel, B., Paredes-Espinosa, M. B., Pazmiño, A. S. R., Castillo, P., Masaquiza, C., Rivera-Olivero, I., Ortiz-Prado, E., Henriquez-Trujillo, A. R., Coronel, B., Galvis, H., Jaramillo, T., Lozada, T., Cruz, M., team, COVID-19, & Garcia-Bereguiain, M. A.
- 2023.
- Massive testing in the Galapagos Islands and low positivity rate to control SARS-CoV-2 spread during the first semester of the COVID-19 pandemic: a story of success for Ecuador and South America. D.O.I: https://doi.org/10.22605/RRH7643

- Factor de impacto: 2.733 (2022). Q1 en la categoría Emergency Medical Services.
- Trabajo de laboratorio, recogida de datos y revisión del manuscrito.

PUBLICACIÓN 9:

- ARTÍCULO
- PUBLICADO
- Vallejo-Janeta, A.P.; **Morales-Jadan, D**.; Freire-Paspuel, B.; Lozada, T.; Cherrez-Bohorquez, C.; Garcia-Bereguiain, M.A.; Ortiz-Prado, E.; Rivera-Olivero, I.; Henriquez, A.R.; Jaramillo, T.; et al.
- 2021.
- COVID-19 outbreaks at shelters for women who are victims of gender-based violence from Ecuador. *International Journal of Infectious Diseases* **2021**, *108*, 531–536. D.O.I: https://doi.org/10.1016/j.ijid.2021.06.012
- Factor de impacto: 10.7 (2022). Q1 en la categoria Infectious Diseases
- Trabajo de laboratorio, recogida de datos y revisión del manuscrito.

PUBLICACIÓN 10:

- ARTÍCULO (letter).
- PUBLICADO
- Garcia-Bereguiain, M.A.; Bruno, A.; **Morales-Jadan, D**.; Vidal, J.E.
- 2022.
- Equitable distribution of SARS-CoV-2 tests. *Bulletin of the World Health Organization* 2022, *100*, 411–412. D.O.I: https://doi.org/10.2471/BLT.21.287398
- Factor de impacto: 13.831 (2021). Q1 en la categoría Public Health, Environmental and Occupational Health.
- Trabajo de laboratorio y escritura de artículo.

PUBLICACIÓN 11:

- ARTÍCULO (letter).
- PUBLICADO
- García-Bereguiain, M.A.; Perez, F.; Parra-Vera, H.; Bruno, A.; Freire-Paspuel, B.; **Morales-Jadan, D**.; Castro-Rodríguez, B.; Rivera-Olivero, I.; Lozada, T.; Orlando, S.A.; et al.
- 2022.
- High SARS-CoV-2 viral load in travellers arriving in Spain with a negative COVID-19 test prior to departure: Ecuador as a model for COVID-19 testing quality in Latin America. Journal of travel medicine 2022, D.O.I: https://doi.org/10.1093/JTM/TAAC120
- Factor de impacto: 39.194 (2023). Q1 en la categoria Public Health, Environmental and Occupational Health
- Recolección de datos y escritura de artículo.

En el caso de las publicaciones anteriores realizadas en coautoría con el estudiante de doctorado, declaro que todos los firmantes que figuran en coautoría han autorizado la

inclusión de estos trabajos para la elaboración de esta Tesis Doctoral presentada como compendio de publicaciones y han renunciado a incluir dichas publicaciones en otra/s Tesis Doctoral/es presentadas como compendio de publicaciones.

En Badajoz, a 13 de junio de 2023.

Fdo.
Director de la Tesis Doctoral

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INDICE

1. RESUMEN

La pandemia a nivel mundial denominada COVID-19 causada por el coronavirus del síndrome respiratorio agudo severo de tipo 2 (SARS-CoV-2) se inició a finales de 2019 en China y; se propagó muy rápidamente a nivel mundial. En el Ecuador, el primer caso de COVID-19 se dio el 28 de febrero de 2020 y los casos fueron incrementando debido al ingreso de personas de países extranjeros donde existía circulación activa produciendo que aparezcan más casos en diferentes ciudades del país.

Conociendo que las pandemias son una realidad ineludible para el ser humano, los países de la región pusieron en marcha acciones para enfrentar la COVID-19. Entre estas medidas podemos mencionar los confinamientos estrictos, seguidos de restricciones a la movilidad, desarrollo de actividades de educación y empleos en modalidad virtual, protección social y sistemas sanitarios priorizados; que en algunos casos fueron muy tardíos debido al gran impacto y devastación que trajo consigo la pandemia. Se pudo confirmar que los sistemas de salud no estaban preparados y colapsaron, hubo deficiencia en el diagnóstico y falla en la comunicación interinstitucional, particularmente en países en vías de desarrollo como Ecuador. A pesar de todos estos inconvenientes, diversas instituciones públicas y privadas de investigación realizaron estudios para mejorar el diagnóstico de SARS-CoV-2, y estuvieron involucradas en el diagnóstico de COVID-19 en Ecuador en apoyo a los centros de referencia del Ministerio de Salud.

Se desarrollaron diversos estudios destinados a mejorar los protocolos diagnósticos de COVID-19 en Ecuador, así como para evaluar la calidad de diferentes pruebas diagnóstico-disponibles en el mercado (tanto pruebas RT-qPCR como pruebas de antígeno). Pudimos mostrar cómo es posible el desarrollo de protocolos de diagnóstico de infección por SARS-CoV-2 más económicos, lo cual tiene particular relevancia en el contexto de países en vías de desarrollo como Ecuador, donde la escasez de recursos para la contención de la pandemia fue constante. Además, pudimos constatar como muchas pruebas para diagnóstico molecular de infección por SARS-CoV-2 de baja calidad fueron comercializadas en Ecuador, mientras que no disponían de autorización para uso clínico en países desarrollados.

Además, nuestro laboratorio en la Universidad de Las Américas estuvo involucrado en diagnóstico de COVID-19 desde abril de 2020, desarrollando una intensa labor de diagnóstico gratuito en comunidades vulnerables de Ecuador. Por lo tanto, pudimos obtener información importante sobre la epidemiología de SARS-CoV-2 en diferentes parroquias desatendidas de las provincias de Esmeraldas, Manabí, Tungurahua, Cotopaxi, Bolívar, Napo, Amazonia y las Islas Galápagos del Ecuador con valores de prevalencia que indican que se dio trasmisión comunitaria de COVID-19 desde los primeros meses de la pandemia en 2020, poniendo de relieve la necesidad de realizar pruebas diagnóstico más allá de los pacientes sintomáticos que acuden a los centros hospitalarios. Además, se analizaron grupos de riesgo como personas que viven en albergues, unidades policiales, trabajadores de funerarias y personal de

hospitales confirmando su mayor vulnerabilidad frente a la infección por SARS-CoV-2. Finalmente, también se desarrollaron estudios sobre coinfecciones con otros patógenos respiratorios en pacientes de hospitales y ambulatorios en Ecuador.

Las publicaciones derivadas de esta tesis doctoral pueden servir como referente para el manejo adecuado de futuras pandemias, poniendo énfasis en la necesidad de garantizar un diagnóstico de calidad en todos los países independientemente de su contexto socioeconómico. Además, nuestros resultados ponen de relieve la necesidad de una masificación del diagnóstico incluyendo a población no hospitalaria para el control de futuras pandemias causadas por agentes patógenos cuya infección puede cursar sin el desarrollo de síntomas.

2. ABREVIATURAS

ACE2: enzima convertidora de angiotensina humana 2

ARCSA: Agencia Nacional de Regulación, Control y Vigilancia Sanitaria

CDC: Centros para el Control y la Prevención de Enfermedades

COVID-19: Enfermedad por Coronavirus de 2019

EPP: Equipos de protección personal

EUA: Autorización de uno de emergencia

FDA: Administración de Drogas y Alimentos de los EE. UU

GIR: Grupo de intervención y rescate

GOE: Grupo de operaciones especiales

IC: Intervalo de confianza

INSPI: Instituto de Investigación en Salud Pública

LoD: límite de detección

NAAT: Pruebas de amplificación de ácidos nucleicos

NP: Nasofaríngeo

OMS: Organización Mundial de la Salud

PDR: Pruebas diagnósticas rápidas

PDR-Ag: Pruebas diagnósticas rápidas basadas en la detección de antígenos.

RT-qPCR: reacción en cadena de la polimerasa cuantitativa de transcripción inversa

SARS-CoV-2: Coronavirus de tipo 2 causante del síndrome respiratorio agudo severo

SRAS: Síndrome de dificultad respiratoria aguda o grave por neumonía severa

UDLA: Universidad de Las Américas

VOC: Variantes de preocupación

VOI: Variantes de interés

VSR: Virus Sincitial Respiratorio

3. INTRODUCCIÓN

La introducción de la tesis doctoral representa en su conjunto todos los artículos presentados dentro de los requerimientos para un compendio de publicaciones, debido a que los mismos están relacionados con estudios de validaciones de métodos alternativos, evaluación de kits comerciales para el diagnóstico de SARS-CoV-2 por RT-qPCR y estudios de epidemiología de la COVID-19 en Ecuador.

La enfermedad del nuevo coronavirus (COVID-19) fue descrita inicialmente a finales de 2019 cuando se informaron los primeros 27 casos en Wuhan-China, causada por el virus denominado SARS-CoV-2 e infectando a más de 765 millones de personas en todo el mundo y causando más de 6 millones de muertes hasta mayo de 2023 [1].

Desde las primeras etapas de la pandemia de COVID-19, la Organización Mundial de la Salud (OMS) hizo una amplia variedad de recomendaciones, entre estas están: usar en el rostro máscaras para reducir la propagación de partículas de aerosol que contienen el virus, distanciamiento social y aislamiento de casos confirmados para frenar la propagación de la enfermedad.

En cuestión de semanas, aumentó la demanda de diagnóstico clínico microbiológico basado en herramientas moleculares. El estándar de oro para la detección del SARS-CoV-2 es la reacción en cadena de la polimerasa cuantitativa de transcripción inversa (RT-qPCR). La OMS respaldó en las primeras etapas de la pandemia varios ensayos de RT-qPCR desarrollados por instituciones públicas como los Centros para el Control y la Prevención de Enfermedades (CDC) en los Estados Unidos de América o el Hospital Charité en Alemania [2].

Además, mientras avanzaba la pandemia la Organización Mundial de la Salud respaldó el uso de pruebas diagnósticas rápidas (PDR) desde el 8 de abril de 2020 al emitir una guia de uso y sobre todo que se pueda garantizar pruebas diagnósticas fiables, menos costosas y más rápidas que detecten antígenos específicos de la infección por el SARS-CoV-2 y la necesidad de seleccionarlas cuidadosamente [3].

3.1. Patogenia de COVID-19

Los coronavirus pertenecen a la superfamilia Nidovirus y a la familia Coronaviridae, que consta de dos subfamilias: Coronavirinae y Torovirinae. Se dividen en cuatro géneros: α, β, γ y δ y son virus de ARN monocatenario dentro de una envoltura viral con una morfología similar a una corona. Los del SARS y el MERS son β-coronavirus, aunque de linajes diferentes [4].

El genoma codifica cuatro o cinco proteínas estructurales: una proteína de las espículas o espiga (S), otra de la envoltura (E), de la membrana (M), de la nucleocápside (N), y en algunos virus una proteína hemaglutinina-esterasa (HE), varios marcos de lectura abierta dispersos por los genes estructurales, así como una poliproteína que se procesa en múltiples proteínas no estructurales. Estas proteínas no estructurales participan en la replicación del virus; pero no se incorporan en el virión [5]. La proteína espiga del SARS-CoV-2 se une con

gran afinidad a la enzima convertidora de angiotensina humana 2, o receptor ACE2, pero también puede interactuar con otros receptores y enzimas [6].

El virus se propaga rápidamente, produce un cuadro respiratorio febril, con síntomas generales, rinorrea, tos intensa y disnea, pueden existir vómitos y diarreas, y entre 10% a 25% sufren un síndrome de dificultad respiratoria aguda o grave por neumonía severa (SRAS) que puede llevar a un fallo de órganos multisistémico con una letalidad elevada, combinada con lesiones en los pulmones y corazón [7,8].

Los coronavirus son la causa de muchas enfermedades tanto en animales salvajes como en animales domésticos. En los seres humanos, las infecciones por coronavirus más prevalentes causan el resfriado común. Sin embargo, los coronavirus asociados con el SARS-CoV-2 han mostrado potencial para enfermedades graves, ya que, después de la infección viral, se produce una serie de alteraciones moleculares y celulares posteriores en el huésped que se han implicado en la progresión de los signos y síntomas observados en pacientes con COVID-19 [9].

La pandemia de COVID-19 generó un impacto dramático en todo el mundo, principalmente en países de bajos y medianos recursos, siendo notorio en el caso de Ecuador. Hasta la fecha, se reportan de marzo de 2020 a mayo de 2023 en Ecuador 1.065.013 casos confirmados de COVID-19 por laboratorio y 67.527 defunciones (confirmadas y probables). El exceso de mortalidad en el primer año de pandemia alcanzó el 55% y en el 2023 es del 4% [10].

Bajo este escenario, varias universidades han jugado un papel importante para mejorar las capacidades de prueba de SARS-CoV-2 en el país al adaptar sus instalaciones de investigación y personal hacia el diagnóstico clínico estimando que al menos 140.000 pruebas fueron realizadas por universidades ecuatorianas hasta el 31 de enero de 2021, lo que representa más del 15% de la prueba de diagnóstico para SARS-CoV-2 en ese momento. Además, se han destacado en su afán por evaluar nuevos protocolos relacionados con el diagnóstico de laboratorio de SARS-CoV-2, incluidos estudios que abordan el uso de hisopos de algodón para la recolección de muestras, un sistema eficiente de alto rendimiento con agrupación de muestras antes de la RT-qPCR, el rendimiento clínico de un enfoque sin extracción de ARN para el diagnóstico de SARS-CoV-2 o un nuevo método multiplex RT-qPCR [11–16].

Debido a la gran importancia y necesidad de conocer la pandemia de COVID-19 en el contexto ecuatoriano, esta tesis esta fragmentada en 2 bloques que describen los diferentes ámbitos a los que se realizó una vigilancia activa. El primer bloque se enfoca en mejoras en el diagnóstico, incluyendo el desarrollo de nuevos protocolos más económicos, así como evaluaciones de kits comerciales para detección de SARS-CoV-2 y virus respiratorios; el segundo bloque se enfoca en la aplicación del conocimiento del bloque anterior para un diagnóstico masivo de calidad para obtener información epidemiológica de la COVID-19 junto a la presencia de otros patógenos.

3.2. Estudios de validación de métodos alternativos y evaluación de kits comerciales para el diagnóstico de SARS-CoV-2 por RT-qPCR.

La pandemia creó una gran demanda de reactivos e insumos comerciales para el diagnóstico de SARS-CoV-2. Algunas de estas pruebas recibieron autorización de uso de emergencia de la Administración de Drogas y Alimentos de los EE.UU (FDA) o se incluyeron en la lista de uso de la OMS. Para otros, la información sobre su desempeño clínico era escasa y no contaban con autorización de uso de emergencia en el país de producción [17–22]. En Ecuador, no se requiere una evaluación experimental local del desempeño clínico antes de usar los ensayos en pacientes para la detección de SARS-CoV-2, ya que no se realizan estudios por la Agencia Nacional de Regulación, Control y Vigilancia Sanitaria (ARCSA), por falta de recursos e infraestructura o debido a la urgencia de un diagnóstico en la pandemia. Por las razones expuestas, varias universidades e instituciones han realizado evaluaciones del rendimiento de varios kits disponibles en el mercado ecuatoriano [23–25].

Nuestro grupo de investigación desde el laboratorio de diagnóstico de SARS-CoV-2 en la UDLA ha mejorado la calidad y accesibilidad al diagnóstico mediante la validación del desempeño clínico y la sensibilidad analítica de kits comerciales para SARS-CoV-2 RT-PCR, uso de cotonetes de algodón en la toma de muestra, validación de técnicas sin extracción previa de RNA, validación de mezclas maestras en el desarrollo de pruebas moleculares, pruebas serológicas y de antígenos. Adicionalmente, la UDLA ha desarrollado el "ECUGEN SARS-CoV-2 RT-PCR kit", el primer kit SARS-CoV-2 RT-PCR fabricado en Ecuador

3.2.1. Desarrollo de protocolos para reducir costos en el diagnóstico por RT-qPCR de SARS-CoV-2

- *Hisopos de plástico con punta de algodón para el diagnóstico y evitar la escasez de suministros* [16].

Los sistemas de salud pública han sido desafiados y se han visto abrumados en países en desarrollo como Ecuador durante la pandemia, presentando un diagnóstico interrumpido por la falta de insumos principalmente en lugares aislados como Las Islas Galápagos, especificamente de hisopos de fibra sintética. El hisopado nasofaringeo (NP) es el método de muestreo de referencia para el diagnóstico de SARS-CoV-2, según lo recomendado por la Organización Mundial de la Salud (OMS) y los Centros para el Control y la Prevención de Enfermedades (CDC) [26–28]. Bajo este escenario, realizamos un estudio de validación para el muestreo de NP para el diagnóstico de SARS-CoV-2 utilizando hisopos de plástico con punta de algodón fácilmente disponibles y no encontramos el efecto de inhibición en la reacción de PCR que ocurre con los de madera y tienen un rendimiento equivalente a los hisopos de rayón. Conociendo que los hisopos de plástico con punta de algodón se producen masivamente en todo el mundo y evitarían la escasez de suministro de hisopos bajo las altas demandas actuales de pruebas de SARS-CoV-2, particularmente en los países en desarrollo.

- *Evaluación analítica y clínica de un método de detección de SARS-CoV-2 por choque térmico sin extracción de ARN* [12].

Los Centros para el Control y la Prevención de Enfermedades (CDC) y la Organización Mundial de la Salud (OMS) recomiendan estrictos protocolos de diagnóstico molecular RT-qPCR SARS-CoV-2 con especificaciones para la recolección de muestras y extracción de ARN [26,27]. Bajo este escenario, los kits de extracción de ARN se encuentran entre los insumos más demandados para el diagnóstico del SARS-CoV-2. El principio de choque térmico se basa en la alteración de la integridad física de los virus a altas temperaturas, lo que permite la liberación de ARN viral para la detección por RT-PCR. El presente estudio evaluó un método de choque térmico para la detección de SARS-CoV-2 sin extracción de ARN utilizando los protocolos de RT-PCR CDC (gen N) y Charite (gen E) [29,30], con un tamaño de muestra significativo. Comparamos el rendimiento clínico y la sensibilidad analítica del método de choque térmico para la detección de SARS-CoV-2 con los resultados obtenidos utilizando un protocolo de kit de extracción de ARN manual basado en columna.

- *"Pools" de muestras de extractos de ARN para acelerar el diagnóstico de SARS-CoV-2* [11].

El ensayo de los CDC llamado 2019-nCoV se basa en sondas N1 y N2 para detectar SARS-CoV-2 y RNaseP (RP) como un control de calidad de extracción de ARN, ha recibido autorización de uso de emergencia (EUA) de la Administración de Drogas y Alimentos de los EE. UU. (FDA) [26]. De acuerdo con el protocolo de los CDC para 2019-nCoV CDC EUA, las 3 sondas están etiquetadas con FAM, por lo que se necesitan 3 reacciones de PCR para cada diagnóstico de muestra. Sin un protocolo de PCR triplex validado para N1, N2 y RNaseP, el protocolo CDC actual reduce la capacidad de procesamiento de muestras diarias para un dispositivo de PCR de placa de 96 pocillos típico. En países en desarrollo como Ecuador, la mayoría de los laboratorios de microbiología clínica que realizan diagnósticos de SARS-CoV-2 operan con un solo dispositivo de PCR en tiempo real. En este escenario, la utilización de "pools" de muestras es una herramienta poderosa para aumentar la capacidad de prueba del SARS-CoV-2 [31,32]. Además, los costos de las pruebas se reducen y la escasez de suministros se puede mitigar mediante el uso de un protocolo de "pools", crucial para respaldar la vigilancia en los países en desarrollo. Este estudio evalúa el rendimiento de un protocolo de RT-qPCR de "pools" de muestras donde las muestras se agrupan después de la extracción de ARN y se cargan en la misma reacción de RT-qPCR para el diagnóstico de SARS-CoV-2, utilizando el kit 2019-nCoV CDC EUA (IDT, EE.UU.).

- *Validación y desempeño analitico de varias enzimas para RT-qPCR usadas para la deteccion de SARS-CoV-2.*

Durante la pandemia del SARS-CoV-2, la ausencia de reactivos ha permitido utilizar varios kits de diagnóstico comerciales, formando diferentes combinaciones con mezclas maestras de enzimas de RT-qPCR que se encuentran disponibles en el mercado, específicamente en Ecuador. La pregunta de cómo esta variación genera cambios en la sensibilidad y especificidad de los protocolos aprobados por la Organización Mundial de la Salud fue de interés para el grupo de investigación. El objetivo de nuestro estudio es indicar cómo influye el uso de una u otra mezcla maestra de un solo paso (TaqMan Fast Virus 1-Step

Master Mix y liofilizado 1-step RT polimerasa mix, Tib-Molbiol) en el rendimiento clínico de kits comerciales para la detección de SARS-CoV-2, utilizando los protocolos CDC y Charité-Berlin en el contexto ecuatoriano.

Entre las medidas adoptadas para desarrollar técnicas más accesibles y económicas se encuentran la optimización de los pasos que limitan la velocidad, ayudan a aumentar la disponibilidad de las pruebas, reducir su tiempo de respuesta y costes y el uso de una mezcla maestra eficiente ayuda a economizar procedimientos. A la vez, debe quedar claro que las mezclas maestras comerciales deben ser validadas para saber si hay variación de sensibilidad en los protocolos ya elaborados y aprobados para de esta manera, mejorar la calidad del diagnóstico y evitar repeticion de ensayos. Este estudio se encuentra en proceso de publicación.

- *Validación del método por homogenización sin extracción previo a la RT-qPCR* [33].

Existen varios métodos de extracción de ARN, tanto químicos como mecánicos, así como kits comerciales que aseguran una extracción óptima de ARN. Todos estos métodos de extracción consumen mucho tiempo y provocan un retraso en el diagnóstico clínico. Además, este paso de extracción de ARN aumenta el costo del análisis al usar más reactivos y suministros desechables [34]. Uno de los métodos de extracción de ácidos nucleicos más convencionales es el aislamiento por cromatografía sólida, comúnmente conocido como kit de extracción de ARN por columnas, que atrapa el material genómico mediante resinas cargadas positivamente, seguido de pasos de elución que producen una muestra pura de alta calidad. Se han propuesto varias alternativas a los kits de extracción de ARN convencionales para la detección del SARS-CoV-2. Esos métodos alternativos combinan tratamiento de choque térmico y/o tampones de lisis, y la sensibilidad reportada varía de 77,5% a 100% [12]. Además, la lisis osmótica y mecánica se ha propuesto previamente como una alternativa a la extracción de ARN para la detección de ácidos nucleicos virales [35]. Sin embargo, hasta donde sabemos, este método rápido y económico aún no se ha probado para el diagnóstico del SARS-CoV-2. Por lo tanto, el objetivo de este estudio fue evaluar el rendimiento clínico de un protocolo simple de detección de SARS-CoV-2 basado en la homogeneización con un molino de bolas seguido de RT-qPCR directa.

3.2.2. Estudios de validación de pruebas diagnóstico RT-qPCR para detección de SARS-CoV-2.

- *La calidad de las pruebas comerciales de ácido nucleico de SARSCoV-2 en Ecuador: lecciones de la pandemia de COVID-19 para avanzar en la equidad social a través de la microbiología* [14,15,18,36].

La pandemia de COVID-19 creó una enorme demanda de NAAT ("nucleic acid amplification test") por parte de los laboratorios de todo el mundo como nunca se había visto. Bajo este escenario, cientos de kits comerciales de NAAT para el diagnóstico de SARS-CoV-2 estuvieron disponibles durante los últimos tres años, algunos de ellos con Autorización de Uso de Emergencia (EUA) por parte de la Administración de Drogas y Alimentos de los Estados Unidos (FDA) u otras agencias reguladoras internacionales, mientras que otros

cuentan con escasa información relacionada con su desempeño clínico y falta de EUA en el país de fabricación.

Afortunadamente, el desempeño de estos kits ha sido evaluado por grupos de investigación a nivel mundial con la posterior publicación de sus resultados en revistas científicas. En Ecuador se evaluaron un total de once kits SARS-CoV-2 NAAT. Hubo diferencias preocupantes en el rendimiento clínico entre esos kits de NAAT para SARS-CoV-2 con valores de sensibilidad que oscilaron entre el 63,4 y el 100 %, en comparación con el estándar de oro, y un límite de detección que osciló entre 500 y 100000 copias/mL. Cabe destacar que cinco de esos kits de NAAT para SARS-CoV-2 arrojaron una sensibilidad sustancialmente más baja (63,4 a 78,9 %) y un límite de detección defectuoso (8000-100 000 copias/mL) que el informado por los fabricantes.

Con base en estos hallazgos, proponemos implementar políticas de salud pública éticamente correctas en países de ingresos bajos y medianos con el objetivo de autorizar la comercialización y el uso de kits SARS-CoV-2 NAAT confiables para aquellos que al menos obtienen EUA en el país donde el fabricante tiene su sede. Esto sería particularmente relevante para países como Ecuador, donde las autoridades locales de salud pública no realizaron evaluaciones experimentales para otorgar EUA a los kits SARS-CoV-2 NAAT.

- *Validación de 2 kits de qPCR fabricados en Sudamérica: Uruguay y Ecuador* [37].

Los kits comerciales se basan en la detección de SARS-CoV-2 al apuntar a diferentes genes como E, S, N o la región orf1ab. El kit FDA EUA 2019-nCoV CDC diseñado por los CDC (IDT, EE. UU.) se basa en objetivos genéticos N1 y N2 para detectar el SARS-CoV-2 y la RNasa P como un control de calidad de extracción de ARN, se considera un estándar de oro en todo el mundo para la detección de SARS-CoV-2 [26,38] La principal limitación del kit de CDC es la necesidad de ejecutar tres reacciones de RT-qPCR por muestra. Para solucionar este inconveniente, inspirados en este protocolo de los CDC, se diseñaron, produjeron y comercializaron dos ensayos multiplex SARS-CoV-2 RT-qPCR en América del Sur.

"COVID-19 RT-PCR Real TM FAST (CY5)" (ATGen, Uruguay) es un ensayo dúplex que incluye el objetivo viral N1 y la RNasa P como control de calidad de extracción de ARN y "ECUGEN SARS-CoV-2 RT-qPCR" (UDLA- STARNEWCORP, Ecuador) es un ensayo tríplex que incluye dianas virales N1 y N2 y también RNasa P como control de calidad de extracción de ARN. Estos kits requieren una sola reacción de PCR para la detección de SARS-CoV-2, lo que reduce el tiempo y el costo del diagnóstico. La evaluación de la sensibilidad analítica y desempeño clínico fue realizada para los dos kits sudamericanos frente al estándar de oro de la CDC en muestras nasofaríngeas.

- *Comparación analítica y clínica de los kits Viasure y ECUGEN CDC RT-qPCR para el diagnóstico de SARS-CoV-2, Influenza A/B y Virus Respiratorio Sincitial.*

De acuerdo con los datos actualizados de 2018 por el Ministerio de Salud Pública, indica 1.280 pacientes confirmados con Influenza, constituyendo el 33% de afecciones respiratorias agudas [39]. Las epidemias de influenza normalmente alcanzaron su punto máximo en

diciembre y enero y las epidemias de Virus Respiratorio Sincitial en marzo. El virus sincitial ocurre principalmente en circulación conjunta con la influenza.

El diagnóstico puede ser problemático, ya que una amplia gama de patógenos puede causar infecciones respiratorias que se presentan con síndromes clínicos similares. Se ha demostrado que los ensayos de PCR en tiempo real son una herramienta de diagnóstico sensible y específica para la detección de los virus SARS-CoV-2, Influenza A/B y virus Sincitial respiratorio (VSR). Es necesario contar con una prueba diagnóstica que nos permita diferenciar el virus respiratorio causal y tomar las medidas adecuadas, de ahí la importancia de este estudio. CerTest Biotec, empresa de España, ha desarrollado kits de detección por PCR en tiempo real para SARS-CoV-2/Flu A, B/RSV que permiten analizar el material de los pacientes y detectar la presencia de estos virus. Nuestra tarea es validar estos kits e informar si son aptos para la detección por el valor de sensibilidad y especificidad. Este estudio se encuentra en proceso de publicación.

3.2.3. Estudio de validación de pruebas de inmunocromatografía de flujo lateral para detección de SARS-CoV-2.

- *Pruebas rápidas de antígenos disponibles en Ecuador* [40].

La mayoría de las pruebas diagnósticas rápidas basadas en la detección de antígenos (PDR-Ag) de la COVID-19 se basan en un método de inmunodetección de tipo sándwich y emplean un formato de prueba de inmunocromatográfica de flujo lateral fácil de usar, que detectan las proteínas de la nucleocápside del virus. Las PDR-Ag suelen consistir en un cartucho de plástico con pocillos para la muestra y el tampón, una tira de matriz de nitrocelulosa con una línea de prueba en la que se han inmovilizado anticuerpos específicos contra los complejos antígeno de interés anticuerpo conjugado y una fila de control en la que se han inmovilizado anticuerpos específicos contra los anticuerpos conjugados [3]. Estas pruebas suelen ser denominadas de varias formas, durante toda esta tesis se usarán las siglas PDR-Ag. Nuestro objetivo fue conocer el desempeño clínico y la sensibilidad analítica de tres marcas comerciales de PDR-Ag disponibles en Ecuador.

A fines de 2020 y durante 2021, las agencias reguladoras internacionales o las autoridades de salud pública respaldaron el uso de varias marcas comerciales de PDR-Ag para la detección del SARS-CoV-2 [41,42]. Los inmunoensayos de flujo lateral para SARS-CoV-2 permiten la identificación en el punto de atención del virus en muestras nasofaríngeas, orofaríngeas o nasales en un período de tiempo de 10 a 30 minutos, según la marca comercial. Además, las PDR-Ag pueden ser realizadas por personal de enfermería o por el propio paciente, considerando que son más baratas y más rápidos constituyen una herramienta poderosa para la vigilancia del SARS-CoV-2 por la detección masiva en comunidades de bajos ingresos [43–46]. La sensibilidad de estas pruebas rápidas es superior al 90 % para cargas virales con > 10^6 copias genómicas de virus/mL [47], siendo aceptable dentro de un escenario como el de la pandemia de COVID-19 porque se acompaña de un aumento de las capacidades de prueba detectando un mayor número de personas positivas [48]. Estas validaciones deben realizarse progresivamente debido a mutaciones que experimenta el virus y la reactividad cruzada con patógenos infecciosos [49–51].

- *Sensibilidad analítica de dos marcas de pruebas rápidas de antígenos para 6 variantes de SARS-CoV-2.*

Los virus de ARN tienen tasas de mutación extremadamente altas, lo que se asocia con una mejor adaptación y la rápida evolución [52–54]. Esta continua evolución viral da lugar a la aparición de numerosas variantes con importantes mutaciones en proteínas estructurales, modificando indirectamente la respuesta inmunitaria del huésped y comprometiendo la eficacia terapéutica de las vacunas, lo que genera preocupación en todo el mundo por el aumento de la transmisión y la mortalidad [55,56], con este antecedente es fundamental comprender la epidemiología de los linajes de SARS-CoV-2 en circulación y compararlos con dos marcas de pruebas rápidas de antígenos ampliamente disponibles en el país, para identificar diferencias en las propiedades de detección de cada prueba asociada a una variante viral específica. Según la OMS, las variantes del SARS-CoV-2 se clasifican como variantes de interés (VOI) o variantes de preocupación (VOC). Este método de clasificación permite realizar un seguimiento de variantes circulantes [57,58]. Hemos desarrollado un estudio comparativo de la sensibilidad de diferentes pruebas de antígenos comerciales para la detección de variantes de SARS-CoV-2, que se encuentra en proceso de publicación.

3.3. Estudios de epidemiología de COVID-19 en Ecuador

El primer caso registrado de contagio de COVID-19 en América Latina y el Caribe fue notificado en la última semana de febrero de 2020 en Sao Paulo (Brasil). Desde entonces, se han reportado contagios en todos los países de la región, llegando a zonas marginadas con pobreza notoria [59,60]. Debido a la débil infraestructura de salud, la falta de personal, la falta de equipos de bioseguridad y la desconfianza en el gobierno público, esta región se vio muy afectada por la pandemia [61].

Ecuador sufrió la ola más letal de la pandemia en el primer trimestre de 2020, reportando un 700% más de muertes per cápita que los Estados Unidos de América en su día más crítico [62,63]. Considerando que hubo un acceso limitado a las pruebas del SARS-CoV-2 debido a que solo los 3 laboratorios del Instituto Nacional de Investigación en Salud Pública (INSPI) realizaban la detección. Esto condujo a una crisis de salud pública como se vio durante marzo-abril de 2020 en Guayaquil, la ciudad más poblada de Ecuador [64], lo que se tradujo en una tasa de pruebas deficiente de 7,46 pruebas PCR por cada 10.000 personas, y una de las tasas de mortalidad por COVID-19 más altas (10,93 muertes por millón de personas) en América Latina [62]. Durante los primeros meses de la pandemia, las pruebas PCR disponibles por parte del Ministerio de Salud Pública apenas alcanzaban 3000/día para una población de casi 17 millones de habitantes [25].

Con la ayuda de las brigadas médicas realizadas por la Universidad de Las Américas se pudieron cubrir 18 de las 24 provincias en Ecuador evaluando principalmente a población asintomática de áreas rurales desatendidas y grupos de riesgo tratando de cumplir la estrategia de un programa de vigilancia exitosa por la OMS para contener la propagación de COVID-19 que se basa en evaluar a la mayor cantidad posible de población y que no más del 5% de las personas analizadas para SARS-CoV-2 deben ser positivas [25].

Nuestro aporte fue particularmente relevante para las provincias de Pichincha, Manabí, Esmeraldas, Chimborazo, Pastaza y en las Islas Galápagos, siendo la última hasta la fecha la provincia con mayor número de pruebas de SARS-CoV-2 per cápita.

Dentro de todos los estudios epidemiológicos realizados es posible dividirlos por localidades ecuatorianas, grupos de riegos, por variables externas como altitud y coinfección.

3.3.1. Diagnóstico de SARS-CoV-2 en comunidades ecuatorianas.

- *Comunidades rurales pobres en la región costera de Ecuador (Manabí y Esmeraldas)* [17,65].

Los estudios realizados se enfocan en conocer la epidemiologia de dos provincias costeras vulnerables, conociéndose que se encuentran en la categoría tercera y octava de población en Ecuador y con el preámbulo de haber sufrido catástrofes naturales recientes (que aún afectan a la infraestructura de salud) como en Manabí, y ser una de las más pobres como el caso de Esmeraldas.

Manabí es la cuarta provincia ecuatoriana más grande con 22 cantones, teniendo una población rural que abarca el 44% [66]. Posee una deficiencia e inoperancia en la

infraestructura de 59 hospitales y algunos centros de salud. En 2020, durante la pandemia solo cuenta con un laboratorio de diagnóstico de SARS-CoV-2 de pequeña capacidad dentro del sistema de salud pública, lo que se traduce en una vulnerabilidad frente a la pandemia. La ayuda en diagnóstico fue realizada en coordinación con la comunidad local de líderes, el Ministerio de Salud y el gobierno regional (Prefectura de Manabí) en comunidades rurales de 15 cantones entre agosto y septiembre de 2020.

Esmeraldas se encuentra al norte de la región costera; es la séptima provincia más grande en superficie con 491.168 habitantes, distribuidos en su mayoría en comunidades rurales de siete cantones. Además, el 43% de su población se identifica como afroecuatoriana, lo que convierte a Esmeraldas en la principal región afroecuatoriana del Ecuador [66,67]. Según el Ministerio de Salud, durante el primer semestre de la pandemia de COVID-19, con un total de 9.129 pruebas SARS-CoV-2 RT-qPCR realizadas, menos del 2% de la población de Esmeralda se realizó la prueba a pesar de la dramática tasa de positividad del 39,8% informada. Por lo que, conocer la situación epidemiológica de la provincia de Esmeraldas durante octubre de 2020 en coordinación con líderes comunitarios locales, colegas de la Universidad Técnica Luis Vargas Torres y el gobierno provincial fue el principal objetivo en la pandemia por SARS-CoV-2.

- *Comunidades indígenas (Sierra y Amazonía) [68–70].*

Sierra: Los grupos vulnerables infectados con COVID-19 incluyen no solo a los ancianos y las personas con comorbilidades, sino también a las poblaciones indígenas históricamente desatendidas [71–74] Hay más de 476 millones de indígenas en el mundo, altamente representados y tradicionalmente desatendidos en América Latina [75,76]. En Ecuador, los indígenas representan más del 7% de la población total y se asocian principalmente con comunidades rurales marginadas [77,78]. Esas comunidades suelen estar aisladas o mal comunicadas y tienen poco acceso a los servicios de salud retrasando la búsqueda de atención médica, lo que complica el manejo temprano y, por lo tanto, genera mayores riesgos de complicaciones y mortalidad en un escenario como el de la pandemia de COVID-19. En Ecuador, el Consejo Nacional para la Igualdad de los Pueblos y Nacionalidades ha exigido la protección de los pueblos indígenas, reportando brotes de COVID-19 entre sus comunidades y reclamando el apoyo de las autoridades de salud pública para contener la pandemia en sus comunidades. Bajo este escenario, siguiendo la solicitud de los líderes comunitarios, llevamos a cabo una prueba de vigilancia de SARS-CoV-2 entre indígenas y mestizos que viven en comunidades en los Andes ecuatorianos de las provincias de Tungurahua, Cotopaxi, Bolívar y Napo, pocas semanas después de que se levantara el confinamiento poblacional en junio de 2020.

Amazonía: Los brotes de COVID-19 en las comunidades indígenas amazónicas son particularmente preocupantes para los pueblos Tagaeri y Taromenane, los últimos grupos no contactados de la Amazonía ecuatoriana en la Zona Intocable del Parque Nacional Yasuní, donde el acceso está restringido debido a la presencia de tribus amazónicas no contactadas y son comunidades que tienen algún contacto con el pueblo Waorani.

Los pueblos Waorani, Siona, Kichwa, Shuar y Kofan, algunos de los grupos más aislados de la zona y a menudo solo accesibles por barco o avión, fueron incluidos en esta intervención de junio a septiembre de 2020. Nuestro objetivo fue conocer la tasa de detección y carga viral de SARS-CoV-2 en comunidades aisladas entre algunos de los grupos indígenas y en peligro de extinción en la Amazonía ecuatoriana. Este trabajo fue realizado con ayuda de organizaciones sin fines de lucro, indígenas, líderes comunitarios y las autoridades regionales de salud pública del Ministerio de Salud.

A todo el personal del equipo de investigación que asistió a las comunidades se les realizó la prueba de SARS-CoV-2 por RT-PCR 24 h antes de viajar. En el trabajo con las comunidades siempre se utilizó el equipo de protección personal requerido. Además, se tomaron muestras de cada comunidad inmediatamente después de su llegada, por lo que se pudo descartar la transmisión del personal científico a las comunidades. Descubrimos que algunos miembros de estas comunidades habían viajado recientemente desde áreas urbanas y suburbanas. Además, antes de que las comunidades supieran del brote de COVID-19, todavía celebraban sus festividades tradicionales como beber chicha fermentada con saliva de una olla para compartir, lo cual es una práctica de alto riesgo de infecciones cruzadas bacterianas o virales [79].

- *Islas Galápagos* [23,80].

Las Islas Galápagos están ubicadas a 972 km de la costa ecuatoriana y comprenden 13 islas, de las cuales 4 se encuentran habitadas. Las Islas Galápagos tienen 25.124 habitantes, distribuidos en las islas Santa Cruz (Población: 15.393), San Cristóbal (Población: 7.330), Isabela (Población: 2.256) y Floreana (Población: 145) [78]. En este territorio, para el 6 de abril de 2020 se contaba con un laboratorio de pruebas de SARS-CoV-2, siendo la única provincia del país que contaba con un laboratorio propio fuera de la red de los centros del Instituto Nacional de Salud Pública e Investigación localizados en las tres principales ciudades del país (Quito, Guayaquil y Cuenca), debido a la colaboración interinstitucional entre el gobierno regional de las Islas Galápagos ("Consejo de Gobierno"), las autoridades locales de Santa Cruz, San Cristóbal e Isabela, las autoridades regionales del Ministerio de Salud del Ecuador, la "Agencia de Regulación y Control para la Bioseguridad y Cuarentena para Galápagos" pudiendo demostrar la utilidad de las pruebas masivas de personas que viven en la comunidad para controlar la propagación del SARS-CoV-2.

3.3.2. Diagnóstico de SARS-CoV-2 en grupos de riesgo.

- *Albergues para mujeres víctimas de violencia* [81].

Puntos críticos para la infección y el contagio de SARS-CoV-2 en albergues para refugiados, viviendas para víctimas de violencia de género, prisiones o centros de detención provisional son el confinamiento y la falta de infraestructura adecuada [82], convirtiéndose en condiciones que promueven la propagación del virus, junto con la deficiencia en la capacidad de realización de pruebas por sistemas de salud débiles de países en desarrollo [83,84].

En este contexto, la Universidad de Las Américas y la oficina del Alto Comisionado de las Naciones Unidas para los Refugiados (ACNUR) en Ecuador coordinaron esfuerzos para otorgar acceso a pruebas PCR de SARS-CoV-2 a los ocupantes de diez albergues para mujeres víctimas de violencia de género localizada en todo el país en septiembre de 2020.

- *Unidades policiales y fuerzas especiales* [85].

La primera medida adoptada por la mayoría de los países en la pandemia por SARS-CoV-2 fue implementar bloqueos en todo el país declarando el estado de emergencia [86]. Las fuerzas de seguridad fueron las responsables de controlar el distanciamiento social y garantizar que la población siguiera las medidas de control para reducir la propagación del COVID-19 [87,88], experimentando un mayor riesgo ocupacional de exposición al virus. Teniendo como antecedente que 145 de los 264 policías que murieron en el cumplimiento de su deber en 2020 fueron víctimas de COVID-19 en los Estados Unidos [89]. En América Latina, se han informado números dramáticos de infecciones por SARS-CoV-2 y muertes relacionadas con COVID-19 entre policías, con 534 víctimas en Perú, 465 víctimas en Brasil y más de 1,000 soldados y policías infectados en Colombia para junio de 2020 [90–92].

Debido a la naturaleza de sus actividades, los agentes de policía corren un mayor riesgo de contagio de persona a persona a través de aerosoles cuando están cerca de alguien que tose, estornuda o habla [93]. En Ecuador, en el primer mes de la pandemia de COVID-19, hubo 248 policías y soldados infectados [62], de ahí el interés de realizar un tamizaje preventivo entre unidades policiales de fuerzas especiales en Quito (GOE/GIR), durante julio de 2020, apenas unas semanas después de que se levantara el confinamiento poblacional.

- *Trabajadores de funerarias [94].*

Las morgues y las funerarias han mantenido miles de cadáveres desde el comienzo de la pandemia. Esta demanda inusual y la incertidumbre de manejar cuerpos potencialmente infecciosos han estableció un riesgo laboral para muchos trabajos de primera línea junto a actividades que podrían resultar en contacto con fluidos corporales y superficies contaminadas conociendo que la incidencia de contaminación del equipo de protección personal después de una autopsia completa oscila entre el 15 - 65% [95–99]. Por esta razón, al ser un grupo vulnerable, nos hemos enfocado en el estudio de la epidemiologia del personal de funerarias que manejan y no manejan cuerpos para conocer las tasas de infección por COVID-19.

- *Personal de hospitales*

Los profesionales de la salud corren el riesgo de contraer una infección mientras brindan atención a los pacientes [100–102]. Miles de trabajadores de la salud en China se infectaron con SARS-CoV-2 debido a la falta de conciencia y al uso incorrecto de equipos de protección personal (EPP) en las primeras etapas de la pandemia de COVID-19. Por lo tanto, el papel de la transmisión nosocomial del SARS-CoV-2 se ha informado en todo el mundo y representa del 12 al 29% de los casos [103]. Además, dado que se estima que las infecciones asintomáticas, presintomáticas y sintomáticas leves por el SARS-CoV-2 representan alrededor de la mitad de todos los casos [104], algunos modelos han sugerido que las pruebas semanales de los

trabajadores de la salud asintomáticos podrían reducir la transmisión posterior en un 16-23 % junto al aislamiento basado en los síntomas [105].

Las infecciones entre los proveedores de atención médica son alarmantes debido a la falta de equipo de protección médica adecuado y detección rápida de infecciones por SARS-CoV-2 [106]. Por esta razón, nuestro objetivo fue conocer la tasa de ataque en profesionales de la salud principalmente asintomáticos y en un grupo administrativo y de trabajadores de 9 hospitales en Ecuador durante la primera ola de la pandemia de COVID-19 para evaluar su riesgo laboral.

3.3.3. Diagnóstico de SARS-CoV-2 considerando variable de altitud [107].

La categorización ampliamente adoptada por la comunidad de medicina de montaña define como baja altitud todo lo que se encuentra por debajo de los 1500 m, altitud moderada o intermedia entre los 1500 m a 2500 m, altitud elevada de 2500 m a 3500 m, altitud muy elevada de 3500 m a 5800 m, más de 5800 m gran altitud extrema y finalmente por encima de los 8000 m se considera la zona de muerte [108]. Existen ciertas interrogantes sobre la transmisión y la carga viral de la COVID-19 a diferentes altitudes [107,109,110]. Desde el comienzo de la pandemia, algunos investigadores han propuesto que la hipoxia hipobárica podría actuar como un factor protector contra el SARS-CoV-2 o mortalidad relacionada con COVID-19 [111,112]. Siendo el Ecuador un país apto para este estudio se planteó investigar esta hipótesis y la posible relación de la altitud con la infección por COVID-19.

Las hipótesis en torno al papel de la altura en la transmisión del SARS-CoV-2 y el impacto de la pandemia en estas poblaciones se pueden clasificar en tres grandes grupos: (1) El papel fisiológico y biológico de los organismos adaptados a la altura en relación con la transmisión del virus o replicación (es decir, el papel de los receptores ACE-2 en la altura), (2) la relación epidemiológica entre los factores sociodemográficos y la incidencia y mortalidad de COVID-19 en la altura (es decir, la densidad de población, las actividades de hacinamiento o la migración) y, por último, (3) las consecuencias directas o indirectas del medio ambiente sobre la virulencia o la transmisión viral [111,113].

3.3.4. Determinación de coinfecciones con otros patógenos respiratorios en individuos infectados por SARS-CoV-2.

Los virus alteran la barrera del epitelio respiratorio y el mecanismo de defensa del huésped, conduciendo a la entrada de microorganismos de manera directa o indirecta y por ende, una infección secundaria [114]. Las coinfecciones bacterianas son dominantes en todos los pacientes con COVID-19 entre 1 y 4 días después de la enfermedad, *S. pneumoniae* es el más común, seguido de *Klebsiella pneumoniae* y *Haemophilus influenzae* [115,116]. También se ha reportado la coexistencia con otros virus respiratorios, el más frecuentemente hallado es el de Influenza, siendo de similar presentación clínica en el mecanismo de transmisión y la coincidencia estacional [117].

- *Coinfección en pacientes ambulatorios y en unidad de cuidados intensivos*

En Ecuador se ha establecido un plan de vigilancia integral de COVID-19 y otros virus respiratorios a través del trabajo del Laboratorio Nacional de Referencia del Instituto Nacional de Investigación en Salud Pública (INSPI), pero no se dispone de datos. Por lo que, identificar la prevalencia de coinfección viral entre personas infectadas por SARS-CoV-2 en Ecuador es primordial para saber las diferentes manifestaciones clínicas en presencia de otros microorganismos concomitantes y proceder con un tratamiento eficaz, junto con la importancia de saber a qué área pertenecen los pacientes y las diferencias que pueden existir en aquellos que son ambulatorios y hospitalizados en la unidad de cuidados intensivos, ya que es un desafío diferenciar clínicamente entre una infección viral aislada relacionada con COVID-19 y una posible infección bacteriana o fúngica agregada [118,119]. Existe evidencia de competencia patógena entre virus respiratorios, esto podría deberse a la interferencia mediada por el sistema inmunitario que da como resultado que algunos virus se regulen a la baja durante el pico de otro virus, un fenómeno que se ha reconocido durante muchas décadas [120,121].

Se han realizado varios estudios indicando diferentes tasas de coinfección, tenemos que alrededor del 5,8 % de los casos positivos por COVID-19 en Wuhan estaban infectados con otros virus respiratorios [122]. Los virus respiratorios fueron los agentes más coinfectados [123], siendo la influenza uno de los virus de mayor preocupación [124,125].

Al menos 26 virus se han asociado con la neumonía adquirida en la comunidad y la causa de las coinfecciones. Entre ellos tenemos virus respiratorio sincitial, rinovirus, virus Influenza A, B y C, Metapneumovirus humano, Virus Parainfluenza tipos 1, 2, 3 y 4, Coronavirus tipos 229E, OC43, NL63, HKU1, adenovirus entre otros. Siendo de importancia las anteriormente mencionadas y evaluadas en este estudio.

4. JUSTIFICACIÓN

La pandemia de la COVID-19 decretada el 11 de marzo de 2020 como emergencia sanitaria, puso de relieve las falencias de los sistemas públicos de salud, sobre todo en países en desarrollo como Ecuador. En América Latina, la crisis fue inmensa; los sistemas de salud fragmentados, las economías más débiles y la alta densidad demográfica, así como la pobreza, eran la fórmula perfecta para un desastre [126,127].

Entre los principales problemas en los sistemas de salud destacó la escasez de suministros tanto insumos como reactivos ante la elevadísima demanda global de pruebas diagnósticas para la detección de SARS-CoV-2. Ante esta necesidad, fue necesario la implementación de protocolos alternativos de bajo costo e independientes de kits comerciales que solo estaban disponibles en mercados internacionales más accesibles a países desarrollados.

Además, debido a esta escasez de suministros, se permitió la comercialización de diversas pruebas diagnóstico de SARS-CoV-2 no contaban con la autorización de uso clínico en su país de fabricación. Por esta razón, universidades e instituciones privadas se enfocaron en realizar estudios de desempeño de kits disponibles en Ecuador.

La estrategia de control de la pandemia de COVID-19 implementadas por el Ministerio de Salud Pública estaba basada en la existencia de sólo 3 laboratorios de referencia a nivel nacional en las ciudades de Quito, Guayaquil y Cuenca, donde se priorizó el envío de muestras para detección del SARS-CoV-2 de pacientes sintomáticos hospitalizados, dejando de lado población asintomática o no hospitalizada. Por esta razón, abordamos estudios epidemiológicos incluyendo a población excluida de los muestreos del Ministerio de Salud Pública para una mejor comprensión de la pandemia de COVID-19 en Ecuador, considerando principalmente diferentes grupos de riesgo y comunidades en contextos de aislamiento y pobreza.

En definitiva, era necesario optimizar los recursos disponibles para el diagnóstico de SARS-CoV-2 para maximizar el número de población con acceso al diagnóstico, como estrategia fundamental para la contención de la pandemia COVID-19.

5. OBJETIVOS

Objetivo general

Optimizar herramientas de calidad y económicas para el diagnóstico de la COVID-19 permitiendo maximizar las pruebas realizadas en un contexto de pandemia y escasez de recursos.

Objetivos específicos

- Evaluar protocolos alternativos de detección para el diagnóstico de SARS-CoV-2 por RT-qPCR.
- Evaluar el desempeño clínico de las pruebas diagnóstico para SARS-CoV-2 disponibles en Ecuador.
- Aplicar los protocolos de diagnóstico desarrollados para maximizar el número de pruebas diagnóstico en población no hospitalizada en comunidades vulnerables y grupos de riesgo de Ecuador.
- Analizar los datos epidemiológicos obtenidos.

6. RESULTADOS: TRABAJOS PUBLICADOS

6.1. Estudios de validación de métodos alternativos y evaluación de kits comerciales para el diagnóstico de SARS-CoV-2 por RT-qPCR.

6.1.1. Rápido, económico y sensible: método sin extracción de ARN basado en homogeneizador para la detección de SARS-CoV-2 mediante RT-qPCR.

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El método de detección del estándar de oro del SARS-CoV-2 es una RT-qPCR con un paso previo de extracción de ARN viral de la muestra del paciente, ya sea mediante el uso de kits de extracción comerciales automatizados o manuales. Este paso de extracción de ARN es costoso y requiere mucho tiempo.

El objetivo de nuestro estudio fue evaluar el desempeño clínico de un protocolo simple de detección de SARS-CoV-2 basado en una homogenización rápida e intensa de la muestra seguida de RT-qPCR directa.

En este estudio se analizaron 388 hisopos nasofaríngeos. 222 de ellos dieron positivo para SARS-CoV-2 por el método de extracción de ARN estándar de oro y RT-PCR, mientras que 166 dieron negativo. 197 de esas 222 muestras positivas también dieron positivo para el protocolo de homogeneización, lo que arroja una sensibilidad del 88,74 % (95 % IC; 83,83 – 92,58). 166 de esas muestras negativas también lo fueron para el protocolo de homogeneización, por lo que la especificidad obtenida fue del 97% (95% IC; 93,11 –99,01). Para valores de Ct por debajo de 30, lo que significa una carga viral de 10^3 copias/uL, solo 4 muestras positivas para SARSCoV-2 fallaron para el método libre de extracción de ARN; para ese límite de detección, el método basado en homogeneizador tenía una sensibilidad de 97,92% (IC 95%; 96,01 – 99,83).

Nuestros resultados muestran que este método de homogeneización rápido y económico para la detección de SARS-CoV-2 por RT-qPCR es una alternativa fiable y de alta sensibilidad para pacientes positivos para SARS-CoV-2 potencialmente infecciosos. Este protocolo sin extracción de ARN ayudaría a reducir el tiempo y el costo del diagnóstico, y a superar la escasez de kits de extracción de ARN experimentada durante la pandemia de COVID-19.

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Fast, cheap and sensitive: Homogenizer-based RNA extraction free method for SARS-CoV-2 detection by RT-qPCR

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Background: The SARS-CoV-2 gold standard detection method is an RT-qPCR with a previous step of viral RNA extraction from the patient sample either by using commercial automatized or manual extraction kits. This RNA extraction step is expensive and time demanding.

Objective: The aim of our study was to evaluate the clinical performance of a simple SARS-CoV-2 detection protocol based on a fast and intense sample homogenization followed by direct RT-qPCR.

Results: 388 nasopharyngeal swabs were analyzed in this study. 222 of them tested positive for SARS-CoV-2 by the gold standard RNA extraction and RT-qPCR method, while 166 tested negative. 197 of those 222 positive samples were also positive for the homogenization protocol, yielding a sensitivity of 88.74% (95% IC; 83.83 – 92.58). 166 of those negative samples were also negative for the homogenization protocol, so the specificity obtained was 97% (95% IC; 93.11 – 99.01). For Ct values below 30, meaning a viral load of 10^3 copies/uL, only 4 SARS-CoV-2 positive samples failed for the RNA extraction free method; for that limit of detection, the homogenizer-based method had a sensitivity of 97.92% (95% CI; 96.01 – 99.83).

Conclusions: Our results show that this fast and cheap homogenization method for the SARS-CoV-2 detection by RT-qPCR is a reliable alternative of high sensitivity for potentially infectious SARS-CoV-2 positive patients. This RNA

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extraction free protocol would help to reduce diagnosis time and cost, and to overcome the RNA extraction kits shortage experienced during COVID-19 pandemic.

KEYWORDS

SARS – CoV – 2, RT-q PCR, homogenization, RNA extraction kit, COVID - 19

Introduction

The world is still undergoing a pandemic called COVID-19 that has affected millions of people since the initial outbreak in December 2019. This is caused by the SARS-CoV-2 virus, that belongs to the β-coronavirus family that causes severe acute respiratory syndrome (SARS). This syndrome affects the human body systemically, through the evasion of various control points of the innate immune system, causing cases ranging from asymptomatic to severe inflammation episodes (Ortiz-Prado et al., 2020). Up to October 2022, more that 625 million COVID-19 cases and more that 6.5 million of COVID-19 related deaths have been reported (Center for Systems Science and Engineering, 2022).

Several molecular methods allow the identification of SARS-CoV-2, but the gold standard is still the RT-qPCR from nasopharyngeal samples, where the viral detection requires a correct extraction of a viable genetic material (Freire-Paspuel et al., 2020). There are several methods of RNA extraction such as chemical and mechanical, as well as commercial kits that assure an optimal RNA extraction (Fomsgaard and Rosenstierne, 2020). All these extraction methods are time consuming and causes a delay in clinical diagnosis. Moreover, this RNA extraction step increases the cost of the analysis by using more reagents and disposable supplies (Morehouse et al., 2021). One of the most conventional nucleic acid extraction methods is isolation by solid chromatography, commonly known as ARN extraction kit by columns, which traps genomic material by resins charged positively, followed by elution steps that yield a high-quality pure sample.

Several alternatives to conventional RNA extraction kits have been proposed for SARS-CoV-2 detection. Those alternatives methods combine heat shock treatment and/or lysis buffers, and the sensitivity reported varies from 77,5% to 100% (Bruno et al., 2021). Moreover, osmotic and mechanical lysis has been previously proposed as an alternative to RNA extraction for viral nucleic acid detection (Moore et al., 2008). However, to the best of our knowledge, this fast and cheap method has not yet been tested for SARS-CoV-2 diagnosis. Therefore, the aim of this study was to evaluate the clinical performance of a simple SARS-CoV-2 detection protocol based in homogenization with a bead mill followed by direct RT-qPCR.

Materials and methods

Sample collection

nasopharyngeal samples were collected in plastic tubes containing 2mL of ultrapure water. A total of 388 nasopharyngeal samples were included in the study (Supplementary Table 1). Those samples were randomly selected as the SARS-CoV-2 positivity rate was really high during the period this study was done. Those samples followed two sequential SARS-CoV-2 diagnosis protocols as illustrated in Figure 1:

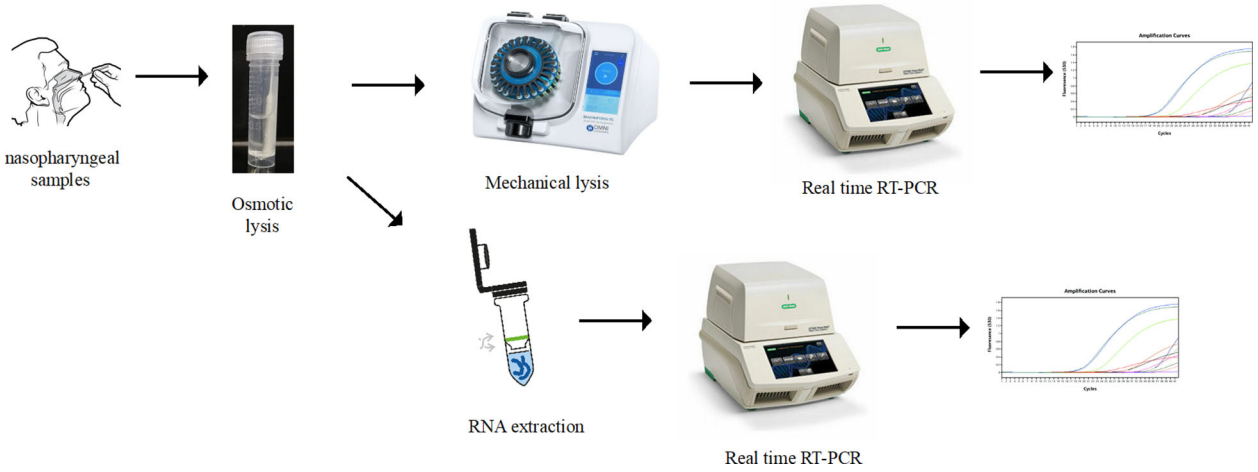

FIGURE 1
Flow chart for nasopharyngeal samples processing for SARS-CoV-2 detection by RT-qPCR for the regular RNA extraction method and for the RNA extraction free protocol based on mechanical lysis with a homogenizer.

a) the standard RNA extraction using commercial column kits; b) a mechanical lysis using a homogenizer without RNA extraction.

Mechanical lysis by sample homogenization

Homogenization was performed in the Bead Ruptor Elite equipment (OMINI International, USA) whose operation is based on the vigorously shaking of the vials that contains sample at a set speed and time to lyse cells. Despite being a bead mill homogenizer, no beads are used in this processing. The homogenization program is regulated at 4 m/s for 30 seconds.

RNA extraction using the Virus DNA/RNA Purification kit (Biocomma, China). RNA extraction was carried out using this commercial kit by following the manufacturer's manual.

SARS-CoV-2 detection by RT-qPCR

Both the homogenized samples and the RNA extractions were processed for SARS-CoV-2 detection by RT-qPCR as we have previously reported (Freire-Paspuel and Garcia-Bereguiain, 2021a; Freire-Paspuel and Garcia-Bereguiain, 2021b; Freire-Paspuel and Garcia-Bereguiain, 2021c; Freire-Paspuel et al., 2021; Santander-Gordon et al., 2021; Freire-Paspuel et al., 2022; Rodriguez-Paredes et al., 2022). The RT-qPCR was performed in a the CFX96 (Biorad, USA) equipment using the commercial kit ECUGEN SARS-CoV-2 RT-qPCR (Starnewcorp-UDLA, Ecuador), a triplex assay based on the CDC protocol that includes the viral targets N1 and N2, and the RNase P gene as a control for RNA extraction quality. According to the manufacturer, its sensitivity is 97.7% and specificity 100% (Freire-Paspuel and Garcia-Bereguiain, 2021a; Freire-Paspuel et al., 2022). As we could not have access to a BSL3 lab for SARS-CoV-2 cultures, we could not address sample tittering with known concentration of SARS-CoV-2 viral particles. So, for viral loads calculation, the 2019-nCoV N positive control (IDT, USA) was used, provided at 200.000 genome equivalents/uL. This positive control is a plasmid including N1 and N2 viral gene targets sequences and it is a SARS-CoV-2 positive control recommended by CDC guidelines (Freire-Paspuel and Garcia-Bereguiain, 2021a; Freire-Paspuel and Garcia-Bereguiain, 2021b; Freire-Paspuel et al., 2022). Serial dilutions of the positive control were included in each set of samples RT-qPCR running, so an internal calibration curve with triplicates of known concentrations of genomic SARS-CoV-2 material was always available. A regression analysis was made for each of those calibration curves taking RT-qPCR Ct values for N1 and N2 targets and viral genomic material concentrations as variables; the equation obtained was used for viral load calculations for each set of clinical, finally expressed of an average of the values for N1 and N2 targets. Regression coefficients over 0.99 were obtained for the viral load calibration curves. The viral loads are expressed in copies/uL of RNA extraction, and the conversion factor for copies/mL of sample media is 200 in our experimental conditions.

Statistical analysis

The sensitivity, specificity, positive predictive value, and negative predictive value were calculated with confidence level of 95%. The t-Student was used to compare the Ct values obtained by each of those values for each protocol. Kappa-Cohen coefficient was calculated to measure the degree of agreement between two methods. All statistical analysis was carried out using SPSS Statistics 23 software.

Results

Clinical performance of the homogenizer-based RNA extraction free method compared to the standard column based RNA extraction

A total of 388 nasopharyngeal samples were included in the study, of which 222 were positive and 166 were negative for SARS-CoV-2 using the column extraction method followed by RT-qPCR. 197 out of 222 SARS-CoV-2 positive samples tested also positive with the alternative homogenizer method. Moreover, 161 SARS-CoV-2 negative samples were also negative for the mechanical lysis method without RNA extraction (See Supplementary Table 1). So, the overall sensitivity was 88.74% (95% IC; 83.83 – 92.58) and specificity was 97% (95% IC; 93.11 – 99.01). Also, Positive Likelihood Ratio was 29.46 (95% CI 12.41 - 69.94); and Negative Likelihood Ratio was 0.12 (95% CI 0.08 - 0.17). Considering the SARS-CoV-2 infection rate obtained in the sample set included in this study, a positive predictive value of 97.52% (IC 95%; 93.3 – 99.6) and a negative predictive value of 86.56% (IC 95%; 81.6 – 91.5) was obtained. The Kappa Cohen value for the comparison between the RNA extraction method and the homogenization method was 0.844, which means a "very good" agreement between both protocols.

In Table 1, the values of the clinical performance parameters for different Ct values thresholds are detailed. For Ct < 25, the sensitivity was 100%. For Ct < 30, the sensitivity was 97.92% (IC 95%; 96.01 – 99.83).

Analytical sensitivity of the homogenizer-based RNA extraction free method compared to the standard column based RNA extraction

The analytical sensitivity for the homogenizer-based RNA extraction free method was addressed by comparing the Ct values obtained for the viral N1 and N2 targets (Figure 2). The mean Ct value for N1 was 21.42 ± 7.10, and for N2 was 19.93 ± 5.8, with the column based RNA extraction protocol. The mean Ct value for N1 was 25.27 ± 4.81, and for N2 was 24.54 ± 5.25, with the homogenizer-based RNA extraction free method. These differences between both methods for the mean N1 and N2 Ct values were statistically significant ($p<0.001$). In Figure 2B, the linear regression analysis for the N1 and N2 Ct values for both protocols used is displayed, showing a significant linear adjustment with R^2 values of 0.63 and 0.46.

In Supplementary Table 2, the Ct values and viral loads (RNA copies/uL) for the 25 false negative samples with the RNA extraction free protocol by homogenization are detailed. Only 4 of those false negative samples have N1 Ct values below 30, meaning viral loads over 10^3 copies/uL. The sensitivity for the homogenizer-based RNA

TABLE 1 Evaluation of the clinical performance of the RNA extraction free homogenization-based method for SARS-CoV-2 detection.

Cycle Threshold (Ct) N1	N	Sensitivity (%)	Specificity (%)	% NPV
≤ **25**	168/168	100	N: 161/166; 96,98 (94.4 – 99.6)	100
≤ **30**	189/193	97.92 (96.01 – 99.83)		97.57 (95.22 – 99.92)
< **40**	197/222	88.74 (84.58 – 92.9)		86.55 (81.6 – 91.45)

Sensitivity, Specificity and Negative Predictive Values (NPV) for different cycle threshold values by RT-qPCR are presented with 95% confidence interval.

extraction free method for SARS-CoV-2 detection by RT-qPCR was 97.92% (189/193) for a Limit of Detection (LoD) of 10^3 copies/uL.

Discussion

Our results endorse that the RNA extraction free homogenization methodology for SARS-CoV-2 detection by RT-qPCR presented in this study is a reliable alternative to the classic column based RNA extraction protocols. We obtained an overall high sensitivity (88.74%) and specificity (97%), but also for Ct values below 30 or viral loads of 10^3 copies/uL of RNA extraction (200,000 copies/mL of sample media) the sensitivity was as high as 97.92%. Considering the viral loads associated to SARS-CoV-2 transmission (Wölfel et al., 2020), this protocol for SARS-CoV-2 detection by RT-qPCR without RNA extraction would potentially detect all the infectious individuals.

In a previous study from our lab, we revised the sensitivity of heat shock based RNA extraction free methods compared to the classic RNA extraction protocols for SARS-CoV-2 detection by RT-qPCR; the sensitivity values for those protocols vary from 54% to 97% depending on the reports. Also, a direct sample to PCR assay protocol without any treatment or RNA extraction have been reported to achieve a sensitivity of 92% (Bruce et al., 2020). Although our protocol includes a step of sample collection in ultra pure water, we could not get a high sensitivity for osmotic lysis, even for samples with Ct values lower than 25; so we decided to include a homogenization step following the osmotic lysis. Our mechanical lysis by homogenization protocol accomplished even a better sensitivity than those other heat shock based methods, up to 97.92% for infectious viral loads. On the other hand, this method has two drawbacks: 1) individuals in the early phase of the infection could be reported as false negative due to the the reduced sensitivity; 2) we cannot totally rule out that the small reduction of specificity could be attributed to cross contamination due to the vigorous homogenization step, although this issue may also happen with the standard RNA extraction method.

The development of sensitive RNA extraction free protocols for SARS-CoV-2 detection by RT-qPCR represents a reliable alternative

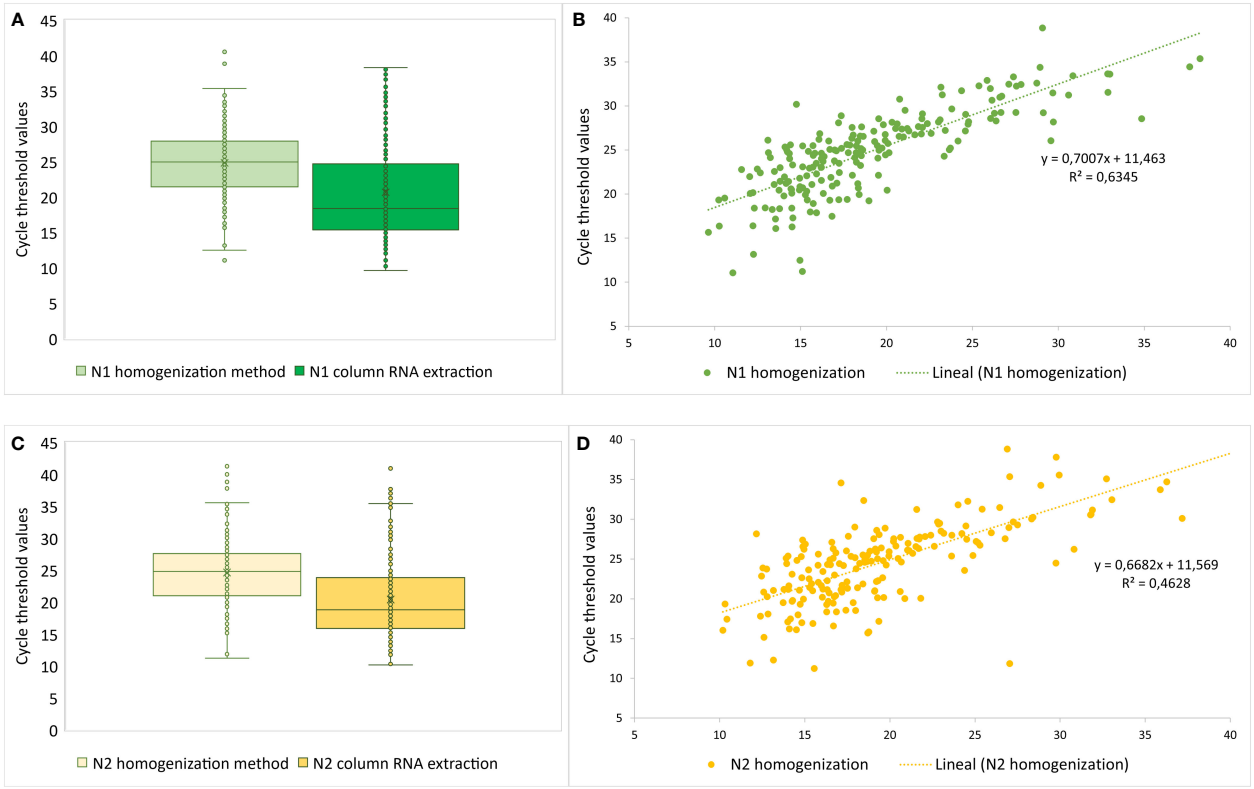

FIGURE 2
(A-C) Average Ct values obtained for N1 and N2 genes using homogenization method vs column RNA extraction (gold standard); **(B-D)** Linear plots for Ct values for N1 and N2 genes for RNA extraction versus homogenization method.

to overcome the main challenges experienced during COVID-19 pandemics, specially at LMICs: 1) Cost reduction of testing by suppressing the RNA extraction with commercial kits; 2) Avoiding RNA extraction kits supply shortage as it was experienced during COVID-19 pandemics; 3) Speeding up the sample processing as RNA extraction methods are time consuming, and testing demands under pandemics scenarios are extremely high.

In conclusion, while keeping the described drawbacks of this method in mind, this fast, cheap and sensitive homogenizer-based RNA extraction free method for SARS-CoV-2 detection by RT-qPCR would support a more equitable COVID-19 testing capacities for public health systems at LMICs. Moreover, it is an important lesson for future pandemics: more flexible protocols that keep a high sensitivity will allow to have a more equity for testing in future pandemics.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The paired samples used for the homogenization protocol were the leftover of the samples collected for routine SARS-CoV-2 diagnosis. Nevertheless, this work is included in a study that was approved by the IRB from the Dirección Nacional de Inteligencia de la Salud (Ministerio de Salud Publica, Ecuador) under the code 008-2020.

Author contributions

All the authors contributed to experimental design and lab work. CR-C, DM-J and MG-B wrote the first draft of the manuscript. MG-B wrote the final version. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1074953/full#supplementary-material

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La pandemia de COVID-19 creó una enorme demanda de NAAT (nucleic acid amplification test) por parte de los laboratorios de todo el mundo como nunca se había visto. Bajo este escenario, cientos de kits comerciales de NAAT para el diagnóstico de SARS-CoV-2 estuvieron disponibles durante los últimos tres años, algunos de ellos con Autorización de Uso de Emergencia (EUA) por parte de la Administración de Drogas y Alimentos de los Estados Unidos (FDA) u otras agencias reguladoras internacionales, mientras que otros cuentan con escasa información relacionada con su desempeño clínico y falta de EUA en el país de fabricación. Afortunadamente, el desempeño de estos kits ha sido evaluado por grupos de investigación a nivel mundial con la posterior publicación de sus resultados en revistas científicas.

Al menos en Ecuador, la urgente necesidad de contener la pandemia de COVID-19 hizo que las autoridades de salud pública eximieran a los kits SARS-CoV-2 NAAT comercializados en el país de presentar una EUA del país de fabricación. No se requirió una evaluación experimental local del rendimiento clínico antes de usar los ensayos para la detección de SARS-CoV-2 en pacientes potencialmente infectados. Esto podría haber generado una subestimación de la carga de la pandemia de COVID-19 en todo el país, lo que habría llevado a una mayor morbilidad y mortalidad.

En Ecuador se evaluaron un total de once kits SARS-CoV-2 NAAT. Nuestro laboratorio de investigación había informado previamente varias evaluaciones de nuevos protocolos relacionados con el diagnóstico de laboratorio de SARS-CoV-2, incluidos estudios que abordan el uso de hisopos de algodón para la recolección de muestras, un sistema eficiente de alto rendimiento con agrupación de muestras antes de RT-qPCR, el rendimiento clínico de un enfoque sin extracción de ARN para el diagnóstico de SARS-CoV-2 o un nuevo método multiplex RT-qPCR.

Un análisis comparativo de los resultados publicados anteriormente sobre el rendimiento clínico y la sensibilidad analítica de once kits de SARS-CoV-2 NAAT disponibles en Ecuador. Este análisis comparativo se basa en varias publicaciones revisadas por pares, incluidos ocho artículos de investigación de nuestro laboratorio. Hubo diferencias preocupantes en el rendimiento clínico entre esos once kits de NAAT para SARS-CoV-2 con valores de sensibilidad que oscilaron entre el 63,4 y el 100 %, en comparación con el estándar de oro, y un límite de detección que osciló entre 500 y 100 000 copias/mL. Cabe destacar que cinco de esos kits de NAAT para SARS-CoV-2 arrojaron una sensibilidad sustancialmente más baja

(63,4 a 78,9 %) y un límite de detección defectuoso (8000-100 000 copias/mL) que el informado por los fabricantes.

Con base en estos hallazgos, proponemos implementar políticas de salud pública éticamente correctas en países de ingresos bajos y medianos con el objetivo de autorizar la comercialización y el uso de kits SARS-CoV-2 NAATS confiables para aquellos que al menos obtienen EUA en el país donde el fabricante tiene su sede. Esto sería particularmente relevante para países como Ecuador, donde las autoridades locales de salud pública no realizaron evaluaciones experimentales para otorgar EUA a los kits SARS-CoV-2 NAAT.

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The quality of commercial SARS-CoV-2 nucleic acid tests in Ecuador: lessons from COVID-19 pandemic for advancing social equity through microbiology

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KEYWORDS

SARS-CoV-2, emergency use authorization, Ecuador, RT-q PCR, RT- LAMP, clinical performance

Introduction

The "coronavirus disease 2019" (COVID-19) pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), challenged the public health systems worldwide since the initial outbreak in China in December 2019 (Gorbalenya et al., 2020; Zhou et al., 2020).

The gold standard for the detection of SARS-CoV-2 is the reverse transcription–quantitative polymerase chain reaction (RT-qPCR), although other nucleic acid amplification tests (NAATs) like the reverse transcription–loop-mediated isothermal amplification (RT-LAMP) are also available. There are several available RT-qPCR assays developed by public institutions such as the Centers for Diseases Control and Prevention (CDC, Atlanta, USA) or the Charité Hospital (Berlin, Germany), endorsed by the World Health Organization for SARS-CoV-2 diagnosis (Corman et al., 2020; Lu et al., 2020; PAHO, 2020). However, the COVID-19 pandemic created a huge NAATs demand from laboratories worldwide not ever seen before. Under this scenario, hundreds of NAATs commercial kits for SARS-CoV-2 diagnosis became available during the last 3 years, some of them with Emergency Use Authorization (EUA) by the US Food and Drug Administration (FDA) or other international regulatory agencies, while others have scarce information related to their clinical performance and the lack of EUA at the country of manufacture (https://www.fda.gov/medical-devices/emergency-situations-medicaldevices/emergency-use-authorizations; FIND, 2020). Due to the COVID-19 pandemic, the EUA of SARS-CoV-2 NAATs kits adopted flexible regulatory protocols. Fortunately, the performance of these kits has been evaluated by research groups worldwide with the subsequent publication of their results in scientific journals (Udugama et al., 2020; Hernandez et al., 2021; Yu et al., 2021).

The weakness of low- and middle-income countries to guarantee the quality of commercial SARS-CoV-2 NAATs: the case of Ecuador

The majority of NAATss for the detection of SARS-CoV-2 are developed by companies from high-income countries. Therefore, middle- and low-income countries have had to import commercial NAATs kits for SARS-CoV-2 diagnosis. For instance, this is the case of countries in South America like Ecuador, where multiple commercial NAATs kits for SARS-CoV-2 produced in the USA, Canada, Europe, China, or South Korea are available. While some of those assays have obtained EUA by reputed governmental agencies in the countries of manufacture, others, for undisclosed reasons, have been denied with a local EUA. Whether with a local EUA granted or not, SARS-CoV-2 NAATs kits have been commercialized in middle- and low-income countries with the understanding that the latter (i.e., EUA not granted) have been unethically distributed. At least in Ecuador, the urgent need for containing the COVID-19 pandemic made public health authorities to exempt SARS-CoV-2 NAATs kits commercialized in the country for presenting an EUA from the country of manufacture. No local experimental evaluation of the clinical performance was required before using the assays for the detection of SARS-CoV-2 in potentially infected patients. This could have generated a country-wide underestimation of the COVID-19 pandemic burden, leading to an increased morbidity and mortality. We emphasize the importance of validating tests with patient samples in the field in the country where those SARS-CoV-2 NAATs were intended to be used. Moreover, we highlight the importance to evaluate the performance of the SARS-CoV-2 NAATs not only prior to their commercialization but also after commercial distribution to follow up that quality was guaranteed.

The role of academia to support a good-quality SARS-CoV-2 diagnosis in Ecuador

To assure the quality of the clinical performance of SARS-CoV-2 NAATss during the COVID-19 pandemic crisis in Ecuador, research laboratories have been actively conducting evaluation studies of SARS-CoV-2 NAATs kits available in the country. As it is summarized in Table 1, a total of 11 SARS-CoV-2 NAATs kits were evaluated in Ecuador (Freire-Paspuel et al., 2022; Freire-Paspuel and Garcia-Bereguiain, 2020; Freire-Paspuel et al., 2020a; Freire-Paspuel and Garcia-Bereguiain, 2021a; Freire-Paspuel and Garcia-Bereguiain, 2021b; Freire-Paspuel and Garcia-Bereguiain, 2021c; Freire-Paspuel et al., 2021; Freire-Paspuel et al., 2021).

Unfortunately, the Ecuador's government agency responsible for clinical use authorization lacks infrastructure to carry out clinical performance evaluations like the ones carried out in research laboratories. Moreover, local clinical microbiology laboratories without prior experience in PCR-based diagnosis began to perform SARS-CoV-2 detection by RT-qPCR, but, in general, those laboratories did not have the expertise to conduct internal evaluations of SARS-CoV-2 NAATs kits.

On the other hand, our research laboratory had previously reported several evaluations of new protocols related to the SARS-CoV-2 laboratory diagnosis, including studies addressing the use of cotton swabs for sample collection, an efficient high-throughput system with sample pooling prior to RT-qPCR, the clinical performance of an RNA-extraction free approach for SARS-CoV-2 diagnosis or a novel multiplex RT-qPCR method (Freire-Paspuel et al., 2020; Freire-Paspuel et al., 2020b; Bruno et al., 2021; Freire-Paspuel and Garcia-Bereguiain, 2021d; Ramirez-Cordova et al., 2023). The same approach was utilized for the clinical performance evaluation of SARS-COV-2 NAATs kits. The SARS-CoV-2 diagnosis gold-standard protocol used in our evaluations was nasopharyngeal swab sample collection followed by manual RNA extraction with column-based commercial kits prior to the detection of SARS-CoV-2 using the CDC-designed "FDA EUA 2019-nCoV CDC kit" (IDT, USA) (Freire-Paspuel et al., 2020; Freire-Paspuel et al., 2020b; Bruno et al., 2021; Freire-Paspuel and Garcia-Bereguiain, 2021d; Ramirez-Cordova et al., 2023). This protocol targets the conserved SARS-CoV-2 N1 and N2 sections of the viral N gene and uses the transcript for the human RNase P as an RNA extraction quality control; it is considered a gold standard for SARS-CoV-2 RT-qPCR diagnosis worldwide (Corman et al., 2020; Lu et al., 2020; PAHO, 2020). We additionally used "TaqMan 2019-nCoV Assay Kit v1" (Thermofisher, USA) as a reference method of a high-quality SARS-CoV-2 NAATs kit with EUA by the FDA.

Having said all the above, we herein present a comparative analysis of previously published results about the clinical performance and analytical sensitivity of 11 SARS-CoV-2 NAATs kits available in Ecuador. This comparative analysis is based on several peer-reviewed publications, including eight research articles from our laboratory. As detailed in Table 1, there were worrisome differences in the clinical performance among those 11 SARS-CoV-2 NAATs kits with sensitivity values ranging from 63.4% to 100%, compared to the gold standard, and the limit of detection ranging from 500 to 100,000 copies/ml. Noteworthy, five of those SARS-CoV-2 NAATs kits yielded substantially lower sensitivity (63.4%–78.9%) and a defective limit of detection (8,000–100,000 copies/ml) than that reported by manufacturers or to that recommended for a reliable SARS-CoV-2 diagnosis (Freire-Paspuel et al., 2020; Freire-Paspuel et al., 2020a; Freire-Paspuel and Garcia-Bereguiain, 2021a; Freire-Paspuel and Garcia-Bereguiain, 2021b; Freire-Paspuel et al., 2021). For the other seven NAATs kits, the available peer-reviewed publications reported a great clinical performance with sensitivity values ranging from 87.7% to 100% and the limit of detection of 500–4,000 copies/ml (Freire-Paspuel et al., 2022; Hur et al., 2020; Iglói et al., 2020; Shen et al., 2020; Wang et al., 2020; Xiong et al., 2020; Yu et al., 2020; Fellner et al., 2021; Freire-Paspuel and Garcia-Bereguiain, 2021b; Freire-Paspuel and Garcia-Bereguiain, 2021c; Freire-Paspuel et al., 2021; Freire-Paspuel et al., 2021; Hernandez et al., 2021; Liotti et al., 2021), in close concordance with the values reported by manufacturers. Two of the evaluation studies reported different values of sensitivity for two of the kits included in Table 1 also evaluated in our laboratory (Salinas et al., 2022; Fellner et al., 2021). However, one of those studies is not properly a clinical evaluation because only artificial SARS-CoV-2 samples from viral cultures were used (Fellner et al., 2021). Moreover, the reference

TABLE 1 Clinical performance and analytical sensitivity of 11 commercial SARS-CoV-2 NAAT kits available in Ecuador. (EUA is referred to clinical authorization use within country of manufacture and/or by FDA).

Detection kit (company, country)	Sensitivity (manufacturer)	Specificity (manufacturer)	LoD (manufacturer)	Sensitivity (observed)	Specificity (observed)	LoD (viral copies/ml of sample) (copies/µl of RNA extraction)	EUA	Ref.
nCoV-QS (MiCo BioMed, South Korea)	NA	NA	1.8 copies/µl of RNA extraction	70.6%–100%	92.9%–100%	10,000 50	**Ecuador**	(Freire-Paspuel et al., 2020a; Salinas et al., 2022)
AccuPower SARS-CoV-2 real time RT-PCR kit (Bioneer, South Korea)	NA	NA	NA	78.9%–100%*	100%	40,000 200	**Ecuador**	(Freire-Paspuel and Garcia-Bereguiain, 2020; Fellner et al., 2021)*
AccuPower SARS-CoV-2 Multiplex RT-PCR kit (Bioneer, South Korea)	100%	100%	2 copies/µl of RNA extraction	73.5%	100%	18,000 90	**Ecuador**	(FIND, 2020; Freire-Paspuel and Garcia-Bereguiain, 2021b)
Isopollo COVID-19 detection kit (Monitor, South Korea)	NA	NA	NA	63.4%	100%	100,000 500	**Ecuador**	(Freire-Paspuel and Garcia-Bereguiain, 2021a)
GenomeCoV19 kit (ABM, Canada)	100%	100%	1 copy/µl of RNA extraction solution	75.0%	100%	8,000 40	**Ecuador**	(Freire-Paspuel and Garcia-Bereguiain, 2021c)
Allplex 2019-nCoV Assay (Seegene, South Korea)	100%	93.07%	12.5 RNA c/µl of RNA extraction solution	87.7%–98.2%	93.75%–100%	2,000–4,000 10–20	S.Korea/ FDA	(Hur et al., 2020; Freire-Paspuel and Garcia-Bereguiain, 2021b; Hernandez et al., 2021; Liotti et al., 2021)
Viasure SARS-CoV-2 (CerTest Biotec, Spain)	100%	97.5%	4 copies/µl of RNA extraction	91.86%	100%	2,000 10	Spain	(Freire-Paspuel et al., 2021c)
COVID-19 (SARS-CoV-2) Nucleic Acid Test Kit (eDiagnosis, China)	95.93%	94.07%	500 copies/ml of sample	100%	94.1%–100%	500 2.5	China	(Hernandez et al., 2021; Freire-Paspuel et al., 2021)
Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (Sansure Biotech, China)	100%	100%	200 copies/ml of sample	83.3%–95.3%	87.5%–100%	484–3,000 0.66-5	China/ FDA	(Iglói et al., 2020; Wang et al., 2020; Xiong et al., 2020; Yu et al., 2020; Freire-Paspuel et al., 2021)
COVID-19 RT-PCR Real TM Fast (Cy5) (ATGen)	NA	NA	NA	96.4%	96%–100%	2,000 10	Uruguay	(Freire-Paspuel et al., 2022)
Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (Da An Gene, China)	100%	100%	500 copies/ml of sample	78.6%–100%	100%	484–3,000 0.16–10	China	(Iglói et al., 2020; Wang et al., 2020; Xiong et al., 2020; Yu et al., 2020; Freire-Paspuel and Garcia-Bereguiain, 2021c)
ECUGEN SARS-CoV-2 RT-PCR kit (UDLA-Starnewcorp; Ecuador)	97.7%	100%	5 copies/µl of RNA extraction	100%	94.6%–100%	500–1,000 2.5–5	Ecuador	(Freire-Paspuel et al., 2022)

* Fellner et al., 2021 reports 100% sensitivity but is not a clinical performance evaluation as only artificial SARS-CoV-2 samples were used.

Sensitivity, specificity, and the limit of detection reported by manufacturers and by scientific publications are detailed (EUA, Emergency Use Authorization at the country of production and by the FDA; FDA, US Food and Drug Administration; Ref, references; NA, not available).

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methods used in this two studies had a lower sensitivity that the gold standard method used in the evaluation carried out in our laboratory, resulting in a potential bias due to the exclusion of low-viral-load samples in those evaluations (Salinas et al., 2022; Fellner et al., 2021).

Emergency use authorization at country of manufacture as a proxy for quality control for SARS-CoV-2 nucleic acid amplification tests in low- and middle-income countries

It was not surprising that the seven SARS-CoV-2 NAATs kits with great clinical performance had been granted with EUA in their country of origin, including EUA granted by the FDA in some cases, whereas those five kits with poor clinical performance lack of EUA at their country of manufacture (Freire-Paspuel and Garcia-Bereguiain, 2020; Freire-Paspuel and Garcia-Bereguiain, 2021a; Freire-Paspuel and Garcia-Bereguiain, 2021b; Freire-Paspuel et al., 2021).

Based on these findings, we propose to implement ethically correct public health policies in low- and middle-income countries aim to authorize the commercialization and use of reliable SARS-CoV-2 NAATs kits to those at least obtaining EUA in the country where the manufacturer has its headquarters. This would be particularly relevant for countries like Ecuador, where the local public health authorities did not carry out experimental evaluations to grant EUA to SARS-CoV-2 NAATs kits. While we acknowledge that these policies should be encouraged, designed, and implemented in-country, ignoring the fact that the issue did exist could have caused unnecessary morbidity and mortality in the affected countries and potentially negative side effects to the control of the COVID-19 pandemic in industrialized countries. For example, to travel to the USA from Ecuador, a negative SARS-CoV-2 NAATs result was requested during 2020–2022. If the SARS-CoV-2 NAATs kit utilized was, for instance, "Isopollo" (see Table 1 for details), then nearly 4 out of 10 SARS-CoV-2 positive travelers would be allowed to enter the country, with a negative impact in the control of the COVID-19 pandemic.

Cheap and sensitive SARS-CoV-2 NAATs developed at low- and middle-income countries as an alternative to the non-equitable distribution of good-quality SARS-CoV-2 NAATs

We call the attention of two of the SARS-CoV-2 NAATss kits detailed in Table 1. "COVID-19 RT-PCR Real TM FAST (CY5)" and "ECUGEN SARS-CoV-2 RT-qPCR" RT-PCR kits are within the group of SARS-CoV-2 NAATs kits with great clinical performance and analytical sensitivity (Freire-Paspuel et al., 2022). Moreover, both of them have equivalent clinical performance than TaqMan 2019-nCoV Assay Kit v1 (Thermo Fisher, USA), a reference of high-quality SARS-CoV-2 NAATs kits (Freire-Paspuel et al., 2022). Both kits are produced in South American countries (Uruguay and Ecuador) and were designed by a consortium between universities and private companies, pointing out the role of the academia to improve SARS-CoV-2 testing, as it has been suggested even in the USA (Mascuch et al., 2020). This is particularly relevant in the context of low- and middle-income countries, as high-quality locally produced SARS-CoV-2 NAATs kits would potentially increase SARS-CoV-2 testing capacities by two main reasons: (a) testing cost reduction as these local SARS-CoV-2 NAATs kits are substantially cheaper than "high-quality" imported ones and (b) SARS-CoV-2 testing supply shortages would be more easily overcome as local production is guaranteed. The local production of reagents and enzymes for SARS-CoV-2 NAATss has already been proposed by others and showed excellent results (Mascuch et al., 2020; Wozniak et al., 2020; Graham et al., 2021), and, although improving a good-quality control strategy for those local products would be mandatory, we believe that this should be the path to follow for a low-cost diagnosis in low- and middle-income countries.

Conclusion: lessons for future pandemics

The COVID-19 pandemic was the worst public health threat that our globalized humanity has faced over the last few decades and needed a global health approach to be defeated. This is also true for future pandemics. This global health approach means that diagnostic quality and testing capacities should be guaranteed for any country in the world. Beyond a matter of human rights, it is the only way to fight infectious disease outbreaks and that should be one of the main lessons learned after the COVID-19 pandemic.

In summary, we suggest to Ecuadorian public health authorities to review the protocols for the EUA of SARS-CoV-2 NAATs kits that were adopted during the COVID-19 pandemic. We also express our concern to companies from high-income countries that were exporting low-quality products to low- and middle-income countries during the COVID-19 pandemic. We encourage the scientific community in low- and middle-income countries to carry out clinical performance evaluation studies for commercial diagnostic kits and publish their results, contributing as sentinels for quality control diagnosis in those settings. Finally, this letter is a call for action to international public health organizations to claim for a fair trade of SARS-CoV-2 NAATs kits and any diagnosis tool in general, based on universal quality standards without income bias.

Author contributions

All the authors contributed to the conceptualization and writing of the manuscript. All the authors also contributed to the clinical performance evaluation studies previously published by our research team.

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Conflict of interest

DMJ, BCR, CVD and MAGB are employees of "Universidad de Las Américas". This University is enrolled in the commercialization "ECUGEN SARS-CoV-2 RT-PCR kit", one of the SARS-CoV-2 NAATs kits referenced in this study. FP is employed by OneLabt.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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6.1.3. Sensibilidad analítica y desempeño clínico de "COVID-19 RT-PCR Real TM FAST (CY5) (ATGen, Uruguay) y 'ECUGEN SARS-CoV-2 RT-qPCR' (UDLA-STARNEWCORP, Ecuador)": Pruebas locales de SARS-CoV-2 para Sudamérica con alta calidad y bajo costo.

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Hay docenas de kits comerciales de RT-qPCR para la detección de SARS-CoV-2 disponibles con o sin autorización de uso de emergencia (EUA) de la FDA u otras agencias reguladoras.

Evaluamos el desempeño clínico de dos kits SARS-CoV-2 RT-PCR diseñados y producidos en Sudamérica, "COVID-19 RT-PCR Real TM FAST (CY5)" (ATGen, Uruguay) y "ECUGEN SARS- CoV-2 RT-qPCR" (UDLA-STARNEWCORP, Ecuador), para la detección de RT-qPCR SARS-CoV2 utilizando "TaqMan 2019-nCoV Assay Kit v1" (Thermofisher, EE. UU.) como técnica estándar de oro.

Reportamos un gran desempeño clínico y sensibilidad analítica para los dos kits sudamericanos con valores de sensibilidad de 96.4 y 100%, especificidad de 100% y límite de detección en el rango de 10 copias/uL de extracción de ARN.

Los kits "COVID-19 RT-PCR Real TM FAST (CY5)" y "ECUGEN SARS-CoV-2 RT-qPCR" son pruebas confiables de SARS-CoV-2 fabricadas en Sudamérica que han sido ampliamente utilizadas en Uruguay, Argentina, Brasil, Bolivia y Ecuador. Estas pruebas de SARS-CoV-2 producidas localmente han contribuido a superar la escasez de suministros y a reducir los costos de diagnóstico, al tiempo que mantienen los altos estándares de calidad de los kits disponibles comercialmente de FDA EUA. Este enfoque podría extenderse a otros productos de diagnóstico para mejorar la vigilancia de enfermedades infecciosas en países de ingresos medios y bajos más allá de la pandemia de COVID-19.

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Analytical sensitivity and clinical performance of "COVID-19 RT-PCR Real TM FAST (CY5) (ATGen, Uruguay) and "ECUGEN SARS-CoV-2 RT-qPCR" (UDLA-STARNEWCORP, Ecuador)": High quality-low cost local SARS-CoV-2 tests for South America

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Abstract

Background

Dozens of commercial RT-qPCR kits for SARS-CoV-2 detection are available with or without Emergency Use Authorization (EUA) by FDA or other regulatory agencies.

Objective

We evaluated the clinical performance of two SARS-CoV-2 RT-PCR kits designed and produced in South America, "COVID-19 RT-PCR Real TM FAST (CY5)" (ATGen, Uruguay) and "ECUGEN SARS-CoV-2 RT-qPCR" (UDLA-STARNEWCORP, Ecuador), for RT-qPCR SARS-CoV2 detection using "TaqMan 2019-nCoV Assay Kit v1" (Thermofisher, USA) as a gold standard technique.

Results

We report a great clinical performance and analytical sensitivity for the two South American kits with sensitivity values of 96.4 and 100%, specificity of 100% and limit of detection in the range of 10 copies/uL of RNA extraction.

Conclusions

"COVID-19 RT-PCR Real TM FAST (CY5)" and "ECUGEN SARS-CoV-2 RT-qPCR" kits are reliable SARS-CoV-2 tests made in South America that have been extensively used in Uruguay, Argentina, Brazil, Bolivia and Ecuador. These locally produced SARS-CoV-2 tests have contributed to overcome supply shortages and reduce diagnosis cost, while maintaining the high quality standards of FDA EUA commercially available kits. This approach could

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Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: The authors of this study are employees of "Universidad de Las Américas". This institution is enrolled in the commercialization "ECUGEN SARS-CoV-2 RT-PCR kit", one of the kits evaluated on this study.

be extended for other diagnostic products to improve infectious diseases surveillance at middle and low income countries beyond COVID-19 pandemic.

Author summary

COVID-19 pandemic is the worst public health crisis that humanity has faced in the last decades. To success controlling the virus spread an unprecedented amount on molecular tests based on the technique called PCR has been necessary. To carry out viral infection tests, commercial kits are normally used by clinical laboratories. Those commercial kits are mainly produced in industrialized countries and that means a disadvantage in the access to COVID-19 testing in developing countries. Here we present the results of the evaluation of two commercial kits produced in South America for local stakeholders, showing how good quality biotech products can help to fight COVID-19 pandemic in low and middle income countries.

Introduction

The "coronaviruses disease 2019" (COVID-19) pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has challenged public health systems worldwide since the initial outbreak in the Chinese city of Wuhan in December 2019 [1,2]. While SARS-CoV-2 vaccination programs progress successfully at high income countries and COVID-19 pandemic is somehow under control, the challenge persists for middle and low income countries in need to improve SARS-CoV-2 testing [3–5]. A wide variety of commercial SARS-CoV-2 RT-qPCR detection kits are available in South America for clinical use, some of them with Emergency Use Authorization (EUA) from the U.S. Food & Drug Administration (FDA) or other international recognized agencies, while others even lack of their country of origin EUA and compromise a reliable diagnosis in these high burden COVID-19 settings [6–13]. Among the SARS-CoV-2 RT-qPCR commercial kits available, "TaqMan 2019-nCoV Assay Kit v1" (Thermofisher, USA) holds FDA EUA and it is considered one of the most reliable SARS-CoV-2 RT-qPCR kits [6]. All those commercial kits are based in SARS-CoV-2 detection by targeting different genes like E, S, N or the orf1ab region.

The CDC designed FDA EUA 2019-nCoV CDC kit (IDT, USA) is based on N1 and N2 gene targets to detect SARS-CoV-2 and RNase P as an RNA extraction quality control, it is considered a gold standard worldwide for SARS-CoV-2 RT-PCR detection [14–16]. The main limitation for the CDC kit is the need to run three RT-qPCR reactions per sample. To solve this inconvenience, inspired on this CDC protocol, two SARS-CoV-2 RT-qPCR multiplex assays have been designed, produced and commercialized in South America. "COVID-19 RT-PCR Real TM FAST (CY5)" (ATGen, Uruguay) is a duplex assay including N1 viral target and RNase P as an RNA extraction quality control. "ECUGEN SARS-CoV-2 RT-qPCR k" (UDLA-STARNEWCORP, Ecuador) is a triplex assay including N1 and N2 viral targets and also RNase P as an RNA extraction quality control. So, for both South American kits, a single PCR reaction is required for SARS-CoV-2 detection, reducing the time and cost of the diagnosis.

We herein present an analytical sensitivity and clinical performance evaluation of "COVID-19 RT-PCR Real TM FAST (CY5)" (ATGen, Uruguay) and "ECUGEN SARS-CoV-2 RT-qPCR" (UDLA-STARNEWCORP, Ecuador) for SARS-CoV-2 RT-qPCR detection from

nasopharyngeal samples using "TaqMan 2019-nCoV Assay Kit v1" (Thermofisher, USA) as a gold standard technique.

Materials and methods

Ethics statement

This study is part of a project approved by the IRB from the Dirección Nacional de Inteligencia de la Salud (Ministerio de Salud Publica, Ecuador) under the code 008–2020.

Study design

119 samples were included in this study. Those samples were leftovers of the RNA extractions of clinical specimens previously processed for SARS-CoV-2 test and stored at -80 C. Those RNA extractions were obtained from nasopharyngeal swabs collected on 0.5mL TE pH 8 buffer from community dwelling individuals attending Universidad de Las Américas for SARS-CoV-2 testing during November and December 2020. Also, negative controls (TE pH 8 buffer) were included as control for carryover contamination, one for each set of RNA extractions. The overall protocol for sample collection and SARS-CoV-2 diagnosis at our laboratory has been previously published [17–20].

All the clinical samples were processed with the same RNA extraction kit "AccuPrep Viral RNA extraction kit" (Bioneer, South Korea) and used for SARS-CoV-2 detection. Only the viral RNA extractions stored at -80 C were used for the three RT-qPCR protocols included in this study. For the RNA extractions, 200μL of TE pH8 buffer that contained the sample were used. At the end of the RNA extraction, RNA was eluted in 40μL elution buffer.

A total of 109 RNA samples were evaluated using ECUGEN SARS-CoV-2 RT-qPCR Kit (UDLA-STARNEWCORP, Ecuador) and a total of 80 RNA samples were evaluated using COVID-19 RT-PCR Real TM FAST (CY5) (ATGen, Uruguay). All the RNA samples of both sets were additionally processed with TaqMan 2019-nCoV Assay Kit v1 as gold standard SARS-CoV-2 detection method.

RT-qPCR for SARS-CoV-2 detection using TaqMan 2019-nCoV Assay Kit v1. All samples were tested following the manufacturer instructions using TaqPath 1-Step Master Mix GC in a reduced reaction volume of 15μL including 4μL of RNA sample. TaqMan 2019-nCoV Control Kit v1 was used as reaction positive control. Following the manufacturer's manual, a viral target with $Ct < 37$ is considered positive and with $37 \leq Ct < 40$ is considered inconclusive. A new PCR reaction was run for inconclusive results, and a $Ct < 40$ is sufficient to considered that run as positive. Samples that showed positive results for at least two of the three SARS-CoV-2 genes (S, N and ORF1ab) and inconclusive samples that showed recurrent positive results for at least one viral gene target were marked as SARS-CoV-2 positive. RT-qPCR assays were performed in a CFX96 Real-Time PCR Detection System (Bio-Rad).

RT-qPCR for SARS-CoV-2 detection using ECUGEN SARS-CoV-2 RT-qPCR Kit. Samples were tested following the manufacturer instructions in a reaction volume of 15μL including 4μL of RNA sample. The criteria for positivity were $Ct \leq 40$ for N1 and N2 targets simultaneously. Also, inconclusive samples where either N1 and N2 were positive, were repeated and if either N1 or N2 target amplified, the sample is considered positive. RT qPCR assays were performed in a CFX96 Real-Time PCR Detection System (Bio-Rad).

RT-qPCR for SARS-CoV-2 detection using COVID-19 RT-PCR Real TM FAST (CY5) (ATGen) kit. Samples were tested following the manufacturer instructions in a reduced reaction volume of 15μL including 3,75μL of RNA sample. Samples that presented a $Ct < 35$ for the viral target gene were considered positive, and samples with values of $35 \leq Ct \leq 40$ were

considered inconclusive. The latter were repeated and consistent results with the first test was sufficient to be considered positive. RT-qPCR assays were performed in a CFX96 Real-Time PCR Detection System (Bio-Rad).

Analytical sensitivity. Limit of detection (LoD) was performed using the commercially available 2019-nCoV N positive control (IDT, USA); provided at 200,000 genome equivalents/uL, it was used for calibration curves to obtain the viral loads of the samples. Viral loads can be expressed as copies/uL of RNA extraction or copies/mL of sample; the conversion factor is 200, as 0.2mL of sample is used for RNA extraction and 40uL is used as final elution volume of RNA extraction.

Statistics analysis. All data was analyzed in Excel and statistics were done using SPSS software.

Results

Clinical performance and analytical sensitivity of ECUGEN SARS-CoV-2 RT-qPCR Kit

109 samples were tested for SARS-CoV-2 with ECUGEN SARS-CoV-2 RT-qPCR Kit using TaqMan 2019-nCoV Assay Kit v1 as a gold standard. For the TaqMan 2019-nCoV Assay Kit v1, 55 samples tested positive, and 54 samples tested negative (Table 1 and S1 Data). 51 out of 54 samples tested negative for the TaqMan 2019-nCoV Assay Kit v1 were also SARS-CoV-2 negative for ECUGEN SARS-CoV-2 RT-qPCR Kit, so the specificity obtained in this study was 94.4% (95% CI = 84.6 to 98.8%). The three "false positive" samples had Ct values > 35 and viral loads of 3.1, 4.72 and 6.84 copies/uL of RNA extraction (Samples 62, 64 and 66 at S1 Data).

For the 55 SARS-CoV-2 positive samples for the TaqMan 2019-nCoV Assay Kit v1, 55 samples tested also positive for ECUGEN SARS-CoV-2 RT-qPCR Kit, resulting a sensitivity of 100.0% (95% CI = 93.51 to 100.00%) (Table 1 and S1 Data). Cohen's κ was run and almost perfect agreement between results was obtained with both kits (κ = 0.945, $p<0.001$). In Fig 1A, the distribution of Ct values of N gene target for SARS-CoV-2 positive samples included in the study for ECUGEN SARS-CoV-2 RT-qPCR Kit and TaqMan 2019-nCoV Assay Kit v1 is show. In Fig 1B the linear regression analysis for Ct values for N gene target for both kits is presented, yielding a $R^2 = 0.9906$.

The limit of detection (LoD) is defined as the lowest viral load in which all replicates are detected (100% sensitivity). As it is detailed in Table 2, after running 15 replicates for viral loads in the range from 500 to 2000 copies/mL, we could set the LoD for ECUGEN SARS-CoV-2 RT-qPCR Kit in 2000 copies/mL of sample (10 copies/μL of RNA extraction). Although N2 target fails for 1 out of 15 replicates at that viral load, as the criteria of positivity only requires the amplification of one of the viral target on the replicate, the LoD was the lowest viral load for 100% sensitivity for N1 target.

Table 1. Clinical performance of ECUGEN SARS-CoV-2 RT-qPCR and COVID-19 RT-PCR Real TM FAST (CY5) kits using TaqMan 2019-nCoV Assay Kit v1 as reference methodology (% values: sensitivity). Only SARS-CoV-2 positive samples included on the study are detailed.

SARS-CoV-2 RT-qPCR kit	Positive Samples	False Negative Samples	Total SARS-CoV-2 positive samples
ECUGEN SARS-CoV-2 RT-qPCR Kit	55 (100.0%)	0	55
COVID-19 RT-PCR Real TM FAST (CY5)	53 (96.36%)	2	55

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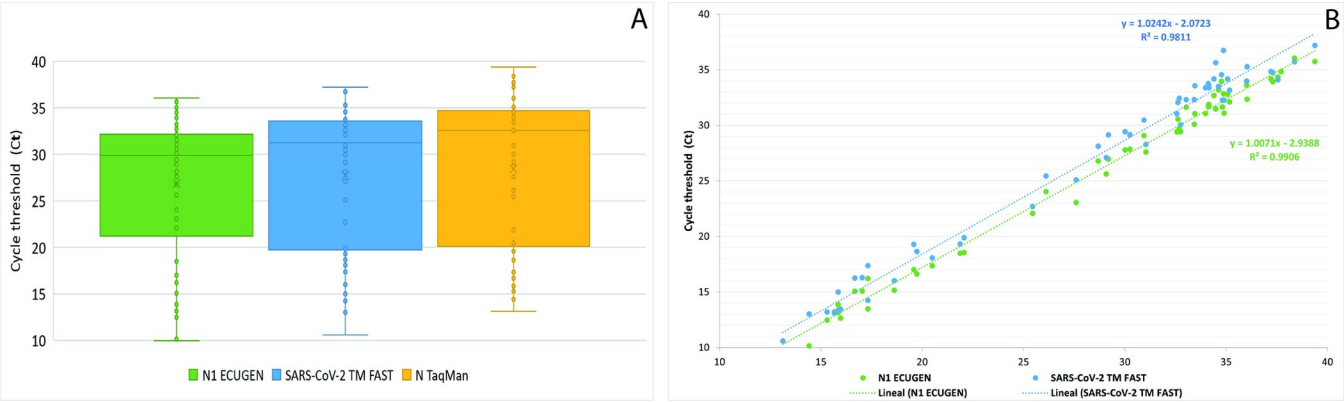

Fig 1. A: Cycle threshold (Ct) distribution for N gene target for SARS-CoV-2 positive samples with the three commercial kits included in this study: ECUGEN SARS-CoV-2 RT-qPCR Kit (green), COVID-19 RT-PCR Real TM FAST (blue) and TaqMan 2019-nCoV Assay Kit v1 (yellow). B: Linear regression for the Ct values for N gene target for ECUGEN SARS-CoV-2 RT-qPCR Kit (green) and COVID-19 RT-PCR Real TM FAST (blue) compared to TaqMan 2019-nCoV Assay Kit v1.

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Clinical performance and analytical sensitivity of COVID-19 RT-PCR Real TM FAST (CY5) kit

80 samples were tested for SARS-CoV-2 with of COVID-19 RT-PCR Real TM FAST (CY5) (ATGen) kit using TaqMan 2019-nCoV Assay Kit v1 as gold standard. For the TaqMan 2019-nCoV Assay Kit v1, 55 samples tested positive, and 25 samples tested negative (Table 1 and S1 Data). 24 out of 25 samples tested negative for the TaqMan 2019-nCoV Assay Kit v1 were also SARS-CoV-2 negative for COVID-19 RT-PCR Real TM FAST Kit, so the specificity obtained in this study was 96.00% (95% CI = 79.6 to 99.9%). The sample 62 (S1 Data) had a Ct value of 34.73 for N target and also presented a positive result with ECUGEN SARS-CoV-2 RT-qPCR Kit, however, this sample tested negative with the TaqMan kit.

From the 55 samples that tested positive with TaqMan 2019-nCoV Assay Kit v1, 53 samples were positive for COVID-19 RT-PCR Real TM FAST Kit, resulting a sensitivity of 96.4% (95% CI = 87.5 to 99.6%) (Table 1 and S1 Data). Samples 59 and 68 amplified for at least one SARS-CoV-2 target in different TaqMan RT-qPCR reactions, therefore, these samples were marked as positive. Cohen's κ was run and almost perfect agreement between results obtained with both kits was found ($\kappa = 0.914$, $p<0.001$). In Fig 1A, the distribution of Ct values of N gene target for SARS-CoV-2 positive samples included in the study for COVID-19 RT-PCR Real TM FAST Kit and TaqMan 2019-nCoV Assay Kit v1 is show. In Fig 1B the linear regression analysis for Ct values for N gene target for both kits is presented, yielding a $R^2 = 0.9811$.

As the limit of detection (LoD) is defined as the lowest viral load in which all replicates are detected (100% sensitivity), our data indicates that the LoD for COVID-19 RT-PCR Real TM FAST (CY5) Kit should be in the range of 5–10 copies/μL of RNA extraction (1000–2000 viral

Table 2. Analytical sensitivity for ECUGEN SARS-CoV-2 RT-qPCR kit. The ratio represents the number of positive replicates for each viral load related to the total number of replicates.

Viral load (copies/mL)	N1 replicates	N1 sensitivity	N2 replicates	N2 sensitivity
2000*	15/15	100%	14/15	93.3%
1500	14/15	93.3%	14/15	93.3%
1000	12/15	80%	12/15	80%
500	12/14	85.7%	10/14	71.4%

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RNA copies/mL of sample), as the 4 samples with viral loads within that range were detected (S1 Data), and positive samples that failed for this kit were below 5 copies/uL.

Discussion

Although the main limitation of our study is the sample size (119 specimens), our results support that "COVID-19 RT-PCR Real TM FAST (CY5) (ATGen, Uruguay) and "ECUGEN SARS-CoV-2 RT-qPCR" (UDLA-STARNEWCORP, Ecuador) kits had a great clinical performance with sensitivity values of 96.4% and and 100%, respectively. Moreover, although a reduction of specificity was found for "COVID-19 RT-PCR Real TM FAST (CY5) (96%) and "ECUGEN SARS-CoV-2 RT-qPCR" (UDLA-Starnewcorp, Ecuador)" (94.4%) kits, we believe that the 3 "false positive" samples would actually be true positives samples as the Ct values obtained indicated that those samples had really low viral loads on the threshold for detection of the gold standard method used. Actually, one of the false positive samples was positive for both "COVID-19 RT-PCR Real TM FAST (CY5) and "ECUGEN SARS-CoV-2 RT-qPCR" kits. Additionally, both kits use the same N viral targets than the CDC protocol that do not have cross reactivity with other respiratory virus [14,15], and those 3 samples were reported as positive for the regular CDC protocol used for clinical diagnosis in our laboratory [14,15]. So, the specificity for "COVID-19 RT-PCR Real TM FAST (CY5)" and "ECUGEN SARS-CoV-2 RT-qPCR" kits could be considered 100%.

We could calculate the LoD for "ECUGEN SARS-CoV-2 RT-qPCR" at a really low viral load of 10 viral copies/uL of RNA extraction (2000 viral RNA copies/mL of sample) that it is equivalent to LoDs of high quality commercial RT-qPCR SARS-CoV-2 kits. Also, a similar LoD was estimated for "COVID-19 RT-PCR Real TM FAST (CY5). Moreover, this LoD is extremely reliable for SARS-CoV-2 diagnosis considering the viral load frequency population distributions [21,22].

In the Table 3, analytical parameters and other features for "COVID-19 RT-PCR Real TM FAST (CY5) and "ECUGEN SARS-CoV-2 RT-qPCR" RT-PCR kits are summarized. Considering the great clinical performance and analytical sensitivity for those locally designed and produced SARS-CoV-2 tests, compared to a high quality commercial kit like TaqMan 2019-nCoV Assay Kit v1 (Thermo Fisher), the current study endorses the use of these kits as a reliable alternative to expensive imported commercial kits. This would potentially allow to increase SARS-CoV-2 testing capacities by two main reasons: a) SARS-CoV-2 testing cost reduction as this locally produced RT-qPCR kits are substantially cheaper than high quality imported ones (less than 10 USD per reaction); b) supplies shortages would not affect SARS-CoV-2 testing capacities as local production is guaranteed.

Finally, we point out a common feature for both South American kits evaluated in this study: they were designed and produced by a consortium between universities and private companies. "COVID-19 RT-PCR Real TM FAST (CY5)" was created by collaboration of "Universidad de La República" (public university), "Instituto Pasteur de Montevideo" (research center) and ATGen (private company); "ECUGEN SARS-CoV-2 RT-qPCR" was created by

Table 3. Description of ECUGEN SARS-CoV-2 RT-qPCR (UDLA-Starnewcorp, Ecuador) and COVID-19 RT-PCR Real TM FAST (CY5) (ATGen, Uruguay) features (LoD means limit of detection in copies/uL of RNA extraction elution).

SARS-CoV-2 RT-PCR kit	Gene Targets	Estimated LoD (copies/uL)	countries of distribution
ECUGEN SARS-CoV-2 RT-qPCR (Ecuador)	N1, N2 (virals) RNaseP (control)	10	Ecuador
COVID-19 RT-PCR Real TM FAST (CY5) (Uruguay)	N (viral) RNaseP (control)	5–10	Uruguay, Argentina, Bolivia, Brasil, Ecuador.

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collaboration of "Universidad de Las Américas" (private university) and STARNEWCORP (private company). In both cases, the role of the Academia has been crucial to improve good quality SARS-CoV-2 testing, as it has been suggested even for USA [23]. Moreover, we describe two cases of cross talk and knowledge transference among the Academia and the private sector, common on high incomes countries but not as usual in the context of South America. We hope that these two successful stories will inspire similar biotechnological developments in the future to improve South American public health systems and reduce the regional overall technological dependency beyond COVID-19 pandemic.

Supporting information

S1 Data. Ct values for all samples included in this study for all the gene targets included in the three commercial SARS-CoV-2 RT-qPCR kits tested.
(XLSX)

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6.1.4. Desempeño clínico de tres pruebas comerciales rápidas de antígeno SARS-CoV-2 para personas que viven en un entorno tropical en la comunidad.

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Durante el segundo año de la pandemia de COVID-19, el uso de pruebas de antígeno de diagnóstico rápido (PDR-Ag) para la detección de SARS-CoV-2 ha aumentado sustancialmente ya que algunas de las marcas disponibles en el mercado fueron certificadas para uso clínico por agencias reguladoras internacionales. Los PDR-Ag son una herramienta rápida y económica para la vigilancia del SARS-CoV-2 con un gran potencial para mejorar las capacidades de prueba en países de ingresos medios y bajos en comparación con el estándar de oro RT-qPCR. Sin embargo, dado que se ha demostrado que el rendimiento clínico de los PDR-Ag varía mucho entre las marcas comerciales disponibles, se necesitan estudios de evaluación. Además, las mutaciones no informadas y/o las nuevas variantes de SARS-CoV-2 pueden comprometer la sensibilidad de PDR-Ag ya que la vigilancia genómica es limitada en estos entornos.

Aquí describimos una evaluación multicéntrica e independiente del fabricante del rendimiento clínico y la sensibilidad analítica de tres marcas diferentes de PDR-Ag disponibles en América del Sur de tres empresas, Rapigen (Corea del Sur), SD-Biosensor (Corea del Sur) y CerTest (España), en comparación con el estándar de oro RT-qPCR. Se incluyeron en el estudio un número total de 1646 hisopos nasofaríngeos de personas que viven en la comunidad, y 379 de ellos dieron positivo para SARS-CoV-2 por RT-qPCR.

La sensibilidad general para cada PDR-Ag fue del 79% (IC95 %: 72 - 86,2), 64,2% (IC95 %: 56,7 - 71,6) y 45,8% (IC95 %: 35,8 - 55,8) para SD-Biosensor, CerTest y Rapigen, respectivamente. La especificidad general para cada PDR-Ag fue del 100 %, 97,7 % (IC95 %: 96,8 - 98,6) y 100 % para SD-Biosensor, CerTest y Rapigen, respectivamente. Sin embargo, el límite de detección (LoD) para lograr una sensibilidad superior al 90% fue sustancialmente más bajo para CerTest (10^2 copias/uL) en comparación con SD-Biosensor (10^3 copias/uL) o Rapigen (10^6 copias/uL), considerando que el método RT-qPCR tiene una alta sensibilidad del 97,7% y un bajo LoD de 5 copias/uL. Además, CerTest también mostró una sensibilidad mejorada de hasta el 79,7% (IC95 %: 70,2 – 89,2) para personas sintomáticas.

Finalmente, la ligera reducción en la especificidad para CerTest solo se asoció con uno de los laboratorios que realizaron este estudio, lo que señala la necesidad de una evaluación de manera local para pruebas rápidas de antígeno como el nuestro realizado en Ecuador. En conclusión, dos de los tres PDR-Ag probados en este estudio son una herramienta rápida, económica y de punto de atención para la vigilancia del SARS-CoV-2 y lo suficientemente confiable como para detectar individuos infectados por el SARS-CoV-2.

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Clinical Performance of Three Commercial SARS-CoV-2 Rapid Antigen Tests for Community-Dwelling Individuals in a Tropical Setting

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During the second year of the COVID-19 pandemic, the use of Rapid Diagnosis Antigen Tests (RDAgTs) for SARS-CoV-2 detection has substantially increased as some of the brands available in the market were certified for clinical use by international regulatory agencies. RDAgTs are a fast and cheap tool for SARS-CoV-2 surveillance with great potential to improve testing capacities in middle- and low-income countries compared to the gold standard RT-qPCR. However, as the clinical performance of RDAgTs has been shown to vary greatly between the commercial brands available, evaluation studies are necessary. Moreover, the available evaluation has been done in high-income countries while SARS-CoV-2 transmission is also actively happening in developing countries, many of which are located in tropical latitudes where cross-reactivity with other infectious agents is highly prevalent, which could compromise RDAgT specificity. Moreover, unreported mutations and/or new SARS-CoV-2 variants may compromise RDAgT sensitivity as genomic surveillance is limited in these settings. Here we describe a multicenter and manufacturer-independent evaluation of the clinical performance and analytical sensitivity of three different RDAgTs brands available in South America from three companies, Rapigen (South Korea), SD-Biosensor (South Korea), and Certest (Spain), compared to the gold standard RT-qPCR. A total number of 1,646 nasopharyngeal swabs from community-dwelling individuals were included in the study, and 379 of them were SARS-CoV-2 positive by RT-qPCR. The overall sensitivity for each RDAgT was 79% (IC95%: 72 - 86.2), 64.2% (IC95%: 56.7 - 71.6), and 45.8% (IC95%: 35.8 - 55.8) for SD-Biosensor, Certest, and Rapigen, respectively. The overall specificity for each RDAgT was 100%, 97.7% (IC95%: 96.8 - 98.6), and 100% for SD-Biosensor, Certest, and Rapigen, respectively. However, the limit of detection (LoD) to achieve a sensitivity over 90% was substantially lower for Certest RDAgT (10^2 copies/uL) compared to SD-Biosensor (10^3 copies/uL) or Rapigen (10^6 copies/uL) RDAgTs, considering that the gold standard RT-

qPCR method used in this study has a high sensitivity of 97.7% and low LoD of 5 copies/uL. Additionally, the Certest RDAgT also showed an improved sensitivity up to 79.7% (IC95%: 70.2 – 89.2) for symptomatic individuals. Finally, the slight reduction in specificity for Certest RDAgTs was only associated with one of the laboratories performing this study, pointing out the need for locally assessed evaluation for RDAgTs like this one carried out in Ecuador. In conclusion, two of the three the RDAgTs tested in this study are a fast, cheap, and point of care tool for SARS-CoV-2 surveillance and reliable enough to detect SARS-CoV-2 infectious individuals.

Keywords: antigen test, RapiGEN® Ag test kit, SD-Biosensor, Certest, SARS–CoV–2, clinical performance

INTRODUCTION

After the initial COVID-19 outbreak in Wuhan, China, in December 2019, SARS-CoV-2 spread rapidly and the World Health Organization declared COVID-19 a pandemic on 11 March 2020, and this pandemic is still ongoing (Wang et al., 2020; Gorbalenya et al., 2020; Zhou et al., 2020). SARS-CoV-2 RNA detection by RT-qPCR was the gold standard for acute infection diagnosis during the first year of the COVID-19 pandemic (Corman et al., 2020). By the end of 2020 and during 2021, the use of several commercial brands of point of care or Rapid Diagnosis Antigen Tests for SARS-CoV-2 detection became endorsed by international regulatory agencies or public health authorities (Cerutti et al., 2020; Pray et al., 2021). However, RT-qPCR is still widely used to confirm SARS-CoV-2 infection though this technique has several limitations for a scenario like the current COVID-19 pandemic: it is not easy to improve as a point of care diagnosis method, it requires sophisticated laboratory infrastructure, it depends on skilled personnel with a molecular biology background, and it is also permanently dependent on reagents that have experienced supply shortages (Freire-Paspuel et al., 2020; Cubas-Atienzar et al., 2021). Moreover, both RT-qPCR effectiveness for triage and contact tracing surveillance strategies are challenged by the need for 24 to 72 hours from sample collection to diagnosis (Kretzschmar et al., 2020). Additionally, RT-qPCR is an expensive diagnostic tool in the context of middle- and low-income countries that compromise their testing capacities (Cuellar et al., 2021; Santander-Gordon et al., 2021).

By contrast, the lateral flow immunoassays for SARS-CoV-2 antigen detection, also known as Rapid Diagnosis Antigen Tests (RDAgTs), allow for the point of care identification of SARS-CoV-2 virus in nasopharyngeal, oropharyngeal, or nasal samples in a time frame of 10 to 30 minutes depending on the commercial brand (Cerutti et al., 2020; Cubas-Atienzar et al., 2021; Iglói et al., 2021; Lee et al., 2021; Pray et al., 2021). Moreover, RDAgTs can either be performed by nursing staff without any laboratory infrastructure requirements or have been validated for patient self diagnosis (Nagura-Ikeda et al., 2020; Marx et al., 2021). Additionally, the cost of RDAgTs diagnosis is substantially cheaper than RT-qPCR diagnosis, as there are currently several RDAgTs commercial brands for self diagnosis sold for less than 5 USD even at grocery stores in the USA and some European countries. As RDAgTs are cheaper, faster, and available for point of care diagnosis, they are a powerful tool for SARS-CoV-2 surveillance, not only for triage in hospital settings for symptomatic individuals but also for the massive screening of community-dwelling individuals in middle- and low-income countries (Iglói et al., 2021; Marx et al., 2021; Pollock et al., 2021; Pray et al., 2021; Tinker et al., 2021).

Studies have addressed the clinical performance of different RDAgT brands compared to the gold standard RT-qPCR (Cubas-Atienzar et al., 2021; Lee et al., 2021). Those studies confirm that RDAgTs have reduced sensitivity and a higher limit of detection compared to RT-qPCR (Cubas-Atienzar et al., 2021; Lee et al., 2021). However, the accuracy of some RDAgTs brands has been suggested to allow the identification of the vast majority of infectious individuals, as the sensitivity is over 90% for viral loads with $> 10^6$ genomic virus copies/ml (Corman et al., 2021; Cubas-Atienzar et al., 2021; Lee et al., 2021). Additionally, under a scenario like the COVID-19 pandemic, a reduction in sensitivity is acceptable as long as it comes with an increase in testing capacities, so the final output is a higher number of SARS-CoV-2 positive individuals detected (Mina et al., 2020). RDAgTs would fulfill these requirements as they are fast, cheap, and accurate enough to allow massive and rapid detection and isolation of new cases to stop transmission chains and reduce the impact of COVID-19 (World Health Organization (WHO), 2020; Andreani et al., 2021; Corman et al., 2021; Cubas-Atienzar et al., 2021; Lee et al., 2021; Pekosz et al., 2021; Weiss and Bellmann-Weiler, 2021).

As we have described above, the SARS-CoV-2 testing capacity in developing countries has been a challenge during the COVID-19 pandemic as it has been relying on the RT-qPCR technique. Moreover, as vaccination programs have been progressing slowly in middle- and low-income countries, SARS-CoV-2 circulation is still happening very actively in those settings, threatening COVID-19 pandemic control and eradication through new SARS-CoV-2 variant appearances (Dhawan et al., 2022). RDAgTs have the necessary features to improve effective SARS-CoV-2 surveillance programs in developing countries (World Health Organization (WHO), 2020; Andreani et al., 2021; Corman et al., 2021; Lee et al., 2021; Pekosz et al., 2021; Weiss and Bellmann-Weiler, 2021). However, the clinical performance evaluation studies for RDAgTs have been done in high-income countries (Albert et al., 2021; Andreani et al., 2021; Baro et al., 2021; Cerutti et al., 2020; Corman et al., 2021; Cubas-Atienzar et al., 2021; Iglói et al., 2021; Lee et al.,

2021; Pérez-García et al., 2021; Pollock et al., 2021; Pray et al., 2021; Tinker et al., 2021; Weitzel et al., 2021). It has been already reported that low-quality COVID-19 diagnosis products are commercialized in developing countries and genomic surveillance in those settings is limited, so the tracking of new mutations or variants of SARS-CoV-2 potentially compromises the sensitivity of RDAgTs for COVID-19 diagnosis (Cota et al., 2020; Freire-Paspuel and Garcia-Bereguiain, 2020; Freire-Paspuel and Garcia-Bereguiain, 2021; Freire-Paspuel and Garcia-Bereguiain, 2021; Freire-Paspuel et al., 2021). Moreover, as the cross reactivity with other infectious pathogens for SARS-CoV-2 serology testing has been described, this phenomenon may also happen for RDAgTs, compromising their specificity in these middle- and low-income tropical countries (Echeverría et al., 2021; Faccini-Martínez et al., 2020; Tso et al., 2021). Considering this scenario, clinical performance evaluation of RDAgTs in the context of middle- and low-income countries are mandatory.

The aim of this work was to address the clinical performance and analytical sensitivity of three RDAgT commercial brands available to community-dwelling individuals in Ecuador.

MATERIALS AND METHODS

Study Design

A total number of 1,646 community-dwelling individuals (COVID-19 asymptomatic or mildly symptomatic) were included in the study performed from 12 January to 8 May 2021 at two independent laboratories: 1,076 samples were taken at a laboratory for SARS-CoV-2 detection at "Universidad de Las Américas" in Quito, Pichincha province, Ecuador (UDLA lab); and 570 samples were taken at "OneLabt" laboratory in Ballenita, Santa Elena province, Ecuador. Overall, the study population included 1,267 individuals who tested negative and 379 who tested positive for SARS-CoV-2 detection by RT-qPCR (29.9% positivity rate).

A single nasopharyngeal swab was collected for each individual and tested for SARS-CoV-2 detection by RT-qPCR following the standard protocol in both laboratories. As the sample collection buffer volume was sufficient to perform RT-qPCR and RDAgTs, the spare sample volume was immediately processed for SARS-CoV-2 detection by RDAgT.

According to Ecuadorian regulations, all the results for SARS-CoV-2 detection made by RT-qPCR must be reported to the Ministry of Health, where a short survey is completed and information regarding COVID-19 related symptoms for individuals is stored. Based on this survey, we could classify our study groups as symptomatic or asymptomatic individuals.

SARS-CoV-2 Detection Using Rapid Diagnosis Antigen Tests

Three different commercial brands of RDAgTs were evaluated in this study: Biocredit Covid-19 Ag Detection Kit (RapiGen, South Korea), SARS-CoV-2 Ag Test (Certest Biotec, Spain), and SARS-CoV-2 Rapid Antigen Test (SD-Biosensor, South Korea). Hereafter, we refer to the different test kits using the names "Rapigen", "Certest", and "SD-Biosensor".

The three RDAgTs included in the study are based on lateral flow immunochromatography. We used the collection buffer provided for each RDAgT for sample collection and follow each manufacturer's instructions to perform the SARS-CoV-2 detection. The reading time for the RDAgT varied from 10 to 30 min depending on the commercial brand.

As only one sample was collected from each patient, there were only paired samples for each RDAgT brand and RT-qPCR: 200 samples for Rapigen; 223 samples for SD-Biosensor; 1,223 for Certest. The variability or bias of the sample size for each commercial brand was due to the total number of RDAgTs that were kindly donated by each Ecuadorian distribution company for each of those brands. For Rapigen and "SD-Biosenseor, all the samples were processed at the UDLA lab. However, for the Certest evaluation, 653 and 570 samples were processed at UDLA lab and Onelabt, respectively.

SARS-CoV-2 Detection Using RT-qPCR

Both laboratories involved in the study performed SARS-CoV-2 detection by RT-qPCR with the same protocol based on an adapted version from the Centers for Disease Control and Prevention (USA) protocol by using a CFX96 BioRad instrument and a triplex PCR assays (Freire-Paspuel et al., 2020; Freire-Paspuel and Garcia-Bereguiain, 2021; Freire-Paspuel et al., 2021; Freire-Paspuel et al., 2021). Briefly, the commercial kit ECUGEN SARS-CoV-2 RT-qPCR kit (UDLA-Startnewcorp, Ecuador) includes a triplex assay for N1 and N2 viral targets to detect SARS-CoV-2 and RNase P as an RNA extraction quality control (Freire-Paspuel and Garcia-Bereguiain, 2021). Also, negative controls (sample collection buffer) were included as a control for carry-over contamination, one for each set of RNA extractions. For viral loads calculation, the 2019-nCoV N positive control (IDT, USA) was used and provided at 200.000 genome equivalents/mL (Freire-Paspuel et al., 2020; Freire-Paspuel et al., 2021).

This positive control is a plasmid including N1 and N2 viral gene targets sequences, and it is a SARS-CoV-2 positive control recommended by CDC guidelines (Freire-Paspuel et al., 2020; Freire-Paspuel et al., 2021). Serial dilutions of the positive control were included in each set of samples RT-qPCR running, so an internal calibration curve with known concentrations of genomic SARS-CoV-2 material was always available. A regression analysis was made for each of those calibration curves taking RT-qPCR Ct values for N1 and N2 targets and viral genomic material concentrations as variables. The equation obtained was used for viral load calculations for each set of clinical samples, finally expressed as an average of the values for N1 and N2 targets. Regression coefficients over 0.99 were obtained for the viral load calibration curves. The RT-qPCR method used in this study has a high sensitivity of 97.7% and a low LoD of 5 copies/uL (Freire-Paspuel et al., 2020; Freire-Paspuel and Garcia-Bereguiain, 2021).

Statistical Analysis

We carried out a descriptive study of the characteristics of the population by sex, age, and presence or absence of symptoms. The

sensitivity, specificity, positive predictive value, and negative predictive value of the three different commercial brands of lateral flow immunochromatography based SARS-CoV-2 Rapid Diagnosis Antigen tests (RDAgT) were calculated in the general population, separating them into symptomatic and asymptomatic individuals at two different laboratories with a confidence level of 95%. Furthermore, Sensitivity and Negative Predictive Values (NPV) for different viral load detection thresholds of Limit of Detection (LoD) by RT-qPCR are presented.

All statistical analysis was carried out using SPSS Statistics 23 software.

RESULTS

A descriptive analysis was performed by age, sex, and presence or absence of symptoms in the total study population (**Table 1**). Most of the population was female (618/1076, 57.4%) and the highest number of participants ranged in age from 20 – 40 years (593/1076, 55.1%). It should be noted that sex and age information from one of the laboratories is not included as it was not collected. Conversely, the distribution of individuals according to the presence or absence of symptoms is provided for the whole population study, with a greater number of asymptomatic patients (1119/1646, 68%), as detailed in **Table 1**.

Overall Clinical Performance for the Three SARS-CoV-2 Rapid Diagnosis Antigen Tests Included in the Study

The analysis of the clinical performance for Rapigen, SD-Biosensor, and Certest RDAgTs is detailed in **Table 2**. The number of samples tested was 200, 223, and 1,223 for Rapigen, SD-Biosensor, and Certest, respectively. The ratio values for the number of positive SARS-CoV-2 samples by each RDAgT compared to RT-qPCR were 44/96, 98/124, and 102/159 for Rapigen, SD-Biosensor, and Certest, respectively. So, the overall sensitivity values for the RDAgTs evaluated in the present work were 45.8% (IC95%: 35.8 - 55.8), 79% (IC95%: 72 - 86.2), and 64.2% (IC95%: 56.7 - 71.6) for Rapigen, SD-Biosensor, and Certest, respectively (**Table 2**).

No SARS-CoV-2 false-positive samples were found for RDAgTs from the Rapigen and SD-Biosensor brands, so the specificity in both cases was 100%. For the Cerstest RDAgT, a total of 1,040 SARS-CoV-2 negative samples out of 1,064 samples were correctly identified, yielding a specificity of 97.7% (**Table 2**).

Evaluation of the Analytical Sensitivity for the Three SARS-CoV-2 Rapid Diagnosis Antigen Tests Included in the Study

In **Table 3**, the analysis of the clinical performance at different limit of detection (LoD) or viral load thresholds for the three RDAgTs evaluated in this study is detailed. The values of LoD for which the sensitivity is over 90% were as follows: 100 copies/uL for Certest (90.8%, IC95%: 85.4 - 96.2), 1,000 copies/uL for SD-Biosensor (94.7%, IC95% 90.2 – 99.2), and 1,000,000 copies/uL for Rapigen (100%). For an LoD of 1,000,000 copies/uL, the sensitivity values for Certest and SD-Biosensor were 100% and 97.4% (IC95%:92.4 – 100), respectively.

In **Figure 1**, the viral load distribution for the SARS-CoV-2 positive samples by RT-qPCR included in each RDAgTs evaluation is detailed. There are statistically significant differences ($p < 0.05$) for the mean viral load between RDAgT positive and RDAgT negative samples for Certest and SD-Biosensor, but not for Rapigen.

TABLE 1 | Characteristics of the population tested with the three different commercial brands of lateral flow immunochromatography based SARS-CoV-2 Rapid Diagnosis Antigen tests (RDAgT) included in this study.

Brand	Total samples	Age (years)			Sex		Symptoms	
		≤ 20	20 and 40	≥40	Female	Male	Symptomatic	Asymptomatic
Rapigen	200	5 (6,8%)	122 (20,6%)	73 (17,8%)	116 (18,8%)	84 (18,3%)	138 (26,2%)	62 (5,5%)
Certest	653	46 (63%)	358 (60,4%)	249 (60,7%)	372 (60,2%)	281(61,4%)	166 (31,5%)*	1057 (94,5%)*
SD-Biosensor	223	22 (30,1%)	113 (19,1%)	88 (21,5%)	130 (21%)	93 (20,3%)	223 (42,3%)	0
Total	1076	73 (6,8%)	593 (55,1%)	410 (38,1%)	618 (57,4%)	458 (42,6%)	527 (32%)	1119 (68%)

**For CerTest, the information on gender and age does not include data from OneLabt laboratory in Ballenita, Santa Elena province, Ecuador.*

TABLE 2 | Clinical performance of the three different commercial brands of lateral flow immunochromatography based SARS-CoV-2 Rapid Diagnosis Antigen tests (RDAgT) included in this study (total samples: number of samples included in the evaluation; positive samples: number of SARS-CoV-2 positive samples included in the evaluation for RDAgTs or RT-qPCR; negative samples: number of SARS-CoV-2 negative samples included in the evaluation for RDAgTs or RT-qPCR; PPV, positive predictive value; NPV, negative predictive value; parentesis includes IC95%.

RDAgT brand	Total samples	Positive samples (RDAgT/RT-qPCR)	Negative samples (RDAgT/RT-qPCR)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Rapigen	200	44/96	104/104	45.8 (35.8 - 55.8)	100	100	66.7 (59.2 - 74)
CerTest	1,223	102/159	1,040/1,064	64.2 (56.7 - 71.6)	97.7 (96.8 - 98.6)	81 (74.1 – 87.85)	94.8 (93.5 - 96.1)
SD-Biosensor	223	98/124	99/99	79 (72 – 86.2)	100	100	79.2 (72 - 86.3)

TABLE 3 | Evaluation of the analytical sensitivity of the three different commercial brands of lateral flow immunochromatography-based SARS-CoV-2 Rapid Diagnosis Antigen tests (RDAgT) included in this study.

LoD (copies/mL)	Rapigen			CerTest Biotec			SD-Biosensor		
	N	Sensitivity (%)	% NPV	N	Sensitivity (%)	% NPV	N	Sensitivity (%)	%NPV
10^2	44/76	57.9 (46.8 - 69)	76.4 (69.3 - 83.6)	99/109	90.8 (85.4 - 96.2)	99 (98.5 - 99.6)	96/108	88.8 (82.8 – 94.7)	84.6 (77.9 – 91.3)
10^3	42/66	63.6 (51.2 - 75.2)	81.2 (74.5 - 88)	86/91	94.5 (89.8 - 99.1)	99.5 (99.1 - 99.9)	90/95	94.7 (90.2 – 99.2)	95 (90.8 - 99.2)
10^4	38/50	76 (64.1- 87.8)	89.6 (84.1 - 95.2)	57/59	96.6 (92 - 100)	99.8 (99.5 - 100)	83/86	96.5 (92.6 – 100)	97 (93.7 – 100)
10^5	29/33	87.9 (76.8 - 99)	96.3 (92.7 – 99.8)	33/34	97.1 (91.3 - 100)	99.9 (99.7 - 100)	64/65	98.5 (95.5 – 100)	99 (97 -100)
10^6	11/11	100	100	10/10	100	100	38/39	97.4 (92.4 – 100)	99 (97 – 100)

Sensitivity and Negative Predictive Values (NPV) for different viral load detection thresholds of Limit of Detection (LoD) by RT-qPCR are presented next to the 95% confidence interval.

Clinical Performance for the Three SARS-CoV-2 Rapid Diagnosis Antigen Tests Included in the Study for Symptomatic and Asymptomatic Individuals

In **Table 4**, the clinical performance of the three RDAgTs in symptomatic and asymptomatic individuals is shown. For Rapigen, the sensitivity values for symptomatic and asymptomatic individuals were 48.7% and 28.6%, respectively. For Certest, the sensitivity values for symptomatic and asymptomatic individuals were 79.7% and 52.2%, respectively. For SD-Biosensor, the sensitivity values were only addressed for symptomatic individuals, as asymptomatic individuals were excluded in this evaluation, so the value and overall sensitivity (79%) are the same as reported above. In **Table 4** we included a sensitivity value of 43.6% that has been reported in another study (Weitzel et al., 2021) for SD-Biosensor with asymptomatic individuals for comparison. There was a significant reduction in sensitivity ($p < 0.05$) for asymptomatic individuals compared to symptomatic individuals for the Certest and Rapigen RDAgTs.

Comparison of the Clinical Performance for the Rapid Diagnosis Antigen Test From Certest at Two Different Laboratories Located in Quito (Pichincha Province, Andean Region of Ecuador) and Ballenita (Santa Elena Province, Coastal Region of Ecuador)

For the clinical performance evaluation of Certest RDAgTs, there were two independent laboratories involved in the evaluation. In **Table 5**, the results of the clinical performance of the RDAgTs are presented for each of those two labs. In the UDLA lab, 653 samples were processed and the values for sensitivity and specificity were 72.2% and 95.9%, respectively. In the Onelabt laboratory, 653 samples were processed and the values for sensitivity and specificity were 57.5% and 100%, respectively.

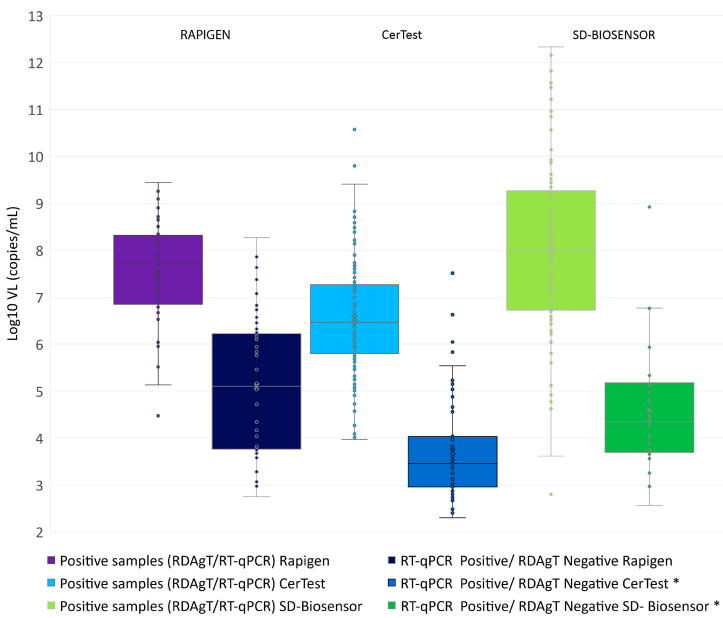

FIGURE 1 | Viral loads distribution for all the SARS-CoV-2 positive samples by RT-qPCR was included in the study. Viral loads (VL) are presented on a Log10 scale. The different sets of samples used for each Rapid Diagnosis Antigen Test (RDAgT) brand are divided into two categories: RDAgT positive and RDAgT negative. *There are statistically significant differences ($p < 0.05$) for VL between RDAgT positive and RDAgT negative only for Certest Biotec and SD-Biosensor brands.

TABLE 4 | Clinical performance of the three different commercial brands of lateral flow immunochromatography based SARS-CoV-2 Rapid Diagnosis Antigen tests (RDAgT) included in this study for symptomatic and asymptomatic individuals (total samples: number of samples included in the evaluation; positive samples: number of SARS-CoV-2 positive samples included in the evaluation for RDAgTs or RT-qPCR; negative samples: number of SARS-CoV-2 negative samples included in the evaluation for RDAgTs or RT-qPCR; PPV: positive predictive value; NPV negative predictive value; next to the 95% confidence interval (IC95%).

Type of individual	RDAgT Brand	Total samples	Positive samples (RDAgT/RT-qPCR)	Negative samples (RDAgT/RT-qPCR)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Symptomatic	Rapigen	138	40/82	56/56	48.7 (37.8 – 59.5)	100	100	57.1 (47.3 – 66.9)
	Certest	166	55/69	94/97	79.7 (70.2 – 89.2)	97 (93.6 – 100)	94.8 (89.1-100)	87 (80.6- 93.3)
	SD-Biosensor	223	98/124	99/99	79 (71.8 – 86.2)	100	100	79.2 (72.1- 86.3)
Asymptomatic	Rapigen	62	4/14	48/48	28.6 (5 – 52.3)	100	100	82.8 (73.1-92.5)
	CerTest	1057	47/90	946/967	52.2 (41.9 – 62.5)	97.8 (96.8 – 98.7)	69.1 (58.1-80.1)	95.6 (94.3-96.9)
	SD-Biosensor*	286	44/101	178/185	43.6 (33.5 – 52.9)	96.2 (93.4 – 98.9)	86 (76.5 – 95.5)	75.7 (70.2- 81.2)

**For SD-Biosensor, as non-asymptomatic individuals were included in our study, we took values from the published report described in reference 26).*

DISCUSSION

In this study, we describe the clinical performance of three commercial RDAgTs brands currently available in several South American countries, including Ecuador. We found differences in terms of the overall sensitivity for the three RDAgTs evaluated. While Rapigen has a substantially reduced sensitivity below 50%, Certest and SD-Biosensor have an equivalent performance of almost 80% sensitivity for symptomatic individuals. Moreover, for a viral load threshold of 100 copies/uL, only the Certest RDAgT had an overall sensitivity over 90%. Both Certest and SD-Biosensor had sensitivity values close to 95% when samples with viral loads lower than 1000 copies/uL were excluded from the analysis. However, the overall sensitivity of Rapigen only reached a value over 90% for samples with viral loads over 10^6 copies/uL. As an approximate LoD of 10^6 copies/ml has been proposed as the minimal analytical sensitivity by the WHO or the Department of Health and Social Care from the United Kingdom (Department of Health and Social Care, 2020; WHO & R&D Blue Print, 2020), only SD-Biosensor and Certest RDAgTs evaluated in this study accomplished that requirement. Moreover, as the viral load is a dynamic parameter that may grow exponentially during the incubation period, our results would support the use of either Certest or SD-Biosensor over Rapigen RDAgTs (Avanzato et al., 2020; Kawasuji et al., 2020; Kleiboeker et al., 2020; Lavezzo et al., 2020; Pekosz et al., 2021; Singanayagam et al., 2020; Walsh et al., 2020; Weiss and Bellmann-Weiler, 2021).

Additionally, we call attention to the variability of sensitivity and specificity among the two labs involved in this evaluation study. As the same protocol for sample collection and RT-qPCR was used in both laboratories, the differences observed in sensitivity were associated at a random event such as a higher number of individuals with low viral loads in one of the locations. This difference in sensitivity occurred considering that more than 500 samples were evaluated in each lab setting, pointing out the need for extensive and multi-center studies for an accurate clinical performance evaluation of commercial RDAgTs. As reflected in **Table 6**, our results are within the range of sensitivity and specificity reported for RDAgTs, but there are substantial differences in the clinical performance between the different studies, even for the same RDAgT commercial brand (Albert et al., 2021; Baro et al., 2021; Cerutti et al., 2020; Corman et al., 2021; Cubas-Atienzar et al., 2021; Iglói et al., 2021; Lee et al., 2021; Nagura-Ikeda et al., 2020; Pérez-García et al., 2021; Pray et al., 2021; Pollock et al., 2021; Tinker et al., 2021). Moreover, the vast majority of clinical performance evaluations for RDAgTs have been carried out in high-income countries. However, SARS-CoV-2 current transmission is also happening in middle- and low-income countries where COVID-19 vaccination programs are progressing slowly. Moreover, SARS-COV-2 genomic surveillance in developing countries is limited, so new mutations or SARS-CoV-2 variants may not be well characterized. Under this scenario, locally assessed studies of the available RDAgT commercial brands are needed, as there is a concern regarding the potential reduction of sensitivity for SARS-CoV-2 variants (Frediani et al., 2021).

In terms of specificity, the three RDAgTs showed a good performance with values of 100% for Rapigen, SD-Biosensor, and also for Certest at one of the laboratories. Interestingly, there was almost a 5% reduction in specificity for the Certest RDAgT only for the UDLA lab evaluation. It is important to note

TABLE 5 | Independent evaluation of the clinical performance of the SARS-CoV-2 Rapid Diagnosis Antigen Test (RDAgT) from Certest Biotec at two different laboratories in Ecuador (total samples: number of samples included in the evaluation; positive samples: number of SAS-CoV-2 positive samples included in the evaluation for RDAgTs or RT-qPCR; negative samples: number of SARS-CoV-2 negative samples included in the evaluation for RDAgTs or RT-qPCR; PPV, positive predictive value; NPV, negative predictive value; parentesis includes IC95%.

Clinical Lab	Total samples	Positive samples (RDAgT/RT-qPCR)	Negative samples (RDAgT/RT-qPCR)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
UDLA	653	52/72	581/605	72.2 (61.9 – 82.5)	95.9 (94.3 – 97.5)	68.4 (57.9 – 78.8)	96.5 (95 – 97.57)
OneLabt	570	50/87	483/483	57.5 (47.1-67.9)	100	100	92.9 (90.7 – 95.1)

TABLE 6 | Comparative analysis of the clinical performance for several SARS-CoV-2 Rapid Diagnosis Antigen Test (RDAgTs) with evaluation studies published in peer review journals (* for Abbott, results from two different commercial RDAgTs are included).

RDAgT Brand	Sensitivity (%)	Specificity (%)	Reference
CerTest (Spain)	53.5-79.7	97.7-100	14,21,27, our study.
Rapigen (South Korea)	28.6-62	100	14,20,25, our study.
SD-Biosensor (South Korea)	43.6-79	96.2-100	14,21,26,27, our study
Abbott (USA)*	20-79.6	100	14,17,18,21,27

that the two labs involved in the study were located in a tropical latitude (Ecuador) but were two environmentally different settings: Quito is in the Andean Region of Ecuador at 2800 meters above sea level and Ballenita is at sea level in the Santa Elena province in the coastal region of Ecuador. As the weather conditions are different among these two locations, cross reactivity with a respiratory virus circulating at the time of this study is a plausible explanation for the differences observed between Quito and Ballenita. A similar phenomenon has been described for anti-SARS-CoV-2 serological tests, particularly in developing countries and tropical regions, due to the higher prevalence of some pathogens compared to high-income countries, where most of the COVID-19 diagnosis tools evaluations are conducted (Cota et al., 2020; Tso et al., 2021; Echeverría et al., 2021). Our results endorse the need for locally assessed evaluation studies in middle- and low-income settings to guarantee a reliable specificity for SARS-CoV-2 detection with RDAgTs.

This clinical performance evaluation has some limitations. For instance, no viral cultures were used to assess the LoDs as no BSL3 facility was available. However, the viral load calculations made by using a tittering of the CDC-designed SARS-CoV-2 positive control, described in the methods, were in agreement with other reports analyzing the same commercial brand RDAgTs (Corman et al., 2021; Lee et al., 2021; Mina et al., 2020; Pérez-García et al., 2021; Weitzel et al., 2021). Another limitation is that SD-Biosensor only included symptomatic patients, although this commercial brand is among the most used worldwide and several evaluation reports have already been published (Cubas-Atienzar et al., 2021; Lee et al., 2021). Moreover, the two laboratories were only involved in Certest RDAgT evaluation as the Ecuadorian representatives for the other two brands could not provide as many tests as requested.

In conclusion, the clinical performance and analytical sensitivity of Certest and SD-Biosensor RDAgT brands tested were within the WHO requirements. These results support the use of RDAgTs as a fast, cheap, and reliable point of care tool for SARS-CoV-2 detection for most COVID-19 contagious individuals. The massive use of RDAgTs would have a tremendous impact on COVID-19 pandemic control in developing countries where SARS-CoV-2 remains at a high level of transmission.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IRB certified by Ministry of Health from Ecuador (code 008-2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DM-J and MG-B wrote the manuscript. All the authors contributed to data collection and analysis, and also to manuscript revision and approval prior to submission.

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6.2. Estudios de epidemiología de COVID-19 en Ecuador

6.2.1. Transmisión Comunitaria y Súper Propagadores de COVID-19 en aldeas rurales de la Provincia de Manabí en la región Costera de Ecuador evaluadas mediante pruebas masivas.

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Las comunidades rurales desatendidas en América Latina son altamente vulnerables al COVID-19 debido a una mala infraestructura de salud, estructura y acceso limitado al diagnóstico del Síndrome Respiratorio Agudo Severo Coronavirus 2 (SARS-CoV-2). Manabí es una provincia de la Región Costa del Ecuador caracterizada por una alta prevalencia de población rural en condiciones de pobreza. En el presente estudio, presentamos el análisis retrospectivo de los resultados de un operativo masivo de pruebas de SARS-CoV-2 en poblaciones no hospitalizadas de Manabí realizado de agosto a septiembre de 2020.

Un total de 4.003 personas de 15 cantones fueron evaluadas para SARS-CoV-2 por RT-qPCR, lo que resultó en una tasa de infección general del 16,13% para SARS-CoV-2, con varias comunidades con valores mayores al 30%. Además, se encontraron 29 individuos que vivían en la comunidad con superpropagadores de SARS-CoV-2 con cargas virales superiores a 10^8 copias/mL.

Estos resultados respaldan que la transmisión comunitaria descontrolada de COVID-19 estaba ocurriendo en Manabí durante el primer semestre de la pandemia de COVID-19. Este informe avala la utilidad de las pruebas masivas de SARS-CoV-2 en población asintomática para el control y vigilancia de la COVID-19.

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COVID-19 Community Transmission and Super Spreaders in Rural Villages from Manabi Province in the Coastal Region of Ecuador Assessed by Massive Testing of Community-Dwelling Population

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Abstract. Neglected rural communities in Latin America are highly vulnerable to COVID-19 due to a poor health infrastructure and limited access to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnosis. Manabí is a province of the Coastal Region of Ecuador characterized by a high prevalence of rural population living under poverty conditions. In the current study, we present the retrospective analysis of the results of a massive SARS-CoV-2 testing operation in nonhospitalized populations from Manabí carried out from August to September 2020. A total of 4,003 people from 15 cantons were tested for SARS-CoV-2 by reverse-transcriptase quantitative polymerase chain reaction, resulting in an overall infection rate of 16.13% for SARS-CoV-2, with several communities $>$ 30%. Moreover, 29 SARS-CoV-2 super-spreader community-dwelling individuals with viral loads above 10^8 copies/mL were found. These results support that uncontrolled COVID-19 community transmission was happening in Manabí during the first semester of COVID-19 pandemic. This report endorses the utility of massive SARS-CoV-2 testing among asymptomatic population for control and surveillance of COVID-19.

INTRODUCTION

Coronaviruses (CoVs) are positive strand RNA viruses contained within a viral envelope with a crown-like morphology. They belong to the Nidovirus superfamily and are the largest known group of RNA viruses. Coronaviruses are the cause of many diseases in wild animals as well as domestic animals. In humans, the most prevalent coronavirus infections cause the common cold; however, severe acute respiratory syndrome (SARS)-associated coronaviruses have shown potential for severe, noteworthy diseases.[1] On January 30, 2020, the WHO declared a "public health emergency of international concern" due to the outbreak of the novel coronavirus SARS-coronavirus 2 (CoV-2) to anticipate a coordinated international response.[2] The SARS-CoV-2 pandemic spread from China to almost every country within months. In Latin America, the first outbreak appeared 4 weeks after Western Europe and 2 weeks after the United States and Canada, reaching marginalized regions with noticeable poverty. Because of the weak health infrastructure, understaffing, lack of biosafety equipment, and distrust in public governance, this region was greatly affected by the pandemic.[3] During the first year of the COVID-19 pandemic, more than 51 million COVID-19 cases and more than 1 million deaths were reported in the Americas.[4] More than 500,000 cases and 32,000 deaths associated with COVID-19 were reported since the first case in February 2020 through September 2021 in Ecuador.[5]

From the early stages of the COVID-19 pandemic, the WHO made a wide variety of recommendations, such as using face masks to reduce the spread of aerosol particles containing the virus, social distancing, and isolation of confirmed cases to slow the spread of the disease. One of the key observations of the WHO is that a successful surveillance strategy to contain the spread of COVID-19 is always based on testing as much of the population as possible. However, the Ecuadorian population has limited access to SARS-CoV-2 testing. In the early stages of the pandemic, only the National Institute of Research in Public Health laboratories, located in the three main cities of Ecuador (Guayaquil, Quito, and Cuenca) performed SARS-CoV-2 detection using reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) within the public health system, which was translated in a poor testing ratio of 7.46 PCR tests per 10,000 people.[6] Up to September 10, 2021, after more than 1 year into the COVID-19 pandemic, 1,786,863 SARS-CoV-2 RT-qPCR tests had been done for 17 million Ecuadorians, with a positivity rate of 28.2%, according to the Ecuadorian Ministry of Health (MoH).[5] The WHO recommends that no more than 5% of the individuals tested for SARS-CoV-2 should be positive to consider a surveillance program to control the spread of the virus. This means that many regions in Ecuador have not been sufficiently tested to control COVID-19 spread.[7]

Manabí is a primarily rural Ecuadorian province with an area of 19,427 km^2 and a total of 1,390,200 inhabitants, of whom 617,880 reside in the rural areas. Manabí is the fourth largest province and the third most populated, with 22 counties.[8] This province had a gross value added of 5,829.023 million USD in 2019 (gross value added for Ecuador was 100,871.577 million USD), giving a gross value added per capita below 5,000 USD (according to the data from Central Bank of Ecuador). On April 16, 2016, a 7.8-magnitude

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earthquake hit Ecuador with its epicenter in Pedernales, Manabí. The earthquake affected 720,000 people, with a total of 660 deaths, 4,605 injuries, 40 people missing, more than 30,000 people displaced, and 9,750 damaged buildings. The infrastructure damage included 59 hospitals and healthcare facilities, which, due to structural damage, were rendered inoperative in most cases. The impacted cities became chaotic with displacement and rural communities highly affected. The effect of the earthquake became visible with damage to the sanitation infrastructure, disruption of healthcare services, and overwhelming social and environmental disturbances. To this date, the province has not fully recovered from the infrastructure damages left by the earthquake.[9] The lack of medical infrastructure, paired with RT-qPCR sampling limited to symptomatic patients attending a health center in urban areas, makes the rural areas of Manabí especially vulnerable to the SARS-CoV-2 pandemic. According to the MoH, during the first half year of the COVID-19 pandemic up to September 12, 2020, a total of 20,598 SARS-CoV-2 RT-qPCR tests done. This means that 1.5% of the Manabí population was tested at that point despite the dramatic 44.9% positivity rate.[10] Up to May 2021, this province only has a small-capacity SARS-CoV-2 diagnosis laboratory within the public health system, meaning this region is still highly vulnerable to SARS-CoV-2 outbreaks.

This study is a retrospective analysis of epidemiological data obtained after massive aid surveillance SARS-CoV-2 testing carried out in coordination with local community leaders, the MoH, and the regional government (Prefectura de Manabi) at rural communities on 15 cantons included in the province of Manabí. This study is a follow-up of a previous short report published in this journal,[11] now including all the community-dwelling individuals tested from August to September 2020.

METHODS

Study design and setting. A total of 4,003 individuals enrolled the surveillance. All samples were taken from community-dwelling, mostly asymptomatic individuals at the communities visited since August 3, 2020 to September 14, 2020, in 15 of 24 cantons of Manabí Province: Rocafuerte, Pajan, Santa Ana, Junín, Tosagua, Olmedo, Portoviejo, Manta, San Vicente, 24 de Mayo, Bolivar, Pedernales, Chone, El Carmen, and Jama (Figure 1).

Because the samples were not collected for a research study but as part of an aid surveillance intervention, the communities were selected by convenience following the recommendations from the local organizations and the provincial government of Manabí that helped us. Rural communities with high levels of poverty and reported cases of COVID-19 were included in the study. Within the community, convenience sampling was carried out where only one family member per household was included on the testing.

Sample collection, RNA extraction, and RT-qPCR for SARS-CoV-2 diagnosis using the CDC protocol. Nasopharyngeal swabs were collected on 0.5-mL Tris-EDTA (TE)

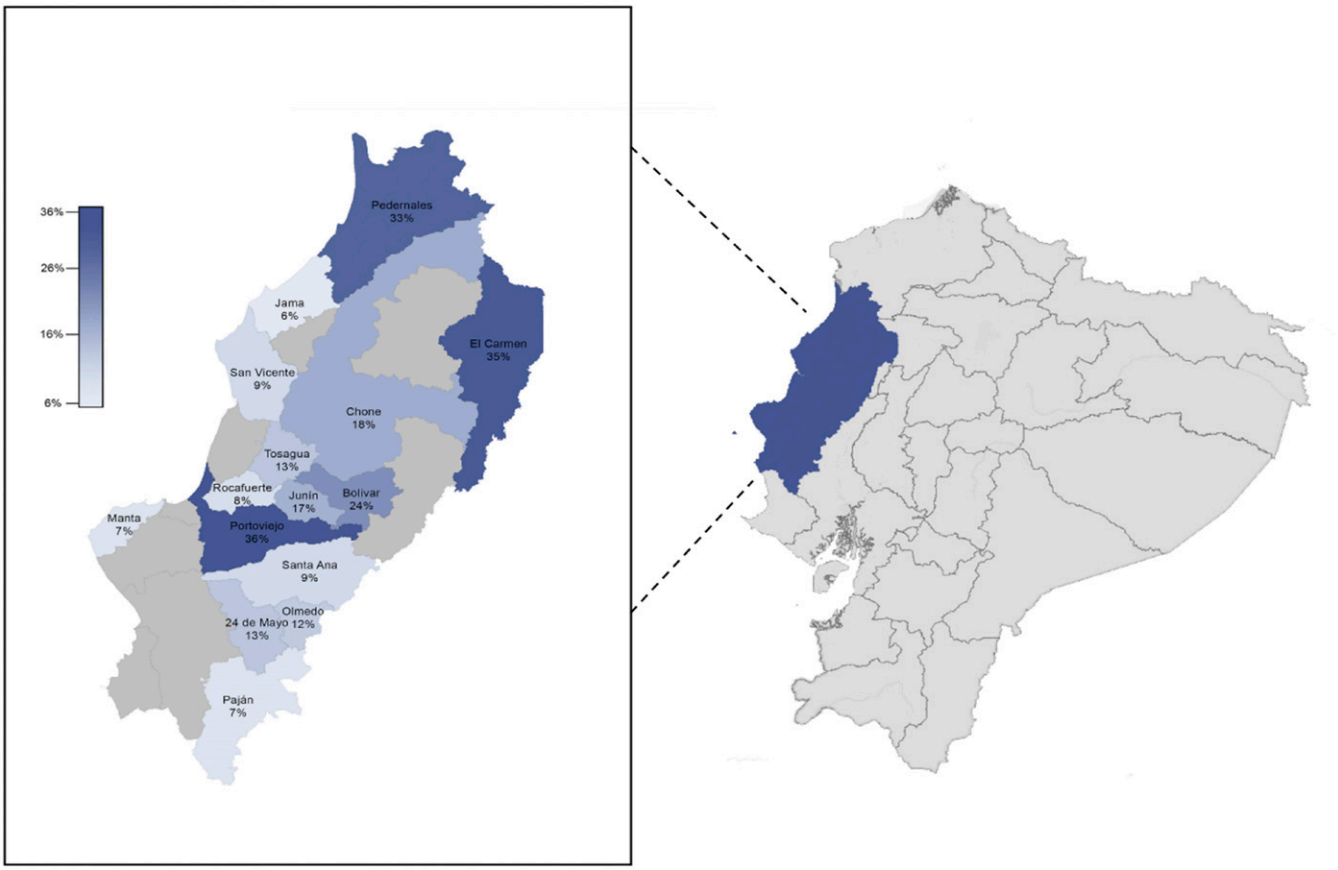

FIGURE 1. Location of Manabí Province in Ecuador. Cantons included in the massive testing during August–September 2020 and their severe acute respiratory syndrome coronavirus 2 infection rates. This figure appears in color at www.ajtmh.org.

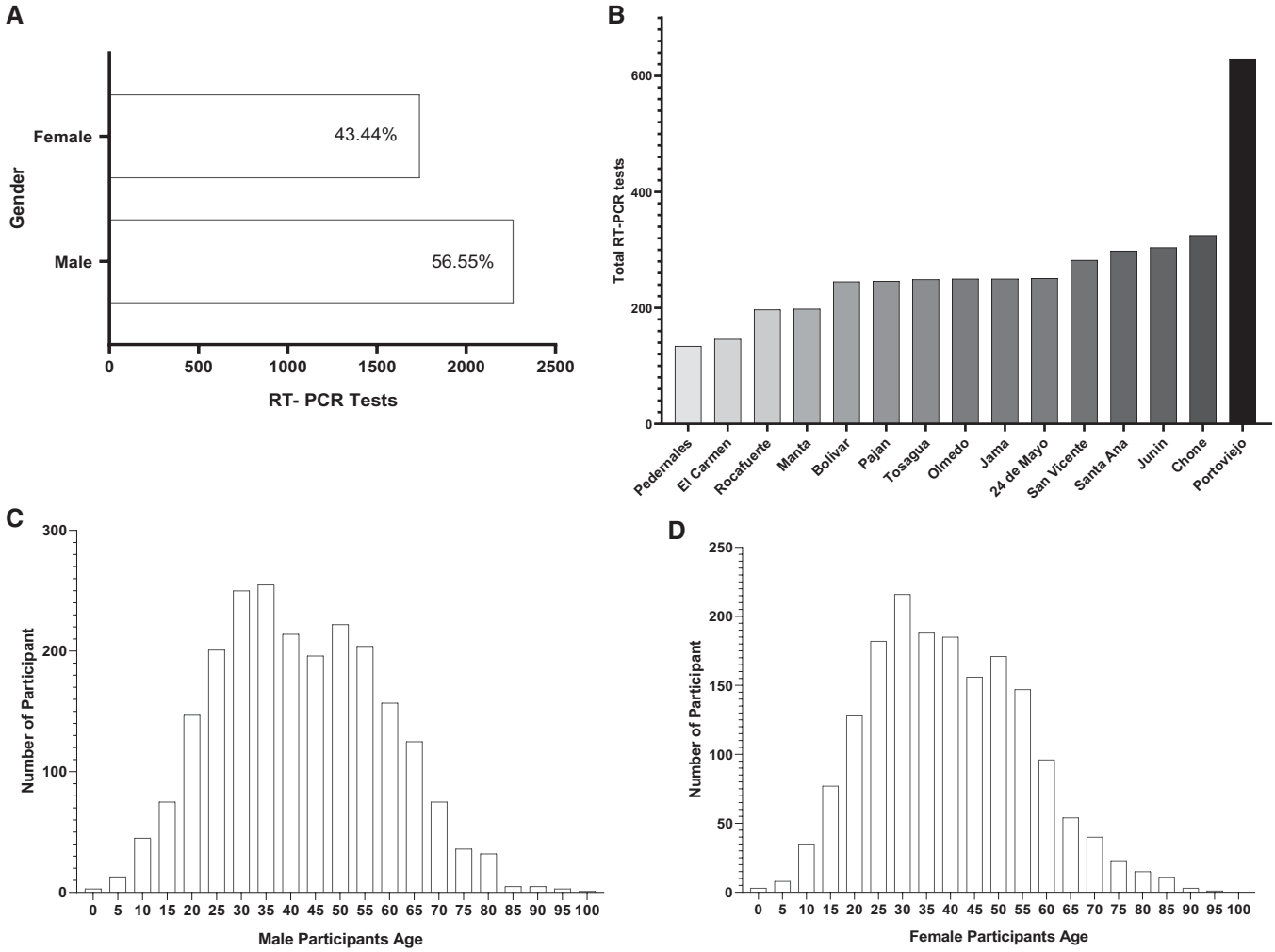

FIGURE 2. Description of the study population from Manabí included in the severe acute respiratory syndrome coronavirus 2 testing. (**A**) Distribution of individuals by sex. (**B**) Number of tests applied in each of the 15 cantons from Manabí Province. (**C** and **D**) Population distribution by age among male and female participants.

pH 8 buffer for SARS-CoV-2 diagnosis by RT-qPCR following an adapted version of the CDC protocol by using PureLink Viral RNA/DNA Mini Kit (Invitrogen, Waltham, MA) as an alternate manual column-based RNA extraction method and CFX96 BioRad (Hercules, CA) instrument.[12–19] Briefly, the CDC-designed RT-qPCR FDA EUA 2019-nCoV CDC kit (Integrated DNA Technologies, Coralville, IA) is based on N1 and N2 probes to detect SARS-CoV-2 and RNase P as an RNA extraction quality control.[18,19] Also, negative controls (TE pH 8 buffer) were included as control for carryover contamination (one for each set of RNA extractions) to guarantee that only true positives were reported. For viral loads calculation, the 2019-nCoV N positive control (Integrated DNA Technologies) was used, provided at 200,000 genome equivalents per microliter, and a factor of 200 was applied to convert the viral loads to genome equivalents per milliliter and then converted to logarithmic scale.

Statistical analysis. For the statistical analysis of data, positivity rates were calculated for each canton, as well as for different age groups and sexes. To assess differences in the positivity rates, χ^2 for comparison of proportions was applied. All statistical analysis was carried out using R software.

RESULTS

This study tested 4,003 people for SARS-CoV-2 using nasopharyngeal swabs and RT-qPCR, out of which 2,264 (56.55%) were male and 1,739 (43.44%) were female (Figure 2). The study was carried out in 15 cantons of Manabí province (Figures 1 and 2). The individuals tested at each canton were distributed as follows: 134 at Pedernales, 146 at El Carmen, 197 at Rocafuerte, 198 at Manta, 245 at Bolivar, 246 at Pajan, 249 at Tosagua, 250 at Olmedo, 250 at Jama, 251 at 24 de Mayo, 282 at San Vicente, 298 at Santa Ana, 304 at Junín, 325 at Chone, and 628 at Portoviejo. Most individuals recruited were between ages 30 and 40 years (males: mean$=$ 37.63 $\pm$ 0.86; females: (mean $=$ 38.42 $\pm$ 0.91), as detailed in Figure 2.

The SARS-CoV-2 infection rates were significantly different ($P < 0.05$) among the cantons included in the study (Figure 1): 33% for Pedernales, 35% for El Carmen, 8% for Rocafuerte, 7% for Manta, 24% for Bolivar, 7% for Pajan, 13% Tosagua, 12% Olmedo, 6% for Jama, 13% for 24 de Mayo, 9% for San Vicente, 9% for Santa Ana, 17% for Junín, 18% for Chone and 36% for Portoviejo.

The overall SARS-CoV-2 infection rate was 16.13%, as 646 individuals tested positive for SARS-CoV-2 RT-qPCR. In

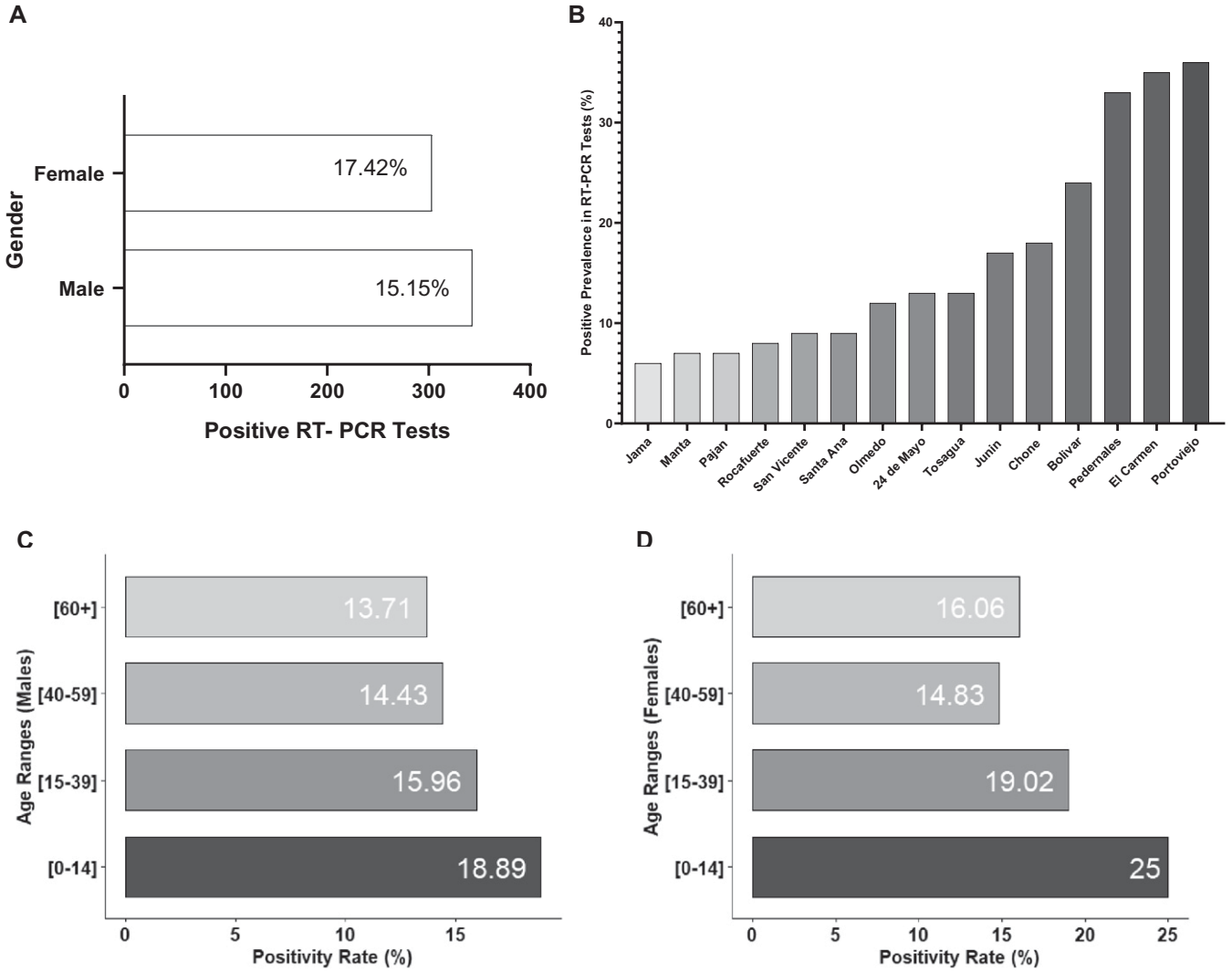

FIGURE 3. Positivity rates in the study population. (**A**) Total number of reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) tests per gender and their infection rate. (**B**) Positive rate of RT-qPCR tests per canton. (**C** and **D**) Positivity rate according to the age and sex of participants.

Figure 3, the SARS-CoV-2 infection rate for male and females is shown, with values of 15.15% and 17.42%, respectively, although this differences were not significant ($P > 0.05$). Also, the highest percentage of SARS-CoV-2 positive cases was distributed among young males (mean = 38.59 ± 0.99) and young females (mean = 37.11 ± 0.96), as detailed on Figure 3.

Viral load distribution did not show any significant difference among sex ($P > 0.05$) or age groups ($P > 0.05$) (Figure 4). Thirty-nine individuals had SARS-CoV-2 viral loads values $> 10^8$ copies/mL.

DISCUSSION

More than 1 year since the first reported case of COVID-19 in Latin American, the epidemiological information available is scarce. Moreover, this information is mainly coming from government reports that are frequently incomplete for Ecuador.[6,7,11,20–23] According to the few scientific reports available, SARS-CoV-2 community transmission was happening in Ecuador[11,21–23] during the first half-year of the COVID-19 pandemic. Not enough testing capacity was available across the country, particularly among rural and indigenous communities.[6,7,20] Furthermore, no information about the epidemiological situation of COVID-19 among community-dwelling asymptomatic individuals beyond the few studies carried out by universities is available.[7,11,21–23] In this context, the present study is a follow-up of a previous short report publish in this journal.[11] Now, with 15 cantons from Manabí Province included and more than 4,000 community-dwelling individuals tested, the SARS-CoV-2 infection rate of 16.13% clearly supports that uncontained SARS-CoV-2 community transmission was happening in Manabí Province during August–September 2020. Our study also highlights the limited SARS-CoV-2 diagnosis capacity installed in this province: the 4,003 samples collected for SARS-CoV-2 testing within a few weeks from August to September 2020 by our medical brigades represents 19.4% of the total 20,598 RT-qPCR test performed at Manabí Province up to September 12, 2020.[10]

Although the overall SARS-CoV-2 infection rate of 16.3% is high, the situation is particularly worrying in El Carmen,

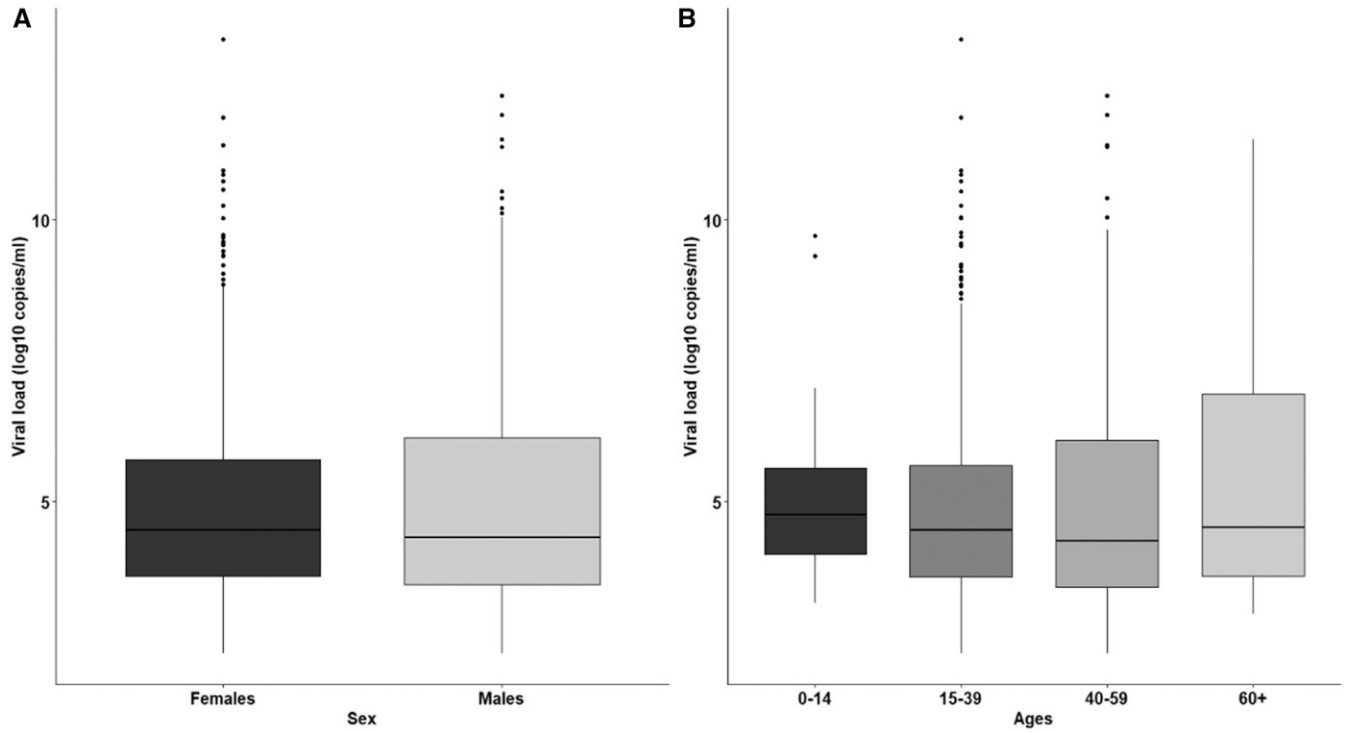

FIGURE 4. Severe acute respiratory syndrome coronavirus 2 viral load distribution among sex (**A**) and age (**B**) ranges (0–14: children; 15–39: young adults; 40–59; adults, 60+: elders).

Portoviejo, and Pedernales cantons, with infection rates $> 30\%$.

There was no clear association between high infection rates and proximity to urban locations, as the two main cities in Manabí Province are Manta and Portoviejo (both with populations greater than 150,000 people), located in cantons where the rural communities had either low or high infection rates, respectively. So far, a geographic trend was not found; high or low infection rates were evenly distributed across the province. All in all, our results confirm COVID-19 community transmission across the Coastal Region of Ecuador. Beyond the dramatic situation broadcasted by the media at Guayaquil during the initial COVID-19 outbreak in March 2020, COVID-19 community transmission has also been described for the coastal provinces of Santa Elena and Esmeraldas.[23,24] Large population seroprevalence studies would be helpful to determine the dimension of COVID-19 spread on these communities from the Coastal Region of Ecuador.

Although any trend regarding SARS-CoV-2 viral loads associated with either sex or age was found at our study population, it is important to note that 39 individuals had viral loads $\geq 10^8$ viral copies/mL and could be considered SARS-CoV-2 super spreaders.[25] Those community-dwelling individuals were either completely asymptomatic or reported some mild symptoms at the time of sample collection. This finding is particularly worrying considering that we did not observe a strong adherence to either mask use or social distancing in the communities surveyed.

The impact of COVID-19 pandemic was dramatic worldwide, but rural communities such as those described in this study for the Manabí Province in Ecuador were even more exposed and at risk of severe consequences from COVID-19 outbreaks. These communities have been traditionally neglected in terms of public health infrastructure. Moreover, the conditions imposed by climate and poverty in rural settings in the Ecuadorian coastal region make those communities prone to the spread of SARS-CoV-2,[23,26–30] which indicates the necessity of the optimal implementation of control and prevention strategies for these neglected populations.

Although the main limitation of our work is that sampling was no randomized because samples were not collected for a research study but for an aid diagnosis program, we believe our results may nonetheless reflect the COVID-19 pandemic situation in Manabí during August–September 2020 for several reasons. First, we covered community spread in multiple cantons throughout the region. Second, several locations were visited within each canton. Third, only one person per household was included in the surveillance to exclude family-clustering bias in the infection rate calculation. Fourth, the sample size is $>$ 4,000 individuals. Fifth, living conditions for the majority of the population in Manabí are similar to those in the communities visited.

In conclusion, we suggest than more resources should be allocated for COVID-19 pandemic containment in Manabí Province, from improving testing capacities to reinforcing hospital capacity to attend to COVID-19 patients under an scenario of community transmission such as that described in our study.

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Disclosure: Written consent was obtained for all the individuals included on the surveillance before sample collection. This retrospective study using anonymized data from SARS-CoV-2 testing study was approved by "Universidad de Las Américas" Institutional Review Board in accordance with the regulations for COVID-19–related research from the Ministry of Health of Ecuador.

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6.2.2. *Transmisión comunitaria sostenida de COVID-19 y posibles eventos de súper propagación en comunidades afroecuatorianas desatendidas evaluadas mediante RT-qPCR masiva y pruebas serológicas de la población que vive en la comunidad.*

Publicado en: Frontiers in Medicine 2022, 9, 2326. Sustained COVID-19 community transmission and potential super spreading events at neglected afro-ecuadorian communities assessed by massive RT-qPCR and serological testing of community dwelling population. D.O.I: https://doi.org/10.3389/fmed.2022.933260

Las minorías étnicas de las poblaciones rurales desatendidas en América Latina son altamente vulnerables a la enfermedad por coronavirus 2019 (COVID-19) debido a la infraestructura de salud deficiente y al acceso limitado al diagnóstico del síndrome respiratorio agudo severo coronavirus 2 (SARS-CoV-2). Esmeraldas es una provincia mayoritariamente rural de la Región de la Costa del Ecuador caracterizada por una alta presencia de población afroecuatoriana que vive en condiciones de pobreza.

Nuestro objetivo es presentar un análisis retrospectivo de la prueba de vigilancia del SARS-CoV-2 en población comunitaria de Esmeraldas realizada por nuestro laboratorio universitario en colaboración con las autoridades regionales de salud durante la primera semana de octubre de 2020, en una región donde no hay acceso público. El laboratorio de detección de SARS-CoV-2 estaba disponible en ese momento.

Un total de 1259 personas se sometieron a la prueba de SARS-CoV-2 mediante la reacción en cadena de la polimerasa cuantitativa de transcripción inversa (RT-qPCR), lo que resultó en una tasa de infección general del 7,7 % (97/1259, IC del 95 %: 6,32– 9.35) para SARS-CoV-2, hasta 12.1% en algunas comunidades. Curiosamente, los súper esparcidores que viven en la comunidad con cargas virales superiores a 10^8 copias/mL representaron el 6,2 % de la población infectada por SARS-CoV-2. Además, se aplicaron pruebas serológicas anti-SARS-CoV-2 IgG al mismo grupo de estudio, lo que arrojó una seroprevalencia general del 11,68 % (IC del 95 %: 9,98–13,62), pero de hasta el 24,47 % en algunas comunidades.

Estos resultados respaldan la transmisión comunitaria activa de COVID-19 en la provincia de Esmeraldas durante el primer semestre de la pandemia de COVID-19 como se ha demostrado para otras comunidades rurales en la Región de la Costa Ecuatoriana.

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Sustained COVID-19 community transmission and potential super spreading events at neglected afro-ecuadorian communities assessed by massive RT-qPCR and serological testing of community dwelling population

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Background: Neglected ethnic minorities from underserved rural populations in Latin America are highly vulnerable to coronavirus disease 2019 (COVID-19) due to poor health infrastructure and limited access to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnosis. Esmeraldas is a mainly rural province of the Coastal Region of Ecuador characterized by a high presence of Afro-Ecuadorian population living under poverty conditions.

Objective: We herein present a retrospective analysis of the surveillance SARS-CoV-2 testing in community-dwelling population from Esmeraldas carried out by our university laboratory in collaboration with regional health authorities during the first week of October 2020, in a region where no public SARS-CoV-2 detection laboratory was available at that time.

Results: A total number of 1,259 people were tested for SARS-CoV-2 by Reverse Transcription quantitative Polimerasa Chain Reaction (RT-qPCR), resulting in an overall infection rate of 7.7% (97/1259, 95% *CI*: [6.32–9.35%]) for SARS-CoV-2, up to 12.1% in some communities. Interestingly, community-dwelling super spreaders with viral loads over 10^8 copies/ml represented 6.2% of the SARS-CoV-2-infected population. Furthermore, anti-SARS-CoV-2 IgG serological tests were applied to

the same study group, yielding an overall seroprevalence of 11.68% (95% *CI*: [9.98–13.62%]) but as high as 24.47% at some communities.

Conclusion: These results support active COVID-19 community transmission in Esmeraldas province during the first semester of the COVID-19 pandemic as it has been shown for other rural communities in the Ecuadorian Coastal Region.

KEYWORDS

SARS-CoV-2, COVID-19, Ecuador, seroprevalence, RT-qPCR, Afro-Ecuadorian population, Esmeraldas, rural communities

Introduction

The outbreak of coronavirus disease 2019 (COVID-19) raised concerns in the global scientific and health communities since the first 27 cases were reported in December 2019 from Wuhan, China (1). The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread readily and quickly around the world, WHO declared a global pandemic, and the first cases in Latin America were reported just 2 months after the original report (1, 2). Until March 2022, more than 480 million COVID-19 cases and more than 6 million deaths were reported worldwide (2). In Ecuador, more than 830,000 cases and 35,000 deaths associated to COVID-19 were reported since the arrival of the first case in February 2020 till August 9 2021 (2)[1] From the early stages of the COVID-19 pandemic, a wide variety of recommendations were handed by the World Health Organization (WHO) like using mask, social distancing, and isolation of confirmed cases to slow down the spread of the disease. Moreover, there was a permanent call on the public media from WHO authorities asking to governments worldwide to carry as many tests as possible as the best strategy to control the virus spread.

The COVID-19 pandemic hits all health systems worldwide. However, its impact was particularly greater in low- and middle-income settings, such as those found in developing countries from Latin America (3, 4). Despite the latter arrival of the pandemic to these countries, and the subsequent time they had to prepare and prevent an eventual health system collapse, the saturation of hospitals and shortage of supplies were inevitable (5). These lead to a public health crisis as seen during March–April 2020 in Guayaquil, the most populated city of Ecuador (4–8). At the beginning of this emergency, only the National Institute of Research in Public Health (INSPI) laboratories, located in the 3 main cities of Ecuador (Guayaquil, Quito, and Cuenca) performed SARS-CoV-2 detection using RT-qPCR within the public health system, which was translated in a poor testing ratio of 7.46 PCR tests per 10,000 people, and one of the highest COVID-19 mortality rates (10.93 deaths per million people) in Latin America (7). During the first 18 months of the COVID-19 pandemic, less than 2 million SARS-CoV-2 RT-qPCR tests have been done for a 17 million Ecuadorian population, with a positivity rate over 20%, according to the Ecuadorian Ministry of Health (MoH) (9). This is clearly higher that the 5% positivity rate recommended for the WHO and it means that SARS-CoV-2 testing in Ecuador was not enough for an effective control and prevention strategy (10).

Esmeraldas is located in the Ecuadorian Northern Coastal Region; it is the seventh largest province on the surface and the eighth most populated one with 491,168 inhabitants, mostly distributed across rural communities in seven cantons; and it is one of the poorest regions in the country (11–13). Additionally, 43% of its population identifies themselves as Afro-Ecuadorian, making Esmeraldas the main Afro-Ecuadorian region of Ecuador (13). According to MoH, during the first semester of the COVID-19 pandemic (up to September 12, 2020), with a total of 9,129 SARS-CoV-2 RT-qPCR tests done, less than 2% of Esmeralda's population (185.9 test per 10,000 inhabitants) was tested despite the dramatic 39.8% positivity rate reported (14).

This study aims to insight into the epidemiological situation of Esmeraldas province in Ecuador during the first wave of the COVID-19 pandemic, where community transmission was suspected to happen for months according to the media reports of public health authorities. We present the retrospective analysis of a surveillance SARS-CoV-2 testing intervention during October 2020, carried out by Universidad de Las Américas (UDLA) in coordination with local community leaders, our colleagues from "Universidad Técnica Luis Vargas Torres" and the provincial government ("Prefectura de Esmeraldas").

Methods

Study design and setting

A total number of 1,259 individuals enrolled in this surveillance. All samples were taken from community-dwelling asymptomatic or mild symptomatic individuals at the

1 https://www.salud.gob.ec/actualizacion-de-casos-de-coronavirus-en-ecuador/.

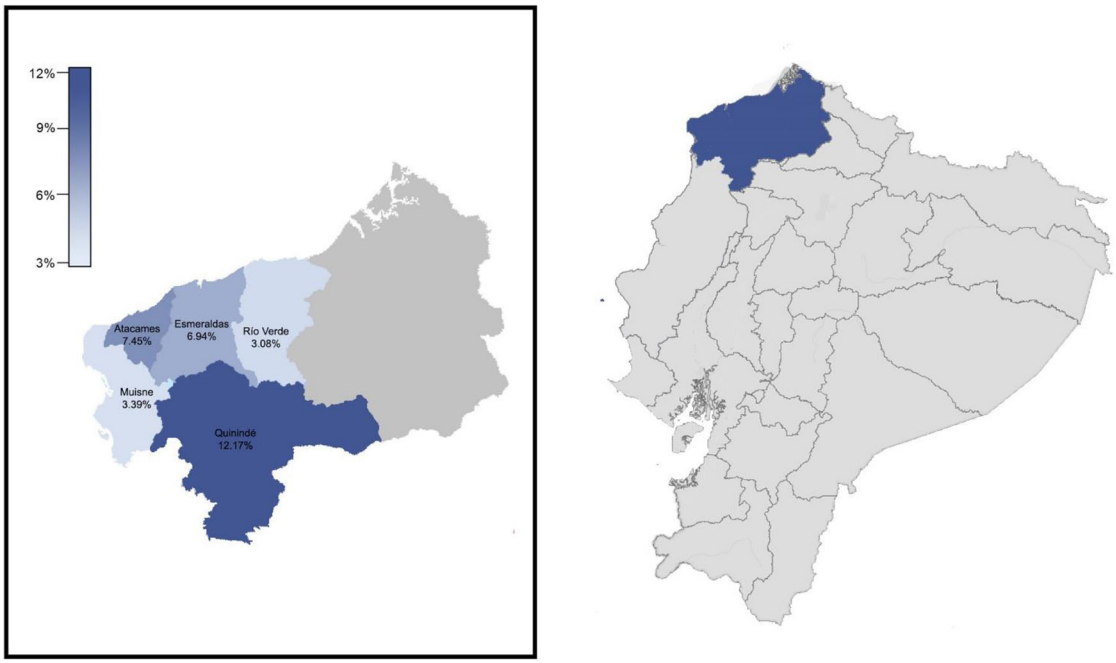

FIGURE 1
Location of esmeraldas province within ecuador. Cantons included in the surveillance carried out during October 2020 and their severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection rates by RT-qPCR.

communities visited from 12th to 19th October 2020 in five out of seven cantons of Esmeraldas province: Esmeraldas, Atacames, Muisne, and Quinindé y Rio Verde (Figure 1). Two cantons were not included in the study (San Lorenzo and Eloy Alfaro) for logistic and security reasons, although those two cantons share the same demographic and socioeconomic features with the five cantons tested. Esmeraldas (capital city of the province with 150,000 inhabitants approximately) is the only canton, such as truly urban communities, where 5 different locations within the city were included for a total of 778 individuals tested. The other 4 cantons are rural ones, and 481 samples out of 7 different communities were included (3 communities for Quininde, 2 communities for Atacames, and 1 community for Muisne and Rio Verde). Although socioeconomic and ethnic information was not collected, the majority of the population enrolling the surveillance was low-income Afro-Ecuadorians.

As the study is a retrospective analysis of the data collected during a COVID-19 surveillance intervention as part of an aid program funded by "Fondo Salvar Vidas" from Banco de Guayaquil, to provide free access to SARS-CoV-2 testing, the communities were selected at convenience following the recommendations from the local organizations and the provincial government of Esmeraldas responsible for the logistics of this surveillance program.

Sample collection, RNA extraction and RT-qPCR for SARS-CoV-2 diagnosis using the CDC protocol

Nasopharyngeal swabs were collected on 0.5 ml TE pH 8 buffer for SARS-CoV-2 diagnosis by RT-qPCR following an adapted version of the CDC protocol by using PureLink Viral RNA/DNA Mini Kit (Invitrogen, USA) as an alternate RNA extraction method and CFX96 BioRad instrument (15–22). Briefly, the CDC-designed RT-qPCR FDA EUA 2019-nCoV CDC kit (IDT, USA) is based on N1 and N2 probes to detect SARS-CoV-2 and RNase P as an RNA extraction quality control (21, 22). Also, negative controls (TE pH 8 buffer) were included as a control for carryover contamination, one for each set of RNA extractions, to guarantee that only true positives were reported. For viral loads calculation, the 2019-nCoV N positive control (IDT, USA) was used, provided at 200.000 genome equivalents/µl, and a factor of 200 was applied to convert the viral loads to genome equivalents/ml and then converted to a logarithmic scale. The individuals with viral loads bigger than 10^8 copies/ml are considered SARS-CoV-2 super spreaders as it has been previously described (23).

Serological test for anti SARS-CoV-2 IgG

A commercially available lateral-flow immunochromatographic anti-SARS-CoV-2 IgG from INNOVITA (TANGSHAN Biological Technology Co. Ltd, Hebei, China) was used. The appropriate sample volume (20 ul of venous whole blood) was transferred to the indicated sample port, followed immediately by provided diluent, following manufacturer instructions. The lateral flow cartridges were incubated for 15 min at room temperature before readings. Results negative or positive according to manufacturer instructions in each cartridge were read for test line intensity by two independent readers blinded to specimen status.

According to the manufacturer manual, the expected sensitivity and specificity for the test are 87.3 and 100%, respectively. We carried out an internal evaluation with 127 serum samples from SARS-CoV-2 positive individuals by RT-qPCR and 40 serum samples prior to the COVID-19 pandemic, and we got a sensitivity of 79.5% (95% CI: 71.5–86.2%) and specificity of 100% (95% *CI*: 91.2–100%) (24).

Statistical analysis

For the statistical analysis of data, positivity rates and viral loads were calculated for each canton, as well as for different age groups, sex, and COVID-19 symptoms status. Seroprevalences were also calculated for all cantons. To assess differences in the positivity rates and viral loads, a non-parametric statistical test (Wilcoxon and Kruskal–Wallis) for comparison of proportions was applied. Confident intervals were calculated for a significance of 95%. All statistical analysis was carried out using R software.

Ethical approval and consent to participate. All participants signed informed consent to participate freely and voluntarily in this SARS-CoV-2 testing surveillance. This study is a secondary analysis of the anonymized laboratory results from a previous surveillance testing done in the context of the COVID-19 pandemic. Nevertheless, the study was approved by the Institutional Review Board from Hospital General San Francisco (Quito) with code CEISH-HGSF-2021-002.

Results

SARS-CoV-2 infection surveillance by RT-qPCR

A total of 1,259 samples for SARS-CoV-2 detection by RT-qPCR were taken in Esmeraldas province, distributed along five cantons: Esmeraldas, Rio Verde, Quininde, Muisne, and Atacames (Figure 1; Supplementary Table 1). The population was evenly distributed between men and women. Most of the individuals were adults (mean = 41.26 ± 0.48 years). The overall SARS-CoV-2 infection rate found in the province was 7.71% (97/1259, 95% *CI*: [6.32–9.35%]). See Supplementary Table 1 for details. The distribution of SARS-CoV-2 RT-qPCR individuals by sex and age is detailed in Supplementary Figure 2. The average age for SARS-CoV-2 positive individuals was 42.11 ± 1.81 years old. The SARS-CoV-2 infection rates for men and women were 6.35% (39/614, 95% *CI*: [4.61–8.66%]) and 8.99% (58/645, 95% *CI*: [6.95–11.54%]), respectively; although this difference was not significant (p-value > 0.05). SARS-CoV-2 prevalence varies among age groups, with values of 4.23% (3/71, 95% *CI*: [1.10–12.67%]) for children (0–14 years), 7.91% (44/556, 95% *CI*: [5.87–10.56%]) for young adults (14–39 years), 7.16% (31/433, 95% *CI*: [4.99–10.11%]) for adults (40–60 years), and 9.55% (19/199, 95% *CI*: [5.99–14.72%]) for elders (>60 years). Nevertheless, neither significant differences nor trends were found between each age group and prevalence of SARS-CoV-2 infection.

The five cantons included in the study were divided into two categories: rural and urban. All the communities from Atacames, Muisne, Rio Verde, and Quininde were rural and 43 SARS-CoV-2 positive individuals out of 481 ones tested were found, yielding an attack rate of 8.94% (95% *CI*: [6.61–11.94%]). All the communities for Esmeraldas canton were urban, and 54 SARS-CoV-2 positive individuals out of 778 ones tested were found, yielding an infection rate of 6.94% (95% *CI*: [5.30–8.66%]). Those values were not statistically different (p-value > 0.05). There are significant differences (p-value < 0.01) in the SARS-CoV-2 infection rates among the cantons included in the study (Figure 1; Table 1): 7.45% (7/94, 95% *CI*: [3.30–15.24]) for Atacames, 6.94% (54/778, 95% *CI*: [5.30–9.02]) for Esmeraldas, 3.39% (2/59, 95% *CI*: [0.59–12.75]) for Muisne, 12.17% (32/263, 95% *CI*: [8.59–16.89]) for Quininde, and 3.08% (2/65, 95% *CI*: [0.54–11.64]) for Rio Verde.

SARS-CoV-2 viral load distribution and super spreaders prevalence

The SARS-CoV-2 viral load distribution between age groups, sex, and COVID-19 symptoms status is presented in Figure 2. We found no significant difference in the viral loads among any of those variables (p-value > 0.05). There are 6 individuals with viral loads bigger than 10^8 copies/ml, representing 6.2% of the SARS-CoV-2 positive population (6/97; 95% *CI*: [2.54–13.50%]) (Figure 2, Table 2) that are considered SARS-CoV-2 super spreaders (23). No association was found between the super spreader condition with sex, age, or presence of symptoms (Table 2).

TABLE 1 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection rate by RT-qPCR and anti-SARS-CoV-2 Ig G seroprevalence for the 5 cantons included on this surveillance study at Esmeraldas province in October 2020.

CANTON	SARS-CoV-2 infection rate (%)	SARS-CoV-2 infection rate per 10 000 inhabitants	anti-SARS-CoV-2 IgG seroprevalence (%)*	anti-SARS-CoV-2 IgG seroprevalence per 10 000 inhabitants	Percentage of population sampled
Esmeraldas	6.94 [5.30–9.02]	2.85	11.10 [9.02–13.57]	4.54	0.41
Quininde	12.17 [8.59–16.89]	2.61	12.21 [8.62–16.95]	2.61	0.22
Atacames	7.45 [3.30–15.24]	1.69	24.47 [16.44–34.61]	5.54	0.23
Muisne	3.39 [0.59–12.75]	0.70	6.78 [2.19–17.27]	1.41	0.21
Rio Verde	3.08 [0.54–11.64]	0.74	1.67 [0.09–10.14]	0.37	0.24
Province overall	7.71 [6.32–9.35]	1.82	11.68 [9.98–13.62]	2.73	0.24

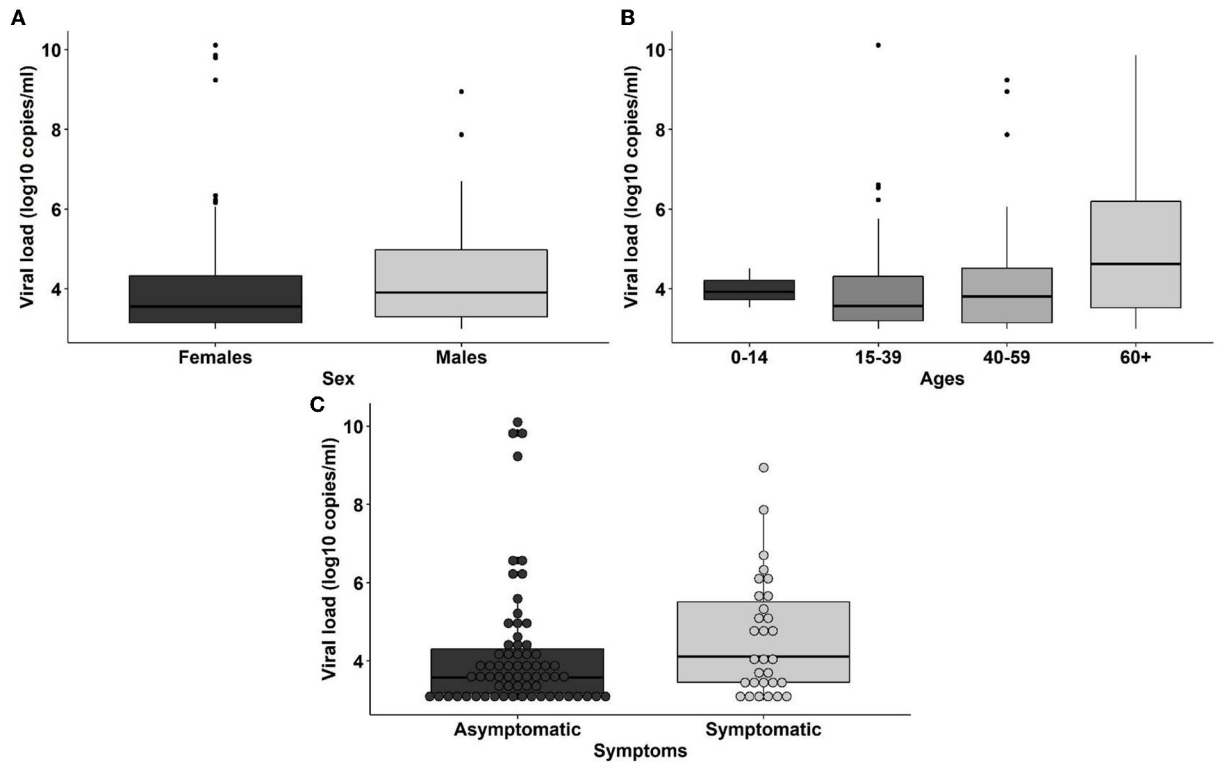

FIGURE 2
Distribution of viral loads among sexes **(A)**, age ranges (0–14: children, 15–39: young adults, 40–59: adults, 60+: elders) **(B)** and coronavirus disease 2019 (COVID-19) symptoms status **(C)** of the individuals.

TABLE 2 Demographic and clinical information from the super-spreaders.

Super-spreader	Age	Sex	Location	Symptoms	Viral Load (log_{10} copies/mL)
1	30	Female	Quininde	Asymptomatic	10.105
2	68	Male	Quininde	Asymptomatic	9.854
3	68	Female	Esmeraldas	Asymptomatic	9.791
4	43	Female	Esmeraldas	Asymptomatic	9.234
5	59	Male	Esmeraldas	Symptomatic	8.947
6	49	Male	Esmeraldas	Symptomatic	7.866

Seroprevalence of anti-SARS-CoV-2 IgG

A total of 1,250 serological tests were applied across the same population considered for the RT-qPCR testing. The overall seroprevalence in Esmeraldas province was 11.68% (95% *CI*: [9.98–13.62%]), with 146 positive IgG tests (Table 1; Supplementary Figure 2). The seropositive population was distributed as follows (Supplementary Figure 2): 58 men and 88 women, average age: 45.25 ± 1.48 years old. Significant differences were found in the seroprevalence of anti SARS-CoV-2 IgG between men (9.53%, 58/608, 95% *CI*: [7.38–12.23%]) and women (13.71%, 88/642, 95% *CI*: [11.19–16.67%]; p-value < 0.05), as well as in the seroprevalence for each age group: children (18.6%, 13/70, 95% *CI*: [10.64–30.02%]), young adults (7.2%, 40/553, 95% *CI*: [5.28–9.80%]), adults (13.2%, 57/431, 95% *CI*: [10.25–16.88%]), and elders (18.4%, 36/196, 95% *CI*: [13.35–24.66%]).

For the rural communities from Atacames, Muisne, Rio Verde, and Quininde, 60 anti-SARS-CoV-2 IgG positive individuals out of 475 ones tested were found, yielding a seroprevalence of 12.63% (95% *CI*: [9.85–16.04%]). For the urban communities from Esmeraldas canton, 86 anti-SARS-CoV-2 IgG positive individuals out of 775 ones tested were found, resulting in a seroprevalence of 11.10% (95% *CI*: [9.02–13.57%]). Those values were not statistically different (p-value > 0.05).

Regarding the anti-SARS-CoV-2 IgG seroprevalence for the different cantons (Table 1), Atacames had the highest value of 24.47% (23/94, 95% *CI*: [16.44–34.61]), followed by Quininde with 12.21% (32/262, 95% *CI*: [8.62–16.95]), Esmeraldas with 11.10% (86/775, 95% *CI*: [9.02–13.57]), Muisne with 6.78% (4/59, 95% *CI*: [2.19–17.27]), and Río Verde with 1.67% (1/60, 95% *CI*: [0.09–10.14]). Significant differences were found between those values (p-value < 0.01).

Discussion

After more than 2 years of the COVID-19 pandemic, the epidemiological information available from low and middle-income countries like those in Latin America is still scarce compared to high-income countries. Moreover, this information is mostly coming from governments' reports that are mainly focused in hospitalized patients as it has been shown for Ecuador (5, 7, 10, 23–27). According to the few scientific reports available detailed in Table 3, SARS-CoV-2 community transmission was happening in rural and remote areas of Ecuador (25–28) during the first semester of the COVID-19 pandemic. Unfortunately, not enough testing capacity was installed across the country (5, 7, 10) and no official information about the epidemiological situation of SARS-CoV-2 among rural and remote vulnerable populations like ethnic minorities was available (10, 25, 27, 28). For instance, the 1,259 samples collected for SARS-CoV-2 testing within a week period in October 2020 by our medical brigades represented 12.3% of the total RT-qPCR performed at Esmeraldas province since the COVID-19 outbreak in February 2020 (10). In this context, the present study addresses the impact of the first wave of COVID-19 pandemic in historically neglected population like the Afro-Ecuadorians from Esmeraldas province (29).

We found an overall SARS-CoV-2 infection rate of 7.71% in Esmeraldas province, with values as high as 12.17% for Quininde canton, confirming active SARS-CoV-2 community transmission as the population targeted was community-dwelling non-hospitalized individuals. This finding confirms SARS-CoV-2 community transmission across rural and remote regions of Ecuador like Manabi, Santa Elena, the Amazonia, and Galapagos Islands, as it is shown in Table 3, where high SARS-CoV-2 infection rates were also found within community-dwelling population (25, 28). Despite the active transmission described from the SARS-CoV-2 RT-qPCR diagnosis displayed, the overall anti-SARS-CoV-2 IgG seroprevalence of 11.68%, with values as high as 24.47% for Atacames canton, also supports that SARS-CoV-2 community transmission has been happening for weeks before October 2020 in Esmeraldas. Moreover, considering that the serological test used on the study had a lack of sensitivity over 20% (24), the percentage of the population exposed to SARS-CoV-2 would be even higher than the observed. However, the SARS-CoV-2 infection rates were similar or higher than seroprevalence values, for instance at Quininde and Rio Verde cantons, indicating that COVID-19 outbreaks were probably a recent event prior to October 2020 in Esmeraldas.

We found striking results when comparing rural and urban communities. While a higher infection rate and seroprevalence would be expected at urban locations in Esmeraldas city (population of around 150,000 people), both parameters were slightly higher for the rural and relatively isolated cantons. However, this is due to the high values obtained for Atacames and Quininde, for either infection rates (7.45 and 12.17%) or seroprevalence (12.21 and 24.47%). Although the communities visited within those cantons were rural, both Atacames and Quininde have certain socioeconomics features that would potentially explain those results. On one hand, Quininde is less than 20 miles away from Santo Domingo de los Taschilas that it is the fourth capital province in population (over 250,000 people) in Ecuador, and also an important commercial and communication hub for the region. On the other hand, Atacames is the host of multiple hotel facilities as its popular beaches received thousands of visitors from the capital city of Quito and other places of Ecuador.

Although any particular trend regarding SARS-CoV-2 viral loads or attack rates associated with either sex, age, or symptoms status was found at our study population, 6 individuals had viral loads in the range from 10^8 to 10^{10} viral copies/ml

TABLE 3 Comparison of SARS-CoV-2 infection rate by RT-qPCR and anti-SARS-CoV-2 Ig seroprevalence for surveillance studies performed in Ecuador during the COVID-19 pandemics (NA: Not available).

Location	Number of samples	SARS-CoV-2 infection rate (%)	anti-SARS-CoV-2 IgG seroprevalence (%)	Reference
Esmeraldas	1,259	7.72	11.68	Present study
Manabi	4,003	16.13	NA	25,35
Santa Elena	673	NA	45.02	28
Galapagos	2,480	5.85	3.30	Manuscript under preparation.
Amazonia	769	49.16	NA	27,36

and could be considered SARS-CoV-2 as super spreaders, representing a striking 6.2% of the infected population (23). Although there are limitations associated to calculate the viral load based on C_t values representing all the viral genomic material on the sample, and infection of cell cultures is used for sample infectivity confirmation, it is a clear association between low C_t values (that mean high viral loads based on genomic material quantification) and infectivity (23). Those super spreader individuals were either completely asymptomatic or reported some minor mild symptoms at the time of sample collection. This finding is particularly worrying considering that we did not observe a strong adherence to neither mask use nor social distancing in the communities visited. The impact of the COVID-19 pandemic was terrific worldwide, but rural communities and ethnic minorities like the Afro-Ecuadorian people from Esmeraldas were even more exposed and in risk of having severe consequences from COVID-19 outbreaks during the first wave of COVID-19 pandemic. As demonstrated by other studies and ours, the conditions imposed by climate and poverty at rural settings in the Ecuadorian Coastal and Amazonian Regions make those communities prone to the spread of SARS-CoV-2 (27, 29–35), which remarks the necessity of the optimal implementation of control and prevention strategies in these neglected territories.

Due to the retrospective nature of this study, there was not a randomized sample collection to include a statistically representative population sampling for Esmeraldas region. This is a strong limitation in our work, as the bias on sample collection could mean that the results obtained were not truly representative of the COVID-19 epidemiological context in this region, but were limited to the communities selected. However, SARS-CoV-2 community transmission was confirmed either by RT-qPCR or serology at all the communities visited; moreover, considering that those communities were quite similar in terms of socio-economical features to most of the communities in Esmeraldas province, we suggest that SARS-CoV-2 community transmission across this region during the first wave of COVID-19 is a plausible conclusion of this surveillance study.

The findings presented in this study of active community transmission and super spreading events among community-dwelling individuals in rural and remote locations would be useful for future pandemics. COVID-19 control and prevention strategies have to focus not only on hospitalized and symptomatic individuals but also including community-dwelling individuals at locations where outbreaks are suspected.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Hospital San Francisco de Quito. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

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Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.933260/full#supplementary-material

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6.2.3. *Altas tasas de infección por SARS-CoV-2 y cargas virales en pobladores de comunidades rurales indígenas y mestizas de los Andes durante la primera ola de la pandemia de COVID-19 en Ecuador.*

Publicado en: Frontiers in Medicine 2023, 10. High SARS-CoV-2 infection rates and viral loads in community-dwelling individuals from rural indigenous and mestizo communities from the Andes during the first wave of the COVID-19 pandemic in Ecuador. D.O.I: https://doi.org/10.3389/FMED.2023.1001679.

Los indígenas y las poblaciones rurales desatendidas en América Latina son altamente vulnerables al COVID-19 debido a la infraestructura de salud deficiente y al acceso limitado al diagnóstico del SARS-CoV-2. La región andina de Ecuador incluye un gran número de comunidades rurales aisladas mestizas e indígenas que viven bajo condiciones de pobreza.

El objetivo del estudio es presentar un análisis retrospectivo de las pruebas de vigilancia del diagnóstico de SARS-CoV2 en poblaciones comunitarias de cuatro provincias de los Andes ecuatorianos, realizadas durante las primeras semanas posteriores al levantamiento del confinamiento nacional en junio de 2020.

Un número total de 1021 personas se sometieron a la prueba de SARS-CoV-2 mediante RT-qPCR, lo que resultó en una alta tasa de infección general del 26,2% (268/1021, IC del 95%: 23,6 – 29%), que fue superior al 50% en varias comunidades. Curiosamente, los super propagadores que viven en la comunidad con cargas virales superiores a 10 copias/mL representaron el 7,46% (20/268, IC del 95%: 4,8–11,1%) de la población infectada por SARS-CoV-2.

Estos resultados respaldan que la transmisión comunitaria de COVID-19 en comunidades rurales de la región andina estaba ocurriendo en las primeras etapas de la pandemia en Ecuador y señalan la debilidad del programa de control de COVID-19. Las personas que viven en comunidades rurales e indígenas desatendidas deben ser consideradas para un programa exitoso de control y vigilancia en futuras pandemias en países de bajos y medianos ingresos.

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High SARS-CoV-2 infection rates and viral loads in community-dwelling individuals from rural indigenous and mestizo communities from the Andes during the first wave of the COVID-19 pandemic in Ecuador

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Background: Neglected indigenous groups and underserved rural populations in Latin America are highly vulnerable to COVID-19 due to poor health infrastructure and limited access to SARS-CoV-2 diagnosis. The Andean region in Ecuador includes a large number of isolated rural mestizo and indigenous communities living under poverty conditions.

Objective: We herein present a retrospective analysis of the surveillance SARS-CoV-2 testing in community-dwelling populations from four provinces in the Ecuadorian Andes, carried out during the first weeks after the national lockdown was lifted in June 2020.

Results: A total number of 1,021 people were tested for SARS-CoV-2 by RT-qPCR, resulting in an overall high infection rate of 26.2% (268/1,021, 95% CI: 23.6–29%), which was over 50% in several communities. Interestingly, community-dwelling super spreaders with viral loads over 10^8 copies/mL represented 7.46% (20/268, 95% CI: 4.8–11.1%) of the SARS-CoV-2 infected population.

Conclusion: These results support that COVID-19 community transmission in rural communities from the Andean region was happening at the early stages of the COVID-19 pandemic in Ecuador and point out the weakness of the COVID-19 control program. Community-dwelling individuals in neglected rural and indigenous communities should be considered for a successful control and surveillance program in future pandemics in low- and middle-income countries.

KEYWORDS

SARS-CoV-2, indigenous people, COVID-19, Ecuador, Andean region

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in China in December 2019 and spread worldwide, causing the COVID-19 pandemic (1). A few weeks after the initial outbreaks, the first COVID-19 cases were reported in Latin America that has since then been deeply affected. For instance, the first case of COVID-19 was confirmed on 29 February 2020 in Ecuador (2), and during the first year of the COVID-19 pandemic, more than 400,000 COVID-19 cases and 20,000 COVID-19-related deaths have been reported by Ecuadorian public health authorities (3).

Vulnerable groups infected with COVID-19 include not only the elderly and individuals with comorbidities but also historically neglected indigenous populations (4–9). There are more than 476 million indigenous people in the world, highly represented and traditionally neglected in Latin America (10, 11). In Ecuador, indigenous people represent more than 7% of the total population and are mainly associated with underserved rural communities (10–13). Those communities are usually isolated or poorly communicated and have poor access to health services. In many cases, such health services have little capacity and limited coverage, which may delay seeking medical attention, complicating early management, and therefore leading to greater risks of complications and mortality under a scenario such as the COVID-19 pandemic (7–9, 13).

From the early stages of the COVID-19 pandemic, there was a call for action to protect indigenous people from the Americas (7–9). In Ecuador, The National Council for the Equality of Peoples and Nationalities has demanded the protection of indigenous people, reporting COVID-19 outbreaks among their communities and claiming support from public health authorities to contain the pandemic in their communities (8, 11). Moreover, several reports have already shown dramatic SARS-CoV-2 outbreaks leading to community transmission in rural and indigenous populations from the Amazonian and Coastal regions of Ecuador (7, 8, 14–19). Under this scenario, following the request from community leaders, we carried out a SARS-CoV-2 surveillance testing among community-dwelling indigenous and mestizo people in the Ecuadorian Andes few weeks after the population lockdown was lifted in June 2020.

This study aimed to carry out a retrospective analysis of the results of our SARS-CoV-2 testing surveillance in mestizo and indigenous communities from the Ecuadorian Andes to show that COVID-19 community transmission had been happening since the early stages of the pandemic.

Materials and methods

Study design and setting

We carried out a retrospective analysis of the data collected from this cross-sectional surveillance to describe the attack rates of SARS-CoV-2 infection among rural indigenous and mestizo communities from the Andean region of Ecuador from June to August 2020. The communities were selected by local public health authorities and community leaders at convenience, using the inclusion criteria of an individual for each household. No random selection of individuals was carried out, so potential bias associated with the sampling cannot be ruled out.

A total of 1,021 community-dwelling individuals were recruited. The communities included in this study belong to the provinces of Chimborazo (communities Lizarzaburu, San Juan, and San Luis at canton Riobamba; community Columbe at canton Colta; and community Penipe at canton Penipe), Tungurahua (communities Benitez, Huambaló, Pelileo, and Salasaca at canton Pelileo), Bolivar (communities Facundo Vela, San Luis, Simiatug, Guaranda, and Veintimilla at canton Guaranda), and Napo (community Oyacachi at canton El Chaco); although Napo is included in the Amazonian provinces of Ecuador, the communities included in this study belong to the highlands area of this province.

In addition, the sociodemographic information was obtained from the official epidemiological record that is mandatory to submit to the local health authority and the Minister of Public Health (MoH) for each sample collected.

Sample collection, RNA extraction, and RT-qPCR for SARS-CoV-2 diagnosis using the CDC protocol

The samples were processed in the BSL2-certified molecular biology laboratory at Universidad de Las Americas. Nasopharyngeal swabs were collected on a 0.5-mL TE pH 8 buffer for SARS-CoV-2 diagnosis by RT-qPCR, following an adapted version of the CDC protocol as it has been previously described by our laboratory. In brief, the CDC RT-qPCR protocol is based on N1 and N2 probes to detect SARS-CoV-2 and RNase P as an RNA extraction quality control (20–28). In addition, negative controls (TE pH 8 buffer) were included as a control for carryover contamination, one for each set of RNA extractions, to guarantee that only true positives were reported. For viral loads calculation, the 2019-nCoV N positive control (IDT, USA) was used, provided at 200.000 genome equivalents/μL, and a factor of 200 was applied to convert the viral loads to genome equivalents/mL and then converted to a logarithmic scale.

Statistical analysis

For the statistical analysis of data, infection rates were calculated for each community and province, and also for sex and age group. To assess differences in the infection rates among communities, provinces, sex, or age group, a chi-square test for comparison of proportions was applied. All statistical analyses were carried out using SPSS Statistics 28 software.

Results

SARS-CoV-2 infection rates

A total of 1,021 indigenous and mestizo individuals from 15 rural communities distributed along four different provinces of the Ecuadorian Andes were tested for SARS-CoV-2 infection (Figure 1A). For Bolivar province, 334 individuals were recruited, distributed in five locations: Facundo Vela, San Luis, Simiatug, Guaranda, and Veintimilla. For Chimborazo province, 322 individuals were recruited, distributed in five locations: Lizarzaburu,

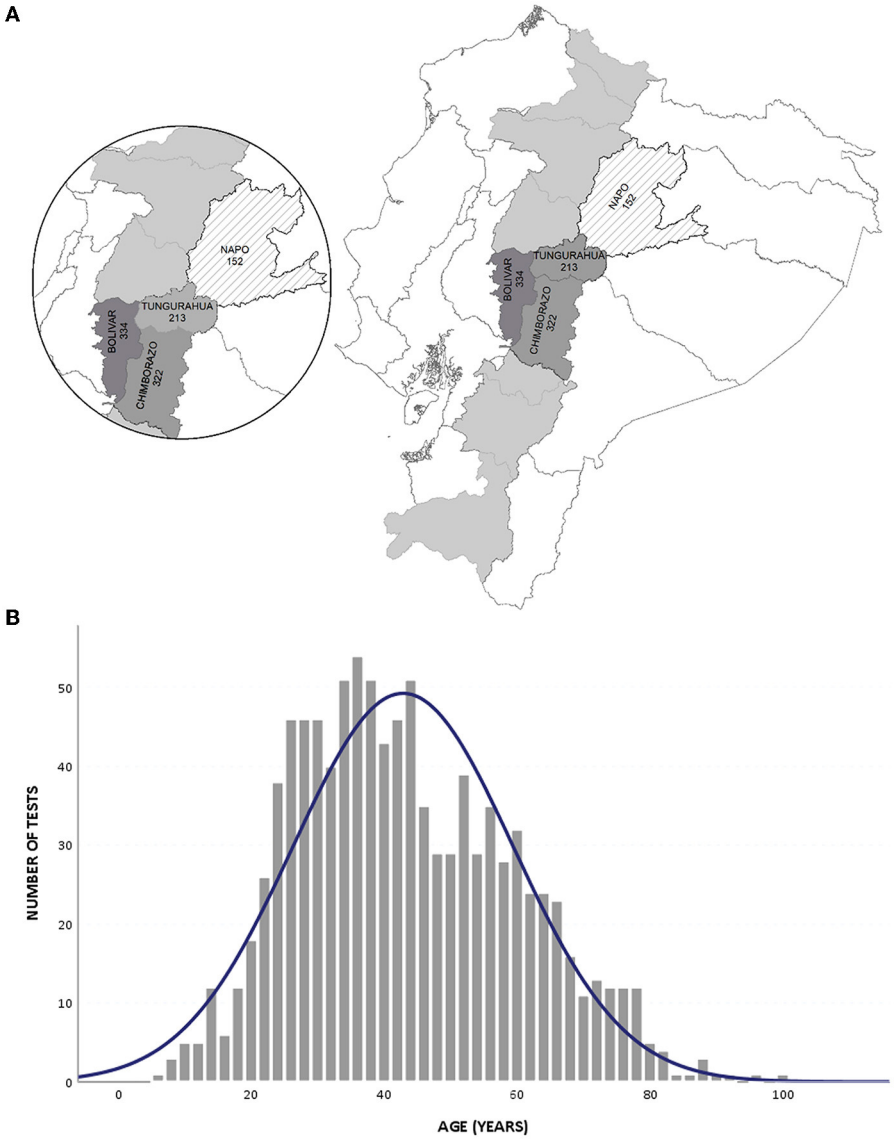

FIGURE 1
Study population. **(A)** Location of the provinces; the gray area indicates the Ecuadorian Andean region. **(B)** Distribution of tests according to the age of participants.

San Juan, San Luis, Columbe, and Penipe. For Tungurahua province, 213 individuals were recruited, distributed in four locations: Benitez, Huambaló, Pelileo, and Salasaca. For Napo province, 152 individuals were recruited from the Oyacachi community. The distribution according to sex was 52.1% (532/1,021) male and 47.9% (489/1,021) female participants. The age distribution for the study population is presented in Figure 1B.

The overall SARS-CoV-2 infection rate was 26.2% (268/1,021, 95% CI: 23.6–29%), with 268 out of 1,021 participants testing positive. The distribution according to sex and age for the individuals infected with SARS-CoV-2 is presented in Table 1. There are no significant differences in the average SARS-CoV-2 infection rate between male and female participants ($p > 0.05$). However, there are significant differences between age groups ($p < 0.05$).

The SARS-CoV-2 infection rates for each province, canton, and community are presented in Table 2. Tungurahua had the highest infection rate value of 139/213, 65.3% (95% IC 58.7–71.4%); followed by Napo with 58/152, 38.2% (95% IC: 30.7–46%); Chimborazo with 54/322, 16.77% (95% IC: 12–21.6%); and Bolivar with 17/334, 5.1% (95% IC: 3.1–7.8%). The SARS-CoV-2 infection rates for cantons comprised Guaranda 5.09% (17/334), Colta 12% (4/33), Penipe 20.6% (13/63), Riobamba 16.4% (37/226), El Chaco 38.2% (58/152), and Pelileo 65.3% (139/213). The SARS-CoV-2 infection rates for communities comprised Simiatug 7.9% (3/38), Veintimilla 7.4% (5/68), Guaranda 4.6% (8/175), San Luis 3.7% (1/27), Facundo 0% (0/26), Columbe 12% (4/33), Penipe 21% (13/63), San Juan 19 % (26/136), San Luis 13% (9/68), Lizarzaburu 9% (2/22), Oyacachi 38% (58/152), Huambaló 74% (58/78), Salasaca 68% (46/68), Benitez 64% (18/28), and Pelileo 44% (17/39). Significant differences were found between those values ($p < 0.01$).

TABLE 1 SARS-CoV-2 infection rates (%) distribution according to sex and age.

	Sex		
Age category years	Male	Female	Total
Infancy: 0–11	0	1 (0.7%)	1 (0.4%)
Adolescence: 10–18	2 (1.6%)	8 (5.7%)	10 (3.7%)
Youth: 19–26	19 (14.8%)	20 (14.3%)	39 (14.6%)
Adulthood: 27–59	86 (67.2%)	92 (65.7%)	178 (66.4%)
Elderly: more 60	21 (16.4%)	19 (13.6%)	40 (14.9%)
Total	128 (47.8%)	140 (52.2%)	268 (26.2%)

TABLE 2 SARS-CoV-2 infection rates for each province, canton, and community included in this study.

Province	Canton	Community	Positive/total; infection rate (%)	Overall infection rate (%)
Bolívar	Guaranda	Facundo	0/26; 0%	17/334; 5.1% (95% IC: 3.1–7.8%)
		Guaranda	8/175; 4.6%	
		San Luis	1/27; 3.7%	
		Simiatug	3/38; 7.9%	
		Veintimilla	5/68; 7.4%	
Chimborazo	Colta	Columbe	4/33; 12.1%	54/322; 16.77% (95% IC: 12–21.6%)
	Penipe	Penipe	13/63; 21%	
	Riobamba	Lizarzaburu	2/22; 9%	
		San Juan	26/136; 19%	
		San Luis R	9/68; 13%	
Napo	El Chaco	Oyacachi	58/152	58/152; 38.2% (95% IC: 30.7–46%)
Tungurahua	Pelileo	Benítez	18/28; 64.3%	139/213; 65.3% (95% IC 58.7–71.4%)
		Huambaló	58/78; 74%	
		Pelileo	17/39, 44%	
		Salasaca	46/68; 68%	
		Overall	268/1021; 26.2%	

SARS-CoV-2 viral loads

The distribution of SARS-CoV-2 viral loads according to sex and age is presented in Figure 2. No significant differences were found ($p > 0.05$). In addition, 20 individuals had viral SARS-CoV-2 load values of above 10^8 copies/mL belonging to the cantons of Penipe (3), Riobamba (5), El Chaco (7), and Pelileo (5). Those individuals represented 7.46% (20/268, 95% CI: 4.8–11.1%) of the individuals infected with SARS-CoV-2.

Discussion

Due to the retrospective nature of this study, there was not a randomized sample collection to include a statistically representative population sampling for these provinces in the Andean region. This is a strong limitation in our study, as the bias on sample collection could mean that the results obtained were not truly representative of the COVID-19 epidemiological context in this region but were limited to the communities selected. However, as the average SARS-CoV-2 infection rate was over 26% (peaking over 50% in several communities) and outbreaks were found at 14 out of 15 communities visited, our results would suggest that non-control COVID-19 community transmission had been happening among rural indigenous communities in the Andes just a few weeks since the national lockdown was lifted. It has been reported that the current health crisis caused by COVID-19 has further aggravated the conditions of vulnerability and social exclusion of indigenous populations in Latin America, and the Andean region would not be an exception (29–33). Similarly, severe COVID-19 outbreaks have been described for Amazonian indigenous people in Brazil and Ecuador (7–9) despite the supposed isolation of those ethnic groups, pointing out the high vulnerability to COVID-19 of those traditionally neglected communities (29–36). In addition, rural communities from the Coastal Region of Ecuador in the provinces of Esmeraldas, Manabí, and Santa Elena were deeply affected by COVID-19 outbreaks during the first wave of the pandemic (14–16, 19). Although widespread, the COVID-19 pandemic has burdened neglected rural and indigenous populations more than others due to limited access to water, poor sanitation of households, lack of information in indigenous languages, and limited access to the healthcare system (32–36).

Interestingly, this study included only community-dwelling non-hospitalized individuals, so either no symptoms or mild symptoms were reported among the individuals infected with SARS-CoV-2. Moreover, 20 individuals from four different cantons had viral loads over 10^8 viral copies/mL and could be considered SARS-CoV-2 super spreaders, representing a 7.46% of the infected population (37). Although there are limitations associated with the calculated viral load based on Ct values representing all the viral genomic material on the sample, and infection of cell cultures is used for sample infectivity confirmation, it is a clear association between low Ct values (that indicates high viral loads based on genomic material quantification) and infectivity (37). As the COVID-19 control and surveillance program in Ecuador was mainly limited to hospitalized individuals, our results clearly endorsed that the strategy was not sufficient to control COVID-19 outbreaks (14–19). Nevertheless, the SARS-CoV-2 testing capacity for the public health system in Ecuador was very limited for a 17-million population (38–40). In addition, no resources were allocated to most of the rural provinces of the country, and the SARS-CoV-2 diagnosis was centralized in the three laboratories from the "Instituto Nacional de Salud Publica e Investigación" located in the three main cities of Ecuador (18). Together with studies carried out in Afro-Ecuadorian communities (19), rural villages from the Manabi province (14, 15), Amazonian indigenous communities (7, 8), women victims of gender-based violence (41), food riders, or funeral home workers (42, 43) from Ecuador, those results highlight the need for active COVID-19 monitoring in community-dwelling individuals from vulnerable groups and neglected communities.

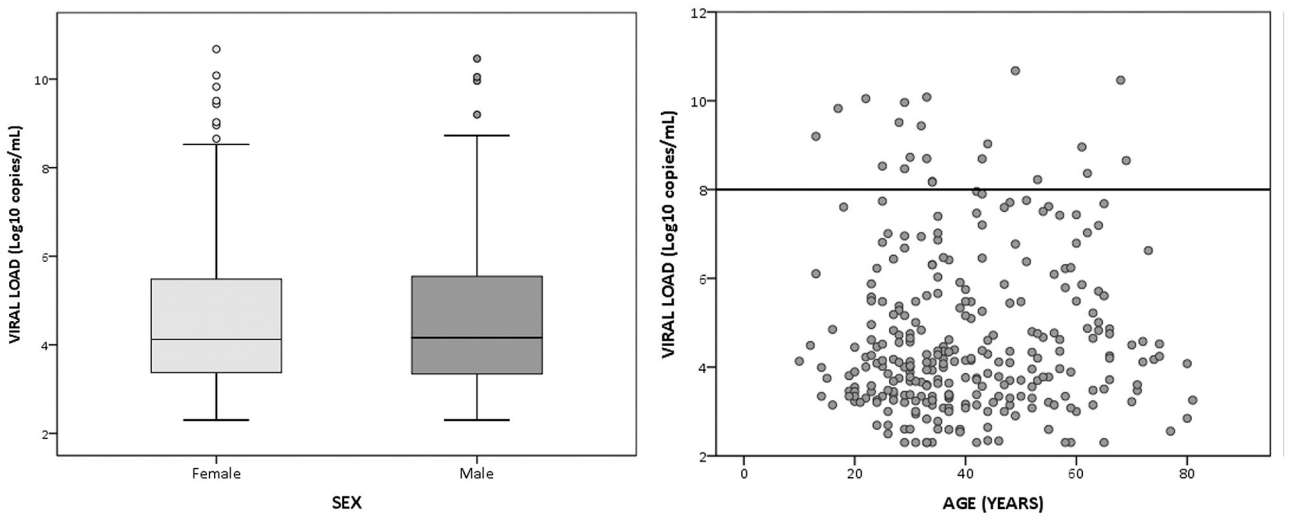

FIGURE 2
Distribution of SARS-CoV-2 viral loads according to sex and age in the study population. Viral load is represented in a log scale.

In conclusion, our findings support that COVID-19 community transmission and super-spreading events were happening among rural mestizo and indigenous communities from the Andean region in Ecuador during the first wave of the COVID-19 pandemic. For the still ongoing COVID-19 pandemic and future ones, our results endorse that control and prevention strategies have to focus not only on hospitalized and symptomatic individuals but also on community-dwelling individuals at locations where outbreaks are suspected.

Author's note

In this study, we described COVID-19 outbreaks in rural indigenous population from the Andean Region of Ecuador. Although several studies regarding COVID-19 and indigenous people have been published from Latin America, this is the first one addressing the situation in the Andean region during the early stages of the pandemics. With a sample over 1,000 community dwelling individuals, high infection rates were found endorsing community transmission during the first wave of COVID-19 pandemic in these neglected population in Ecuador.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The paired samples used for the homogenization protocol were the leftover of the samples collected for routine SARS-CoV-2 diagnosis. Nevertheless, this work is included in a study that was approved by the IRB from the Dirección Nacional de Inteligencia de la Salud (Ministerio de Salud Publica, Ecuador) under the code 008-2020.

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Author contributions

DM-J and MG-B wrote the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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6.2.4. *Pruebas masivas en las Islas Galápagos y baja tasa de positividad para controlar la propagación del SARS-CoV-2 durante el primer semestre de la pandemia de COVID-19: una historia de éxito para Ecuador y Sudamérica.*

Publicado en: Rural and remote health, 2023. Massive testing in the Galapagos Islands and low positivity rate to control SARS-CoV-2 spread during the first semester of the COVID-19 pandemic: a story of success for Ecuador and South America. D.O.I: https://doi.org/10.22605/RRH7643

Durante los primeros meses de la pandemia de COVID-19 en América Latina, varios países como Ecuador, Perú o Colombia experimentaron escenarios caóticos con sistemas de salud pública colapsados y falta de capacidad de prueba para controlar la propagación del virus. En ciudades importantes como Guayaquil en Ecuador, situaciones dramáticas como cadáveres en las calles fueron transmitidas internacionalmente. Sin embargo, mientras la pandemia de COVID-19 estaba devastando Sudamérica, la transmisión del SARS-CoV-2 se manejó con éxito en las Islas Galápagos debido a la implementación de una estrategia de detección masiva que combinaba población hospitalizada y comunitaria, y las restricciones de viaje facilitadas por su ubicación geográfica (972 km lejos del territorio continental ecuatoriano). Por ejemplo, la isla Floreana fue uno de los pocos lugares del mundo que permaneció libre de COVID-19 durante 2020.

En este estudio, analizamos retrospectivamente los datos relacionados con las campañas de pruebas masivas de SARS-CoV-2 de abril a septiembre de 2020 en las Islas Galápagos. Se presentó la tasa de positividad más baja de América del Sur (con un rango de 4.8 a 6.7%).

La baja tasa de positividad en las Islas Galápagos se debió a que este territorio tuvo la tasa de pruebas más alta entre las provincias ecuatorianas (9,87% de la población, es decir, 2.480 de 25.124 habitantes) durante la primera ola de la pandemia de COVID-19.

Esta historia de éxito fue posible gracias a la colaboración interinstitucional entre el gobierno regional de las Islas Galápagos ("Consejo de Gobierno"), las autoridades locales ("Gobiernos Autónomos Descentralizados de Santa Cruz, San Cristóbal e Isabela"), el gobierno regional autoridades del Ministerio de Salud de Ecuador, la "Agencia de Regulación y Control de la Bioseguridad y Cuarentena para Galápagos" y la "Universidad de Las Américas".

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ORIGINAL RESEARCH

Massive testing in the Galapagos Islands and low positivity rate to control SARS-CoV-2 spread during the first semester of the COVID-19 pandemic: a story of success for Ecuador and South America

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Miguel Angel Garcia-Bereguiain: Massive testing in the Galapagos Islands to control SARS-CoV-2

EARLY ABSTRACT:

Introduction: During the first months of COVID-19 pandemic in Latin America, several countries like Ecuador, Peru or Colombia experienced chaotic scenarios with public health systems collapsed and lack of testing capacity to control the spread of the virus. In main cities like Guayaquil in Ecuador, dramatic situations like body corpses in the streets where internationally broadcasted. However, while COVID-19 pandemic was devastating South America, SARS-CoV-2 transmission was successfully managed at Galapagos Islands due to the implementation of a massive screening strategy combining hospitalized and community dwelling population, and travel restrictions facilitated by its geographical location (972 km far from the Ecuadorian continental territory). For instance, Floreana Island was one of the few locations in the world that remained COVID-19 free during 2020.
Methods: In this study, we retrospectively analyzed the data related to SARS-CoV-2 massive testing campaigns from April to September 2020 in the Galapagos Islands.
Results: Galapagos Islands had the lowest positivity rate in South America (ranging from 4.8 to 6.7%).
Discussion: The low positivity rate in Galapagos Islands was due to the fact that this territory had the highest testing ratio among Ecuadorian provinces (9.87% of the population, meaning 2,480 out of 25,124 inhabitants) during the first wave of COVID-19 pandemic.
Conclusion: This story of success was possible thanks to the inter institutional collaboration between the regional government of Galapagos Islands ("Consejo de Gobierno"), the local authorities ("Gobiernos Autonomos Descentralizados de Santa Cruz, San Cristobal and Isabela"), the regional authorities from Ecuadorian Ministry of Health, the "Agencia de Regulación y Control de la Bioseguridad y Cuarentena para Galápagos" and "Universidad de Las Américas".

Massive testing in the Galapagos Islands and low positivity rate to control SARS-CoV-2 spread during the first semester of COVID-19 pandemic: a story of success for Ecuador and South America.

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Abstract

During the first months of COVID-19 pandemic in Latin America, several countries like Ecuador, Peru or Colombia experienced chaotic scenarios with public health systems collapsed and lack of testing capacity to control the spread of the virus. In main cities like Guayaquil in Ecuador, dramatic situations like body corpses in the streets where internationally broadcasted. However, while COVID-19 pandemic was devastating South America, SARS-CoV-2 transmission was successfully managed at Galapagos Islands due to the implementation of a massive screening strategy combining hospitalized and community dwelling population, and travel restrictions facilitated by its geographical location (972 km far from the Ecuadorian continental territory). For instance, Floreana Island was one of the few locations in the world that remained COVID-19 free during 2020. In this study, we retrospectively analyzed the data related to SARS-CoV-2 massive testing campaigns from April to September 2020 in the Galapagos Islands, which make this territory to have the lowest positivity rate in South America (ranging from 4.8 to 6.7%) and the highest testing ratio among Ecuadorian provinces (9.87% of the population, meaning 2,480 out of 25,124

inhabitants) during the first wave of COVID-19 pandemic. This story of success was possible thanks to the inter institutional collaboration between the regional government of Galapagos Islands ("Consejo de Gobierno"), the local authorities ("Gobiernos Autonomos Descentralizados de Santa Cruz, San Cristobal and Isabela"), the regional authorities from Ecuadorian Ministry of Health, the "Agencia de Regulación y Control de la Bioseguridad y Cuarentena para Galápagos" and "Universidad de Las Américas".

Introduction

The rising of a novel coronavirus named SARS-CoV-2 in the Chinese province of Hubei in December 2019 lead to the Coronavirus Disease 2019 (COVID-19) outbreak that made to the World Health Organization (WHO) to declare a pandemic by March 11th 2020 (1,2). Up to March 3rd 2023, more than 676 million cases and 6.8 million deaths have been reported, with the Americas as one of the most affected regions with millions of cases and deaths. For instance, only Brazil and USA account for more than 1.8 million of deaths (https://coronavirus.jhu.edu/map.html).

In Ecuador, more than 1 million of cases and 36,000 deaths were reported up to March 2023 (https://coronavirus.jhu.edu/map.html). The first COVID-19 case reported in the country was identified in Guayaquil on February 29th 2020, with an elder woman who arrived in a flight from Spain. During the following weeks, the transmission of SARS-CoV-2 across the country growth exponentially and put unprecedented pressure and overwhelmed the Ecuadorian healthcare system. The most dramatic consequences were observed in Guayaquil during April 2020, where images of corpses in the streets of low income neighborhoods were broadcasted worldwide. All of this happened despite the efforts of the Ecuadorian government to contain the SARS-CoV-2 virus spread with a complete lockdown for all non-essential services (except for pharmacies, groceries, security and health services), and mobility restrictions (3). Although those measures helped to control COVID-19 transmission, once the measures were relaxed since July 2020 to avoid the collapse of the economy, new COVID-19 outbreaks were observed countrywide leading to an uncontrolled community transmission (4-8).

However, the epidemiological scenario was different for one of the provinces of Ecuador, the Galapagos Islands. Although the first case of COVID-19 was reported as early as March 2020, a low prevalence and deaths associated to COVID-19 were maintained during the first wave of the pandemic. Galapagos Islands are located at 972 km from the Ecuadorian coast and comprise 13 main islands, of which only four are inhabited. The Galapagos Islands have 25,124 inhabitants, distributed along Santa Cruz (Population: 15,393), San Cristobal (Population: 7,330), Isabela (Population: 2,256) and Floreana (Population: 145) (9). The political organization of the population is in three cantons with a population density (individuals/km^2) of 8.8, 8.5 and 0.4 for Santa Cruz, San Cristobal (this canton also includes Floreana as a rural parish) and Isabela, respectively. Moreover, considering that 98% of the Galapagos Island area is included in the National Park, human population is concentrated in a main town in each of the four populated islands, and a few rural parishes. The demographic characteristics in Galapagos are very similar to continental Ecuador, with a young population of 29 years old in average according to the last national census. Regarding the health infrastructure in Galapagos Islands, there are primary health facilities like health centers, a basic hospital in Santa Cruz Island and a general hospital in San Cristobal Islands; overall, the public health system in Galapagos Islands has the issues associated to low and middle income countries, and in this particularly case the situation is aggravated by the geographic location faraway from mainland Ecuador where more specialized hospital facilities are located.

However, by April 6th 2020 there was a SARS-CoV-2 testing laboratory in site, due to the inter institutional collaboration between the regional government of Galapagos Islands ("Consejo de Gobierno"), the local authorities ("Gobiernos Autonomos Descentralizados de Santa Cruz, San Cristobal and Isabela"), the regional authorities from Ecuadorian Ministry of Health, the "Agencia de Regulación y Control para la Bioseguridad y Cuarentena para Galápagos" and "Universidad de Las Américas". By contrast, in continental Ecuador the only laboratories within the public health system at that early stages of COVID-19

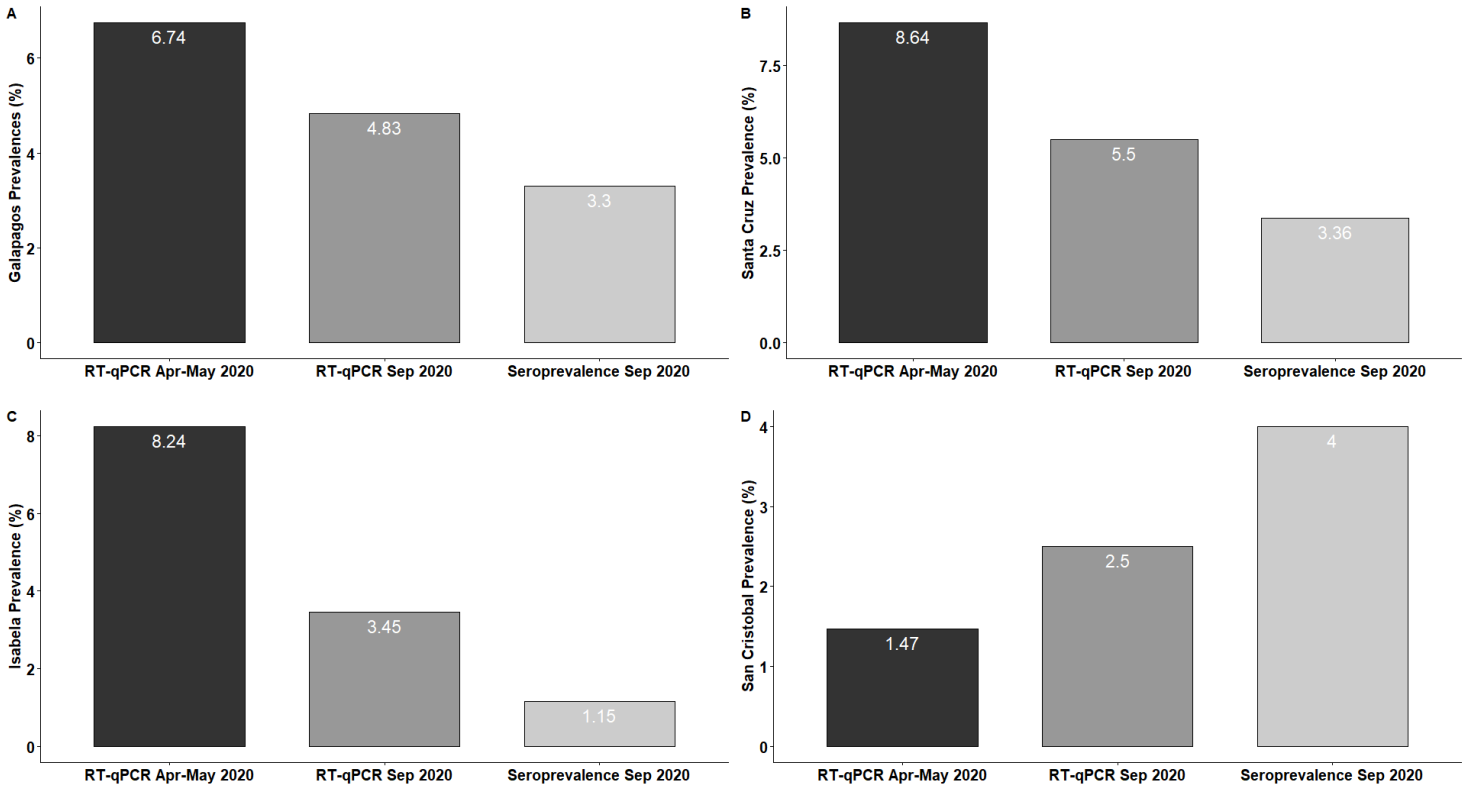

Figure 5: Comparison of SARS-CoV-2 infection rates and anti-SARS-CoV-2 IgG seroprevalence in Galapagos Islands for the surveillance interventions during April-May 2020 and September 2020. A. Overall infection rate and seroprevalence in Galapagos Islands. B-D. Infection rate and seroprevalence for Santa Cruz, Isabela and San Cristobal.

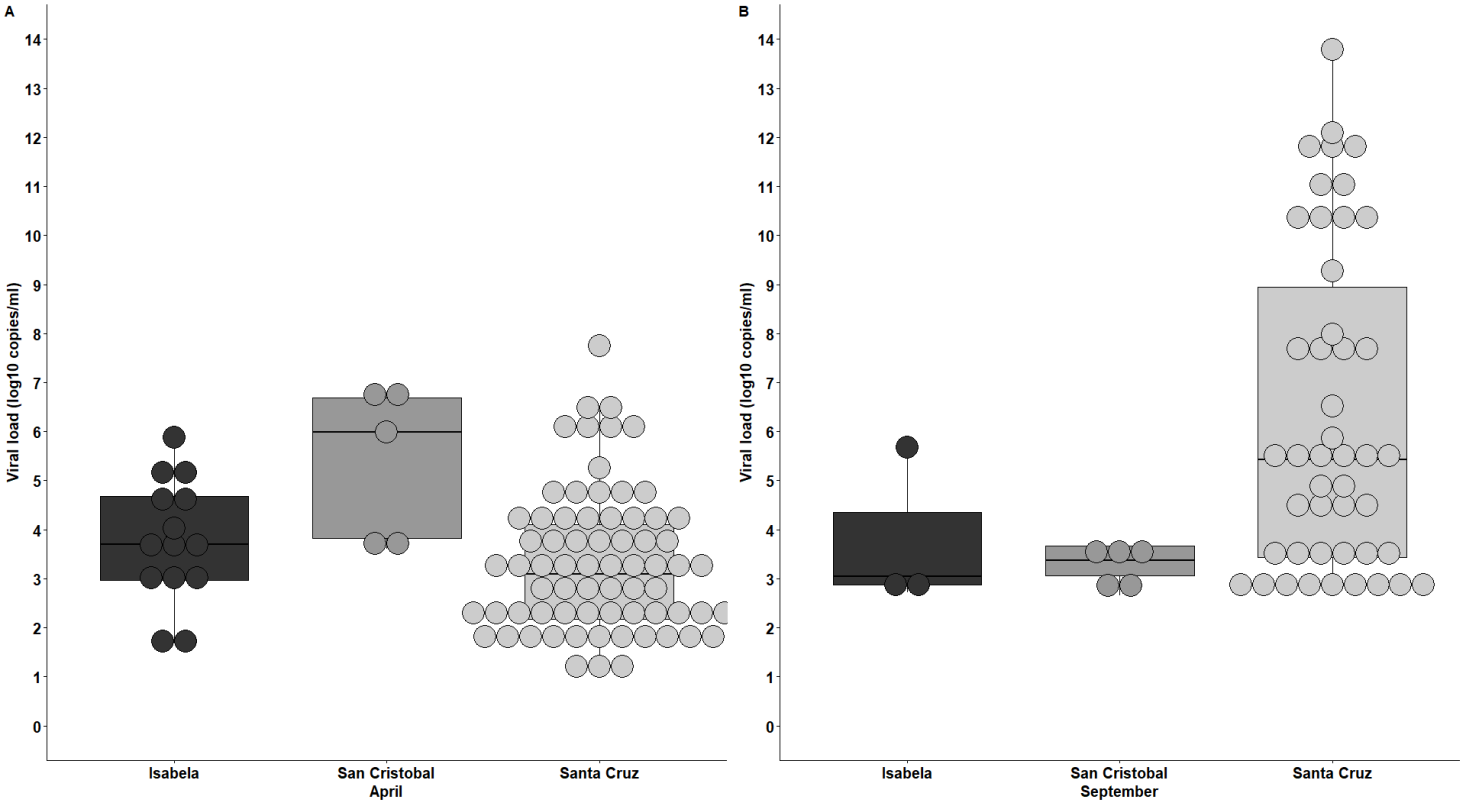

Figure 6. SARS-CoV-2 Viral loads distribution among positive individuals for each island for the surveillance intervention in April-May 2020 (A) and September 2020 (B).

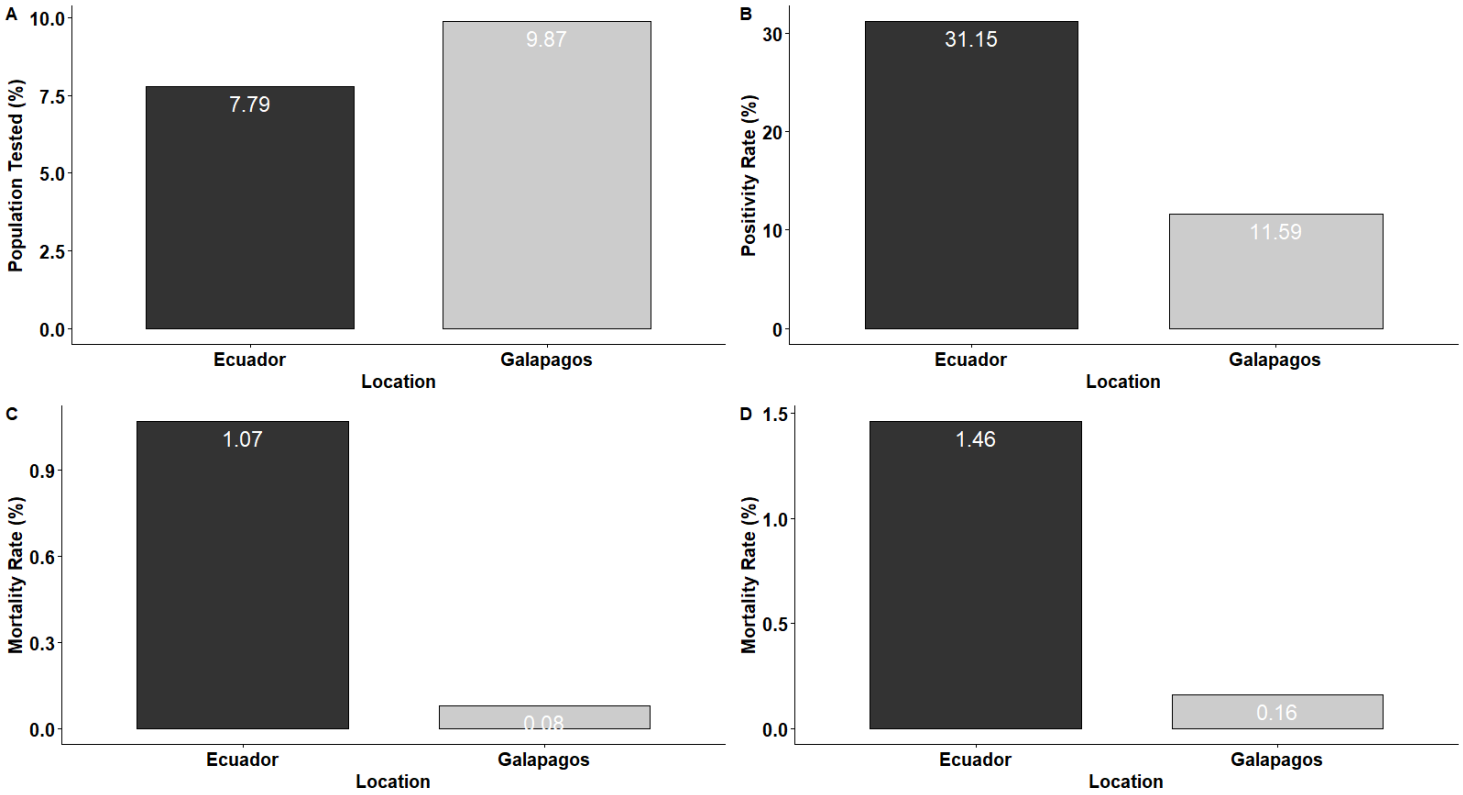

Figure 7. Comparisons of population tested, positivity and mortality rates between Galapagos Islands and Ecuador. A. Percentage of tested population. Data for Ecuador retrieved from MoH Report (May 27th, 2021). B. Positivity rate from Galapagos and Ecuador until May 27th, 2021. Data from MoH report. C. Mortality rates for Ecuador and Galapagos relative to the confirmed deaths from SARS-CoV-2. D. Mortality rates for Ecuador and Galapagos relative to the confirmed and probable deaths from SARS-CoV-2.

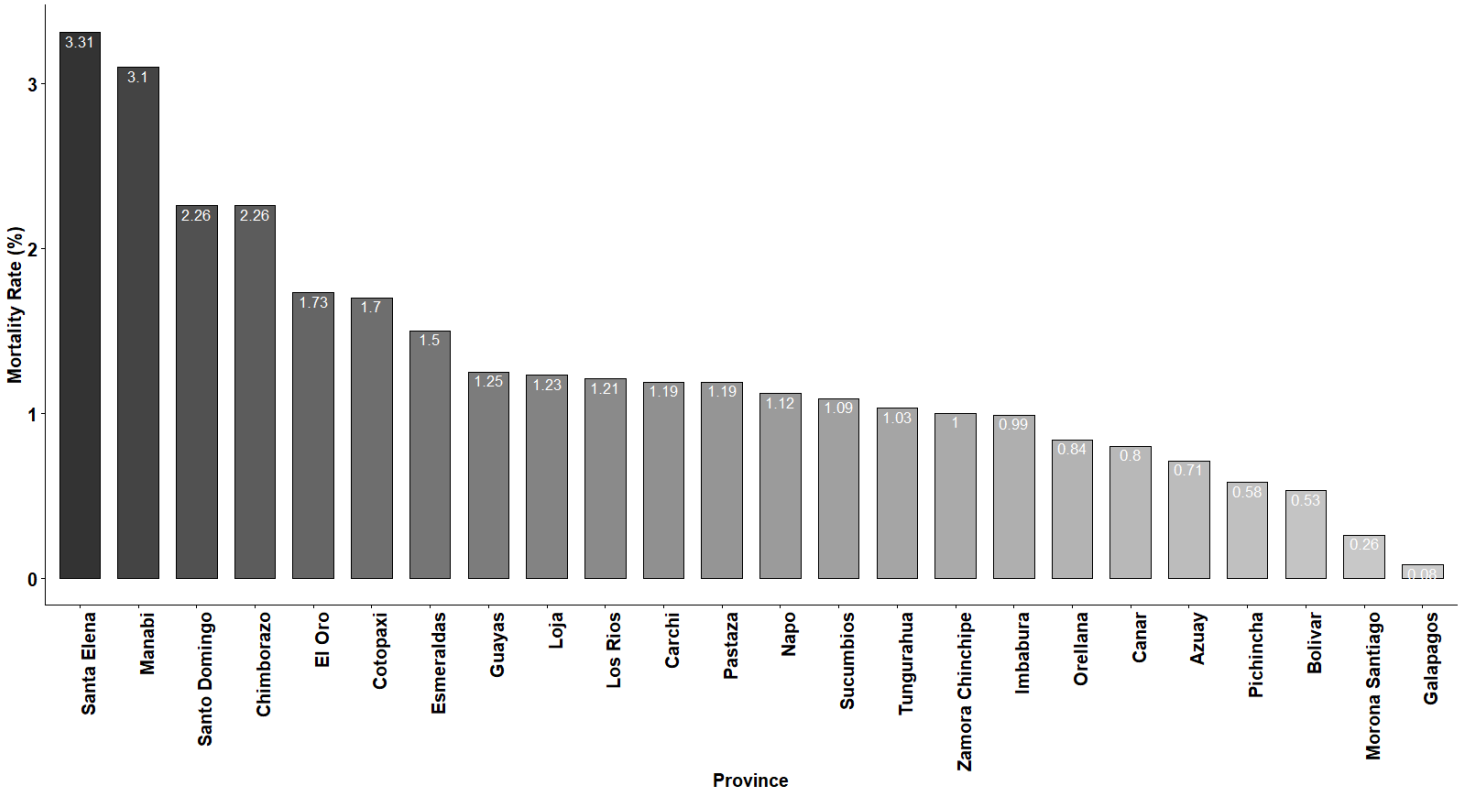

Figure 8. Mortality rate for all Ecuadorian provinces calculated as confirmed deaths from SARS-CoV-2 infection per total positive RT-qPCR tests performed (Data obtained from MoH report for May 27th 2020).

6.2.5. Brotes de COVID-19 en albergues para mujeres víctimas de violencia de género de Ecuador.

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Una de las limitaciones para contener el impacto de la pandemia de COVID-19 en Ecuador es la limitada capacidad de prueba, especialmente en poblaciones de alto riesgo, como las personas que viven en albergues humanitarios.

La oficina del "Alto Comisionado de las Naciones Unidas para los Refugiados" en Ecuador en colaboración con la "Universidad de Las Américas" realizó un tamizaje de vigilancia en albergues para mujeres víctimas de violencia de género. Se les había otorgado acceso a las pruebas RT-qPCR para el diagnóstico de SARS-CoV-2 desde julio de 2020, unas semanas después de que se levantara el bloqueo de la población general.

Un total de 411 personas fueron analizadas, de las cuales, 52 dieron positivo para SARS-CoV-2, lo que arroja una alta tasa de ataque general del 12,65 %. Además, se encontraron brotes de COVID-19 en nueve de los 11 refugios que se incluyeron en el estudio. Si bien las tasas de ataques variaron entre los refugios, no se encontró asociación con la ocupación.

Este estudio es clave para esclarecer la situación epidemiológica de esta población altamente vulnerable en América Latina. Destaca la importancia del testeo masivo más allá de la población sintomática.

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COVID-19 outbreaks at shelters for women who are victims of gender-based violence from Ecuador

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ABSTRACT

Background: One of the constraints in containing the impact of the COVID-19 pandemic in Ecuador is limited testing capacity, especially in high-risk populations such as people living in humanitarian shelters.

Objectives: The "United Nations High Commissioner for Refugees" office in Ecuador in collaboration with "Universidad de Las Américas" performed surveillance screening at shelters for women victims of gender-based violence. They had been granted access to RT-qPCR tests for SARS-CoV-2 diagnosis since July 2020, a few weeks after the general population lockdown was lifted.

Results: From 411 people tested, 52 tests were SARS-CoV-2 positive, yielding an overall high attack rate of 12.65%. Moreover, COVID-19 outbreaks were found in nine of 11 shelters that were included in the study. While attacks rates varied among shelters, no association was found with occupancy.

Conclusion: This study is key to clarifying the epidemiological situation in this highly vulnerable population in Latin America. It highlights the importance of mass testing beyond the symptomatic population to prevent the spread of COVID-19.

Introduction

The outbreak of coronavirus disease 2019 (COVID-19) raised alerts in the global scientific and health communities since the first 27 cases were reported in December 2019 from Wuhan, China. SARS-CoV-2 spread readily and quickly around the world, and the first cases in Latin America were reported just two months after the original report (PAHO, 2021). Until March 2021, more than 51 million COVID-19 cases and more than 1 million deaths were reported in the Americas region; and more than 290,000 cases were reported in Ecuador since the arrival of the first case in March 2020 (PAHO, 2021). However, with limited SARS-CoV-2 testing capacity and a positivity rate over 30%, those numbers are far from the real ones.

During the first months of the pandemic, a wide variety of recommendations were endorsed by the World Health Organization (including social distancing, mass testing, and isolation of confirmed cases) to slow down the spread of the disease. However, those measures are difficult to meet in some specific settings, including: refugee shelters, housing for victims of gender-based violence, prisons or provisional detention centres, among others. Confinement and prevention of spread in those settings is nearly impossible due to the lack of adequate infrastructure and the high occupation of some shelters (Peate, 2020; Wood et al., 2020).

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Moreover, confinement represents harm for the economic needs of the people living in those spaces. Testing capacity is also lower in those settings when compared with middle-income or high-income environments (Wang et al., 2020), and even worse with weak healthcare systems (e.g., in developing countries) (Inter-Agency Standing Committee (IASC), 2020).

Many humanitarian shelters are generally occupied to their full capacity at times of economic crisis, like the current one, making hotspots for SARS-CoV-2 infection and contagion. During the highest peak of the COVID-19 pandemic in Ecuador, the need for mass testing throughout the country was urgent to complement the effort of the public health system and to reach sectors of the population for whom PCR tests were inaccessible (Ortiz-Prado et al., 2021a,b, Freire-Paspuel et al., 2020). In this context, "Universidad de Las Américas" (UDLA) and the United Nations High Commissioner for Refugees (UNHCR) office in Ecuador coordinated efforts for granting access to SARS-CoV-2 PCR tests for the occupants of ten shelters for women victims of gender-based violence located across the country. By September 2020, when this study was finished, according to the official information from the Ecuadorian Ministry of Health (https://www.salud.gob.ec/wp-content/uploads/2020/09/Boletin-196_Nacional_MSP.pdf), a total number of 116,451 SARS-CoV-2-positives cases were reported in the country. However, with a positivity rate of 40.03%, the need to increase SARS-CoV-2 testing is mandatory.

This study aimed to highlight the epidemiological situation of those women's shelters in Ecuador, the importance of testing in such humanitarian settings, and to assess whether the occupancy level of each shelter is related to the positivity rate. Thus, a description of the study population is provided, as well as a comparison between the attack rates at the different shelters and the occupation levels.

Methods

Study design and setting

A study to evaluate the burden of SARS-CoV-2 infection among all the occupants (women victims of gender-based violence and their children) and staff working (total number of 411 individuals) in 11 shelters was performed in nine different Ecuadorian cities from July to September 2020. The UNHCR office in Ecuador provided logistics for sampling in the different cities and the samples transportation to the UDLA Laboratory located in Quito, Ecuador. Also, UNHCR coordinated with "Red Nacional de Casas de Acogida" to select the shelters to be included in the study. The shelters considered were: "Casa de Acogida Manos Unidas Tejiendo Progreso" (Tulcan), "Casa Mama Zoila Espinoza" (Ibarra), "Casa Tránsito Amaguaña" (Cotacachi), "Casa Amiga" (Lago Agrio), "Casa Paula" (Francisco de Orellana), "Casa de la Mujer" and "Casa Matilde" (Quito), "Casa de Acogida Cotopaxi" (Salcedo), "Casa María Amor" (Cuenca), and "Hogar de Nazareth" (Guayaquil). Also, a group of 100 women refugees and their children who were migrating from Colombia and Venezuela were tested at a temporary shelter in Lago Agrio.

Sample collection, RNA extraction, and RT-qPCR for SARS-CoV-2 diagnosis using the CDC protocol

Nasopharyngeal swabs were collected on 0.5 mL TE pH 8 buffer for SARS-CoV-2 diagnosis by RT-qPCR following an adapted version of the Centers for Disease Control and Prevention (CDC) protocol by using "AccuPrep Viral RNA extraction kit" (Bioneer, South Korea) as an alternate RNA extraction method and CFX96 BioRad instrument (Freire-Paspuel and Garcia-Bereguiain, 2020; Freire-Paspuel et al., 2020b,c,d; Freire-Paspuel et al., 2021; Freire-Paspuel and Garcia-Bereguiain, 2021; CDC, 2021; Lu et al., 2020). Briefly, the CDC designed RT-qPCR FDA EUA 2019-nCoV CDC kit (IDT, USA) is based on N1 and N2 probes to detect SARS-CoV-2 and RNase P as an RNA extraction quality control (CDC, 2021; Lu et al., 2020). Negative controls (TE pH 8 buffer) were included as control for carryover contamination, one for each set of RNA extractions, to guarantee that only true positives were reported. For viral loads calculation, the 2019-nCoV N positive control (IDT, USA) was used, provided at 200.000 genome equivalents/μL, and a factor of 200 was applied to convert the viral loads to genome equivalents/mL and then converted to logarithmic scale.

Statistical analysis

For the statistical analysis of data, positivity rates were calculated for each shelter and occupancy rates were provided by UNHCR. To assess differences in the positivity rates among shelters, Chi-squared for comparison of proportions was applied, and a Pearson's correlation test was performed to evaluate correlation between positivity rates and occupancy levels. All statistical analysis was carried out using R software.

Results

Overall SARS-CoV-2 attack rates and viral loads

A total of 411 people from refugee shelters were tested for SARS-CoV-2 using nasopharyngeal swabs. The shelters were distributed along the different geographical regions of Ecuador in nine cities: Cotacachi, Cuenca, Ibarra, Latacunga, Quito, and Tulcan in the Andean Region; Guayaquil in the Coastal Region; and Francisco de Orellana and Lago Agrio in the Amazon Region (Figure 1A). Most of the samples were taken from females (283/411, 68.86%) aged between 30–40 years (mean 31.55 ± 0.73 years) inhabiting those shelters (Figure 1B,C). The overall attack rate of SARS-CoV-2 in all the shelters was 52/411 (12.65%) (Figure 1D). SARS-CoV-2 outbreaks were found in nine of the 11 shelters visited.

The subjects who tested positive for SARS-CoV-2 were distributed as shown in Figure 2, with a lower positivity ratio in females (33/283, 11.66%) than in males (19/128, 14.84%). Furthermore, more cases were distributed among the younger population (aged < 40 years) with an average age of 36.02 ± 2.11 years.

Data for viral loads (VL) were available for 32 of the 52 positive individuals in this study; thus, 32 individuals were considered for differences in VL between age groups and sex. There were no significant differences in the average VL among different sex or age groups ($p > 0.05$). However, higher VL were distributed within young adults (aged 15–39 years) and in females (Figure 3). The latter could be attributable to the fact that most of the occupants of different shelters were women and their children.

SARS-CoV-2 attack rates at different locations and shelters

The location of each shelter and its SARS-CoV-2 attack rate are detailed as follows and in Table 1:

- Lago Agrio city shelters (Sucumbios province, Amazonian Region). Two shelters were tested at Lago Agrio. First, a temporary shelter with 100 women refugees and their children coming from Colombia and Venezuela. In this sample, 100 RT-qPCR tests were performed on 56 females and 44 males. The SARS-CoV-2 attack rate was 20.0%. No occupation rate information was available for this refugee shelter. Second, the foster home "Casa Amiga" in Lago Agrio, with 72% capacity occupation was tested. Thirty-six individuals were sampled in this location and no SARS-CoV-2 positive cases were found.

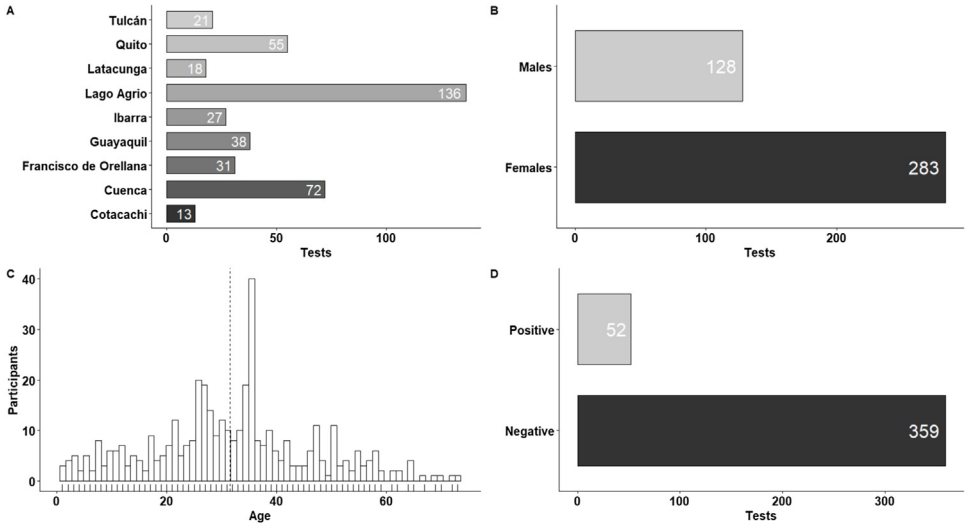

Figure 1. Description of study population. (A) Number of SARS-CoV-2 RT-qPCR tests applied in different shelter locations. (B) Distribution of individuals tested by sex. (C) Distribution of individuals tested according to age. (D) Distribution of individuals who tested positive and negative.

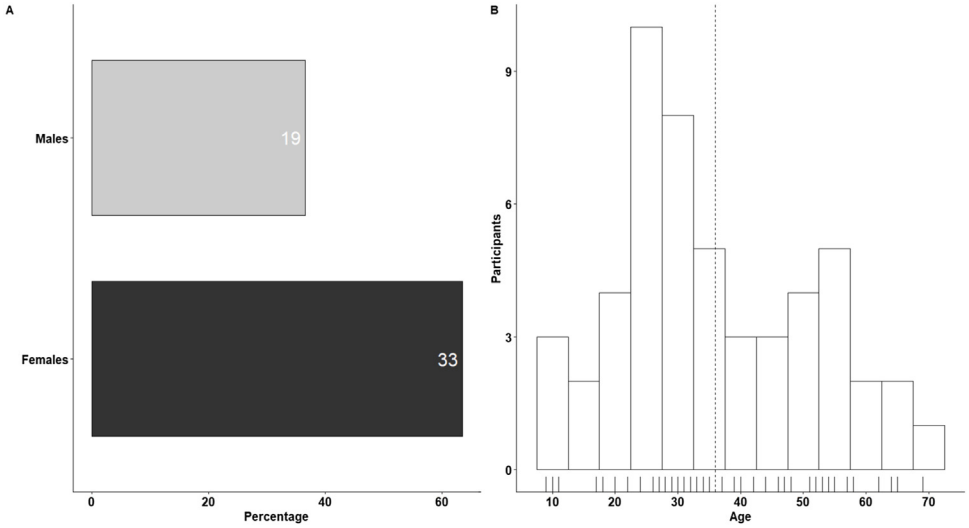

Figure 2. Distribution of SARS-CoV-2 RT-qPCR-positive cases according to sex (A) and age (B) at the shelters for women victims of gender-based violence included in the study.

- Francisco de Orellana shelter (Orellana province, Amazonian Region). In the capital city of Orellana province, "Casa Paula" shelter was considered for this study. Thirty-one people (27 females and four males) were tested, and seven had positive results, resulting in a SARS-CoV-2 attack rate of 22.6%. This shelter was occupied at 100% of its capacity.
- Tulcan city shelter (Carchi province, Andean Region). Twenty-one samples were taken in a shelter in Tulcán, distributed between 16 females and five males, with one male individual being positive, giving a SARS-CoV-2 attack rate of 4.8%. The shelter "Casa Manos Unidas Tejiendo Progreso" was occupied at 30% of its capacity.
- Quito city shelters (Pichincha province; Andean Region). Two shelters from the Ecuadorian capital city were considered in the study: "Casa de la Mujer" and "Casa Matilde". "Casa de la Mujer" had an occupancy percentage of 55%, and 27 samples from 26 females and one male were considered: no SARS-CoV-2-positive individuals were found. In contrast, "Casa Matilde" was occupied at 73% of its capacity, and seven individuals of 28 living in the shelter (22 females and six males) had positive tests, giving a SARS-CoV-2 attack rate of 25.0%.
- Latacunga city shelter (Cotopaxi province, Andean Region). From "Casa de Acogida Cotopaxi" in Latacunga, 15 women and three men were tested for SARS-CoV-2, with three positive individuals, yielding an attack rate of 16.7%. This shelter was at 110% of its capacity.
- Ibarra city shelter (Imbabura province, Andean Region). Twenty-seven individuals were tested with four being positive, giving a SARS-CoV-2 attach rate of 14.8%. No shelter occupancy information was available.
- Cotacachi city shelter (Imbabura province, Andean region). Thirteen individuals were tested with four being positive, giving a SARS-CoV-2 attack rate of 30.8%. No shelter occupancy information was available.
- Cuenca city shelter (Azuay province; Andean region). "Casa María Amor" was overcrowded at 118% of its full capacity. Forty-nine women and 23 men were tested in this shelter, and three individuals were positive, giving an attack rate of 4.17% for this centre.

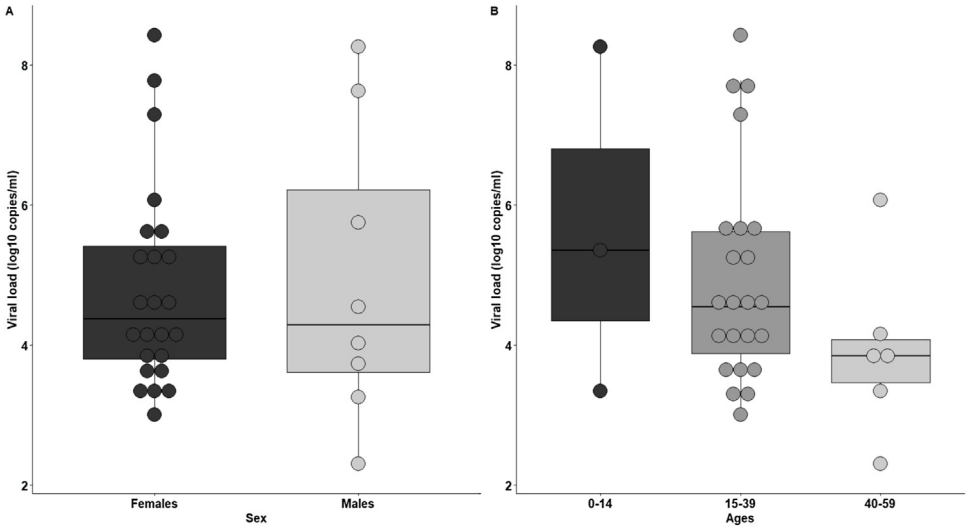

Figure 3. SARS-CoV-2 viral load distribution according to sex (A) and age ranges (0–14: children, 15–39: young adults, 40–50: adults); (B) for the positive individuals included on the study (viral load is expressed as $\log_{10}$ copies/ml).

Table 1
SARS-CoV-2 attack rates, number of individuals tested, and occupation rates from the shelters for women victims of gender-based violence included in the study.

Shelter	SARS-CoV-2 attack rate (%)	Sample size	Occupation (%)
Casa María Amor	4.2	72	118
Casa de Acogida Cotopaxi	16.7	18	110
Casa Paula	22.6	31	100
Casa Matilde	25	28	73
Casa Amiga	0	36	72
Casa de la Mujer	0	27	55
Casa Hogar de Nazareth	7.9	38	37
Casa Manos Unidas	4.8	21	30
Ibarra Shelter	14.8	27	NA
Cotacachi Shelter	30.8	13	NA
Refuges Lago Agrio	20	100	NA

- Guayaquil city shelter (Guayas province, Coastal Region). From the 38 individuals sampled in "Casa Hogar de Nazareth" in Guayaquil, 26 were females and 12 were males, with three individuals being positive for SARS-CoV-2. The attack rate for this shelter was 7.89% and it was occupied at 37%.

SARS-CoV-2 infection attack and shelter occupation rates

The occupation rates for the different shelters with the available information are detailed in Table 1. Although there are significant differences ($p < 0.05$) in the SARS-CoV-2 attack rates for different shelters, no significant linear correlation was found with the number of individuals at the shelter or the occupation rate ($R = 0.36$, $p = 0.39$, 95% CI −0.466, 0.848).

Discussion

Since the early stage of the COVID-19 pandemic in Ecuador, the strategy for prevention or mitigation of the impact of COVID-19 displayed by the Ministry of Health was limited to test symptomatic patients attending hospital facilities. Moreover, despite huge efforts from the National Reference Laboratories for SARS-CoV-2 surveillance from "Instituto Nacional de Salud Pública e Investigación" to keep up the diagnosis, a limited daily testing capacity below 200 PCR tests per million habitants was installed across the country (Ortiz-Prado et al., 2021c; Torres and Sacoto, 2020). However, the few reports about the epidemiological situation of SARS-CoV-2 among vulnerable populations suggest that community transmission has been happening since the population lockdown was lifted in June 2020 in Ecuador (Ortiz-Prado et al., 2021a,b,d; Freire-Paspuel et al., 2020a; Del Brutto et al., 2020). Under this scenario, in collaboration with UNHCR, this surveillance program was implemented at shelters for women victims of gender-based violence, and COVID-19 outbreaks were reported at nine out of 11 shelters visited. It is believed that this is the first publication to date addressing SARS-CoV-2 surveillance at shelters for women victims of gender-based violence.

After the arrival of the SARS-CoV-2 infection to Ecuador, a study revealed that women were less prone to acquire the infection compared with men (Ortiz-Prado et al., 2021c). This is in accordance with the findings in the overall SARS-CoV-2 attack rates for males and females in the current study. The overall high SARS-CoV-2 attack rate of 12.65% is also in agreement with other reports showing SARS-CoV-2 community transmission among community-dwelling individuals in Ecuador (Ortiz-Prado et al., 2021a,b,d; Freire-Paspuel et al., 2020a; Del Brutto et al., 2020).

A wide variety of studies sustain the idea that crowded household settings constitute an important site for COVID-19 outbreaks (Del Brutto et al., 2020; Leclerc et al., 2020; Ortiz-Prado et al., 2020; Rothan and Byrareddy, 2020; Madewell et al., 2020; Karb et al., 2020; Ly et al., 2021). The current study showed that nine of 11 shelters had active COVID-19 outbreaks, confirming that these kind of facilities are at high risk for SARS-CoV-2 transmission. However, a clear relationship between the occupancy level of each shelter and the attack rates was not found; those rates varied from

0 to 30.8%. The characteristics of the shelters could explain such variations in attack rates, as the majority of shelters participating in the screening were "long-stay shelters" and the rotation of housed people was low, the exposure of each resident or working-staff being lower in comparison with "short-stay shelters". In fact, one study performed in Rhode Island (USA) found that homeless shelters in densely populated areas with more transient resident populations had more SARS-CV-2 incidences than shelters with stable residents. The same study recommended resident stability to reduce COVID-19 cases (Karb et al., 2020). Nevertheless, another study carried out at shelters in France also suggested the risk of collective housing for SARS-CoV-2 transmission and found a SARS-CoV-2 attack rate of 7% (Ly et al., 2021). Interestingly, the current study described how the occupation of some included shelters reached or even surpassed the shelter capacity, according to data provided by UNHCR. Considering that COVID-19 outbreaks were found at most of the shelters, recurrent screening for SARS-CoV-2 infection should be implemented.

Regarding the gender approach of this study, most of women included in this study were victims of gender-based violence, which increased during the strict lockdown implemented in the country. In addition to this, gender inequalities have deepened during COVID-19, which is reflected not only in the increase in gender-based violence, but also in worst posttraumatic stress effects after lockdown, or a deeper aftermath from SARS-CoV-2 infection (Silveira Campos et al., 2020; Ayittey et al., 2021). Studies like the current one have been recommended to prevent a deeper impact of the COVID-19 pandemic and guarantee health rights for women victims of gender-based violence (Ayittey et al., 2021).

The main limitation of this study is the fact that sampling took place only at one time point during the highest peak of the pandemic in Ecuador. Additional screening months after the first one would be useful to assess the dynamics of the infection in such humanitarian settings and implement control strategies to prevent the spread; however, a lack of funds did not allow this project to continue. Further prevention strategies in shelters for women and children could include: opportune case reports, mass testing for control of existing cases, proper isolation of affected residents wherever the infrastructure allows it as new residents are accepted, and testing for routine control of staff who are highly exposed (PAHO et al., 2020).

In conclusion, this study constitutes an important report to show the epidemiological situation of COVID-19 in humanitarian shelters in Latin America. Moreover, the findings endorse the importance of mass testing in the prevention of COVID-19 outbreaks, and the importance of closing the breach in SARS-CoV-2 testing, making them accessible to disadvantaged sectors of society.

Ethical approval and consent to participate

Written consent was obtained for all the individuals included in the surveillance. The study is included on a project that was approved by IRB from Universidad de Las Américas.

Consent for publication

Not applicable.

Availability of supporting data

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

All authors contributed to data collection and analysis. MAGB and PVJ wrote the manuscript.

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6.3. Comunicaciones cortas

6.3.1. Distribución equitativa de las pruebas de SARS-CoV-2.

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La pandemia de la enfermedad por coronavirus 2019 creó una gran demanda de laboratorio y más de 300 pruebas comerciales de amplificación de ácido nucleico para el diagnóstico de SARS-CoV-2 estuvieron disponibles durante el primer año del brote. Algunas de estas pruebas recibieron autorización de uso de emergencia de la Administración de Drogas y Alimentos de los EE.UU o se incluyeron en la Lista de uso de emergencia de la OMS. Para otros, sin embargo, la información sobre su desempeño clínico era escasa y no contaban con autorización de uso de emergencia en el país de producción.

Debido a la urgencia causada por el nuevo virus, las agencias reguladoras adoptaron protocolos regulatorios más flexibles para la autorización de uso de emergencia de los kits de amplificación de ácido nucleico del SARS-CoV-2. La mayoría de los kits se desarrollan por empresas de países de altos ingresos. Por lo tanto, muchos países de bajos y medianos ingresos han tenido que importar estos kits de diagnóstico. Los fabricantes dieron a los países de altos ingresos acceso prioritario a kits de buena calidad; por lo tanto, algunas empresas que venden kits que no tienen autorización de uso de emergencia en el país de origen y que no están incluidas en la lista de uso de emergencia de la OMS tienen como objetivo mercados con acceso inadecuado a pruebas de calidad.

Por lo tanto, los kits de calidad subóptima fabricados en países de ingresos altos se han distribuido en países de ingresos bajos y medianos durante la pandemia. De nuestra experiencia en Ecuador, no se requiere una evaluación experimental local del desempeño clínico antes de usar los ensayos en pacientes para la detección de SARS-CoV-2. Por lo tanto, el Ministerio de Salud probablemente esté subestimando la morbilidad y mortalidad por COVID-19 en todo el país.

Ademas se pueden aprender lecciones importantes para enfrentar futuras pandemias con respecto a las capacidades de prueba. Por ejemplo, las autoridades de salud pública de los países de ingresos bajos y medianos podrían haberse basado en la investigación realizada por muchas instituciones y universidades para evaluar el rendimiento de los ensayos de detección del SARS-CoV-2 durante la pandemia.

Ignorar que la evaluación inadecuada del desempeño de estos kits es un problema en muchos países podría causar morbilidad y mortalidad innecesarias en esos países y en países de altos ingresos, debido a casos importados que podrían conducir a nuevos brotes. Finalmente, llamamos a las organizaciones internacionales de salud pública a actuar para garantizar un comercio justo de pruebas de SARS-CoV-2, basado en estándares de calidad universales y sin sesgo de ingresos de los países.

Equitable distribution of SARS-CoV-2 tests

Miguel Angel Garcia-Bereguiain,[a] Alfredo Bruno,[b] Diana Morales-Jadan[a] & Jorge E Vidal[c]

Since its outbreak in 2019, the coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has challenged public health systems worldwide.[1] In a matter of weeks, demand for clinical microbiology diagnosis based on molecular tools increased. The gold standard for the detection of SARS-CoV-2 is the reverse-transcription quantitative polymerase chain reaction (RT-qPCR), although other nucleic acid amplification tests, such as RT-loop-mediated isothermal amplification, are available. Several available RT-qPCR assays (with different sets of primers and probes), developed by public institutions such as the Centers for Disease Control and Prevention in the United States of America or the Charité Hospital in Germany, were endorsed by the World Health Organization (WHO) in the early stages of the pandemic.[2,3] The pandemic created a huge laboratory demand and more than 300 commercial nucleic acid amplification tests for SARS-CoV-2 diagnosis became available during the first year of the outbreak. Some of these tests were given emergency use authorization by the US Food and Drug Administration or were included in WHO's Emergency Use Listing. For others, however, information regarding their clinical performance was scarce and they did not have emergency use authorization in the country of production.[4–10] Due to the urgency caused by the novel virus, regulatory agencies adopted more flexible regulatory protocols for the emergency use authorization of SARS-CoV-2 nucleic acid amplification kits.

Most of the kits are developed by companies from high-income countries. Therefore, many low- and middle-income countries have had to import these diagnostic kits. While some of those assays have obtained emergency use authorization by reputed federal agencies in the countries of manufacture or are included in WHO's emergency use list, others have been denied local emergency use authorization. In Ecuador, manufacturers of kits not receiving authorization have not disclosed the reasons for the denial.[4,6,7,9] Because of the high demand for COVID-19 diagnosis, all countries experienced supply shortages, whether of sample collection swabs or nucleic acid amplification kits. Manufacturers gave high-income countries priority access to good quality kits; therefore, some companies selling kits that do not have emergency use authorization at country of origin and are not included in WHO's emergency use list have targeted markets with inadequate access to quality tests. By taking over the market with those low-quality kits and creating dependency on these kits through extended contracts, manufacturers compromised the access of many of these countries to high-quality COVID-19 diagnostic tools.[6] Some kits of suboptimal quality manufactured in high-income countries have therefore been distributed in low- and middle-income countries during the pandemic. This practice is unethical because SARS-CoV-2 diagnosis kits should have the same quality standards in all countries.

In many countries lacking local technology for the detection of SARS-CoV-2 during the global supply shortage of diagnostic kits, public health authorities exempted companies marketing these kits in their countries from proving they had clinical use authorization in the country of production.[4,6,7] These kits might have given false negative testing results, leading to inadequate control of the outbreak. From our experience in Ecuador, no local experimental evaluation of the clinical performance is required before using the assays on patients for the detection of SARS-CoV-2.[6] Therefore, the health ministry is probably underestimating the countrywide COVID-19 morbidity and mortality.

Two years into the COVID-19 pandemic, important lessons can be learnt to face future pandemics regarding testing capabilities. For instance, public health authorities in low- and middle-income countries could have relied on research conducted by many institutions and universities to assess the performance of SARS-CoV-2 detection assays during the pandemic.[11–13] Although the government agency responsible for clinical use authorization in Ecuador lacks funding to carry out proper clinical performance evaluations, Ecuadorian universities and research centres conducted research on clinical performance evaluations on the kits that were available for clinical use in the country. The evaluations showed worrisome differences in clinical performance among commercial kits, with several kits not reaching a reliable sensitivity for an accurate COVID-19 diagnosis. Those kits that were shown to have poor clinical performance lack emergency use authorization at their country of production and/or are not included in WHO's emergency use list.[4–10]

Based on these findings, we propose that public health authorities in countries lacking appropriate policies to endorse emergency use authorization for diagnostic tests implement stricter policies. At the minimum, only those kits having emergency use authorization in the country where the manufacturer is headquartered should be allowed, and choice of kits should not be based on price, but on quality. Doing so is particularly relevant for countries such as Ecuador, where local public health authorities do not perform experimental evaluations to grant emergency use authorization to market SARS-CoV-2 nucleic acid amplification kits. Adopting such policies should be encouraged, developed and implemented in-country.

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Ignoring that inadequate performance assessment of these kits is an issue in many countries could cause unnecessary morbidity and mortality in those countries and in high-income countries, because of imported cases that could lead to new outbreaks.

The COVID-19 pandemic is one of the most serious public health threats of the last decades and will only be contained through a global health approach. The same is true for future pandemics. This global health approach means that all countries should have diagnostic quality and testing capacities. Ensuring these capacities are available and equally distributed is a human right – that of health – as well as the only way to fight infectious disease outbreaks. This conclusion should be one of the lessons learnt from the COVID-19 pandemic.

We urge public health authorities in low- and middle-income countries to review their protocols for the emergency use authorization of SARS-CoV-2 nucleic acid amplification kits, and revoke authorizations given to under-performing diagnostic kits. We also express our concern to companies exporting low-quality products to low- and middle-income countries and encourage researchers in these countries to contribute with clinical performance evaluation of such detection assays to ensure independent quality control. Finally, we call international public health organizations to act to ensure a fair trade of SARS-CoV-2 tests, based on universal quality standards and without country-income bias. ■

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6.3.2. *Ecuador como modelo de calidad de prueba de COVID-19 en Latinoamérica: Alta carga viral de SARS-CoV-2 en viajeros que llegan a España con test de COVID-19 negativo previo a la salida.*

Publicado en: Journal of travel medicine, 2022**.** High SARS-CoV-2 viral load in travellers arriving in Spain with a negative COVID-19 test prior to departure: Ecuador as a model for COVID-19 testing quality in Latin America. D.O.I: https://doi.org/10.1093/JTM/TAAC120

Leemos con interés el artículo de Molero-Salinas et al. sobre la enfermedad denominada COVID-19 sobre viajejos falsos negativos que llegan a España en 2021. Este estudio desafía la eficacia de la prueba del síndrome respiratorio agudo severo coronavirus 2 (SARS-CoV-2) antes de la salida del vuelo para controlar la propagación de COVID-19. Los autores informan 196 pasajeros falsos negativos de COVID-19 con un valor PCR Ct promedio de 20.3, lo que significa una carga viral alta. Nos parece llamativo que el 90,8% de esos falsos negativos procedían de países latinoamericanos. Sin embargo, quisimos resaltar aquí el hecho de que no se detectaron casos falsos negativos en viajeros que llegan desde Ecuador. La falta de falsos negativos de Ecuador contrasta fuertemente con los casos detectados de Colombia (con 114 casos) y Perú (con 12 casos) que concentraron el 64,3% del total de falsos negativos. Esto es particularmente interesante ya que esos tres países tienen una población migrante extensa viviendo en España en 2021 (297729 colombianos; 126868 ecuatorianos y 112042 peruanos) y una proporción significativa de los pasajeros que vuelan a España proviene de esos países.

Nuestro objetivo es indicar la explicación más plausible de la buena calidad de las pruebas de COVID-19 que evitó casos de falsos negativos entre los ecuatorianos que viajaron a España de abril a junio de 2021. Ecuador, Colombia y Perú son países de ingresos medios-bajos (LMIC) con fuertes similitudes en capacidades tecnológicas para el diagnóstico de laboratorio.

Entonces, planteamos la hipótesis de que las razones de que los certificados de COVID-19 de los viajeros ecuatorianos fueron más confiables debido a la dificultad de falsificar certificados de prueba en Ecuador y la disponibilidad de estas pruebas a tarifas asequibles para los ecuatorianos.

Para abril de 2021, la EMoH fijó un precio máximo de 45,08 USD para una prueba PCR de SARS-CoV-2, lo que representa el 11,3% del salario mínimo (400 USD/mes) en Ecuador. Durante el mismo período, el costo de una prueba PCR en Colombia era de aproximadamente 50 USD y 60 USD en Perú, valores cercanos al 25% del salario mínimo mensual en esos países. La lección aprendida de estos hechos es que una política de salud pública dedicada a hacer que las pruebas de COVID-19 sean asequibles evitaría que los certificados falsos sean usados. En conclusión, al hacer que las pruebas de COVID-19 sean asequibles y al mejorar una evaluación proactiva de certificados de prueba utilizando estrategias simples como el escaneo de códigos QR con un teléfono celular en los aeropuertos de salida, incluso los LMIC como Ecuador podrían contribuir a contener la propagación de COVID-19.

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High SARS-CoV-2 viral load in travellers arriving in Spain with a negative COVID-19 test prior to departure: Ecuador as a model for COVID-19 testing quality in Latin America

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We read with interest the paper by Molero-Salinas *et al*. on coronavirus disease of 2019 (COVID-19) false negative travellers arriving to Spain in 2021.[1] This study challenges the effectiveness of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing prior to flight departure to control COVID-19 spread. The authors report 196 COVID-19 false negative passengers with an average PCR Ct value of 20.3, meaning a high viral load.

It seems striking that 90.8% of those false negative cases came from Latin American countries. However, we wanted to highlight here the fact that no false positive cases were detected in travellers arriving from Ecuador. The lack of false positive cases from Ecuador strongly contrasts with the cases detected from Colombia (with 114 cases) and Peru (with 12 cases) that accounted for 64.3% of the total false negative tests. This is particularly interesting since those three countries have an extensive migrant population living in Spain in 2021 (297 729 Colombians; 126 868 Ecuadorians and 112 042 Peruvians) and a significant proportion of the passengers flying to Spain comes from those countries.[2]

Molero-Salinas *et al*. suggested two explanations for the false negative tests observed among those travellers: (i) low sensitivity or lack of validity of the test implemented in the country of origin; (ii) fake test certificates.[1] Nevertheless, we aim to described the most plausible explanation for the good quality of COVID-19 testing that prevented false negative cases among the Ecuadorians travelling to Spain from April to June 2021.

Ecuador, Colombia and Peru are low-middle income countries (LMICs) with strong similarities in technological capabilities for laboratory diagnosis. Moreover, diagnostics supplies from transnational companies are usually the same within this region.[3–5] Although low sensitivity SARS-CoV-2 PCR kits have been distributed in LMICs, this has been a similar challenge for those countries.[3,5] So, we hypothesized that the reasons Ecuadorian travellers' COVID-19 certificates were more reliable is because the difficulty to fake test certificates in Ecuador and the availability of these test at affordable fees for Ecuadorians.

The Ecuadorian Ministry of Health (EMoH) strictly regulated the COVID-19 test certificate format, including QR codes for laboratory identification and even direct access to laboratory results, allowing easy screenings for fake certificates at the departure airports, or even calling to the laboratory to confirm the test results during the airport check in for travellers to the Galapagos Islands.

By April 2021, the EMoH set a maximum price of 45.08 USD for a SARS-CoV-2 PCR test, representing 11.3% of the minimum wage (400 USD/month) in Ecuador. During the same period, the cost of a PCR test in Colombia was approximately 50 USD and 60 USD in Peru, values close to 25% of the minimum monthly wage in those countries. The lesson learnt from these facts is that a public health policy devoted to make COVID-19 testing affordable would prevent fake certificates to travel.

In conclusion, by making COVID-19 testing affordable and improving a proactive test certificate screening using simple strategies like QR code scanning with a cell phone at departure airports, even LMICs like Ecuador could contribute to contain COVID-19 spread.

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Conflict of interest

All the authors have declared no conflicts of interest.

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7. DISCUSIÓN

7.1. Estudios de validación de métodos alternativos y evaluación de kits comerciales para el diagnóstico de SARS-CoV-2 por RT-qPCR.

La necesidad de estudios extensos y multicéntricos para una evaluación precisa del rendimiento clínico de kits comerciales, su fabricación y el conocimiento de nuevas estrategias como paso previo a la qPCR es de prioridad en nuestro contexto. Por esta razón, varias opciones para superar los desafíos que la pandemia ha ido generando a su paso son: a) Reducción de costos de las pruebas al suprimir la extracción de ARN con kits comerciales; b) Evitar la escasez de suministro de kits de extracción de ARN y; c) Acelerar el procesamiento de muestras, ya que los métodos de extracción de ARN son demorados y las demandas de pruebas en escenarios de pandemias son extremadamente altas. Junto con la necesidad de realizar trabajo conjunto entre universidades y empresas privadas, sobre todo por el déficit de infraestructura tecnológica.

7.1.1. Desarrollo de protocolos para reducir costos en el diagnóstico por RT-qPCR de SARS-CoV-2.

Es importante destacar que, durante la pandemia, el grupo de investigación de la Universidad de Las Américas ha ido desarrollando estudios conforme las necesidades fueron surgiendo, teniendo claros ejemplos como son la validación de los insumos para la toma de muestra nasofaríngea, como son el uso de hisopos de plástico con punta de algodón, conociéndose que hasta ese momento solo eran permitidos los de fibra sintética. Este estudio permitió buscar alternativas de calidad a la gran demanda de pruebas diagnósticas [16]. Al igual, se evaluó un método de detección de SARS-CoV-2 por choque térmico sin extracción de ARN para los genes N y E [12], y la recolección de muestras de extractos de ARN para acelerar el diagnóstico de *SARS-CoV-2 con el kit CDC FDA EUA RT-qPCR* [11].

- *Validación y desarrollo analitico varias enzimas para RT-qPCR usadas para la detección de SARS-CoV-2.*

Se han realizado ensayos triplex de RT-PCR en tiempo real de un paso en 165 muestras clínicas. El protocolo CDC + TaqMan Fast Virus 1-Step Master Mix fue la metodología de referencia, obteniendo así resultados de sensibilidad del protocolo Charite/Berlin + TaqMan Fast Virus del 87,7% (IC 95% = 79,56 - 93,64), del CDC + Tib Molbiol Protocolo mix del 61,54% (IC 95% = 44,62 - 76,64) con una $p<0,001$ y coeficiente kappa de Cohen de 0,60 y el protocolo Charite/Berlin + Tib Molbiol Mix del 71,79% (IC 95% = 55,1-85), $p<0,005$ y coeficiente kappa de 0,71.

Existen variaciones en el uso de mezcla maestra en relación a los Ct > 35, lo que indica que los pacientes que están iniciando la enfermedad o saliendo de ella pueden no ser detectados según el uso de una u otra enzima. En nuestro caso concreto, existe una pérdida de sensibilidad en el protocolo estándar CDC al utilizar la enzima Tib-Molbiol MM, pasando su valor del 100% al 61,54%, esto dificulta la prueba diagnóstica, ya que se observa que con Ct ≥26 existen detecciones erróneas. Estos son los resultados que se han encontrado en este

estudio de enzimas, hay que indicar que su publicacion sera posterior a la sustentación de la tesis, pero es importante incorporarlo y demostrar que la mezcla maestra utilizada puede afectar la capacidad para detectar muestras clínicas positivas de bajo nivel; sin embargo, a la vez se demuestra que el rendimiento general de la reacción es bueno en un amplio rango de concentraciones virales.

- *Validación del método por homogenización sin extracción previo a la RT-qPCR* [33].

En el estudio de validación del método por homogenización sin extracción previo a la RT-qPCR se avala esta metodología como una alternativa confiable a los protocolos clásicos de extracción de ARN basados en columnas. Hubo una alta sensibilidad general (88.74 %) y especificidad (97 %), pero también para valores de umbral del ciclo (Ct: cycle threshold) por debajo de 30 o cargas virales de 10^3 copias/uL de extracción de ARN (200000 copias/mL de medio de muestra), la sensibilidad fue tan alta como 97.92%. Teniendo en cuenta las cargas virales asociadas a la transmisión del SARS-CoV-2, este protocolo detectaría potencialmente a todos los individuos infecciosos [128]. Además, se ha informado que una muestra directa al protocolo de ensayo de PCR sin ningún tratamiento o extracción de ARN logra una sensibilidad del 92% [129]. Este método tiene dos inconvenientes: los individuos en la fase temprana de la infección podrían reportarse como falsos negativos debido a la sensibilidad reducida; y no podemos descartar por completo que la pequeña reducción de la especificidad pueda atribuirse a la contaminación cruzada debida al vigoroso paso de homogeneización, aunque este problema también puede ocurrir con el método estándar de extracción de ARN.

Con estos estudios, se puede resumir como se optimizaron costos y se redujo la dependencia de suministros que comenzaron a escasear durante la pandemia, a la vez, desarrollar protocolos de calidad necesarios en paises de medianos y bajos ingresos, donde la deficiencia en los sistemas de salud fue alarmante.

7.1.2. Estudios de validación de pruebas diagnóstico RT-qPCR para detección de SARS-CoV-2.

- *La calidad de las pruebas comerciales de ácido nucleico de SARSCoV-2 en Ecuador: lecciones de la pandemia de COVID-19 para avanzar en la equidad social a través de la microbiología* [14,15,18,36].

En Ecuador se evaluaron un total de once kits basados en pruebas de amplificacion de acidos nucleicos para deteccion de SARS-CoV-2, de los cuales 8 fueron realizados en la Universidad de Las Américas. Este estudio se basa en un análisis comparativo sobre el rendimiento clínico y la sensibilidad analítica de kits disponibles en Ecuador. Se puede encontrar valores de sensibilidad que oscilaron entre el 63.4 y el 100 %, en comparación con el estándar de oro, y un límite de detección que osciló entre 500 y 100000 copias/mL. Generando una preocupación por el desempeño analítico presentado, conociendo que cinco kits arrojaron una sensibilidad sustancialmente más baja.

- *Validación de 2 kits de qPCR fabricados en Sudamérica: Uruguay y Ecuador* [37].

En la validación de 2 kits de qPCR fabricados en Sudamérica con un tamaño de muestra de 119 especímenes se respaldan que "COVID-19 RT-PCR Real TM FAST (CY5) (ATGen, Uruguay) y "ECUGEN SARS-CoV-2 RT-qPCR Los kits "(UDLA-STARNEWCORP, Ecuador) tuvieron un gran desempeño clínico con valores de sensibilidad de 96,4% y 100%, respectivamente. Además, aunque se encontró una reducción de la especificidad para "COVID-19 RT-PCR Real TM FAST (CY5) (96%) y "ECUGEN SARS-CoV-2 RT-qPCR" (UDLA-Starnewcorp, Ecuador)" (94,4%) kits, en muestras con cargas virales realmente bajas en el umbral para la detección del método estándar de oro utilizado. Los dos kits usan los mismos N objetivos virales que el protocolo de los CDC que no tienen reactividad cruzada con otros virus respiratorios [30]. Por lo tanto, la especificidad de los kits estudiados podría considerarse del 100 %. Podríamos calcular el LoD para "ECUGEN SARS-CoV-2 RT-qPCR" a una carga viral realmente baja de 10 copias virales/uL de extracción de ARN (2000 copias de ARN viral/mL de muestra) que es equivalente a LoD de kits comerciales de alta calidad, similar valor para "COVID-19 RT-PCR Real TM FAST (CY5). Además, este LoD es extremadamente confiable para el diagnóstico de SARS-CoV-2 considerando las distribuciones de población de frecuencia de carga viral [130]. Teniendo en cuenta el gran rendimiento clínico y la sensibilidad analítica de las pruebas de SARS-CoV-2 diseñadas y producidas localmente, en comparación con un kit comercial de alta calidad como TaqMan 2019-nCoV Assay Kit v1 (Thermo Fisher), el estudio actual respalda el uso de estos kits como una alternativa confiable a los costosos kits comerciales importados.

- *Comparación analítica y clínica de los kits Viasure y ECUGEN CDC RT-qPCR para el diagnóstico de SARS-CoV-2, Influenza A/B y Virus Respiratorio Sincitial.*

En relación con la validación de SARS-CoV-2 en los 4 kits, se puede sustentar el buen desarrollo de los kits Viasure en comparación con el protocolo estándar CDC. La detección de SARS-CoV-2 utilizando los objetivos ORF1ab y N, presenta valores similares de sensibilidad (86,1% vs 86,04%) y especificidad del 100% en ambos casos, cabe señalar que este estudio utilizó muestras de hisopado nasal y el tamaño muestra fue mucho mayor (989 frente a 156 muestras). La nueva versión para la detección de dianas N1 y N2 de SARS-CoV-2 presenta valores de sensibilidad y especificidad en los 3 kits superiores al 95% a dos dianas, además, de contar con controles internos. Nuestros resultados coinciden con otros estudios similares tanto en el realizado con el apoyo de University Hospitals Birmingham [131] y en el Reino Unido [132]. En Nueva Zelanda, se observaron valores medios de Ct más bajos para Viasure que para Panther Fusion LDT [133]. Si bien este estudio no cuenta con un tamaño de muestra mayor para la detección de los virus influenza A, B y RSV, brinda información importante para considerar la necesidad del uso de kits de detección por PCR en tiempo real para el diagnóstico de enfermedades que clínicamente se asemejan a los síntomas en su formato multiplex.

Este estudio será publicado posteriormente a la defensa de la tesis, cabe destacar que en su totalidad refleja la importancia de la validación analítica y clínica de kits comerciales para

contar con reactivos de calidad en el diagnóstico sobre todo en países de medianos y bajos recursos, donde prima priorizar recursos.

Después de detallar estos estudios se pude concluir que es una obligación el realizar evaluaciones de todas las pruebas comerciales disponibles, previo a su uso, más aún en este contexto de pandemia por SARS-CoV-2. Además, la importancia de desarrollar kits locales de bajo costo y de calidad sobre todo en países como Ecuador.

7.1.3. Estudio de validación de pruebas de inmunocromatografía de flujo lateral para detección de SARS-CoV-2.

- *Pruebas rápidas de antígenos disponibles en Ecuador* [40].

Se evaluó el desempeño clínico de tres marcas comerciales de pruebas rápidas de antígenos para la detección de SARS-CoV-2, actualmente disponibles en varios países de América del Sur, incluido Ecuador. Solo las marcas SD-Biosensor y CerTest cumplieron con el límite de detección (LoD) de 106 copias/mL de sensibilidad analítica determinado por la OMS o el Departamento de Salud y Atención Social del Reino Unido [134,135] con un 80 % de sensibilidad para las personas sintomáticas. En cuanto a la especificidad las tres marcas mostraron un buen desempeño con valores del 100% Además, dado que la carga viral es un parámetro dinámico que puede crecer exponencialmente durante el período de incubación, nuestros resultados respaldarían el uso de CerTest o SD-Biosensor sobre Rapigen [130,136–138]. La variación de especificidad en los dos laboratorios incluidos en el estudio (Quito y Ballenita) podemos atribuirla a la reactividad cruzada con virus respiratorios circulantes en el momento de este estudio. Se ha descrito un fenómeno similar para las pruebas serológicas anti-SARS-CoV-2, particularmente en países en desarrollo y regiones tropicales [24,51]. Nuestros resultados están dentro del rango de sensibilidad y especificidad reportado para PDR-Ag, pero existen diferencias sustanciales en el desempeño clínico entre los diferentes estudios, incluso para la misma marca comercial [43,46,139].

- *Sensibilidad analítica de dos marcas de pruebas rápidas de antígenos para 6 variantes de SARS-CoV-2.*

Finalmente, se desarrolló un estudio donde se compara la sensibilidad analítica de dos marcas comerciales de pruebas rápidas de antígenos disponibles en Ecuador frente a variantes del SARS-CoV-2 que circulan en el país en el período comprendido entre 2021 y 2022: alfa, gamma, delta, lambda, mu y ómicron. Se incluyeron en el estudio un total de 328 hisopos nasofaríngeos, y todos ellos dieron positivo para SARS-CoV-2 por RT-qPCR. Los valores de sensibilidad observados para CerTest y SD-Biosensor resultaron ser similares al detectar cada una de las variantes, obteniendo valores superiores al 75% para las variantes alfa, gamma, delta y ómicron, mientras que lambda y mu mostraron tasas más bajas. Las variantes no detectadas muestran valores de Ct dentro del amplio rango de 13 a 29 ciclos, lo que implica que el desempeño de las pruebas rápidas de antígenos no depende de la carga viral. Las bajas tasas de sensibilidad pueden estar asociadas con la aparición de mutaciones que permiten evadir la detección de PDR-Ag, como la variante lambda C.37 con la mutación L452Q en la proteína de espiga [140], almacenamiento incorrecto de muestras que dificultan la integridad

del espécimen [141], actividad neutralizante de vacunas en diferentes cepas [142], similitud de mutaciones en diferentes variantes (es decir: gamma con alfa y beta) [143], la prevalencia local de SARS-CoV-2 y la estrategia de prueba de antígeno implementada [144]. Este estudio se enfoca en la investigación del desempeño de las pruebas de antígenos y el efecto de las mutaciones que se pueden encontrar en las variantes circulantes en el Ecuador, específicamente con una gran diferencia en mu y lambda conociendo que los PDR-Ag para SARS-CoV-2, actualmente disponibles se basan en la detección de la proteína N [145]. Este trabajo será publicado en una etapa posterior a la sustentación de la tesis, priorizando su utilidad en la validación actualizada de pruebas rápidas de diagnóstico conforme avanza la pandemia y se conocen las mutaciones que va experimentando el virus.

Estos artículos se enfocan en la importancia de validar pruebas de diagnóstico rápido por la detección de antígenos, sobre todo en el contexto de países con bajos recursos. Siendo necesario el reconocer marcas comerciales que detectan valores de sensibilidad y especificidad aceptados por las entidades reguladoras y sobre todo por la finalidad que proporcionan, velocidad y detección de una mayor cantidad de población, incluyendo pacientes asintomáticos y no hospitalizados que propagan aceleradamente el contagio de SARS-CoV-2.

7.2. Epidemiología de COVID-19 en Ecuador.

7.2.1. Diagnóstico de SARS-CoV-2 en diferentes comunidades ecuatorianas.

Ecuador es un país que no estuvo listo para la pandemia producida por SARS-CoV-2 debido a que no contaba con un sistema de salud eficiente y amplio que abarque comunidades urbanas y rurales desatendidas, bajo una misma realidad que en países sudamericanos [146,147]. Los muestreos que se realizaron en las provincias descritas posteriormente no fueron aleatorios porque el fin de esta intervención fue un diagnóstico de ayuda, con un posterior estudio retrospectivo para indicar resultados que reflejen la situación de la pandemia de COVID-19 en provincias ubicadas en las 4 regiones de Ecuador durante la primera ola, específicamente en personas asintomáticas, de las cuales no hay información epidemiológica descrita [148,149], y se confirmó una transmisión comunitaria descontrolada en la mayoría de las provincias. Se tomaron varias recomendaciones al momento de la toma de información, en la mayoría de los muestreos:

1. Cubrir la difusión de la comunidad en varios cantones de la región.
2. Visitar varios lugares dentro de cada cantón.
3. Considerar una persona por hogar.
4. Mantener que las condiciones de vida de la mayoría de la población sean similares en las comunidades visitadas.

- *Comunidades rurales pobres en la región costera de Ecuador (Manabí y Esmeraldas)* [17,65].

En Manabí se muestrearon 15 cantones con un total de 4.000 personas, la tasa de infección por SARS-CoV-2 fue del 16,13%, siendo superior al 30% en cantones como El Carmen, Portoviejo y Pedernales. Dentro de este grupo estudiado, 39 individuos tenían cargas virales de 10^8 copias virales/mL y podrían considerarse súper propagadores de SARS-CoV-2. Es importante señalar que no hubo adherencia al uso de mascarillas ni al distanciamiento social en las comunidades encuestadas.

En Esmeraldas, 1259 muestras recolectadas para la prueba de SARS-CoV-2 con una tasa general de infección por 7,71% en octubre 2020, con valores tan altos como 12,17% para el cantón Quinindé. En las pruebas de seroprevalencia general de IgG anti-SARS-CoV-2 hay un 11,68%, con valores tan altos como 24,47% para el cantón de Atacames. Además, considerando que la prueba serológica utilizada en el estudio tenía una falta de sensibilidad superior al 20% [146], el porcentaje de población expuesta al SARS-CoV-2 sería incluso superior al observado. Encontramos resultados sorprendentes al comparar comunidades rurales y urbanas debidos a las características socioeconómicas, con parámetros ligeramente superiores para los cantones rurales y relativamente aislados. Hubo 6 individuos con cargas virales en el rango de considerarse como súper propagadores, representando un llamativo 6,2% de la población infectada.

- *Comunidades indígenas (Sierra y Amazonía) [68–70].*

En provincias de la Sierra ecuatoriana la tasa promedio de infección por SARS-CoV-2 superó el 26% de los 1021 pacientes evaluados, teniendo picos de más del 50% en varias comunidades y se encontraron brotes en 14 de las 15 comunidades visitadas. Además, 20 individuos de cuatro cantones diferentes tenían cargas de súper propagadores del SARS-CoV-2, lo que representa un 7.46% de la población infectada [150].

En la región amazónica se analizó un total de 853 personas que viven en la comunidad para detectar la infección por SARS-CoV-2 mediante RT-qPCR a partir de hisopos nasofaríngeos. Se reclutaron todas las personas dispuestas a hacerse la prueba en las 21 comunidades visitadas, solo una aldea Waorani permaneció libre de COVID-19, con una tasa general de infección por SARS-CoV-2 superior al 50%, con un pico de hasta el 90% en algunas aldeas y un 6.2% de personas super propagadoras. No hubo protección relacionada con el nivel de aislamiento de estas comunidades, ya que la mayoría de ellas solo eran accesibles por barco o avión [151] y hay un reporte de visitas frecuentes a otras comunidades. Se informaron hallazgos similares en Brasil y Colombia, donde se informaron brotes de COVID-19 en las áreas de esos países incluidos dentro de la cuenca del Amazonas [152]. Este estudio se publicará posteriormente a la defensa de tesis, pero es importante destacar como nuestros hallazgos confirman la hipótesis de que las comunidades indígenas de las Américas, particularmente las aisladas como en la Amazonía, corren un alto riesgo de sufrir consecuencias devastadoras por pandemias como la del COVID-19. Como también son minorías étnicas desatendidas y en peligro, se recomiendan acciones especiales de las autoridades regionales de salud pública para garantizar su supervivencia.

- *Islas Galápagos [23,80].*

En las Islas Galápagos durante las dos evaluaciones masivas, el 9.8% de la población fue evaluado en pocas semanas con una tasa de positividad de 11.59% en comparación con el 31.15% obtenido por Ecuador. En las primeras semanas del brote de COVID-19, la prueba diaria de 100 a 200 muestras para una población de menos de 30 000 personas desde las primeras semanas del brote de COVID-19 [23] permitió mantener a esta región dentro de una vigilancia aceptable. Llamamos la atención sobre el escenario epidemiológico en la isla Floreana que con 145 individuos optó por autoaislarse de las otras islas, siendo uno de los pocos lugares del mundo que estuvieron libres de COVID-19.

Estos estudios nos han permitido conocer la epidemiologia de la mayoría de las provincias en el Ecuador debido a que no se instaló suficiente capacidad de pruebas en todo el país por parte de organizaciones públicas de salud en una población de 17 millones. Además, las condiciones impuestas por el clima y la pobreza en los entornos rurales hacen que estas comunidades estudiadas sean propensas a la propagación del SARS-CoV-2 [148,149].

Aunque existen limitaciones asociadas para calcular la carga viral basada en valores de Ct que representan todo el material genómico viral en la muestra, y la infección de cultivos celulares que se utilizan para confirmar la infectividad, existe una asociación clara entre

valores bajos de Ct (que significan valores altos de carga viral) e infectividad [153]. Esos individuos súper propagadores en todas las provincias estaban completamente asintomáticos o informaron algunos síntomas leves menores en el momento de la recolección de muestras, siendo importante trabajar con pruebas masivas y la implementación de pruebas de laboratorio en el sitio.

La pandemia de COVID-19 ha afectado a las poblaciones rurales e indígenas desatendidas más que a otras debido al acceso limitado al agua, el saneamiento deficiente de los hogares, la falta de información en lenguas indígenas y el acceso limitado al sistema de salud [154,155]. En el caso específico de los pueblos indígenas, hay estudios relacionados a las tasas de infección en Brasil y Ecuador [70]. Al igual que varios informes, que han avalado que la transmisión comunitaria ha estado ocurriendo, afectando a personas asintomáticas o sintomáticas leves que viven en la comunidad, ya sea de grupos urbanos de riesgo laboral o de comunidades rurales e indígenas no solo en Ecuador sino también en Brasil o Colombia [156].

Toda la vigilancia epidemiológica realizada en provincias y parroquias desentendidas de Ecuador en los estudios anteriores nos indica que hubo una transmisión comunitaria por todo el país con la presencia de un gran número de supercontagiadores entre la población asintomática y ambulatoria; siendo una excepción Galápagos, donde la estrategia de una detección masiva gracias a la implementación de un laboratorio exclusivo para esta población permitió tasas de positividad bajas. Este escenario fue común durante todos estos años de la pandemia, por lo que hacemos un llamado de atención al gobierno y a las políticas de salud vigentes.

7.1.4. Diagnóstico de SARS-CoV-2 en grupos de riesgo

La pandemia de COVID-19 genero una inestabilidad en toda la población, pero principalmente nuestro afán fue determinar si hubo grupos de riesgo laboral que estaban más expuestos a contraer la enfermedad debido al contacto cotidiano con personas infectadas, ya que, el total de casos y muertes por COVID-19 en todo el mundo se había monitoreado y notificado regularmente. Esto es particularmente preocupante para los trabajadores esenciales, como los policías o el ejército, personal de salud y trabajadores de funerarias ya que los brotes graves de COVID-19 entre estos grupos pueden comprometer su capacidad para trabajar por el bien común, al igual que un estudio en personas que viven en albergues y son víctimas de violencia de género debido a su condición de vivir en lugares en hacinamiento.

Bajo este escenario, se deben reforzar las medidas adicionales para evitar la propagación del COVID-19, como la sanitización de los equipos utilizados en el trabajo, la limpieza de la ropa y artículos personales antes de regresar a casa, y el uso de EPP desechables adecuados, como lo recomienda la CDC de EE.UU. o INTERPOL [157] junto con la detección masiva de personas asintomáticas. Teniendo como preámbulo que las superficies más contaminadas se consideran los dispensadores de desinfectante para manos (100 %), equipos médicos (50 %) y pantallas táctiles de equipos médicos (50%) [158]. Junto con la evidencia de que el contagio

con SARS-CoV-2 en el aire es posiblemente más alto en la primera semana de la enfermedad [159].

- *Albergues para mujeres víctimas de violencia* [81].

En los albergues para mujeres víctimas de violencia se reportaron brotes de COVID-19 en 9 de los 11 albergues visitados, la alta tasa general de ataque del SARS-CoV-2 fue del 12.65 %. Una amplia variedad de estudios sostiene la idea de que los entornos domésticos hacinados constituyen un sitio importante para los brotes de COVID-19 [160] pero en este estudio no se encontró una relación clara entre el nivel de ocupación de cada albergue y las tasas de ataque; las tasas varían de 0 a 30.8%. La posible razón se debe a que la mayoría de los refugios eran de larga estancia, es decir, con residentes estables. Comparándolo con otro estudio realizado en Francia se sugirió el riesgo de las viviendas colectivas para la transmisión del SARS-CoV-2 y encontró una tasa de ataque de SARS CoV-2 del 7% [161].

- *Unidades policiales y fuerzas especiales* [85].

En las unidades policiales y fuerzas especiales los resultados confirman brotes graves de COVID-19 con una alta tasa de infección del 12.3 % en Ecuador. Además, un 10% de la población infectada presentó cargas virales altas considerados como super propagadores. Este estudio es el único disponible en Ecuador sobre riesgo laboral a partir de datos oficiales del Ministerio de Salud, nuestro grupo de investigación encontró que el personal policial y militar era casi dos veces más propenso a morir por COVID-19 que la población general dentro del mismo rango de edad [62]. Al compararlo con datos de Perú, donde 524 muertes de policías se registraron hasta mayo de 2021, en la India se reportaron 428 muertes de policías en agosto de 2020 y en los Estados Unidos, más de 2000 agentes del orden ya habían dado positivo por SARS-CoV-2 en abril de 2020 [162–164].

- *Trabajadores de funerarias* [94].

En el personal de las funerarias en la ciudad de Quito, la tasa de infección fue hasta de un 20.7% por SARS-CoV-2 en junio de 2020. Aunque no se pudo concluir el riesgo ocupacional relacionado con el manejo de cadáveres no se podría descartar el riesgo laboral porque durante la pandemia tuvieron el contacto no solo con fómites infectados sino con personas que fueron familiares de víctimas de COVID-19 [165]. Un estudio en trabajadores de la morgue de Qatar informó una alta tasa de infección por SARS-CoV-2 de hasta el 14.9 % informando que la trasmisión comunitaria es más que el riesgo ocupacional para los trabajadores [166], muy similar a los resultados obtenidos en Ecuador. Entre las limitaciones tenemos una deficiencia en el rastreo de contactos para los individuos positivos y el reducido número de trabajadores de la funeraria que completo la encuesta ocupacional.

- *Personal de hospitales*

Entre mayo y octubre de 2020, se recolectaron hisopados nasofaríngeos en el lugar de trabajo de 1671 personal hospitalario principalmente asintomático de 9 hospitales para llevar a cabo el diagnóstico de SARS-CoV-2 (1243 profesionales de la salud y 428 personal complementario) por RT-qPCR en 4 ciudades: Quito 66 %, Santo Domingo 16%, Chone 13% y Riobamba 5%.

Durante el estudio realizado en 9 hospitales se pueden conocer la tasa general de ataque por SARS-CoV-2 del 12% en el personal de salud. Aunque también se obtuvieron porcentajes para profesiones de la salud y el personal administrativo y obrero de cada uno de los hospitales estudiados, valores que se encuentran entre 5 – 35% para el primer grupo y del 11 – 50% para el segundo. Se han informado resultados similares en personal sanitario de todo el mundo: 11.1 % (213/1911) en España [167], 12.6 % (41/326) en Bélgica [168] y 11.33% (164/1447) en Italia [169], teniendo un tamaño de muestra variable. Entre las limitaciones esta que nos todas las instituciones tienen acceso a la detección de SARS-CoV-2 por RT-qPCR por lo tanto el tamaño de la muestra no cubre la mayoría de los hospitales ecuatorianos. Este trabajo será posteriormente publicado enfocándose principalmente en indicar que el personal hospitalario es un grupo de alto riesgo de infección por SARS-CoV-2. Las pruebas periódicas de SARS-CoV-2 deberían ser obligatorias para este grupo, ya que incluso se pueden detectar superpropagadores de SARS-CoV-2 asintomáticos.

Este apartado se enfoca en los principales grupos de riesgo durante la pandemia. Hubo tasas de ataque elevadas para SARS-CoV-2 en personal asintomático, permitiendo hacer así un llamado de atención al Ministerio de Salud y a las diferentes entidades en priorizar las medidas de protección para su personal. Protegiendo servicios que durante cualquier pandemia deben estar activos y expuestos a cualquier eventualidad para mantener a la población segura y cubierta sus necesidades.

7.2.3. Diagnóstico de SARS-CoV-2 considerando variable de altitud [107].

Ecuador es un país que posee una gran variación de población ubicada en lugares a diferentes alturas, por lo que, es un ejemplar a investigar sobre la relación de altitud con la enfermedad de COVID-19, teniendo como razones de peso algunas investigaciones que relacionan las condiciones de vivir a cierta altura entre estas tenemos: hipoxia hipobárica sobre la viabilidad del virus [111] junto con que la susceptibilidad del virus a la temperatura puede verse afectada por el clima [170]. Aunque las temperaturas frías se han asociado con un mayor riesgo de desarrollar algunas enfermedades respiratorias como la influenza u otros virus respiratorios, está claro que el clima tiene un factor más importante que influye en el comportamiento de las personas [171]. Un estudio del 2021 uso un método matemático SEIR, para indicar que la trasmisión viral es menor a gran altura y sus hallazgos respaldan la hipótesis de disminución de la virulencia del SARS-CoV-2 en tierra altas [110].

Nuestros resultados exploratorios no encontraron diferencias significativas entre las personas que viven a gran altura y las que viven en lugares de baja altitud en términos de carga viral de SARS-CoV-2, los cuales fueron realizados en 62 ciudades ubicadas en altitudes bajas, moderadas, altas y muy altas de Ecuador entre muestras de hisopado nasofaríngeo provenientes de una cohorte de 4929 pacientes con una prueba RT-qPCR positiva para SARS-CoV-2.

7.2.4. Determinación de coinfecciones con otros patógenos respiratorios en individuos infectados por SARS-CoV-2.

Nuestro interés radica en conocer como pacientes positivos por SARS-CoV-2 en dos áreas de los hospitales estudiados: pacientes ambulatorios y hospitalizados en la unidad de cuidados intensivos, presentaban colonización y/o coinfección con bacterias y virus. A nivel de literatura mundial hay varios estudios con pacientes ambulatorios, pero hay dificultades en comparaciones con aquellos ingresados en UCI, cabe indicar que la perdida de información en la historia clínica fue una de las limitaciones del estudio.

- *Coinfección en pacientes del área de cuidados intensivos*

En este estudio describimos la prevalencia del SARS-CoV-2 en 24 pacientes del área de cuidados intensivos (UCI) del Hospital Pablo Arturo Suárez de la ciudad de Quito, Ecuador durante el año 2021. Durante el análisis se compararon las fechas de cultivos microbiológicos positivos y PCR SARS-CoV-2 positivo, y se examinó cada caso para evaluar si la infección podía considerarse adquirida en la comunidad o nosocomial (más de 7 días).

Nuestro estudio indica que 13 de los 16 pacientes analizados presentan infecciones del torrente sanguíneo en la UCI (tasa de ataque 81,25%) durante un período de 5 a 46 días en la unidad de cuidados; este valor está por encima de los valores estimados entre el 25-50% de las infecciones adquiridas en la unidad de cuidados intensivos, durante un período de 15-30 días de riesgo [172]. Además, en nuestro estudio la tasa de coinfección fue del 28,5% (IC 95% 12,89 - 53,11), siendo muy superior a las encontradas en Guangzhou y Seattle-EEUU, del 2,1% (42/1996) [173] y la ausencia de virus respiratorios [174], respectivamente. Nuestro estudio es muy similar al realizado en Francia (26/92; 28%) [175] y cercano al 44,4% en Cuba [176].

En este estudio se pueden encontrar pacientes con uno o más patógenos asociados a la coinfección por SARS-CoV-2, observándose que *Klebsiella pneumoniae* (33,3%) fue el principal microorganismo identificado seguido de *Staphylococcus aureus* y *Enterococcus faecalis* con cuatro casos cada uno, información proporcionada por la historia clínica, mientras que por diagnóstico molecular *S. pneumoniae* (19,04%) fue la más frecuente. Esta información está relacionada con el estudio que indica que los patógenos más comunes en pacientes con neumonía grave por SARSCoV-2 son *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae* y Enterobacteriaceae [175]. Se destaca que la atención a nivel de UCI se debe a la presencia de una coinfección respiratoria viral, por lo que se sugiere prevenir el diagnóstico perdido y la transmisión del SARS-CoV-2 [177].

Durante el colapso del sistema de salud en la pandemia de COVID-19, se estima que podría obviarse la necesidad de un diagnóstico por otro patógeno productor de bacteriemia, siendo la principal causa de muerte. Los pacientes con COVID tienen una mayor proporción de bacteriemia nosocomial (95.5%) que los pacientes sin COVID (30.5%) debido a la tasa de contaminación [178], a pesar de que no tuvimos pacientes ingresados en UCI negativos para COVID, nuestros resultados indican que el 33% de los pacientes con COVID fallecieron.

- *Coinfección en pacientes ambulatorios*

Nuestro estudio descriptivo identificó bajas tasas de coinfección bacteriana y viral confirmadas por estudios moleculares en pacientes con COVID-19. Este estudio permite conocer el porcentaje de prevalencia de coinfección y colonización de 12 patógenos virales y 1 bacteriano con el SARS-CoV-2. Nuestro estudio es el primero en demostrar la magnitud de la expansión del SARS-CoV-2 junto a patógenos que pueden o no estar estacionarios durante los años 2020-2021 en la ciudad de Quito-Ecuador.

Algunos reportes muestran que la tasa de coinfección del SARS-CoV-2 con otros virus no es alta [179] como en las coinfecciones bacterianas, específicamente neumococo, siendo la más típica en la neumonía adquirida. La razón de esto puede ser que la ventaja competitiva juega un papel importante en la coinfección.

Nuestro estudio indicó que la prevalencia de coinfección por SARS-CoV-2 con patógenos respiratorios fue Influenza A con 4.4 % (IC 95 %: 1.2 – 7.6), seguida de *S. pneumoniae* 3.7 % (IC 95 %: 0,76-6,64), Respiratorio virus sincitial con 3.1% (IC 95%: 0,4 – 5,8) y finalmente con 1.2% a Rhinovirus y HCoV-NL63 en una población de 5 a 62 años, lo que sugiere que es importante que los pacientes con infección respiratoria se realicen tamizaje para SARS-CoV-2 y otros patógenos respiratorios virales y bacterianos. Entre las razones que pueden contribuir a una mayor prevalencia de Influenza A, puede deberse a que el virus aumenta significativamente la infectividad del SARS-CoV-2 al impulsar la entrada viral en las células y elevar la carga viral, lo que causa daño pulmonar, esta afirmación es apoyado por estudios llevados a cabo en células cultivadas, así como en ratones [180].

La influenza A es uno de los patógenos respiratorios coinfectados más comunes e importantes y se han encontrado varios estudios de detección. De ahí la importancia de su vacunación en épocas estacionales de julio a agosto de cada año. La prevalencia en la mayoría de los estudios oscila entre 0.08 - 4.9% de Influenza A en pacientes positivos de COVID-19. Entre estos estudios tenemos tasas de positividad del 4,9 % (4/89) entre marzo y abril de 2020 en Luisiana [181], 4,35 % (5/115) en China [182], 0,9% (1/116) en el norte de California [123] y 0,08% en Nueva York, incluso no se encontró coinfección en Wuhan – China [183]. Por el contrario, en Qingdao, China, hasta el 60 % para la influenza A y el 53 % para la influenza B, tenga en cuenta que los tamaños de muestra fueron considerablemente pequeños en este estudio en particular [184].

Aunque la incidencia es baja, recomendamos realizar pruebas de SARS-COV-2 y otros patógenos respiratorios comunes para garantizar un diagnóstico preciso, un tratamiento rápido del paciente y un aislamiento adecuado.

Estos estudios hacen énfasis a las posibles coinfecciones, colonizaciones o superinfecciones que se pueden presentar en pacientes hospitalizados como ambulatorios con SARS-CoV-2, lo que nos permite conocer la importancia de un correcto diagnóstico y el hecho de implementar pruebas de diagnóstico para otros patógenos, focalizando tratamientos y evitando disipar recursos en sistemas de salud que carecen de los mismos.

8. CONCLUSIÓN

Mediante los estudios recopilados en esta tesis de doctorado, se puede destacar el uso de la bioquímica clínica aplicada al diagnóstico microbiológico de económico y de calidad para una detección de patógenos emergentes, que permite maximizar el número de pruebas realizadas como estrategia fundamental para la contención de la COVID-19 o futuras pandemias. De forma más detallada, estas son las principales conclusiones de esta tesis doctoral:

1. Es posible la optimización de costos y la reducción a la dependencia de suministros para el diagnóstico molecular de SARS-CoV-2, que comenzaron a escasear durante la pandemia de COVID-19. Se deben por tanto validar y aplicar protocolos más económicos y versátiles que mantengan una alta sensibilidad y especificidad, como los reportados en esta tesis.
2. Es fundamental realizar evaluaciones experimentales de todas las pruebas comerciales disponibles de diagnóstico molecular de SARS-CoV-2 previo a su uso.
3. Las pruebas rápidas de antígeno son una herramienta de punto de atención rápida, económica y confiable para la detección de SARS-CoV-2 con alta sensibilidad en pacientes potencialmente contagiosos.
4. Existió una transmisión comunitaria descontrolada de COVID-19 desde los primeros meses de la pandemia en 2020 en población asintomática no hospitalizada en Ecuador, incluyendo la presencia de supercontagiadores; esta situación pone de relieve la importancia de incluir muestreos poblacionales masivos de población no hospitalizada como parte de la estrategia de contención de COVID-19 (los cuales no siempre fueron realizados en contextos de países en desarrollo como Ecuador ante la escasez de recursos).
5. Las elevadas tasas de positivas para SARS-CoV-2 en grupos de riesgo ocupacional durante los primeros meses de la pandemia COVID-19 reportados en esta tesis doctoral ponen de relieve la necesidad de haber implementado un esquema frecuente de pruebas preventivas de SARS-CoV-2 en dichos grupos.

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