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Genotipificación del virus de Hepatitis B y Hepatitis C en el Ecuador hasta el 2019.

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RESUMEN

El virus de la hepatitis B (HBV) y el virus de la hepatitis C (HCV) representan un grave problema de salud pública a nivel mundial, pueden causar tanto infección aguda como crónica en humanos. En el caso de HBV la infección crónica tiene una prevalencia de 257-291 millones de personas a nivel mundial y es responsable de 890 mil muertes anuales, una tendencia que parece estar en aumento, según reportó la organización mundial de la salud en el 2019. De igual forma el HCV presenta 1.5 millones de casos nuevos anualmente y tiene una mortalidad anual del 290 mil personas a nivel mundial.

El HBV, capaz de infectar a humanos, se agrupa en 10 diferentes genotipos (A-J) subdivididos en varios subgenotipos, las características genéticas de HCV despliegan una excepcional diversidad genética que lo divide en 8 genotipos y 67 subgenotipos. El tratamiento, prevalencia de infección crónica, desarrollo de hepatocarcinoma (HCC) y mortalidad dependen de una gran cantidad de factores virales, incluyendo la determinación del genotipo y subgenotipo que poseen gran importancia.

En Ecuador existe un vacío en la información con respecto a los genotipos y subgenotipos de HBV y HCV que circulan en el territorio; en tal contexto, el propósito de este trabajo radica en proveer una primera aproximación de los genotipos y subgenotipos de HBV y HCV que se encuentran circulando en territorio ecuatoriano. Como resultados se obtuvo que tras la amplificación de 44 muestras de pacientes infectados con HBV distribuidos en la Costa, Sierra y región Amazónica del Ecuador, dos genotipos fueron encontrados: el genotipo F (n=42, 95,5%) y genotipo E (n=2, 4,5%). De los 42 pacientes con genotipo F los subgenotipos encontrados se distribuyeron de la siguiente forma: F3 (n=35, 83.3%), F4 (n=5, 11.9%), F2 (n=1, 2.4% y F1b (n=1, 2,4%). De la amplificación de las 11 muestras obtenidas de pacientes infectados con HCV en territorio ecuatoriano los genotipos y subgenotipos se presentaron de

la siguiente manera: 6 muestras correspondientes a subgenotipo 2b (n=6 54,5%) subgenotipo 1a (n=2 18,2%) subgenotipo 4d (n=2 18.2%) y subgenotipo 1b (n=1 9,1%). Los resultados obtenidos en estos estudios representan la primera aproximación epidemiológica de la distribución de genotipos y subgenotipos de HBV y HCV en Ecuador, contribuyendo en un mejor tratamiento a los pacientes afectados con estas patologías. Se alienta al Ministerio de Salud Pública del Ecuador (MSP) al desarrollo de mejores estrategias para la identificación y seguimiento de estos pacientes.

Palabras clave: HVB, HCV, genotipo, subgenotipo, Ecuador

ABSTRACT

The Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are a public health problem worldwide, in humans they cause acute or chronic infection. Chronic HBV infection has a prevalence of 257-291 million people worldwide and is responsible for 890 thousand deaths per year, a trend that seems to be on the rise as reported by the World Health Organization in 2019. Similarly, HCV is responsible for 1.5 million new infections, and around 290 thousand deaths worldwide.

The HBV, capable of infecting humans, is clustered in 10 different genotypes (A-J) subdivided in several subgenotypes. HCV genetic characteristics display a remarkable genetic diversity which divides HCV into 8 genotypes and 67 subgenotypes. The treatment, the probability of chronic HBV/HCV infection, the presence of hepatocarcinoma (HCC) and mortality depends on numerous virologic factors, including the genotypes and subgenotypes that are particularly important.

In Ecuador, there is a lack of information regarding the HBV and HCV genotypes and subgenotypes circulating; thus, the aim of this study is to provide a first approximation of the HBV and HCV genotypes and subgenotypes circulating in Ecuador. After the amplification of 44 samples from patients distributed in the Andean, the Coast, and the Amazon regions of Ecuador, two mayor genotypes were found: genotype F (n=42, 95,5%) and genotype E (n=2, 4,5%). The identified subgenotypes from genotype F samples were: F3 (n=35, 83,3%), F4 (n=5, 11,9%), F2 (n=1, 2,4%) and F1b (n=1, 2,4%). After the amplification of 11 samples from patients with HCV infection living in the Ecuadorian territory, the genotypes of HCV obtained were distributed as follow: subgenotype 2b (n=6 54,5%), subgenotype 1a (n=2 18,2%), subgenotype 4d (n=2 18,2%) and subgenotype 1b (n= 9,1%). The results of the present study represent the first epidemiological approach to genotype distribution in the Ecuadorian

territory, contributing to the improvement of patient management with HBV infection. We encourage the development of better strategies by the Healthcare Ministry of Ecuador (MSP) for the identification and tracking of HBV patients.

Keywords: HBV, HCV, genotype, subgenotype, Ecuador

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PARTE 1: INTRODUCCIÓN GENERAL

VIRUS DE LA HEPATITIS B

El virus de la hepatitis B (HBV), perteneciente a la familia *Hepadnaviridae*, del género Orthohepnavirus, es un virus envuelto, su DNA es de doble cadena parcial, su genoma es de 3200 pares de bases y se compone de una cadena de sentido negativo que contine cuatro marcos de lectura abiertos (ORF): P, X, preC/C y preS/S. El ORF preS/S es el sector más variable del genoma del HBV. Se encuentra entre los nucleótidos 2848-155, siendo el responsable de codificar tres diferentes glicoproteínas de la envoltura: antígeno de superficie S, M y L, usadas para la determinación de la presencia del virus de la hepatitis B y sus genotipos (Li et a.l, 2020 & Glebe et al., 2021). Este virus que puede causar infección aguda o crónica, representa un grave problema de salud pública al presentarse como infección crónica en 257-291 millones de personas a nivel global (Lim et al., 2020). Según reportes de la OMS la prevalencia de este virus es del 3.5% de la población general, en ese mismo año 890 mil personas fallecieron por causas relacionadas a la infección por HBV (OMS, 2019).

El virus de la hepatitis B se encuentra distribuido en 10 genotipos (A-J), que presentan una distribución geográfica muy marcada, demostrada por análisis de epidemiología molecular (Kyaw et al., 2020; Lin et al., 2017). La división por genotipos está dada por una diferencia de 7.5% en las secuencias de nucleótidos, y posteriormente subdividida en subgenotipos por una divergencia mayor al 4% en la secuencias de nucleótidos (Velkov et al., 2018). La patogénesis, curso natural de la infección por el virus de la hepatitis B y supervivencia de los pacientes, se encuentra influenciada por varios factores virales, pero probablemente el más importante y ampliamente estudiado es el rol de los genotipos de HBV (Lin et al., 2017). El diagnóstico basado en técnicas moleculares para determinar el genoma de HBV ha probado ser revolucionario y de gran utilidad en el manejo clínico de personas que cursan con infección

por este virus. En países desarrollados, la determinación del genotipo de HBV en pacientes positivos para presencia del virus de la hepatitis B en pruebas serológicas es una práctica estandarizada (Roman et al., 2014).

DISTRUBUCIÓN GEOGRÁFICA DE GENOTIPOS DE HBV

Se ha mostrado que el 96% de las infecciones crónicas de HBV a nivel mundial están causadas por los genotipos A al E (Velkov et al., 2018). En el análisis de literatura realizada por Velkov y colaboradores se pudo obtener información interesante que muestra como existe una interrelación entre los genotipos de HBV que afectan a la población y las barreras geográficas como océanos y desiertos. Un llamativo ejemplo de esto es como los genotipos que predominan en el África subsahariana son el A y E, mientras que el norte del mismo continente donde el comportamiento de se asemeja más al oeste del continente asiático, el genotipo predominante es el D (Reed & Tishkoff, 2006). En la mayor parte de Europa, los genotipos A y D fueron los más prevalentes, con predominio de A en el noroeste del continente; mientras que en Europa oriental, se presentaba el genotipo D como el más frecuente. Del mismo modo, en el centro, sur y norte del Asia, así como en el norte del África el genotipo D es el más prevalente. En Gran Bretaña, Dinamarca, Australia y Oceanía se vió una supremacía por parte de los genotipos B y C. Esta alta prevalencia de genotipos B y C también se mostró reportada en países del sureste asiático, incluyendo a China.

En América Latina se pudo observar alta prevalencia de genotipos F, H y G, los cuales son poco comunes en otras partes del mundo. En la gran mayoría de países de America Latina, el genotipo F es el más prevalente, sin embargo, hay países que no se comportan igual, como Brasil donde los genotipos A y D dominan y México donde los genotipos G y H fueron encontrados en números importantes. América del Norte y el Caribe muestran un comportamiento diferente al de América Latina; en América del Norte los genotipos A, B, C y

D son los más prevalentes, mientras que en el Caribe el genotipo A es el más frecuente y, en menor medida, el genotipo D. Esta información sacada del análisis realizado por Velkov y colaboradores muestra que la distribución de genotipos de HBV tiene patrones similares entre países y regiones relacionadas, siendo a su vez muy diferentes al comparar distintas partes del mundo (Velkov et al., 2018).

DISTRIBUCIÓN DE GENOTIPOS DE HBV EN AMÉRICA DEL SUR

Durante los primeros años del uso de epidemiología molecular para hepatitis B, en la región se mostró al genotipo F como el más prevalente. Esto presenta una estrecha relación con la ancestría de indigenas americanos en países como: Brasil (Mello et al., 2013), Paraguay (Mojsiejczuk et al., 2019), Argentina (Piñeiro y Leone et al., 2008), Bolivia (Huy et al., 2008), Perú (Ramírez-Soto et al., 2018), Colombia (Cortes-Mancera et al., 2011) y Venezuela (Jaspe et al., 2014). En América del Sur se han reportado genotipos adicionales que guardan relación con movimientos migratorios a los diferentes países de la región. Dentro de Argentina y Brasil, en ciudades con alta incidencia de inmigración europea, se reporta presencia del subgenotipo A2 y genotipo D (Spitz et al., 2019; Barros et al., 2014). De igual forma, los genotipos B y C se han relacionado con inmigración de población asiática a la región (Khan et al., 2008). En Colombia, en poblaciones afroamericanas se ha reportado la presencia del genotipo E (Alvarado-Mora, 2010). El genotipo G ha sido reportado en Brasil, Argentina y Venezuela (Bottechia, 2008); finalmente el genotipo H ha sido reportado en Argentina (Flichman et al., 2009).

El Genotipo F del HBV se encuentra presente principalmente en la región americana. Esta es una cepa autóctona en comunidades nativo-americanas que ha sido encontrada desde grupos poblacionales en Alaska hasta el centro y sur de América, siendo el genotipo más prevalente en la mayoría de países de América Latina (Mojsiejczuk et al., 2020). En todos los países de

Sudamérica que tienen estudios de epidemiología molecular para HBV se ha demostrado la presencia del genotipo F. En Venezuela se encontró en población Yucpa y Warao, en las cuales el análisis mostró al subgentipo F3 y F2 como prevalentes (Nakano et al., 2001; Blanco et al., 2018). En Colombia, un estudio en pacientes con patología hepática en etapa terminal reveló que todos los pacientes con etiología de infección de HBV correspondieron al genotipo F (Cortes-Mancera et al., 2011). En cuanto a Perú, un estudio realizado en Abancay, una zona endémica, se pudo observar la presencia de genotipo F, específicamente del subgenotipo F1b, como el más prevalente en esa parte del país y un caso aislado de genotipo F4 entre las 14 muestras estudiadas (Ramírez-Soto et al., 2018). En un trabajo de Huy y colaboradores (2008) sobre la caracterización de seis secuencias completas de HBV en Bolivia, se determinó que las 6 muestras pertenecían al subgenotipo F4. En otro trabajo en el mismo territorio se observó que12 de 22 muestras estudiadas fueron del genotipo F, con la particularidad que las muestras pertenecientes a otros genotipos eran de pacientes inmigrantes nipones (Khan et al., 2008). En Chile el 67.5% de los pacientes estudiados por Di Lello y colaboradores (2009) fueron HBV/F. En la región norte de Argentina el 91,7% de las muestras pertenecían a HBV/F, lo que contrasta con la zona metropolitana del país donde el genotipo F no es el más prevalente con tan solo un 30% de los casos (Piñeiro y Leone et al., 2008). Otros estudios relacionados mostraron que el genotipo F deja de ser el más predominante en lugares como Buenos Aires y Misiones (Pezzano et al., 2011; Mojsiejczuk et al., 2016). En Uruguay el estudio de nueve secuencias mostró que el 55% eran genotipo F (López et al., 2015), mientras que en Paraguay el 81.7% de 60 donadores de sangre estudiados que presentaron infección por HBV formaban parte del grupo con genotipo F (Mojsiejczuk et al., 2019). Los resultados de estudios realizados en Brasil han demostrado que el genotipo F no es el más prevalente a nivel país con aproximadamente un 13% de los casos, siendo el subgenotipo F2a el más común con un 92.5% dentro de los casos con infección por HBV genotipo F (Mello et al., 2007; Mello et al., 2013).

Análisis espacio temporales han sugerido que el genotipo D ha llegado a Sudamérica en procesos migratorios masivos de población europea durante el siglo XIX e inicios del siglo XX (Spitz et al., 2019). Los países donde se ha reportado el genotipo D son principalmente: Chile, Argentina y Brasil (Spitz et al., 2019), sin embargo, también se pudo observar en Amerindios Japreira en Venezuela (Blanco et al., 2018). En Chile, el genotipo D presenta una prevalencia de 12.5% para el año 2009 y no ha existido una actualización de epidemiología molecular al respecto (Di Lello et al., 2009). En Argentina, con excepción de la región norte del país, donde el genotipo D solo fue de 2 de 48 muestras estudiadas, existe una alta prevalencia para el genotipo D de entre el 42.5% al 31.7% en zonas metropolitanas como Buenos Aires, a un 65.4% en Misiones, donde se encontró los subgenotipos D3 y D2 principalmente (Piñeiro y Leone et al., 2008; Pezzano et al., 2011; Mojsiejczuk, 2016). En Brasil, de sus cinco regiones geográficas, el genotipo D se encontró en todas ellas, siendo la más común en dos de estas: región central con una prevalencia de 47.6% y región sur con una prevalencia de 84.2%. Y es el segundo más prevalente a nivel nacional (38.5%) (Mello et al., 2007; Spitz et al., 2015, Spitz et al., 2019)

Los subgenotipos A1,A3 y A4 del genotipo A y el genotipo E se encuentran principalmente distribuidos en territorio Africano. En el caso del genotipo E se localiza en la región oeste de África subsahariana (Lago et al., 2014; Velkov et al., 2018). La hipótesis más manejada es que estos genotipos fueron introducidos a América del Sur entre los siglos XVI y XIX con el comercio de esclavos africanos durante la colonización. En Brasil, el genotipo A presenta alta prevalencia en la población general con un 48.5% de los casos, donde la gran mayoría pertenecen al subgenotipo A1 (Mello et al., 2007). Mientras que el genotipo E solo ha sido reportado por primera y única ocasión, en América de Sur, en Colombia en pacientes afrodesendientes (Alvarado-Mora et al., 2010).

VIRUS DE LA HEPATITIS C

El virus de la hepatitis C (HCV), perteneciente a la familia *Flaviridae*, del género Hepacivirus, es un virus envuelto, su RNA es de cadena simple de sentido positivo, su genoma es de 9600 nucleótidos (Tellinghuisen & Rice, 2004). El gen NS5B es una polimerasa de RNA dependiente de RNA localizado entre los nucleótidos 7597 y 9413. Este gen presenta dos características muy importantes: 1) es el más variable del genoma, por lo cual, es utilizado para la determinación del genotipo viral y 2) carece de capacidad de corrección de errores en transcripción. Esta falta de capacidad de corrección genera una importante diversidad genética en el virus, que divide al HCV en 8 genotipos y 67 subgenotipos (Qiu et al., 2002; Tsukiyama-Kohara & Kohara, 2018; Tong et al., 2015). Los genotipos se diferencian uno al otro en un 30% al 35% de los nucleótidos de la secuencia, y los subgenotipos se diferencian en menos del 15% de los nucleótidos (Anderson et al., 2003).

De todos los pacientes infectados con el virus de la hepatitis C entre el 15% al 45% logran estar libres de infección en 6 meses sin recibir ningún tratamiento. Mientras que el porcentaje restante de los pacientes puede presentar enfermedad crónica que puede desencadenar en cirrosis y carcinoma hepatocelular (OMS, 2021; Petruzziello et al.,2016). El compromiso en la salud de la población general del HCV se muestra en aproximadamente 1.5 millones de nuevas infecciones por año y 290 mil muertes reportadas en el año 2019 (OMS, 2021). Dentro de las estrategias planteadas por por la OMS para combatir esta patología se destaca la intención de reducir el 90% de los nuevos casos de hepatitis viral, reducción en el 60% de la mortalidad relacionada a hepatitis viral y que por lo menos el 80% de los pacientes con HCV crónico se encuentre con un plan claro y establecido de tratamiento (OMS, 2021; Blach, 2017). Mostrando la importancia de la identificación, seguimiento y tratamiento adecuado de los pacientes que cursan con esta infección viral. En territorio ecuatoriano no se han realizado estudios de epidemiología molecular sobre HCV, sin embargo, existe información de los genotipos

circulantes en países vecinos. El genotipo 1 es el más prevalente en Colombia (88.5%) y Perú (86%) (Petruzziello et al., 2016). En Venezuela, por otro lado, se ha reportado una alta prevalencia (34.4%) de genotipo 2 en los casos estudiados (Petruzziello et al., 2016)

DISTRIBUCIÓN GEOGRÁFICA DE GENOTIPOS DE HCV

En el año 2016 Petruzziello y colaboradores realizan una actualización de la literatura disponible correspondiente a distribución y circulación de genotipos del virus de la hepatisis C a nivel global. A continuación se resumirá la información obtenida en ese trabajo.

El continente Africano muestra una distribución de genotipos de HCV altamente variable en las cuatro macro áreas en las que generalmente se lo divide: Norte (área Sahariana), central, este, oeste y central. Por ejemplo, el genotipo 4 (G4) en África central presenta una prevalencia de 82.9%, mientras que en el centro del oeste del continente G4 solo representa el 0.6%. En el sur del África, G5 se encuentra en el 35.7% de los casos, mientras que en el oeste y centro los pacientes con este genotipo son casi inexistentes; para el G2, África occidental presenta una prevalencia de 62.9%, mientras que en el sur solo es del 1.2%. El genotipo que no presenta mayor variabilidad entre áreas geográficas en el continente africano es G1 con 36.2%, 31.4% y 25.5% para el este, sur y oeste respectivamente, pero sí con una diferencia en el África central con 12.3% de los casos.

En América, dividida en tres macro áreas, Norte América, Caribe y América Latina, los genotipos más representativos en el continente en general son: G1 con el 74.5% de los casos, G3 con el 10.6%, G2 con el 10.2% y G4 con el 1.7%. Sin embargo, nuevamente se ve alta variabilidad en la distribución de los genotipos en las macro áreas. G1 se presenta en 83% de los casos en el caribe y 66.3% en América del norte. G2 presenta una distribución variable dentro de América Latina, en países de América central una prevalencia de 21.6%, mientras

que en la zona de los andes la prevalencia es de 2% de los casos. En Norte América el 15.7% de los pacientes presenta G3, mientras que en el Caribe este genotipo solo representa el 2.1%.

Asia está dividida en cinco zonas geográficas: Pacífico, central, este, sur y sudeste. En la población general de pacientes con HCV en el continente, G1 es predominante con un 46.6% de los casos, G3 22.4%, G2 18.6% y G6 el 7%. Nuevamente, la variabilidad en la presencia de los genotipos entre macro áreas es alta: fluctúa entre 70.4% en Asia central y 15.5% en el sudeste para el genotipo1; 39.7% en área del pacífico y 1.9% en el sudeste para G2; en el sudeste el 66.7% y 0.4% en el pacífico para G3; 30.8% en el sudeste y 0.5% en el pacífico y sur de Asia para G6.

En Australia y Nueva Zelanda el genotipo más común es G1 con un 55% de los casos, seguido por G3 con un 36% y G2 con un 6.6%. Finalmente, en Europa dividida en tres áreas principales, Europa central, Europa occidental y Europa oriental, la variabilidad en la distribución de los genotipos en las macro áreas no parece ser de importancia: siendo G1, con un 64.4%, el más predominante, seguido por G3 con el 25.% de los casos, G2 con 5.5% y G4 con el 3.7% (Petruzziello et al., 2016).

PARTE 2: ARTÍCULOS CIENTÍFICOS

Completing the information gap, Hepatitis B genotypes in Ecuador 2020.

Introduction:

The Hepatitis B virus (HBV) belongs to the *Hepadnaviridae* family, classified in the genus Orthohepnavirus. In humans, the infection of HBV causes acute or chronic illness. Chronic HBV infection could lead to cirrhosis and finally to hepatocarcinoma (HCC) (Li et al., 2020). Chronic HBV infection has a prevalence of 257 - 291 million people worldwide (Lim et al., 2020). In 2015, the World Health Organization (WHO) reported a global prevalence of 3.5% of the general population for HBV; by the same year, HBV infection was responsible for 890 thousand deaths, and it seems that this trend is on the rise (WHO, 2019). The HBV capable of infecting humans is clustered in 10 different genotypes (A-J) (Kyaw et al., 2020) that have remarkable differences in their geographical distribution as shown by molecular epidemiologic analysis (Lin & Kao, 2017). HBV is an enveloped, with a 3,200 bp genome, it is a partially double stranded DNA virus, composed of a coding negative sense strand that contains four overlapping open reading frames (ORF): P, X, preC/C, and preS/S (Li et al., 2020; Lin et al 2017). preS/S ORF (nt 2848-155) is responsible for encoding three different enveloped glycoproteins: S, M and L surface antigen (HBsAg) used for diagnosis and identification of the Hepatitis B Virus and its genotypes (Li et al., 2020; Glebe et al., 2021). In the pathogenesis and natural course of HBV infection, several virological factors influence the disease progression and survival in patients with chronic HBV infection, among them the genotypes.

The role of genotypes has been vastly studied; patients with genotypes C, D and F carry a higher risk of cirrhosis and hepatocarcinoma development when compared to genotypes A and B. Mutations present in some genotypes are associated with immune scape to immunoglobulins or vaccine immunity (Lin et al., 2017). In most Northwestern European countries, genotypes are A and D are the most prevalent. In Eastern Europe, North and Western Africa, North, South and Central Asia, genotype D is the most frequently found. Genotype C is the most frequent in East Asia and Oceania, followed by genotype B. In East Africa, Middle Africa, and South Africa (Sub-Saharan Africa), genotype E and genotype A are predominating. In North America genotype A, B, C and D were the most prevalent. Latin American countries show a higher prevalence of genotype F, but there are some differences between countries; for example, Brazil exhibits a higher prevalence of genotypes A and D whereas in Mexico genotype H is more prevalent (Velkov et al., 2018). In South America, some countries, such as Ecuador, do not have information available regarding the distribution of HBV genotypes. The aim of this study is to provide a first approximation of the HBV genotypes and subgenotypes circulating in Ecuador.

Methods:

The present study was designed as a cross-sectional and descriptive using the ministry of Ecuador (MSP) database of Hepatitis B (HBV), in which all HBV infected patients that have been identified are reported, for the present study patients identified from 2017 to 2019 were taken into consideration. After calculating an aleatory representative sample, patients were recruited and a team for sample collection went to the provinces were patients confirmed their participation. Samples of a total of 44 individuals with previous diagnosis of HBV were

obtained; these patients were identified as carriers of chronic HBV and candidates for antiviral treatment. The inclusion criteria were: 1) had a previous diagnosis of the studied pathology and be part of the MSP database 2) patient must have adult age (over 18 years old) 3) Patient must sign a written informed consent provided by the investigation team. A single whole blood sample was taken using an appropriate venipuncture technique. Samples were later centrifuged, the plasma was placed in Eppendorf tubes and transported in a nitrogen tank that was later stored at -80 °C.

Ethical statement

The study approval was carried out by the Human Research Research Ethics Committee, CEISH-USFQ San Francisco de Quito University and the Public Health Ministry of Ecuador with the code 2018-241IN.

Primers selection

Primers for preS/S gene amplification of the HBV genome were selected from the work of Kyaw, et al (Kyaw et al., 2020). The primer sets used were the following: PF 5′ TTG GAC TCA CAA GGT GGG AA 3′; PR 5′ GTC CAC CAC GAG TCT AGA CTCT 3′; NF 5′ TCA TTT TGT GGG TCA CCA TAT 3′; NR 5′ C 3′.

HBV DNA extraction

The MagMAXTM Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher Scientific Inc) was used for DNA extraction following the manufacturer's instructions.

HBV preS gene fragment amplification by PCR

HBV preS gene fragments of each sample were amplified with nested PCR using the primer sets (PF-PR and NF-NR), PlatiniumTM II Hot-Start Green PCR Master Mix was used following ThermoFisher Scientific Inc instructions. The amplification mixture contained: 2µl of the HBV extracted DNA, 0.4µl of each of the primers, 4µl of enhancer, 3.2µl of water. The PCR was performed under the following conditions: 94 °C for 5 min; 94 °C for 30s, 51 °C for 30s, 72°C for 45s for 30 cycles; 72°C for 10min. Samples that did not show amplification the first time were subjected to another entire cycle. The resultant amplicons were amplified by nested PCR with the second set of primers (NF-NR), the nested PCR was performed under the following conditions: 94 °C for 5 min; 94 °C for 30s, 54 °C for 30s, 72°C for 45s for 35 cycles; 72°C for 10min. Then, the amplicons were quantified using the EPOCH Microplate Spectrophotometer Reader. After quantification, the amplicons were sent to Macrogen, South Korea for Sanger sequencing.

Phylogenetic analysis

The obtained sequences from Macrogen were compared to identify local similarity regions between sequences using BLAST. Phylogenetic trees were built using the Neighbor-Joining method, and as an outgroup a sequence of HBV from a Woolly monkey (AFO046996), using the software Geneious prime 2022.0.1. Reference sequences added to the phylogenetic tree were obtained by searching in the NCBI GenBank using the key words (Country Name) AND ("Hepatitis B" or "HBV") AND ("complete genome"). All the included countries were from South American continental territory, which had available information about the circulating genotypes. Countries like Ecuador, Guyana, Suriname, and French Guiana did not have any information. From all the sequences obtained, only complete genome sequences were selected. Then, the sequences were downloaded in FASTA format and differentiated by color coding depending on the country of origin for their processing. On the phylogenetic trees obtained, the color differentiation was based on the HBV genotype. An additional phylogenetic tree for HBV genotype E was built using the sequences previously reported from the South American region and from sub-Saharan countries.

Results:

Distribution of the HBV samples

From 451 patients identified in the MSP data, 44 of these patients attended to MSP recruitment and met the inclusion criteria. All 44 HBV positive samples obtained were PCR positive,

amplified and were included for genotype analysis; 26 females (59.1%) and 18 males (40.9%); mean age 32 ± 16.2. The ethnic group distribution was as follows: 28 mestizo (63.6%), 8 Afro-American (18.2%), 7 indigenous (15.9%) and 1 montubio (2.3%). Finally, the geographical distribution of the samples was: Quito 36.4% (Andean region); San Lorenzo 6.8%, Rio Verde 2.3%, Esmeraldas 2.3%, Portoviejo 11.3%, Guayaquil 6.8% (Coast region); El Coca 11.3%, Loreto 2.3%, Puyo 6.8%, Yukitais 2.3%, Patuca 2.3%, Logroño 4.5%, Tiwintza 2.3%, Gualaquiza 2.3% (Amazon region) (Table. 1).

Table 1. Sociodemographic characteristic of HBV Ecuadorian patients

		n	%
Sex			
	Male	18	40.9
	Female	26	59.1
Ethnic group			
	Afro-American	8	18.2
	Indigenous	7	15.9
	Montubio	1	2.3
	Mestizo	28	63.6
City of residence			
	Quito	16	36.4
	San Lorenzo	3	6.8
	Rio Verde	1	2.3
	Esmeraldas	1	2.3
	Portoviejo	5	11.3
	Guayaquil	3	6.8
	El Coca	5	11.3
	Loreto	1	2.3
	Puyo	3	6.8
	Yukitais	1	2.3
	Patuca	1	2.3

	Logroño	2	4.5
	Tiwintza	1	2.3
	Gualaquiza	1	2.3
Age			
	18-29	16	36.4
	30-65	23	52.3
	>65	5	11.3

HBV genotypes and subgenotypes distribution

In this study, two mayor genotypes were found, genotype F (n=42, 95,5%) as the predominant circulating genotype in Ecuadorian territory and genotype E (n=2, 4.5%) as the other circulating genotype (fig. 1). From the 42 genotype F samples identified, the distribution of circulating subgenotypes was as follows: F3 (n=35, 83.3%), F4 (n=5, 11.9%), F2 (n=1, 2.4%) and F1b (n=1, 2.4%) (fig. 3). In the coast region: In Esmeraldas province 4 samples (80%) corresponded to genotype F, subgenotype F3, and 1 sample (20%) to genotype E; Guayas and Manabi exhibited a 100% of genotype F, subgenotype F3. In the Andean region, the city of Quito, Capital of Ecuador, showed the most diverse results: Genotype E n=1 sample (6.25%), subgenotype F1b n=1 (6,25%), subgenotype F3 n=12 (75%), subgenotype F4 n=2 (12.5%). In the Amazon region: In Orellana province 1 sample (16.7%) corresponded to subgenotype F2, 4 samples (66.6%) to subgenotype F3 and 1 sample (16.7%) to subgenotype F4; in Pastaza, 100% of the patients presented subgenotype F3; in Morona Santiago the subgenotypes found were F3 n=4 (66.6%) and F4 n=1 (33.4%) (fig. 2).

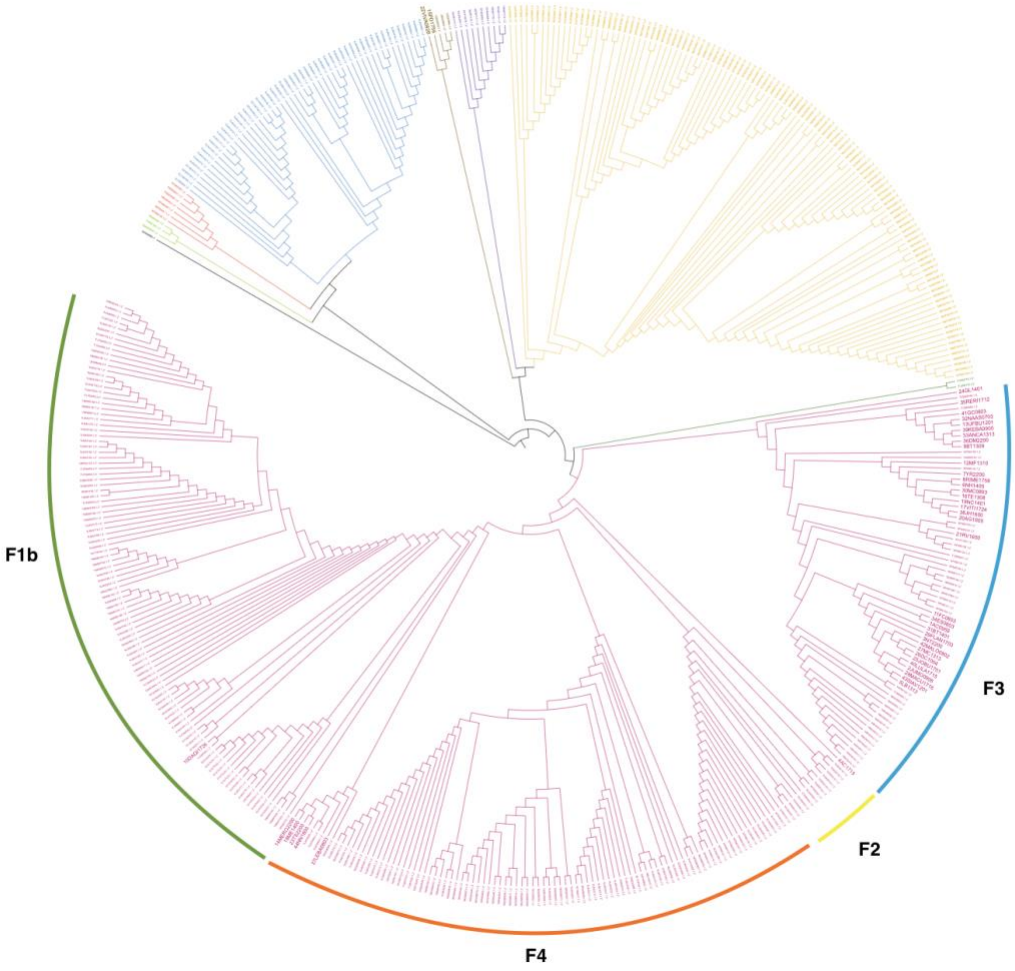


Fig. 1: Phylogenetic tree of South American HBV isolates obtained in the NCBI GenBank and Ecuadorian isolates obtained in this study. The tree was built using the Neighbor-Joining method. From all the isolates, the preS gene region was obtained from the whole genome and a sequence of HBV from a Woolly monkey (AFO046996) was used as the outgroup. The genotypes were color coded: A blue, B green, C red, D yellow, E brown, F pink, G violet, H dark green, outgroup black. Color code for subgenotypes: F1b green, F2 yellow, F3 blue, F4 orange.

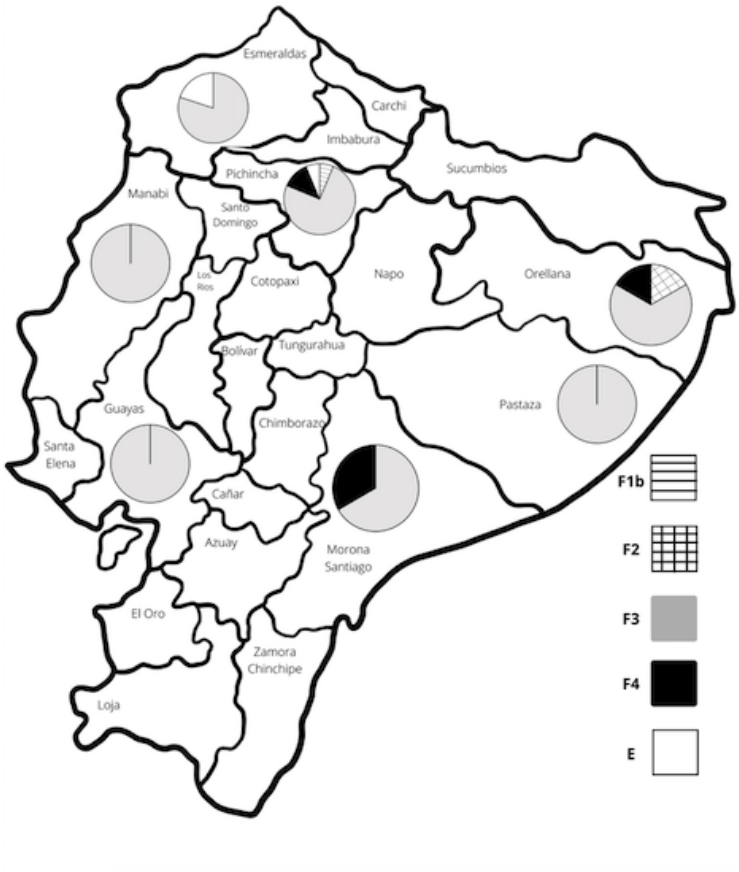


Fig. 2: Geographical distribution of circulating genotypes and subgenotypes in the Ecuadorian territory.

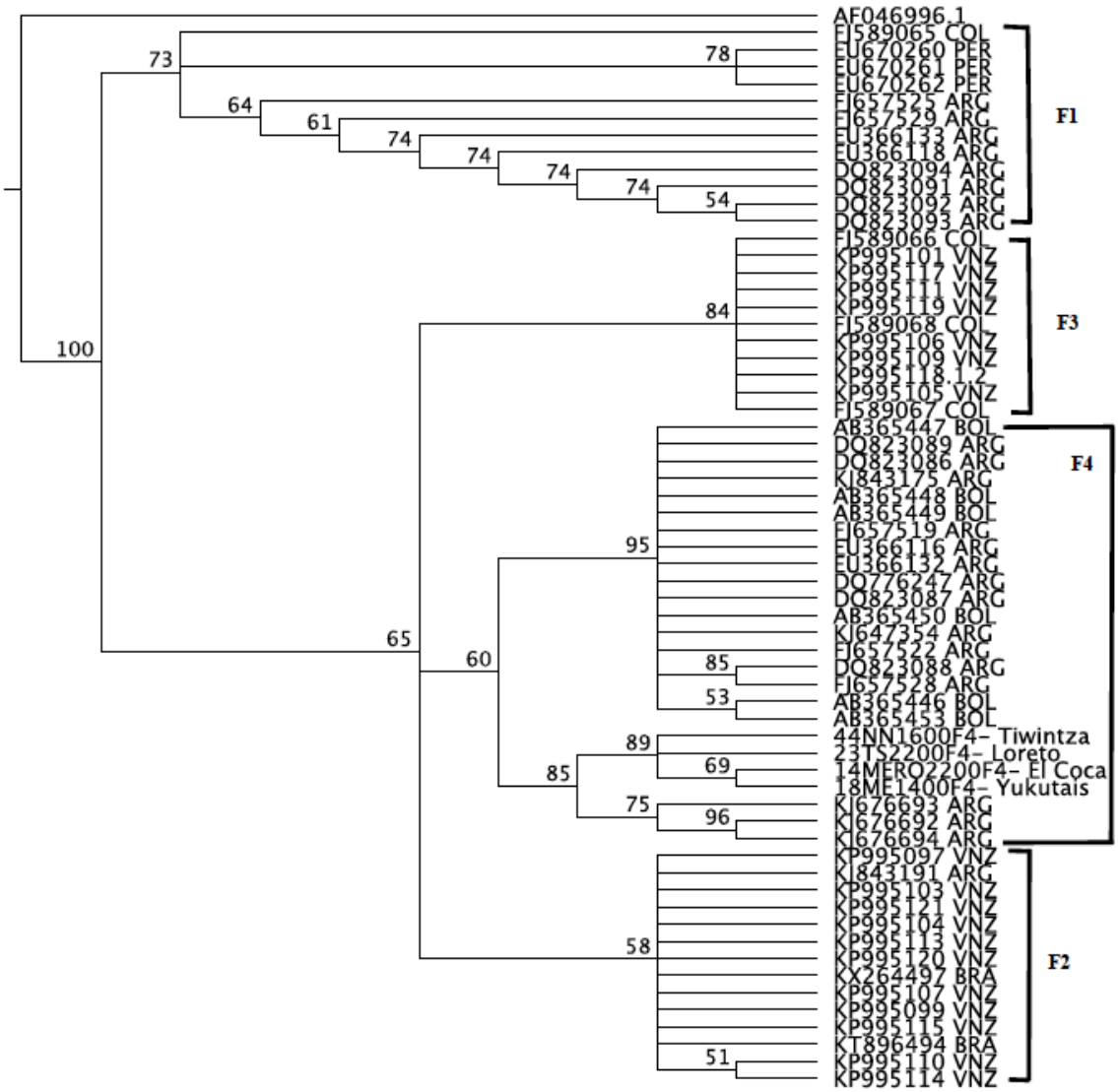


Fig. 3: Phylogenetic tree of HBV genotype F isolates obtained in the NCBI GenBank and Ecuadorian subgenotype F4 isolates obtained in this study. The tree was built using the Neighbor-Joining method. From all the isolates, the preS gene region was obtained from the whole genome and a sequence of HBV from a Woolly monkey (AFO046996) was used as the outgroup. Boot-strap values are shown for each node.

Discussion:

According to PAHO Ecuador has one of the highest prevalence of HBV cases in South America only below Peru and Colombia (PAHO, 2016), however it is the only one country in the region without genotyping studies. With this study we try to provide a national wide analysis of HBV genotypes structure.

Genotype F was identified as the most prevalent (95,5%), followed by genotype E (4.5%). These results are in agreement with Velkov et al (Velkov et al., 2018), who stated that genotype F is the most predominant in the entire South American territory, especially in Venezuela (Jaspe et al., 2014), Colombia (Devesa et al., 2008), Peru (Ramírez-Soto et al., 2018), and Bolivia (Huy et al., 2006). Amerindian population represents an important genetic contribution for the general population of South America and this statement is applicable in the Ecuadorian general population as well (Nagar et al., 2021). A strong adaptation has been demonstrated between genotype F and native American population (Mojsiejczuk et al., 2020).

Subgenotypes of genotype F reported in this study showed F3 (83.3%) as the most prevalent, and present the highest prevalence in all sample's locations, a similar prevalence has been reported in our neighbor countries: Colombia in general population and Venezuela in Amerindians as was reported by Devesa et al (Devesa et al., 2008; Blanco et al., 2018). Our subgenotype F3 sequences have a close phylogenetic relationship with F3 isolates from Venezuela (fig.1).

F1b, F2 and F4 were also found. We identified a subgenotype F1b sequence in the samples analyzed in our territory. Sequences from Argentina and Colombia had phylogenetic proximity to the F1b sequence found in our study (Ramírez-Soto et al., 2018; Sevic et al., 2017) (fig.1)

Also F1b has been reported in Peru (Ramírez-Soto et al., 2018), Alaska and southern South America (Cortes-Mancera et al., 2011; López et al., 2015).

Subgenotype F2 identified sequence of this study comes from the Amazon region, and it had a close phylogenetic relationship with F2 isolates from Venezuela. This genotype has mainly been reported in isolated native ethnic groups (Japreira, Warao, Yanomami, Piaroa and Alaskan Native population) (Devesa et al., 2008; Blanco et al., 2018, Hayashi et al., 2019) (fig.1). A strong risk to develop hepatocarcinoma associated with infection of F2 subgenotype is linked (Pujol et al., 2020).

Subgenotype F4 was the second highest prevalence (11.9%) in Ecuador. The majority of these come from the Amazon region (Yukitais n=1, Loreto n=1, Twintza n=1, El Coca n=1) and one isolate is of a patient from Quito (Ecuador´s capital). Phylogenetic analysis identified that Amazon F4 isolates were closely related to Bolivia and Argentina samples (Huy et al., 2006; Pujol et al., 2020; Pezzano et al., 2011; Barbini et al., 2013) (fig. 3). F4 subgenotype has mainly been reported in central and southern South America including Brazil, Argentina, and Bolivia (Alvarado-Mora & Rebello, 2013). F4 subgenotype infection usually has a benign disease progression (Pujol et al., 2020).

We also found genotype E in a sample from an Afro-American patient resident in Esmeraldas, and has a phylogenetic relationship with the isolates from Quibdo department of Colombia and West African countries like Namibia, Gambia, Nigeria, Cameroon among others (Olinger et al., 2006; Alvarado-Mora et al., 2010) (fig. 4).The presence of this genotype in Esmeraldas (Ecuadorian border with Colombia) supports the hypothesis postulated by Alvarado of the

introduction of this genotype during Spanish colonization related with the slave trade made between the XVI and XIX century (Alvarado-Mora et al., 2010). There is limited information about disease progression and drug resistance of HBV genotype E, nevertheless, there are reports of immunized children with breakthrough infections (Mendy et al., 2008).

In despite of 2.1 million people live with HBV infection in Latin America and the Caribbean, where there is a high prevalence of genotype F, HBV drug treatment effectiveness assays are mainly focused on genotypes prevalent in Europe and the United States, and there is a clear lack of evidence regarding genotype F drug susceptibility. There are limited studies suggesting that INF has a good outcome in patients treated who have an HBV genotype F infection (Hayashi et al., 2019; Marciano et al., 2013; Venegas et al 2015).

Furthermore, hepatocarcinoma and cirrhosis are more prevalent in patients with genotype F infection, and it has been demonstrated that subgenotypes F1b and F2 are highly susceptible to develop deathly outcomes (Hayashi et al., 2019). In our study we identified the presence of sub genotypes F2 and F4 mainly in Amazonic areas which could suggest a high prevalence of these subgenotypes in the Amerindian ethnic groups. A previous study done in Waroa population from Venezuela by Blanco et al., claimed the possibility of a displacement of the F2 subgenotype by the F3 subgenotype (Blanco et al., 2018). The presence of F2 and F4 sub genotypes in difficult accessing localities and a high prevalence of F3 in all localities included in this study, may suggest a similar phenomenon happening in the Ecuadorian territory. It should be recommended that further studies are carried out in difficult access localities in the Amazon region and the Colombian border to analyze HBV diversity and a possible displacement of the HBV subgenotypes currently circulating by subgenotype F3.

Therefore, this information justifies the development of strategies to control HBV endemic infection in Amazonic communities that have difficult access. Also, public health measures in main cities need to be applied to stop the propagation of F3 subgenotype, which can become predominant. Information related to genotyping has to be taken in consideration because of immunological vaccine escape described with F3, F4 and F1b, and reduction of efficacy of diagnostic tests in F4 and F1b (Limeres et al., 2019; Schlabe et al., 2018).

In summary, the present work completes the gap of information in South America about genotypes and subgenotypes circulating. It is noteworthy that the sample number was limited because of the global pandemic situation during sample collection. We need to emphasize the importance of further studies to get more information on the HBV genotypes and subgenotypes in our country.

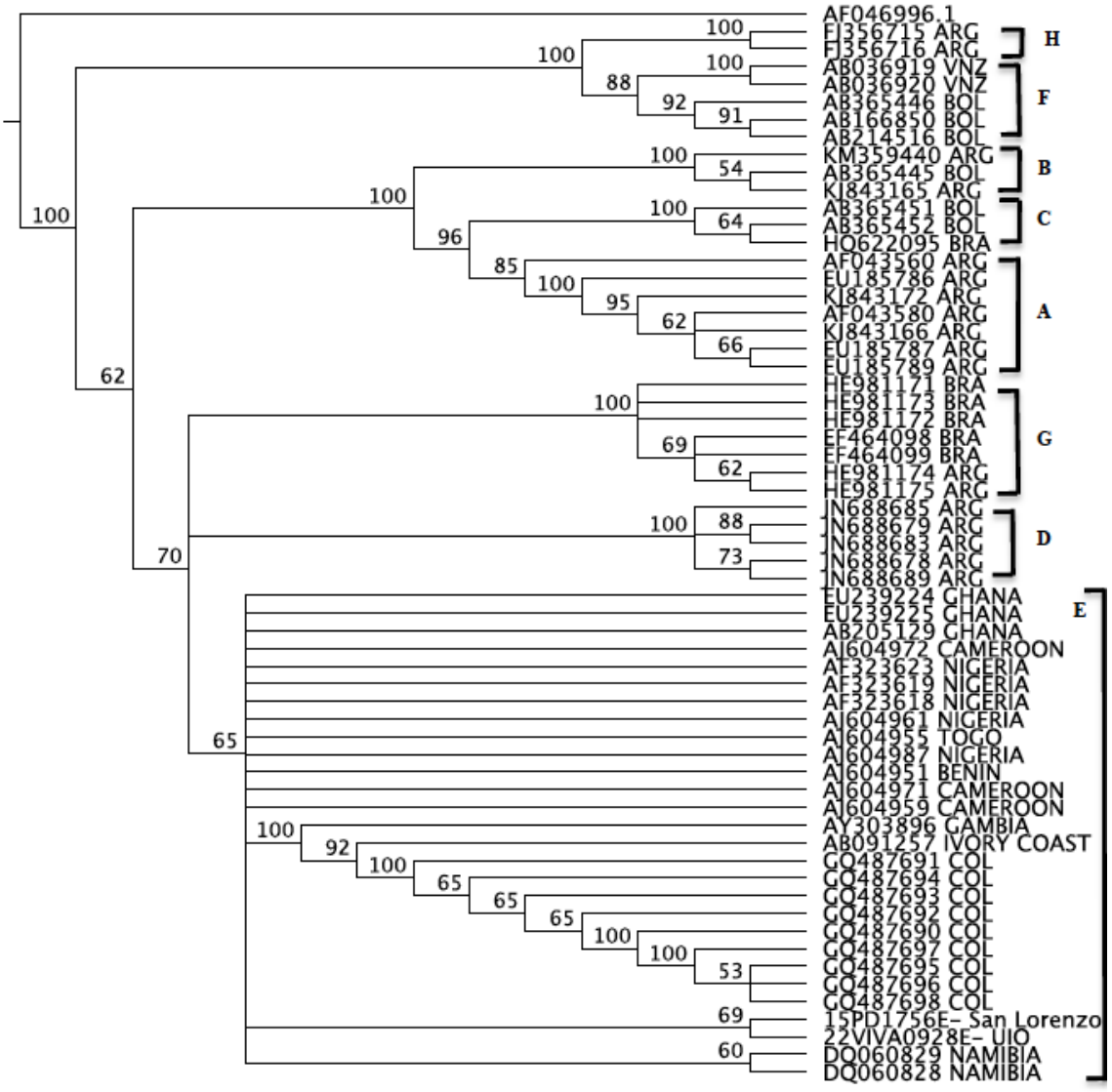


Fig. 4: Phylogenetic tree of HBV isolates obtained in the NCBI GenBank and Ecuadorian genotype E isolates obtained in this study. The tree was built using the Neighbor-Joining method. From all the isolates, the preS gene region was obtained from the whole genome and a sequence of HBV from a Woolly monkey (AFO046996) was used as the outgroup. Boot-strap values are shown for each node.

Identification of the genotypes circulating in the Ecuadorian population infected with the Hepatitis C virus (HCV).

Introduction

Hepatitis C virus (HCV) is a single-stranded, positive-sense RNA virus of approximately 9600 nucleotides in length (Tellinghuisen & Rice, 2004). The NS5B gene is an RNA-dependent RNA polymerase located between the 7597 and 9413 base pairs; this gene lacks proofreading ability. Therefore, HCV represents a remarkable genetic diversity that divides HCV into 8 genotypes and 67 subgenotypes (Qiu et al., 2002; Tsukiyama-Kohara & Kohara, 2018).

Between 15% to 45% of patients infected with HCV can clear it in about 6 months without receiving any treatment (WHO, 2021). The remaining patients might develop, within 20 years, a chronic phase compromising cirrhosis or hepatocellular carcinoma (WHO,2021; Petruzziello et al., 2016). According to the World Health Organization (WHO), HCV is responsible for approximately 1.5 million new infections per year, and around 290 thousand deaths in 2019 (WHO, 2021). HCV represents a significant public health problem, and in 2016 WHO declared it a global target, with different strategies to control HCV have been developed, highlighting a reduction of 90% in new cases of viral hepatitis, a reduction of 60% in deaths related hepatitis by 2030, 80% of eligible patients with chronic HCV with ongoing treatment (Blach et al., 2017).

In addition, it is crucial to identify the genotyping in HCV because treatment depends on genotype and subgenotype, and it can also influence the treatment duration, and clearance rate

(Messina et al., 2015). However, in Ecuador currently, there is no data about HCV circulation genotypes but in neighboring countries like Colombia and Peru genotype 1 is prevalent (88.5% and 86%, respectively) (Petruzziello,2016), while in other like Venezuela genotype 2 represents 34.4% of the cases (Petruzziello,2016). Therefore, this study aimed to provide a first approximation of the main genotypes circulating in Ecuador.

Methods

In a cross-sectional and descriptive study using the Ecuadorian Ministry of Health registry of patients already diagnosed with Hepatitis C (HCV) between 2017 and 2019, and under the following inclusion criteria: 1) confirmed diagnosis of chronic HCV infection with persistent viral charge after 6 months, 2) age over 18 years old, and 3) written informed consent. From 35 patients identified by health ministry, blood samples from a total of 15 subjects (named HCV1 to HCV15) were collected using an appropriate venipuncture technique. Pandemic related circumstances avoid reaching all patients identified by health ministry. Samples were later centrifuged, the plasma was placed in Eppendorf tubes and transported in a nitrogen tank, and later stored at -80 °C at the School of Medicine in the Universidad San Francisco de Quito.

Ethical statement

The study approval was carried out by the Human Research Research Ethics Committee, CEISH-USFQ San Francisco de Quito University and the Public Health Ministry of Ecuador with the code 2018-241IN.

Primers selection

Primers for the amplification of the NS5B section of the HCV genome were selected from the work of Tong et al (Tong et al., 2015). We used primers named F1,R1 to F4,R4 of the NS5B protein. (Fig. 1).

HCV RNA extraction

The MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit was used for RNA extraction following the manufacturer's instructions (ThermoFisher Scientific Inc).

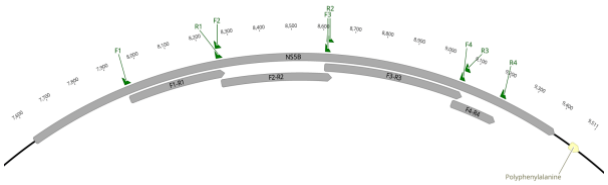
cDNA synthesis

The Thermo Scientific RevertAID First Strand cDNA Synthesis Kit was used to synthsized complementary DNA (cDNA), using the HCV RNA previously obtained following the manufacturer's instructions (ThermoFisher Scientific Inc).

PCR amplification of the HCV NS5B gene fragment

The HCV cDNA template was used for the amplification of the NS5B gen fragment by PCR as figure 1. The PCR was performed using Invitrogen™ Platinum™ II Hot-Start Green PCR Master Mix following the manufacturer's instructions (ThermoFisher Scientific Inc). The PCR conditions used were the following: 94°C for 3 min; 94°C for 30 seg, 56°C for 40 seg, 72°C for 60 seg, 35 cycles; 72°C for 10 min. The temperature of annealing of 56°C was selected as described by Tong, et al. Then, the amplicons were quantified using the EPOCH Microplate Spectrophotometer Reader. After quantification, the amplicons were sent to Macrogen, South Korea for Sanger sequencing. Nucleotide sequences obtain were entered into GenBank under accession numbers: ON540736, ON540737, ON540738, ON540739, ON540740, ON540741 ON540742, ON540743, ON540744, ON540745 and ON540746.

a) b)



Primer Name	Primer sequence (5´ a 3´)	Genome Localization
F1	TTAACCACATCMRCTCCGTGTG	7944-7965
F2	ACACCCGCTGYTTYGACTC	8256-8274
F3	GCCTTCACGGARGCYATG	8609-8626
F4	CATGCCTCAGRAARCTTGG	9057-9075
R1	GTCAAAGCAGCGGGTRTCRTA	8272-8252
R2	GTACCTGGTCATAGCYTCCGTRAA	8635-8612
R3	GGGACCCCAAGYTTYCTGAG	9081-9062
R4	GCCGGCATTGGAGTGAGYYT	9219-9200

Fig. 1: a) Graphic made using Genious Prime 2022.1.1, location of primers used on HCV NS5B protein region b) primers used for NS5B gene amplification as described by Tong YQ, Liu B, Liu H, Zheng HY, Gu J, Liu H, et al. Accurate genotyping of hepatitis C virus through nucleotide sequencing and identification of new HCV subtypes in China population. Clin Microbiol Infect. 2015;21(9):874.e9-874.e21.

Phylogenetic analysis

Sequences reported were compared to identify local similarity regions between sequences using BLAST. Phylogenetic trees were built using the Neighbor-Joining method, Tamura-Nei genetic distance model using the software Geneious prime 2022.0.1. Reference sequences added to the phylogenetic tree were obtained by searching in the NCBI GenBank using the keywords “HCV” AND “NS5B” OR “complete genome”.

Results

From the 51 patients with HCV reported by the MSP data during 2017 to 2019, 15 patients met the inclusion criteria and attended to MSP recruitment; 9 females (60%) and 6 males (40%); mean age 52 ± 24.5. Patient ethnic group self-identification was 100% mestizo. Geographical distribution of samples was Quito 60% and Guayaquil 40% (Table 1). Only 11 of the 15 samples amplified NS5B section. The genotype and subgenotype of 8 samples (HCV1 HCV3, HCV4, HCV7, HCV10, HCV11, HCV13, HCV14), were determined using primers F1-R1 (forward and reverse; fig. 2). Then, using the F2-R2 forward and reverse fragments of the sequences was possible to determine that one subject (HCV9) belonged to the subgenotype 2b (Supplementary figure 1).

Table 1. Sociodemographic characteristic of HCV Ecuadorian patients

		n	%
Sex			
	Male	6	40%
	Female	9	60%
Ethnic group			
	Mestizo		
		15	100%
City of residence			
	Quito	9	60%
	Guayaquil	6	40%
Age			
	18-29	4	26.7%
	30-65	10	66.7%
	>65	1	6.6%

The genome sections R1F1-F1 (forward) for subject HCV2 and R1F1-R1 (reverse) for the subject HCV15 were obtained from the GenBank of the NCBI and aligned via Muscle. Then, all the aligned sequences were trimmed; for HCV2, the obtained sequences had a length of 253 pb (base 8135 to base 8357), while for HCV15 had a length of 265 pb (base 8144 to base 8408). Later, obtained sections were used to create individual phylogenetic trees and the results showed that subject HCV2 belonged to the subgenotype 2b (Supplementary figure 2) and the subject HCV15 did to subgenotype 1a (Supplementary figure 3).

Samples of six subjects corresponded to subgenotype 2b (54.5%), two samples to subgenotype 1a (18.2%), two samples to subgenotype 4d (18.2%), and one sample belonged to subgenotype 1b (9.1%).

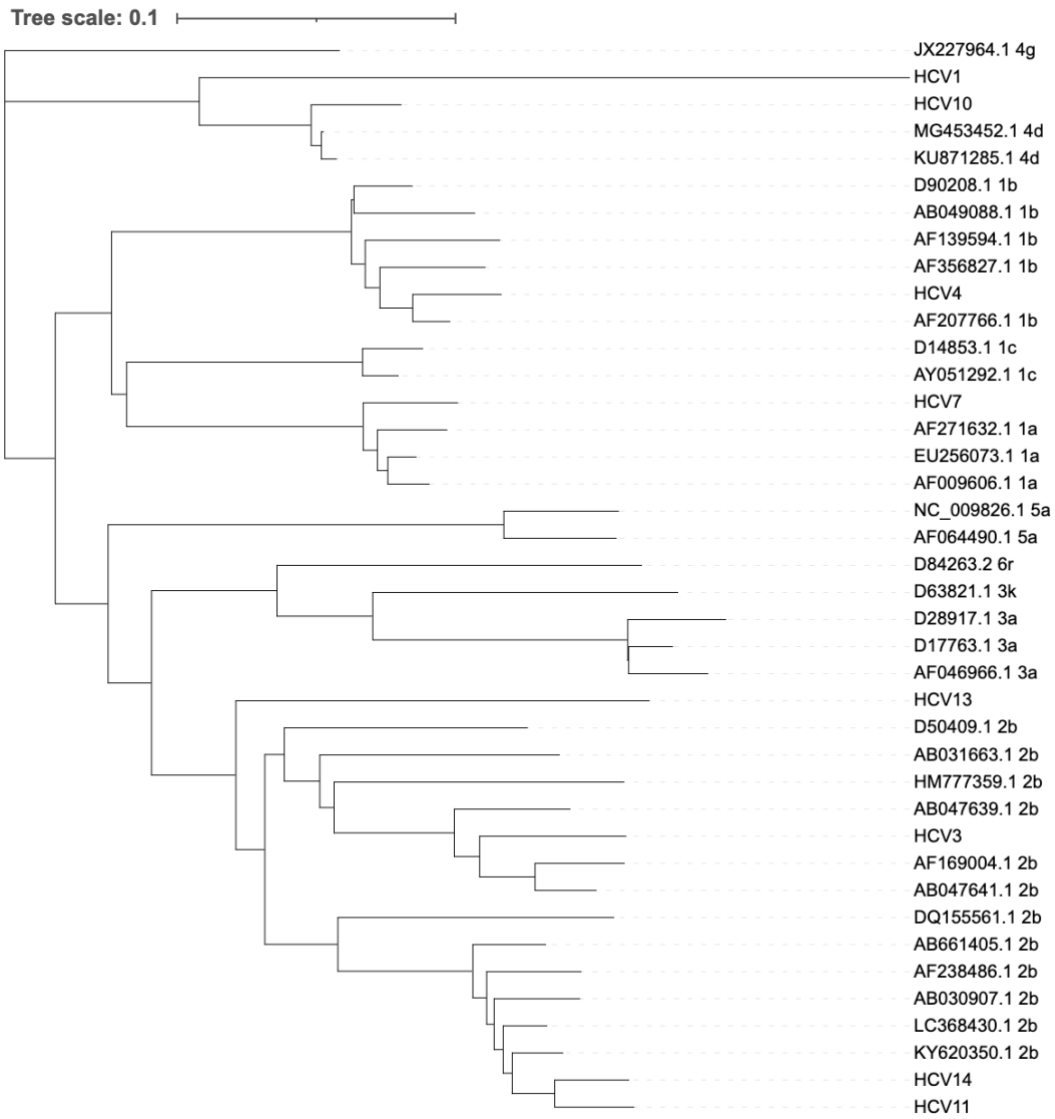


Fig. 2: Phylogenetic tree based on fragments F1-R1 forward and reverse, determination of genotypes and subgenotypes.

Discussion

This report is showing for the first time that HCV subgenotype 2b is prevalent in the Ecuadorian population (54.5%), something in contradiction with data from Latin America compiled by

Petruzziello et al (Petruzziello,2016), where genotype 1 (74.3%) is prevalent. Genotype 2 highest prevalence was found in Venezuela (34.4%), Argentina (24.7%), and Mexico (21.8%) (Petruzziello,2016) but none as high we found.

Although in Latin America, it is estimated that 0.5% of its total population (i.e., 3.8 million people) are infected with HCV (Blach et al., 2017), in Ecuador, according to the Ministry of Health from 2017 to 2019 there were 51 new cases (MSP, 2019). Therefore, the official prevalence of HCV in Ecuador period 2017- 2019 according to the health authority was 0.0003%. This data is far below the reported in Colombia (1.1%) (Maaroufi et al., 2017) and Peru (0.2%), the two neighboring countries of Ecuador. Also, compared with data from other countries, where prevalence ranges from 0.3 to 3.3% (Maaroufi et al., 2017), this data needs further studies to elucidate the real situation of HCV in Ecuador.

On the other hand, response to treatment, particularly to interferon (INF) is different between genotypes 1 and 2 (Splenger, 2018; Jakobsen et al., 2017). Particularly, 50% of subjects with subgenotype 2a shows completed response to INF while those subjects with subgenotype 1b respond only in 11.1% of the cases (Splenger, 2018; Jakobsen et al., 2017). However, the combination of ribavirin with INF leads to a sustained virologic response, mainly in genotypes 2 and 3 (76-82%) than in genotypes 1, 4, 5, or 7 (42-52%) (Splenger, 2018; Jakobsen et al., 2017).

The development of a revolutionary treatment for HCV infected patients known as multiple direct-acting antivirals (DAAs), whose therapeutic target and mechanism of action defines them as a protease inhibitor of nonstructural proteins 3/4A (NS3/4A) (PIs), a polymerase inhibitor of NS5B nucleoside (NPIs), a polymerase inhibitors of NS5B non-nucleoside

(NNPIs), and inhibitor of NS5A (Jakobsen, 2017), have improved the live of HCV patients and have reduced the mortality related with this viral hepatitis. However, in most developing countries, like Ecuador, the high cost of DAAs limited their use, and the lack of accurate epidemiologic information interferes with proper monitoring and treatment of patients.

The main limitation of our report was the small number of samples, however, taking into consideration the reported prevalence, we were able to get a huge proportion of the infected subject. It is important to emphasize that this initial study, with enough methodological rigor, raises the need to improve active diagnostic strategies and at the same time, the urgent need for DAAs availability to improve the quality of life for the patients.

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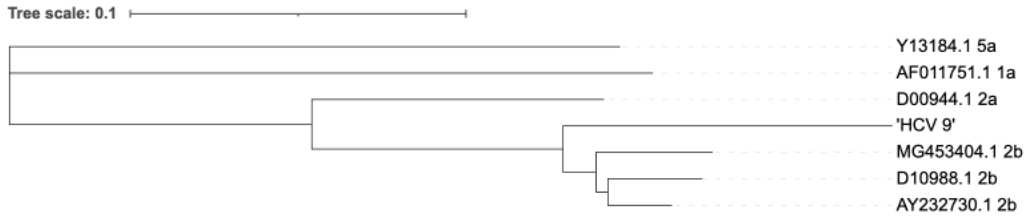
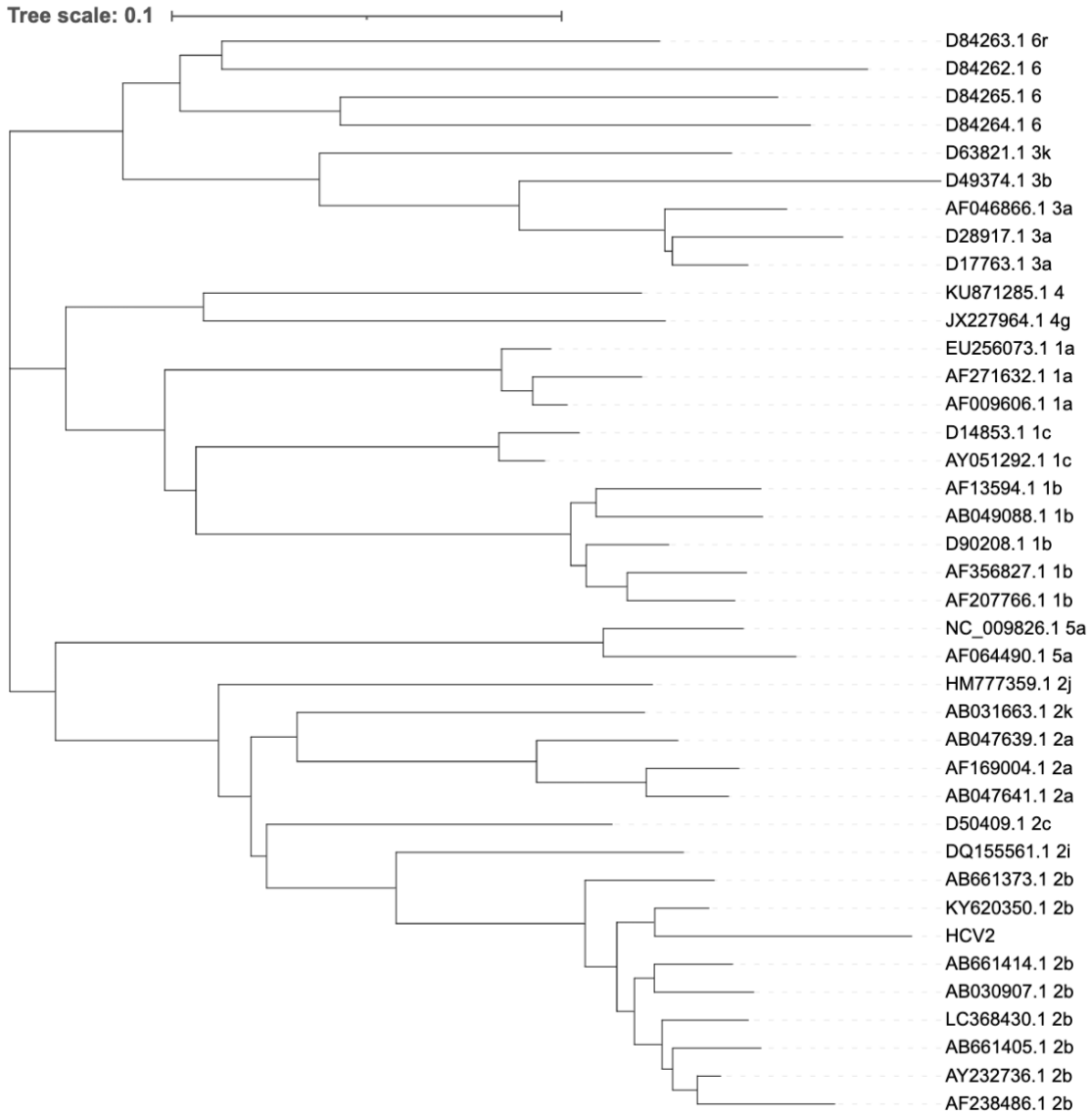
ÍNDICE MATERIAL SUPLEMENTARIO

CODE	SEX	AGE	NACIONALITY	CITY	COMORBILITY	ETHNIC GROUP	HEALTH CENTER	GENOTYPE	VIRAL LOAD	AST	ALT	GGT	ALKALINE PH	TOTAL BILIRUBIN	DIRECT BILIRUBIN	INDIRECT BILIRUBIN
OC0917	MASCULINE/ TRANSGENDER	41	EC	SIMON BOLIVAR/ GUAYAS	DIABETES	MESTIZO	HOSPITAL GUAYAQUIL	2B								
RG0912	FEMENINE	51	EC	GUAYAQUIL/ GUAYAS	HBP,CKD	MESTIZO	CS SIMON BOLIVAR	2B								
CM0909	FEMENINE	58	EC	QUITO/ PICHINCHA	/	MESTIZO	EUGENIO ESPEJO	NO AMPLIFICA	50.866	36	27	16.3	158	0.31	0.18	0.13
JM1704	MASCULINE	65	EC	QUITO/ PICHINCHA	CIRRHOSIS, SCHIZOPHRENIA	MESTIZO	EUGENIO ESPEJO	1A	50.866/135.967	179	275	29	100	0.76	0.31	0.45
EPH431	FEMENINE	75	CU	QUITO/ PICHINCHA	CIRRHOSIS, HBP	MESTIZO	EUGENIO ESPEJO	NO AMPLIFICA	1.315.320	162	145	28	81	1.46	0.94	0.52
JE1707	MASCULINE	57	EC	QUITO/ PICHINCHA	/	MESTIZO	EUGENIO ESPEJO	NO AMPLIFICA	2.211.600	41	53	25	71	0.56	0.21	0.35
BP1759	MASCULINE	29	VNZ	QUITO/ PICHINCHA	HIV	MESTIZO	EUGENIO ESPEJO	2B	11.814	32	46	27.6	85	0.63	0.28	0.35
MR1002	FEMENINE	65	EC	QUITO/ PICHINCHA	HEART FELIURE	MESTIZO	EUGENIO ESPEJO	4D	44.000	26	22	16	60	0.42	0.18	0.26
LU1758	FEMENINE	48	EC	QUITO/ PICHINCHA	REUMATHOID ARTHRITIS	MESTIZO	EUGENIO ESPEJO	1B								
LG1722	FEMENINE	29	EC	QUITO/ PICHINCHA	POLYCYSTIC OVARIE	MESTIZO	EUGENIO ESPEJO	2B	4.818	67	96	61.1	66	0.82	0.28	0.54
MZ0915	FEMENINE	48	EC	GUAYAQUIL/ GUAYAS	/	MESTIZO	HOSPITAL DE INFECTOLOGÍA	2B								
GATE1706	MASCULINE	61	EC	QUITO/ PICHINCHA	/	MESTIZO	CARLOS ANDRADE MARÍN	NO AMPLIFICA								
LERE1314	MASCULINE	29	EC	GUAYAQUIL/ GUAYAS	/	MESTIZO	HOSPITAL GUAYAQUIL	4D								
ROFA0912	FEMENINE	54	EC	GUAYAQUIL/ GUAYAS	/	MESTIZO	HOSPITAL GUAYAQUIL	2B								
DEZA1316	FEMENINE	26	EC	GUAYAQUIL/ GUAYAS	/	MESTIZO	HOSPITAL GUAYAQUIL	2B								

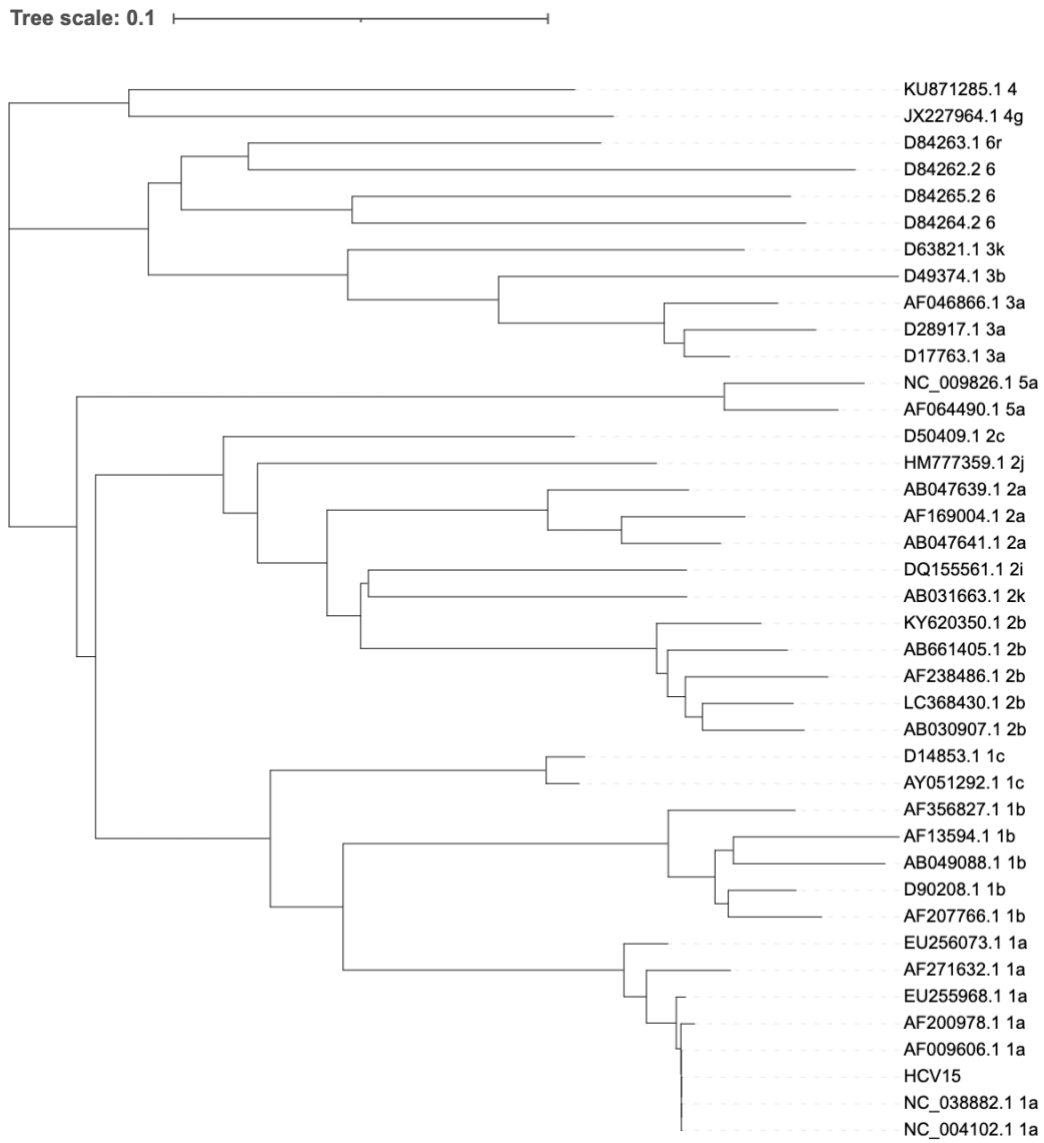
Supplementary table 1: Information obtain from the 1A form and data provided by the MSP



Supplementary figure 1: Phylogenetic tree based on fragments F2-R2 forward and reverse, determination of genotypes and subgenotypes.



Supplementary figure 2: Phylogenetic tree based on fragments R1F1-F1, determination of genotypes and subgenotypes.



Supplementary figure 3: Phylogenetic tree based on fragments R1F1- R1, determination of genotypes and subgenotypes.