Infection, Genetics and Evolution 85 (2020) 104488

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

High prevalence of Hepatitis C Virus infection among people who use crack cocaine in an important international drug trafficking route in Central-West Region Brazil

Vivianne de Oliveira Landgraf de Castro[a,*], Saleem Kamili[b], Joseph C. Forbi[b], Andréa Cristina Stabile[a], Elizeu Ferreira da Silva[a], Sandra Maria do Valle Leone de Oliveira[a], Paula Renata Tedesco de Carvalho[a], Marco Antonio Moreira Puga[a], Tayana Serpa Ortiz Tanaka[a], Bárbara Vieira do Lago[c], Mary Luiza Ibanhes[d], Aufra Araujo[b], Alexandra Tejada-Strop[b], Yulin Lin[b], Guo-Liang Xia[b], Amanda Sue[b], Sheila Araújo Teles[e], Ana Rita Coimbra Motta-Castro[a,f]

[a] Federal University of Mato Grosso do Sul, Avenida Senador Filinto Mueller, s/n, Laboratório de Imunologia Clínica, FACFAN, Campo Grande, MS 79070-900, Brazil
[b] Centers for Disease Control and Prevention, 1600, Clifton Road NE, BlDG 18 MS-A33, Atlanta, GA 30330, USA
[c] Oswaldo Cruz Foundation, Helio and Peggy Pereira Pavillion-Ground Floor-Room B09, FIOCRUZ Av. Brasil, Manguinhos, 4365, Rio de Janeiro, RJ 210360-040, Brazil.;
[d] Public Health Laboratory of Mato Grosso do Sul, Avenida Senador Filinto Mueller, 1600, Campo Grande, MS 79080-320, Brazil
[e] Federal University of Goiás, Rua 227, Viela Q. 68, S / N - Setor Leste Universitário, Goiânia, GO 74605-080, Brazil
[f] Oswaldo Cruz Foundation, Rua Gabriel Abrão, 92 - Jardim das Nações, Campo Grande, MS 79081-746, Brazil

ARTICLE INFO

Keywords:
Hepatitis C virus
Crack cocaine
Molecular epidemiology
Brazil

ABSTRACT

In this study, the prevalence rate, associated risk factors and genetic diversity of hepatitis C virus (HCV) infection were determined among people who use crack from an international drug trafficking route in Central-West, Brazil. Blood samples were collected from 700 users of crack from Campo Grande and two border cities of Mato Grosso do Sul State and tested for HCV infection using serological and molecular testing methodologies. Anti-HCV was detected in 31/700 (4.5%, 95% CI: 2.9–6.0%) and HCV RNA in 26/31 (83.9%) of anti-HCV positive samples. Phylogenetic analysis of three HCV sub-genomic regions (5'UTR, NS5B and HVR-1) revealed the circulation of 1a (73.9%), 1b (8.7%) and 3a (17.4%) genotypes. Next-generation sequencing and phylogenetic analysis of intra-host viral populations of HCV HVR-1 showed a significant variation in intra-host genetic diversity among infected individuals, with 58.8% composed of more than one sub-population. Bayesian analysis estimated that the most recent common HCV ancestor for strains identified here was introduced to this region after 1975 following expansion of intravenous drug use in Brazil. Multivariate analyses showed that only 'ever having injected drugs' was independently associated with HCV infection. These results indicate an increasing spread of multiple HCV strains requiring public health intervention, such as harm reduction, testing services and treatment among crack users in this important border region of Central Brazil.

1. Introduction

Hepatitis C virus (HCV) infection is a global health problem and one of the major global causes of mortality and morbidity (Petruzziello et al., 2016; Thrift et al., 2017). It's estimated that 2.5% of the world population (177.5 million adults) have been infected with HCV and a global viraemic rate of 67% which is equivalent to 118.9 million of HCV RNA positive cases (Petruzziello et al., 2016). Additionally, HCV is one of the main causative agent of liver related disorders including cirrhosis and hepatocellular carcinoma (Westbrook and Dusheiko, 2014).

HCV is a single-stranded, positive sense RNA virus that belongs to the *Flaviviridae* family. HCV RNA-dependent RNA polymerase (RdRp) lacks proof-reading mechanisms that result in high rates of mutations during replication, leading to a continuous diversification of the intra-host viral population (Lohmann, 2013). As a result, each infected individual contains a diverse population of HCV variants or quasispecies (Smith et al., 2014; Welzel et al., 2017). The N-terminus of the E2 protein has the hypervariable region 1 (HVR-1), one of the most heterogeneous regions of the HCV genome. This region is known to contain neutralizing epitopes, and is subject to a strong selection pressure.

* Corresponding author.
E-mail address: vikalandgraf@hotmail.com (V.d.O.L.d. Castro).

https://doi.org/10.1016/j.meegid.2020.104488
Received 14 April 2020; Received in revised form 13 July 2020; Accepted 28 July 2020
Available online 31 July 2020

Thus, it is used to assess intra-host HCV heterogeneity (Bukh, 2016; Farci, 2011).

HCV is mainly transmitted through contact with infectious blood, such as sharing needles and syringes during injection drug use. Other routs of transmission and spread of HCV are unsafe sexual behavior, lack of harm-reduction measures such as sterile tatooing equipment and sharing of paraphernalia used during drug consumption (Scheinmann et al., 2007; von Diemen et al., 2010a).

The consumption of crack cocaine (crack) has become a significant public health problem in Brazil (Bastos and Bertoni, 2014). The availability of this drug is facilitated by the fact that Brazil shares half of its 16,000 km-long border with the world's three biggest cocaine producers, Bolivia, Colombia and Peru. Mato Grosso do Sul (MS) State has 7 municipalities, two of which - Ponta Porã and Corumbá - share borders with two major marijuana and cocaine producers - Paraguay and Bolivia, respectively. Thus, MS state is an important drug trafficking route for cocaine smuggling to other countries and to Brazil's urban centers (Confederaçao Nacional dos Municipios, 2016).

In 2012, a National Survey on Crack Use estimated that there are approximately 370,000 people who use crack (PWUC) in Brazil. These individuals were mostly men (78.7%), with an average age of 30 years and non-whites (80%). Over 70% of them reported sharing their drug paraphernalia, which increases a risk for transmission of infections, especially hepatitis viruses. The HCV infection prevalence among PWUC was 2.63% (CI 95% 1.69–4.07) (Bastos and Bertoni, 2014).

Considering that the studied region borders with two countries, phylogenetic and molecular studies among the border population can be used to understand the importance of cross-border movement and local transmission dynamics of this infectious disease. The World Health Organization (WHO) is developing strategies for the prevention and control of viral hepatitis infection with the central goal of the elimination of viral hepatitis as a public health threat by 2030 World Health Organization, 2016. Thus, a better understanding of the HCV infection prevalence rate, associated risk factors and HCV genetic diversity among key populations, such as PWUC, is important for developing screening and prevention strategies.

2. Methods

This cross-sectional study was carried out between November 2013 and July 2015. The study recruited 782 individuals that use crack in three cities of Mato Grosso do Sul State, Central-West region of Brazil. Of them, 585 were from Campo Grande, capital of Mato Grosso do Sul State; 197 from border cities, Corumbá (n = 113) and Ponta Porã (n = 84), which borders Bolivia and Paraguay, respectively (Fig. 1). PWUC were recruited from drug use treatment centers and the “drug scene” (streets). They were approached by the study team in clinics, shelters and streets, introduced by health and mental health care workers from the rehabilitation clinics and *Consultório na Rua* (healthcare staff that provide on-site assistance to the homeless). Participation in the study was voluntary and no compensation was provided.

Participants were required to meet the following inclusion criteria before providing written informed consent: i) self-reported use of crack regularly as defined by the Pan American Health Organization's CODAR (consumidores de drogas con alto riesgo - high-risk drug users) criteria (for at least 25 days in the last 6 months); ii) age 18 years or older, and iii) competence to provide written informed consent.

After providing informed consent, all participants were interviewed face-to-face by trained health professionals using a standardized questionnaire to collect the data of interest, including socio-demographic status, sexual, and drug use behaviors. After completing the interview, blood samples were collected from all participants in order to perform molecular and serological analysis. All participants were screened for HCV antibodies (anti-HCV) by enzyme-linked immunosorbent assay (ELISA-Murex Diagnostics, UK). Anti-HCV positive and samples with indeterminate results were tested for HCV RNA by real-time RT-PCR (m2000 system, Abbott Molecular Inc., Des Plaines, IL).

The study protocol was approved by the Ethical Committee of the Federal University of Mato Grosso do Sul (approval number 1.802.787).

2.1. HCV molecular characterization

Total nucleic acid was extracted from all anti-HCV positive plasma samples using the Roche MagNA Pure LC instrument and MagNA Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics, Indianapolis, IN), and eluted with 50 μL buffer according to the manufacturer's instructions. To obtain cDNA, the high temperature capability SuperScript1 VILOTM cDNA Synthesis Kit (Invitrogen, Life Technologies, Carlsbad, CA) was used. The reverse transcription PCR conditions were 25 °C for 10 min, 42 °C for 90 min, and 85 °C at 5 min.

Amplification of HCV NS5B, 5’UTR and HVR-1 gene region was done by a nested-PCR targeting the NS5B, 5’UTR and the E1/E2 junction region, respectively. The nested PCR reaction was done using specific primers and thermal cycling condition as previously described (Forbi et al., 2012; Ramachandran et al., 2011; Tejada-Strop et al., 2015).

Amplicons derived from the nested PCR amplifications were sequenced using their respective nested primers and BigDye v3.1 chemistry sequencing kit (Applied Biosystems, Foster City, CA) by an automated sequencer (ABI 3130xl, Applied Biosystems, Foster City, CA). Sequencing PCR reaction involved 25 cycles, each cycle consisting of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min.

2.2. Pyrosequencing of the HVR-1 region

All HCV RNA samples from which HCV HVR-1 region was detected in nested PCR reactions were further used to sequence the intra-host HCV HVR-1 variants. They were analyzed using the Next-generation Sequencing technology (454/Roche GS FLX platform). Each sample was amplified independently with fusion primers including the 454-primer key (A and B for forward and reverse primers, respectively), a different multiple identifier (MID) for each sample and HCV-specific sequence. The PCR products were purified using 2% size select gel (Invitrogen, Life Technologies, Carlsbad, CA), and quantified with the Agilent 2200 TapeStation system (Agilent Technologies, Santa Clara, CA). PCR amplicons were mixed at equimolar concentrations and diluted to a final concentration of 10^7 molecules ml^{-1} prior to being subjected to emulsion (em)PCR, which was performed following the instructions supplied with the kit and enriched beads were subjected to pyrosequencing (titanium chemistry) using the 454/Roche GS FLX instrument (Skums et al., 2012). The sequences generated were analyzed as previously described (Forbi et al., 2014).

2.3. Phylogenetic analysis

Preliminary sequence analysis was conducted using SeqMan and MEGALIGN programs from the Lasergene DNA (version 10.1.2, DNASTAR Inc., Madison, WI) for all consensus sequences obtained by Sanger sequencing. All sequences were aligned using ClustalW implemented in MEGA7 software (Kumar et al., 2016). HCV genotypes/subgenotypes were classified based on the NS5B, 5’UTR and HVR-1 sequences and by comparing each sequence with published reference sequences from GenBank. The list of the GenBank accession numbers of the isolates and countries of origin is shown in Table S1. Phylogenetic trees were inferred for cleaned HVR-1 quasispecies sequences from 454 using the Neighbor-joining method from MEGA 7.0.26 with Kimura 2-parameter nucleotide substitution model. Analyses of minimum distances, maximum distances, nucleotide diversity, were performed on the intra-host HVR-1 variants using MEGA7 software. For the targets obtained by Sanger sequencing, the maximum likelihood method from PhyML (v. 3.0) (Guindon et al., 2010) with GTR + G + I nucleotide

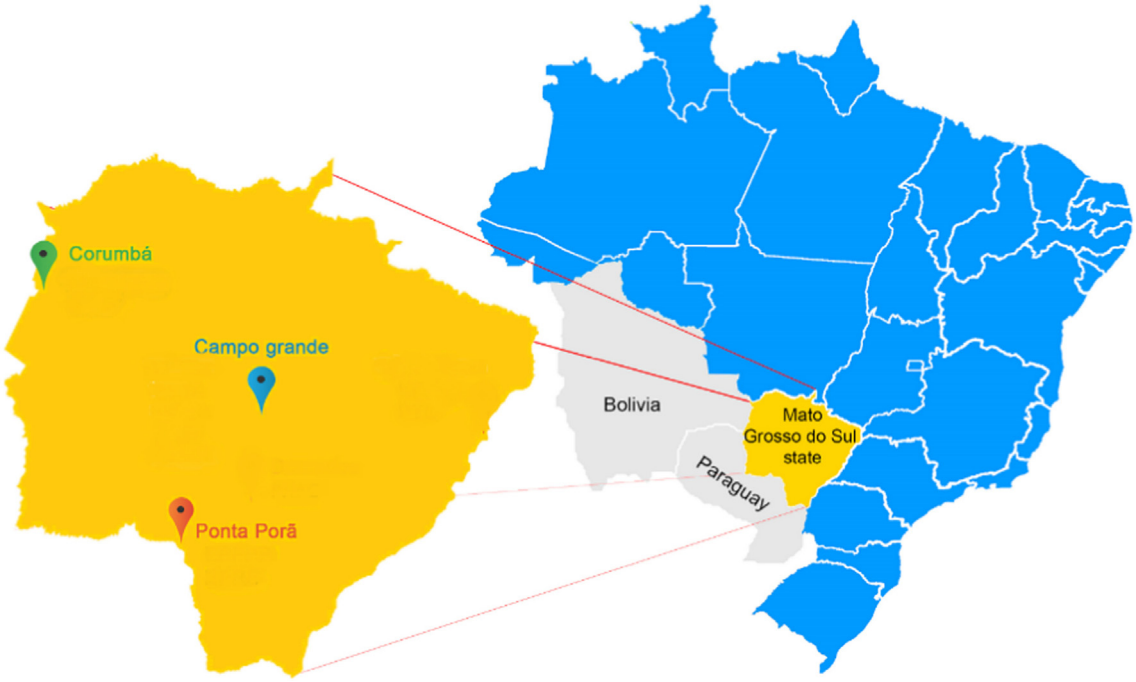

Fig. 1. Geographic location of cross-sectional study (Adapted from Puga et al., 2017).

substitution model obtained at Smart Model Selection (Lefort et al., 2017) was used.

The 5'UTR, NS5B and consensus HVR-1 sequences reported in this study were submitted to GenBank under accession numbers MG818168-MG818186 and MG818188-MG818227.

2.4. Molecular clock

The evolutionary history of HCV genotypes 1 (HCV-1) and 3 (HCV-3) was accessed using the Bayesian coalescent approach as implemented in BEAST (v. 1.8.4) (Drummond and Rambaut, 2007), with the GTR + G + I nucleotide substitution model and a lognormal relaxed (uncorrelated) molecular clock model, which was selected as the best-fit model based on the most relevant Bayes factor support.The date of the most recent common ancestor (MRCA) was calculated using 362 nt NS5B sequences, with a substitution rate of 5×10^{-4} substitutions/site/ year (Pybus et al., 2001). A Markov Chain Monte Carlo (MCMC) was run for 1 + E08 generations. The convergence of parameters was resolved using Tracer v. 1.6 (http://beast.bio.ed.ac.uk/Tracer) was used with a 10% burn-in to ensure convergence was achieved. The effective sample size (ESS) values were checked and only values higher than 200 for all the parameters were accepted. Uncertainties are reflected in the 95% highest posterior density intervals. The maximum clade credibility tree was accessed using TreeAnnotator implemented in the BEAST package and visualized with FigTree v1.4.2 program.

2.5. Statistical analysis

Prevalence data was calculated with 95% confidence intervals (CI). Student's-test (quantitative variables), Chi-square test and Fisher exact test (categorical variables) were used to compare variables and to evaluate the association between the presence of HCV infection and risk factors (2-sided *p* value). Odds ratios and 95% CI were used as measures of the strength of the association between anti-HCV positivity (outcome) and independent variables. Those variables associated with outcome ($p < 0.10$) were entered into a logistic regression backward stepwise model, and a *p*-value less than 0.05 was considered significant. Statistical analysis used SPSS statistical software (Statistical Package for Social Sciences, Chicago, IL) version 17.0 for Windows.

3. Results

Of the 782 PWUC recruited, 80 (10.2%) individuals declined to participate in the study, and 2 (0.3%) refused blood collection. 700 individuals agreed to be interviewed and provided blood samples. Of these, 524 were from Campo Grande (74.9%) and 176 from border cities (25.1%). Most participants were male, > 26 years old, non-white, single, with 5 or less years of education and with history of incarceration. Half of the participants received less than $200/month of family income.

One-third used illicit drugs for the first time at < 15 years of age. Two-thirds used crack for more than 7 years and most of them used drugs daily. Sharing of crack smoking apparatuses were reported by 66% of PWUC, and 45.6% had ever reported oral sores or burns in the mouth area. A small fraction (13.3%) of participants reported a history of injection drugs, and of those 44.0% ever shared needles/syringes. There were no significant differences between the PWUC from Campo Grande and border cities in socio-demographic status, sexual habits and drug use behavior, excepting daily crack consumption and previous history of incarceration (Table 1).

The prevalence of anti-HCV was 4.5% (95% CI: 2.9–6.0%): 4.4% in Campo Grande and 4.6% in two border cities of MS State. Univariate analyses of socio-demographic status, sexual habits and drug use behavior among PWUC revealed that age over 35 years, length of crack use, ever having injected drugs, history of blood transfusion and history of sexually transmission infections (STIs) were statistically associated with HCV exposure. After multivariate analysis, ever having injected drugs, blood transfusion and STI were independent risk factors associated ($p < 0.05$) with HCV exposure (Table 2).

Of the 31 anti-HCV seropositive samples, HCV RNA was detected in 26 (83.9%) using real time PCR. Of them, 5'UTR and HVR-1 were sequenced from 23 (88.5%) and NS5B from 13 (50%) samples. Phylogenetic analysis showed that HCV belongs to genotypes 1a ($n = 17$, 73.9%), 1b ($n = 2$, 8.7%) and 3a ($n = 4$, 17.4%). Concordant genotype results were obtained using HVR-1 and NS5B genomic regions

Table 1
Socio-demographic characteristics and risk behaviors among crack users from Central-West, Brazil, 2013–2015 (n = 700).

	Border cities (n = 176)		Campo Grande (n = 524)		p - value
Variables	Total n (%)	Missing n	Total n (%)	Missing n	
Sociodemographic					
Age, years, mean ± SD	33 ± 10	-	33 ± 10	-	-
Male sex	147 (83%)	-	446 (85%)	-	0.612
Marital status, single	149 (85%)	-	402 (77%)	2	0.070
Low education[b]	129 (73%)	-	346 (66%)	-	0.074
Pattern of crack/similar and psychoactive substances use					
Age at first illicit drug, mean (IQR)[a]	16.6 (8.6)	-	32.6 (32.7)	-	-
Crack use time (months), median (IQR)	13.7 (9.3)	2	13.3 (12.0)	11	-
Daily crack consumption	129 (73%)	-	322 (61%)	-	**0.005**
Share pipe	98 (56%)	16	316 (60%)	52	0.144
Alcohol[c]	139 (79%)	-	412 (79%)	-	0.922
Marijuana[c]	121 (69%)	-	359 (68%)	-	0.953
Sniff cocaine[c]	90 (51%)	-	274 (52%)	-	0.791
Injecting drug[d]	27 (15%)	-	66 (13%)	-	0.353
Sexual risks					
Exchange sex for money and/or drugs[c]	46 (26%)	-	119 (23%)	1	0.361
Sexual violence	26 (15%)	-	77 (15%)	3	0.998
Number of steady partners, median (IQR)	39 ± 10	-	41 ± 10	-	-
Number of eventual partners, median (IQR)	6	73	5	225	-
Anal sex - any intercourse sexual with irregular condom use [c]	132 (75%)	1	372 (71%)	1	0.272
Steady partner - any intercourse sexual with irregular condom use	27 (15%)	89	91 (17%)	272	0.391
Eventual partner- any intercourse sexual with irregular condom use	74 (42%)	73	208 (40%)	228	0.762
STI history[c]	62 (35%)	2	146 (28%)	105	0.855
Other Risk Behavior					
Previous prison[d]	136 (77%)	-	285 (54%)	-	< **0.001**

bolded numbers $p < 0.05$.
[a] SD: standard deviation.
[b] Lower education level was defined as Elementary and Middle Schools (completed or not); STI: Sexually Transmitted Infections.
[c] Previous six months.
[d] Lifetime;

(Fig. 2). No multiple HCV genotypes were detected among the study samples. Despite the small number of HCV sequences isolated from PWUC from border cities (n = 6), most of them were classified as HCV-1a (83.3%). There was no evidence of inter-genotype recombination based on the genomic fragments used.

Analysis of intra-host variants (Fig. 3) showed no evidence of sharing strains among these individuals. Many HCV strains in the PWUC are composed of several distinct subpopulations of intra-host variants. While many samples showed a limited intra-host HCV diversity from 1% (sample 133) to 2% (samples 257, 295 and 480), the maximum genetic distance of more than 10% was observed among the intra-host HVR-1 clusters from samples 261, 307, 556 and 601 (Fig. 3). The nucleotide diversity of HCV genotype 1a strains ranged from 0.5% - 7.5% (Mean 2.2%), while HCV genotype 1b strains ranged from 0.6% - 5.8% (Mean 2.8%) and of HCV genotype 3a strains from 1.5%–3.7% (Mean 2.7%). The maximum distances among intra-host HCV HVR-1 variants varied from 1.2% -27.2%, 1.6%–13.1% and 3.6%–9.1% for HCV genotype 1a, 1b and 3a strains, respectively. The inter-host diversity was 17.2% for HCV genotype 1a, 22.4% for HCV genotype 1b and 18.1% for HCV genotype 3a.

The most recent common ancestor for the sampled HCV-1 strains is estimated to have spread in MS in 1989 (1976–1993, 95% HPD) and for HCV-3 in 2004 (1998–2010, 95% HPD).

4. Discussion

This is the first report about HCV exposure in PWUC from an important border region in Central-West Brazil. The prevalence of anti-HCV positivity found in this study (4.5%; 95% CI: 2.9–6.0%) was higher than among the Brazilian general population (1.38%) and 26 times higher than among the blood donors (0.17%) of Campo Grande, Mato Grosso do Sul state (Ministério da Saúde, 2011; Torres, 2004). Conversely, it was similar to that found in other studies conducted among PWUC in Brazil (Bastos and Bertoni, 2014; Del-Rios et al., 2019; Nunes et al., 2007a; de Sá et al., 2013; Santos Cruz et al., 2013a, 2013b), and lower than frequencies reported from studies conducted in other countries among individuals who do not inject drugs, which has been documented as high as 35.2% (Inciardi et al., 2006a; Nelson et al., 2011). Studies reporting high prevalence (> 20%) tended to have small to modest sample sizes (n = 20 to 260; median = 83) and took place in East Asia, which is one of the regions with a higher prevalence of HCV infection and a great number of people who inject drugs (PWID) (von Diemen et al., 2010b). The present findings could be explained in part by the low rate of injecting drug use in Brazil. Many current PWUC in Brazil cited fear of HIV infection as the principal reason of changing from cocaine injecting into smoking in the last 20 years (Inciardi et al., 2006b; von Diemen et al., 2010a).

Although this study was conducted in an important drug trafficking route, there was no significant difference in prevalence of HCV exposure between PWUC from Campo Grande and border cities. We hypothesize that this is due to similar socio-demographic characteristics and risk behaviors observed among PWUC in both regions.

The prevalence of current HCV infections (detectable HCV RNA) was high (83.9%) among anti-HCV positive PWUC (26/31), highlighting their potential to transmit HCV. Although hepatitis C treatment is available through Brazilian Public Health System Ministério da Saúde, 2017, medical management of HCV in this hard-to-reach and hard-to-treat population represents an important challenge, due to lack of adherence to therapy. Thus, HCV care and treatment, management of addiction and harm reduction programs are needed to reduce the transmission of HCV among this challenging patient population.

As observed in previous studies, the majority of anti-HCV positive PWUC (64.5%; 20/31) are “baby boomers” (people born between 1945 and 1965) (Moore et al., 2019). It is estimated that approximately

Table 2
Univariable and multivariable regression analysis of risk factors associated with HCV prevalence among crack users, Central-West, Brazil, 2013–2015 (n = 700).

Variables	N	Anti-HCV Positive (n = 31)	%	Anti-HCV Negative (n = 669)	%	p- value	Adjusted OR* (95% CI)**	p-value
Socio-demografic characteristics								
Male sex	593	28	4.7	565	95.3	0.375		
Age ≥ 35 yearsold	524	30	5.7	494	94.3	0.004¶	3.61 (0.43–30.42)	0.240
Non-white race (self-report)[a]	477	20	4.2	457	95.8	0.658		
Single	553	28	5.1	525	94.9	0.113		
Low level of education†	476	22	4.6	454	95.4	0.717		
Previously incarcerated	421	21	5.0	400	95.0	0.377		
Patter of drug use								
Age of first use illicit drugs (< 15 years old)	244	13	5.3	231	94.7	0.398		
Length of crack use (> 12 months)[b]	252	19	7.5	233	92.5	0.001¶	1.21 (0.42–3.46)	0.726
Daily crack consumption	451	19	4.2	432	95.8	0.709		
Shared crack smoking equipment	417	18	4.3	399	95.7	0.846		
Oral sores, wounds, burns in the mouth area[c]	318	15	4.7	303	95.3	0.746		
Ever injected drugs	93	23	24.7	70	75.3	< 0.001¶	38.29 (12.41–118.12)	< 0.001§
Piercings/tattooing	468	18	3.8	450	96.2	0.287		
History of blood transfusion[d]	86	11	12.8	75	87.2	< 0.001¶	3.62 (1.31–10.0)	0.013§
Sexual risks								
Homosexual intercourse in the last six months[e]	117	7	6.0	110	94.0	0.193		
Sex with non-regular sex partners in the last six months	402	19	4.7	383	95.3	0.656		
No condom with regular sex partner in the last six months	221	4	1.8	217	98.2	0.651		
No condom with non-regular sex partner in the last six months	117	4	3.4	113	96.6	0.561		
Trading sex for money or drug in the last six months[f]	165	9	5.5	156	94.5	0.467		
Had sex with HIV infected person in the last year[g]	47	3	6.4	44	93.6	0.354		
Sexual violence[h]	103	4	3.9	99	96.1	0.764		
History of STIs‡[i]	208	19	9.1	189	90.9	< 0.001¶	2.8 (1.05–7.32)	0.040§

* Odds Ratio** Confidence interval; Variables entered on step 1: age, infected drugs, length of crack use, blood transfusion and STI. †Lower education level was defined as Illiterate, Elementary and Middle Schools, completed or not. ‡Sexually Transmitted Infections; ¶ p < 0.1 in univariate analysis;§ Statistically significant (p < 0.05).

[a] Missing:2.
[b] Missing: 39.
[c] Missing: 2.
[d] Missing: 108;
[e] Missing: 110.
[f] Missing: 1.
[g] Missing: 54.
[h] Missing: 3.
[i] Missing: 10.

three-quarters of all persons chronically infected with HCV worldwide are “baby boomers”, many of whom are unaware of their infection status (Smith et al., 2012), and very likely were infected decades ago and are at high risk of developing progressive liver disease (Moore et al., 2019; Smith et al., 2012).

Hepatitis C is the most common chronic bloodborne infection and there is a high risk of infection by HCV through infected blood and blood products (Moore et al., 2019). In this study, history of blood transfusion was associated with HCV exposure in PWUC. In Brazil, the blood screening tests for anti-HCV nationwide implementation was in 1994, signaling the beginning of a decline in HCV transmission through blood transfusion (Freitas et al., 2014a). After that, in 2013 the HIV and HCV nucleic acid amplification test screening became mandatory in all Brazilian blood banks, consequently, decreasing the risk of HIV and HCV transmission and improving the quality of blood products (Vieira et al., 2017). Results of this study also indicate an increase in the risk of HCV infection among those who have had STIs. Crack-cocaine use has been directly associated with STIs (Guimarães et al., 2017; Nunes et al., 2007b; Terrault et al., 2013). The consumption of crack and alcohol is associated with higher levels of disinhibition and impulsivity factors that increase levels of sex risk-taking such as prostitution, multiple sexual partners, inconsistent condom use, and co-infection of some STIs, may contribute to sexual transmission of HCV in vulnerable populations (Guimarães et al., 2017; Terrault et al., 2013).

Although the pattern of drug use has changed in Brazil (Dunn and Laranjeira, 1999; Inciardi et al., 2006a; von Diemen et al., 2010a), in this study, history of injection drug use (IDU) was significantly associated with HCV exposure. Injecting drug and needle/syringe sharing behavior is one of the most important routes of transmission for bloodborne infections, such as hepatitis C (De Angelis et al., 2009; Degenhardt et al., 2016). This study supports previous studies which found an association between HCV exposure and ever having shared needles/syringe, and other paraphernalia, such as cookers (spoons or containers for dissolving the drug), ‘cottons’ (filters), and wash water used to rinse needles and syringes and dissolve drugs (Ministério da Saúde, 2011; Nunes et al., 2007b; Pybus et al., 2001; Roy et al., 2012; de Sá et al., 2013; Torres, 2004). In addition, among individuals who reported a history of ever having injected drugs, 78.3% had HCV RNA levels ≥5log IU/mL and, of them, 72.2% reported ever having shared (data not shown). Furthermore, sharing of paraphernalia used to smoke crack could also expose these users to bloodborne infections. This calls for specific preventive strategies including harm reduction programs. Some authors have shown that harm reduction services show positive effects on preventing bloodborne diseases by reducing needle and syringe sharing among PWID (Larney et al., 2015; Li et al., 2014). Interventions such as hepatitis C testing, treatment and prevention are vital

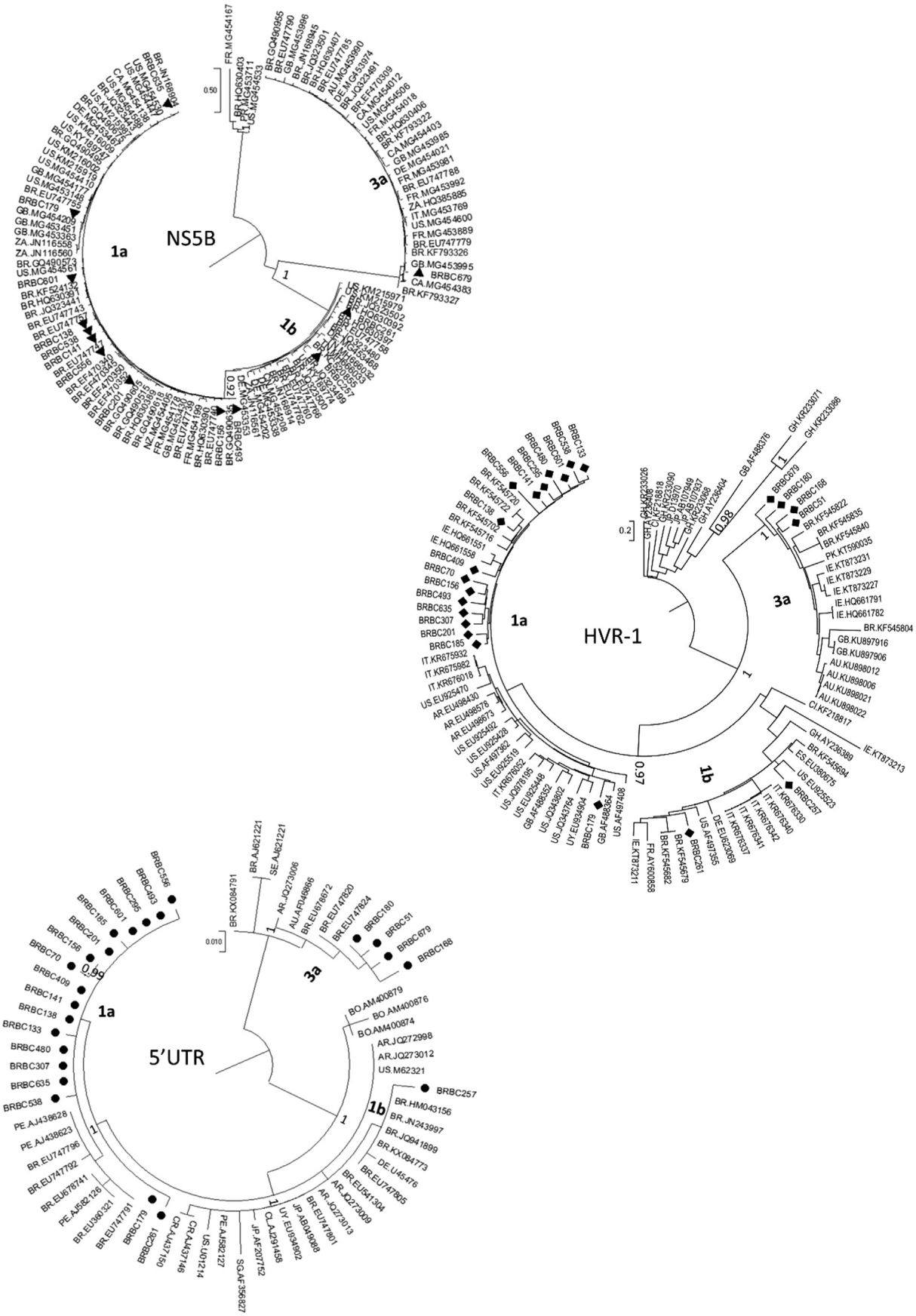

Fig. 2. Phylogenetic tree analysis of the NS5B, HVR-1 and 5'UTR regions of HCV.
NS5B isolates studied are shown in triangles, HVR-1 in diamonds, and 5´UTR in circles. The phylogenetic tree was constructed by maximum likelihood method using PhyML Approximate likelihood-ratio test (aLRT) for branches, which compares the likelihoods of the best and the second best alternative arrangements around the branch of interest. (aLRT values are shown as decimal numbers at select branches in the tree). Reference sequences were obtained from GenBank (see table S1).

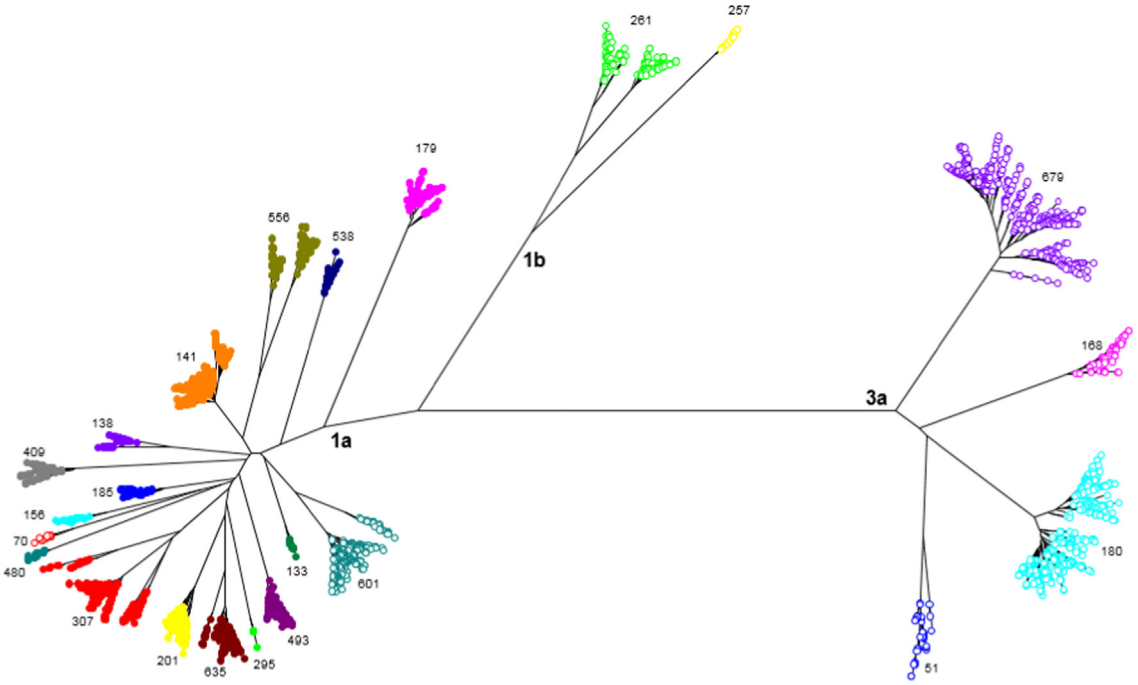

Fig. 3. Maximum likelihood tree reconstructed with intra-host variants of HVR-1 gene sequences of HCV genotypes. Only unique haplotypes are shown. Individual samples are labeled with different colors.

to preventing the spread of HCV infection.

The genotypes found in this study (genotype 1a followed by genotype 3a and 1b) are consistent with those previously found in Brazil (Campiotto et al., 2005; Lampe et al., 2013; Mendes-Correa et al., 2008), among PWUC (Del-Rios et al., 2019; Lopes et al., 2009) and other groups infected with HCV in the same region (Freitas et al., 2014b; Martins et al., 2006). No significance difference was found between intra-host HCV divergence and HCV RNA levels, age, time of drug use, or other variable collected. Individual patient intra-host HCV populations, assessed using HVR-1 sequences (Fig. 3), were, in general, composed of highly related variants and were not shared among other patients. The degree of HVR-1 heterogeneity has been shown to increase significantly after 1–3 years of infection (Ramachandran et al., 2011). However, some intra-host HCV populations were very homogeneous (for example, 133, 257, 295 and 480), indicating a potentially short duration of infections in the individuals. By contrast, some patient HCV isolates (261, 307, 556 and 601) contained more than one distinct subpopulation of intra-host variants, which were distant from each other (maximal genetic difference higher than 10%), suggesting that some of the PWUC were infected for a long time (Forbi et al., 2012; Ramachandran et al., 2011).

Worldwide, it has been estimated that epidemic HCV subtypes 1a, 1b, and 3a emerged only about 100–150 years ago and started to spread exponentially during the 20th century (Khan et al., 2009; Magiorkinis et al., 2009; Pybus et al., 2001). Our study suggests that HCV has been probably introduced into this important international drug trafficking route in Central-West Brazil between 1989 (1976–1993, 95% HPD) for HCV-1 and 2004 (1998–2010, 95% HPD) for HCV-3,which coincides with the expansion of intravenous drug use in the country.

The limited number of HCV-3 samples ($n = 4$) might be a limitation, once it potentially increases the likelihood of error estimation. However, these findings are consistent with a previous study that found the major local chains of HCV transmission in Brazil did not originate until 1975–1980, and it seems to coincide with the expansion of intravenous drug use and the sharp increase in the number of hemodialysis patients in the country (Lampe et al., 2010).

This study has limitations due to cross-sectional and convenience sampling, since participants were recruited from the streets and treatment facilities. Additionally, some risk behaviors may have been underreported due to fear of discrimination and stigma, leading to potential underestimation of associations with these variables and HCV exposure. Despite these limitations, our findings have important implications for future research and prevention efforts targeting PWUC in Brazil.

In conclusion, our study revealed high HCV exposure among PWUC from an important international drug trafficking route in Central-West, Brazil. Despite the association between a history of intravenous drug use and HCV exposure, the limited intravenous drug user sample size in this study suggests that HCV can be transmitted through other mechanisms of HCV dissemination, even among non-intravenous drug users, who have a higher risk of acquiring HCV than the general population. Therefore, the presence of high current HCV infection and risky behaviors in PWUC highlight the need for improved hepatitis C testing and prevention interventions among this unique population.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado do Mato Grosso do Sul, FUNDECT-MS (n.59/300.110/2015). Vivianne de Oliveira Landgraf de Castro was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES fellowship (99999.006313/2015-09).

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2020.104488.

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