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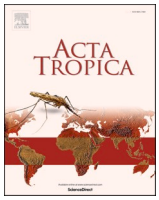

Diversity, geographical distribution and predictive factors of Hepatitis C virus genotypes and subtypes in Rwanda

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ABSTRACT

Background: Existing data on the prevalence of hepatitis C virus (HCV) genotypes and subtypes in Rwanda need to be strengthened. The aim of this study was to identify HCV genotypes and subtypes among HCV-infected patients, as well as their geographical distribution in Rwanda, and to identify the social and economic factors that could influence HCV epidemiology which would make it possible to target national preventive and management actions for infected patients.

Methods: This study included 560 patients with confirmed chronic HCV infection. Patients were recruited from various health facilities in the four provinces of Rwanda as well as in the City of Kigali and had never received treatment with direct-acting antiviral (DAAs). HCV viral loads were measured using Cobas® AmpliPrep/Cobas® TaqMan® HCV Quantitative Test, version 2.0. HCV genotyping was performed using an in-house sequencing protocol targeting the NS5B central region. Genotypic HCV prevalence was correlated with patient geographic location, sociodemographic, behavioral, lifestyle, and clinical factors.

Results: HCV genotype 4 was detected in 99.3% of the patients, while genotype 3 was identified in 0.7%. A total of eight (8) HCV subtypes were detected, with 4k being the predominant subtype nationwide (49.5%), followed by subtypes 4r (21.2%), 4q (16.2%), 4v (7.9%), 4b (2.0%), 4l (1.8%), 4c and 3h represent 0.7% each. Our findings reveal subtype distribution variations among provinces. Subtype 4k was prevalent across regions, particularly in Kigali (64.0%) and the Eastern Province (61.6%). Subtype 4q was more common in the northern province (40.7%), 4r in the southern (43.9%) and western provinces (37.1%), and 4v in the eastern province (17.8%). Farmers exhibit a distinct infection profile compared to other occupations, showing a lower prevalence of subtype 4k but a higher prevalence of subtype 4r.

Conclusions: Our study revealed that HCV infection is unevenly distributed in Rwanda, dominated by HCV genotype 4, with considerable heterogeneity in the repartition of the different subtypes. We found potential associations between rural/urban lifestyles and HCV subtype profiles. Determined HCV distribution and diversity can serve as basis not only for HCV infection awareness and prevention campaigns, but also success and guidance for personalized treatment.

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1. Background

According to the World Health Organization (WHO), hepatitis C virus (HCV) infection remains a major public health problem. WHO estimated in 2022, 50 million people chronically affected with HCV worldwide with around 1 million new infections annually and about 240,000 annual deaths related to HCV-complications. (Cui et al., 2023; WHO Global hepatitis report, 2024). Considering WHO regions, the highest number of chronic HCV infections is in the Eastern Mediterranean Region (12 million people), in the south-East Asia Region (9 million), European Region (9 million), Western Pacific Region (7 million), African Region (8 million), and 5 million in the region of the Americas (WHO Global hepatitis report, 2024). A recent meta-analysis indicated that the prevalence of HCV infection worldwide is estimated to be 2.5%, ranging from 2.9% in Africa to 1.3% in the Americas (Petruzziello et al., 2016). The estimated prevalence of HCV in Rwanda ranges from 3% to 8% (Musafiri et al., 2024; Nisingizwe et al., 2023; Sonderup et al., 2017).

In 2016, the World Health Assembly set an ambitious goal of eliminating HCV by 2030 (Sonderup et al., 2017). Guidance strategies included the ongoing collection and analysis of HCV data, increasing screening and treatment coverage, sustainable and scalable funding, and the development of new affordable diagnostics, vaccines and treatments (Nisingizwe et al., 2023; WHO, 2021). With this in mind, in 2017, the Rwandan government launched a nationwide mass screening and treatment program, as well as a 5-year HCV elimination plan (Umutesi et al., 2019; Zhong et al., 2024).

Recently, HCV treatment and therapeutic options have improved considerably, with the approval of direct-acting antivirals (DAAs), which can cure the disease and prevent long-term liver damage (Pfaender et al., 2014). DAAs appear to be highly effective and well-tolerated, but combined with viral testing, remains very costly and therefore limited for low-income countries (Van Nuil et al., 2021).

Phylogenetic analyses indicate that the HCV can be classified into eight major genotypes (GTs) and approximately 100 distinct subtypes (Jia et al., 2021; Smith et al., 2014). HCV GT1, 2, and 3 have a global distribution with different relative prevalence from one geographic region to another, but the distribution of HCV genotypes and subtypes is complex and variable worldwide (Alvarado-Mora et al., 2012; Iles et al., 2014; Panasiuk et al., 2013). Other GTs have a more restricted geographical distribution: GT4s are most commonly found in the Middle East and North and Sub-Saharan Africa but are also commonly observed in Rwanda (Gupta et al., 2019a; Sonderup et al., 2017). GT5 is frequently found in South Africa, and GT6 is frequently found in Southeast Asia and Southeast Africa (Alvarado-Mora et al., 2012; Charlotte Hedskog et al., 2015; Janahi et al., 2015; Panasiuk et al., 2013). To date, GT7 has been isolated from immigrants originating from the Democratic Republic of Congo (DRC), suggesting a Central African origin, and GT8 has been isolated from India (Jia et al., 2023).

All HCV GTs and subtypes are hepatotropic and can lead to chronic infection. However, the significant variability between circulating strains of HCV can influence the response and duration of treatment, according to the treatments used (European Association for the Study of the Liver, 2014). DAAs are suitable for virtually all patients, and WHO guidelines are now recommending the use of DAAs as the first-line therapy for all patients. However, a recent study in Rwanda showed that subtype 4r was associated with high rates of treatment failure with the ledipasvir®-sofosbuvir® combination (Gupta et al., 2019b).

Determination of HCV genotypes and subtypes are important to predict response to antiviral therapy for the efficacy and barrier to resistance of non-pangenotypic DAAs (Hayes et al., 2022). In developing countries like Rwanda, identification of HCV genotypes and subtypes across country is still incomplete. Therefore, we sought to bridge this gap by genotyping and subtyping chronic HCV naïve patients' samples collected in four provinces of Rwanda and Kigali City to assess the distribution, diversity, and predictive factors of HCV strains, which could also be used as a good tool for decision making of an optimal treatment regimen.

2. Methods

2.1. Design of the study and sampling

For this study, we conducted a retrospective cross-sectional survey of 560 patients from the four provinces of Rwanda and the city of Kigali (Fig. 1), diagnosed with chronic HCV infection and about to receive treatment. Patients were recruited between July 2017 and December 2021 during Rwanda's national mass HCV screening campaign in the general population (Zhong et al., 2024). Participants of this study were recruited through their medical follow-up centers. During this campaign, HCV screening was carried out free of charge in public places by trained nurses. Samples were initially tested at the sampling sites for HCV antibodies using rapid diagnostic tests (RDT). RDT-positive samples and laboratory requisition forms were transported to the nearest health facilities for Antibody ELISA testing where positive ELISA samples were confirmed by RT-PCR. Plasma samples were obtained from whole blood collected in vacutainer EDTA K2–4 ml anticoagulant blood collection tubes. After centrifugation at 4000 rpm for 10 minutes, the supernatants were carefully collected and stored at -80 °C. The patient inclusion criteria were as follows: Confirmed HCV positive patients with an HCV viral load $\geq$ 1000 UI/ml, who had never received DAA therapy, who were eligible for treatment and consented to participate in this study. The patient exclusion criteria were as follows: patients whose HCV infection status was unknown, who had an undetectable viral load, who had previously received DAA treatment, who were foreigners, who were nonresidents or who did not agree to participate in the study.

2.2. Data collection

The demographic, socio-economic, professional, educational, clinical comorbidities, HCV/HIV and/orHBV coinfection status, history of HCV status and treatment, lifestyle and HCV-related behavior and risk factor data were collected and recorded for all participants. An electronic and paper information form were completed by trained mentors. The socio-economic categories evaluated in this study were defined by the *Ubudehe* system (The Local Administrative Entities Development Agency (LODA), 2020). *Ubudehe* rankings refer to the economic standard of living of Rwandan households, with level 5 being assigned to the richest and level 1 to the least wealthy.

2.3. HCV RNA viral load determination

The HCV RNA viral loads test were determined within the Rwanda Biomedical Center, National Reference Laboratory Division using Cobas® AmpliPrep/Cobas® TaqMan® HCV Quantitative Test, version 2.0 (Roche Diagnostics) according to the manufacturer's instructions. The dynamic range of quantification was 15 to 100 million IU/mL with a lower limit of detection of $\geq$15 IU/mL

2.4. HCV genotyping

2.4.1. HCV RNA extraction, NS5B amplification and sequencing

HCV viral RNA was extracted from 140 μL of plasma using a QIAmp viral RNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was reverse-transcribed using the SuperScript™ III One-Step RT-PCR System with Platinum™ Taq High Fidelity DNA Polymerase (Invitrogen by Life Technologies, Van Allen Way Carlsbad, CA, USA) mixed with random hexamers (Thermo Fisher Scientific) to generate cDNA. A RT 9700 ABI thermal cycler (Thermo Fisher Scientific) was used with the following cycling conditions: 30 °C for 5 min, 42 °C for 5 min, 95 °C for 15 S, cooling and cDNA storage at 4 °C.

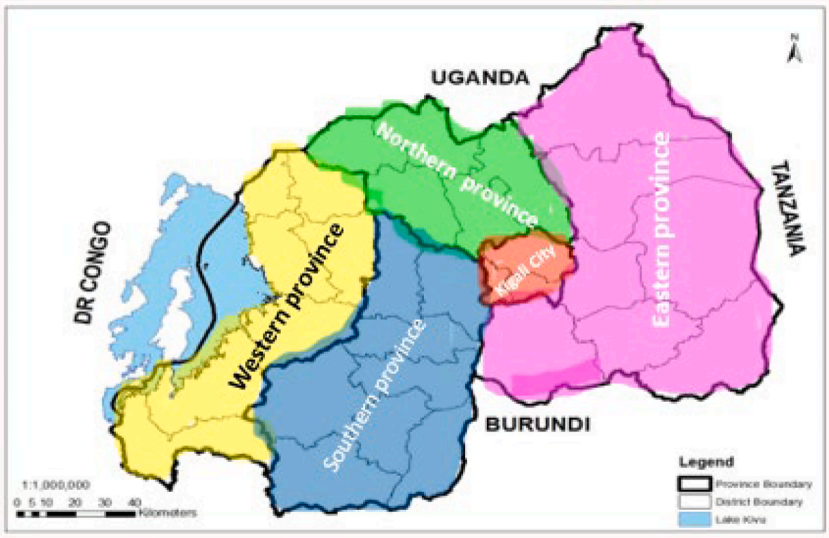

Fig. 1. Locations of Rwanda's four provinces and the city of Kigali.

The HCV NS5B region of 396 bp was amplified based on specific primers PR3 and PR4 as previously described by Laperche (Laperche et al. 2005). The universal M13 forward (5′-TGT AAA ACG GCC AGT-3′ and M13 reverse: 5′-CAG GAA ACA GCT ATG ACC-3′) sequence (Eurogentec) was added to the 5′ end of both the PR3 and PR4 primers to facilitate further sequencing. The thermocycling conditions included a denaturation step of 94 °C for 5 minutes, followed by 45 cycles of 94 °C for 15 s, 53 °C for 1 min, 72 °C for 1 min; and a final extension stepof 72 °C for 10 min. Cooling and DNA storage at 4 °C.

The length of the fragment to be sequenced was determined using the MultiNa Quick Gel Electrophoresis System (Shimadzu) for DNA/RNA analysis. PCR products were purified using ExoSAP-IT® technology. Briefly, ExoSAP-IT was added to the PCR products and loaded into a Veriti 96-well thermocycler (Applied Biosystems) under the following conditions: 37 °C for 15 min and 80 °C for 15 min. Purification was followed by sequencing reaction with the following cycling conditions: 96 °C for 3 min, followed by 25 cycles of 96 °C for 15 s, 50 °C for 15 s and 60 °C for 4 min. Cooling at 10 °C.Sequencing products were then purified to remove excess ddNTPs using a clean Seq kit (Agincourt) and controlled on an automated Caliper Sciclone® workstation (PerkinElmer) running with Maestro software (Life Sciences). The purified sequencing products were further sequenced by Sanger method using an ABI 3500 XL Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's instructions.

2.4.2. Sequence analysis: genotyping and subtyping prediction

Nucleotide sequences obtained in NS5B region were visualized and manually analyzed using Sequence Scanner software 2 (Applied Biosystems). The analyzed reverse sequence was submitted to the online Harvard algorithm software tool (Reverse and/or complement DNA sequences, n.d.) to automatically obtain reverse complementary sequences. The two sequences were submitted to NCBI BLAST® and aligned to obtain a consensus sequence which was then submitted to the Geno2Pheno HCV V1.0 for genotyping, subtyping and prediction tool ("Geno2pheno hcv," n.d.). Phylogenic analysis was performed using the Geneious Prime® 2022.2.1 Software. To make the trees easier to read, only a subset of NS5b HCV genotype 4 sequences were used (143 from the global population and 122 sequences from the "farmers" population). HCV genotype 3 as well as those carrying ambiguous nucleotides were excluded. A Clustal Omega alignment of the selected sequences including 18 HCV genotype 4 subtypes reference sequences retrieved from GenBank was performed and a RAxML (Randomized Axelerated Maximum Likelihood) tree was generated using 1000 replicates and 500,000 parsimony random seed. Genotype and subtype sequence used as references was identified through International Committee on Taxonomy of Viruses (ICTV) and Geno2pheno HCV. Sequences used to generate phylogenic analysis were submitted to Genbank and accession numbers were provided (**see Appendix 1**).

2.4.3. Ethics approval, informed consent and data protection

This study was carried out according to the principles of the declaration of Helsinki. Management approval for each study site was sought prior to the start of the study. The study was approved by the Rwanda National Ethics Committee (RNEC) (130/RNEC/2017) and the National Health Research Committee (NHRC) (NHRC/2017/PROT/009). Written informed consent was obtained from each patient in accordance with the principles of the Rwanda National Ethics Committee. The data collected in this study were strictly confidential, in accordance with the codes of medical ethics, conduct and confidentiality. Data have been rendered anonymous for data analysts and publication purposes.

2.5. Statistical analysis

The data collected were analyzed with the Statistical Package for Social Science (SPSS) Version 25.0. Descriptive statistics such as frequencies, percentages, means and standard deviations were calculated to summarize basic characteristics according to HCV subtypes. The chi-square test was used to assess the associations between different patient characteristics and HCV subtypes. A P value < 0.05 was considered significant.

3. Results

3.1. HCV patient characteristics

The following five hundred and sixty (560) Rwandan patients with chronic HCV infection throughout Rwanda were included in the analysis (Table 1): 35.3% from the city of Kigali, 20.4% from the Southern province, 15.9% from the Western province, 15.4% from the Northern province, and 13.0% from the Eastern province. The majority (59.3%) of the study participants were women, while men accounted for 40.7%. Most of the participants (45.5%) were aged between 50 and 69 years, followed by those aged $\geq$ 70 years (33.9%), with an average age of 62.3 years. The majority (71.1%) of the participants were married, and 23.2% were widowers or divorced. Most of the participants in our study were farmers (65.2%) or retired/unemployed (19.1%), followed by self-

Table 1
Distribution of HCV subtypes according to sociodemographic and economic factors.

Attributes	Total, n(%)	HCV different subtypes by NS5B sequencing								p value
		3h, n(%)	4b, n(%)	4c, n(%)	4k, n(%)	4l, n(%)	4q, n(%)	4r, n(%)	4v, n(%)	
Sex										
Female	332(59.3)	4(1.2)	6(1.8)	2(0.6)	154(46.4)	6(1.8)	61(18.4)	71(21.4)	28(8.4)	0.411
Male	228(40.7)	0(0.0)	5(2.2)	2(0.9)	123(53.9)	4(1.8)	30(13.2)	48(21.0)	16(7.0)	
Age in years										
<30	15(2.7)	0(0.0)	1(6.7)	0(0.0)	10(66.7)	0(0.0)	2(13.3)	2(13.3)	0(0.0)	0.815
30 to 49	100(17.9)	1(1.0)	1(1.0)	1(1.0)	52(52.0)	1(1.0)	17(17.0)	19(19.0)	8(8.0)	
50 to 69	255(45.5)	2(0.8)	2(0.8)	1(0.4)	121(47.5)	5(2.0)	39(15.3)	64(25.0)	21(8.2)	
≥70	190(33.9)	1(0.5)	7(3.7)	2(1.1)	94(49.5)	4(2.1)	33(17.4)	34(17.9)	15(7.9)	
Mean (SD)	62.3(15.4)	59.0(13.5)	68.0(17.0)	64.7(18.2)	61.8(16.5)	68.5(11.4)	63.4(15.1)	61.6(14.0)	62.5(13.8)	0.745
Province										
Kigali	198(35.3)	0(0.0)	7(3.5)	3(1.0)	126(64.0)	6(3.0)	26(13.0)	23(12.0)	7(3.5)	<0.001
Western	89(15.9)	1(1.1)	0(0.0)	0(0.0)	32(36.0)	2(2.2)	13(14.6)	33(37.1)	8(9.0)	
Eastern	73(13.0)	0(0.0)	2(2.7)	0(0.0)	45(61.6)	1(1.4)	4(5.5)	8(11.0)	13(17.8)	
Southern	114(20.4)	0(0.0)	2(1.7)	1(0.9)	38(33.3)	1(0.9)	13(11.4)	50(43.9)	9(7.9)	
Northern	86(15.4)	3(3.5)	0(0.0)	0(0.0)	36(41.9)	0(0.0)	35(40.7)	5(5.8)	7(8.1)	
Marital status										
Single	32(5.7)	0(0.0)	0(0.0)	0(0.0)	21(65.6)	0(0.0)	2(6.2)	6(18.8)	3(9.4)	0.737
Married	398(71.1)	3(0.8)	8(2.0)	2(0.5)	186(46.7)	7(1.8)	71(17.8)	89(22.4)	32(8.0)	
Widower/ divorced	130(23.2)	1(0.8)	3(2.3)	2(1.5)	70(53.9)	3(2.3)	18(13.8)	24(18.5)	9(6.9)	
Occupation										
Farmer	365(65.2)	4(1.1)	6(1.6)	0(0.0)	162(44.4)	6(1.6)	66(18.1)	88(24.1)	33(9.1)	0.005
Civil/private servant	22(3.9)	0(0.0)	2(9.0)	1(4.6)	15(68.0)	1(4.6)	1(4.6)	1(4.6)	1(4.6)	
Self-employee	66(11.8)	0(0.0)	2(3.0)	2(3.0)	33(50.0)	1(1.5)	10(15.2)	12(18.2)	6(9.1)	
None or retired	107(19.1)	0(0.0)	1(0.9)	1(0.9)	67(62.6)	2(1.9)	14(13.1)	18(16.9)	4(3.7)	
Education level										
None	216(38.6)	1(0.4)	7(3.2)	1(0.4)	103(47.7)	4(1.9)	36(16.7)	44(20.4)	20(9.3)	0.627
Primary	187(33.4)	1(0.5)	1(0.5)	2(1.1)	94(50.3)	2(1.1)	34(18.2)	38(20.3)	15(8.0)	
Post primary	98(17.5)	2(2.0)	1(1.0)	0(0.0)	45(45.9)	2(2.0)	15(15.4)	27(27.6)	6(6.1)	
Secondary	31(5.5)	0(0.0)	2(6.4)	0(0.0)	18(58.1)	1(3.2)	3(9.7)	6(19.4)	1(3.2)	
University	28(5.0)	0(0.0)	0(0.0)	1(3.6)	17(60.7)	1(3.6)	3(10.7)	4(14.3)	2(7.1)	
Social-economic level/status*										
Social category 1	189(33.8)	1(0.5)	2(1.1)	2(1.1)	94(49.7)	3(1.6)	37(19.6)	36(19.0)	14(7.4)	0.699
Social category 2	112(20.0)	2(1.8)	2(1.8)	0(0.0)	58 (51.7)	2(1.8)	15(13.4)	21(18.8)	12(10.7)	
Social category 3	209(37.3)	1(0.5)	5(2.4)	1(0.5)	103(49.3)	3(1.4)	35(16.7)	48(23.0)	13(6.2)	
Social category 4	50(8.9)	0(0.0)	2(4.0)	1(2.0)	22(44.0)	2(4.0)	4(8.0)	14(28.0)	5(10.0)	
Health insurance										
Community base	512(91.4)	4(0.8)	10(2.0)	3(0.5)	256(50.0)	7(1.4)	84(16.4)	109(21.3)	39(7.6)	0.08
Public/private servant	36(6.4)	0(0.0)	1(2.8)	0(0.0)	16(44.4)	3(8.3)	5(14.0)	8(22.2)	3(8.3)	
Private insurance	10(1.8)	0(0.0)	0(0.0)	1(10.0)	5(50.0)	0(0.0)	1(10.0)	2(20.0)	1(10.0)	
Uninsured	2(0.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	1(50.0)	

* According to Rwanda's *Ubudehe* classification (The Local Administrative Entities Development Agency (LODA), 2020)

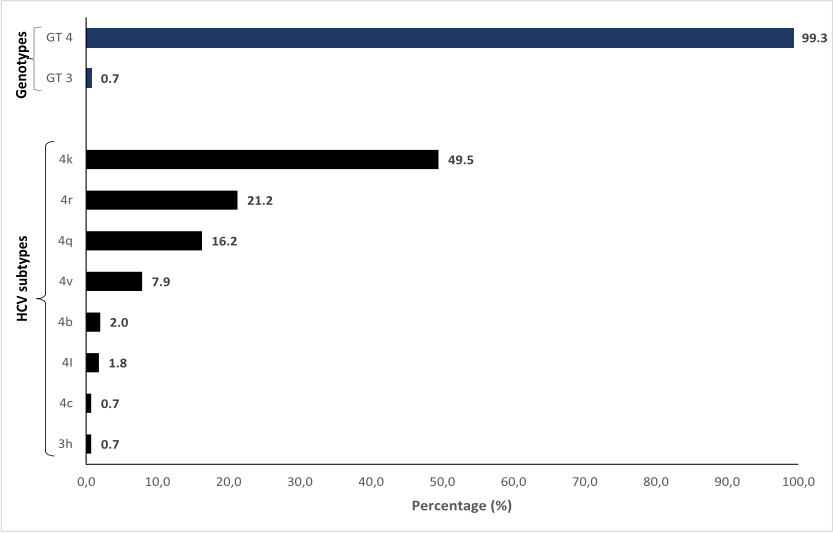

Fig. 2. Proportion (%) of HCV genotypes and subtypes circulating in Rwanda.

employed workers (11.8%). The majority of our participants reported having no formal education (38.6%) or only primary education (33.4%). Only 5% had university training. They were 33.8% in the lowest social category 1, followed by 20%, 37.3% and 8.9% in categories 2, 3 and 4, respectively, according to Rwanda's Ubudehe classification (The Local Administrative Entities Development Agency (LODA), 2020). The vast majority (91.4%) of the participants in this study reported having only basic community health insurance.

3.2. Geographical distribution of HCV genotypes and subtypes

Two (2) HCV genotypes, GT3 and GT4 as well as eight (8) HCV subtypes were identified (Fig. 2). The most frequent HCV genotype was GT4, representing 99.3% of the patients included in our study. GT3 was observed in 0.7% of participants. The predominant HCV subtypes identified were subtype 4k, accounting for 49.5% of patients, followed by 4r (21.2%), 4q (16.2%), 4v (7.9%), 4b (2%) and 4l (1.8%). The least frequent subtypes were 4c and 3h, each representing less than 1%.

The distribution of HCV subtypes in terms of prevalence varied according to province in Rwanda ($p<0.001$) (Fig. 3 and Table 1). Subtype 4k HCV infections were common in all four provinces but predominated in the city of Kigali (64.0%) and in the Eastern Province (61.6%), followed by the Northern Province (41.9%). The Western and Southern provinces had HCV subtype 4k prevalence of 36.0% and 33.3%, respectively. Subtype 4r, on the other hand, was most prevalent in the southern (43.9%) and western (37.1%) provinces. In the city of Kigali and the eastern provinces, we observed a proportion of subtype 4r around 12.0%. The northern province had the lowest proportion (5.8%) of subtype 4r. Subtype 4v was mainly found in the Eastern Province (17.8%), while its prevalence was 9.0% in the Western Province, 8.1% in the Northern Province, 7.9% in the Southern Province and only 3.5% in the city of Kigali. Subtype 3h was only found in the northern and western provinces and represented only 3.5% and 1.1%, respectively.

3.3. HCV subtypes and sociodemographic and economic factors

We observed that subtype 4k was significantly represented among participants employed in the private sector and in the unemployed/retired group than in the farmers' group. In contrast, farmers had a significantly greater percentage of patients with the HCV 4r subtype ($p<0.005$) than did those in the other groups. In this category, we found no other significant variations in relation to age, gender, education or marital status.

RAxML (Randomized Axelerated Maximum Likelihood) trees built with some of the HCV sequences as well as 18 Genbank HCV subtypes 4 reference sequences reflect the diversity of the sequences identified in this study, especially for HCV genotype 4 subtypes in the general population studied as well as in the farmers category (Fig. 4).

3.4. HCV subtypes, clinical and lifestyle factors

The overall analysis of the results related to the clinical and lifestyle factors of the participants (Table 2) shows that a significant percentage of respondents resorted to traditional practices such as scarification (28.4%) or had unprotected sex with multiple partners (27.3%). However, no significant differences between HCV subtypes and the clinical and lifestyle factors evaluated were highlighted.

3.5. HCV subtypes, comorbidities and HIV/HBV coinfections

The distribution of HCV subtypes according to comorbidities and HIV or HBV coinfections showed no significant differences (Table 3).

4. Discussion

In our study, we found significant variations between the geographical localities and occupational activity profiles of HCV-

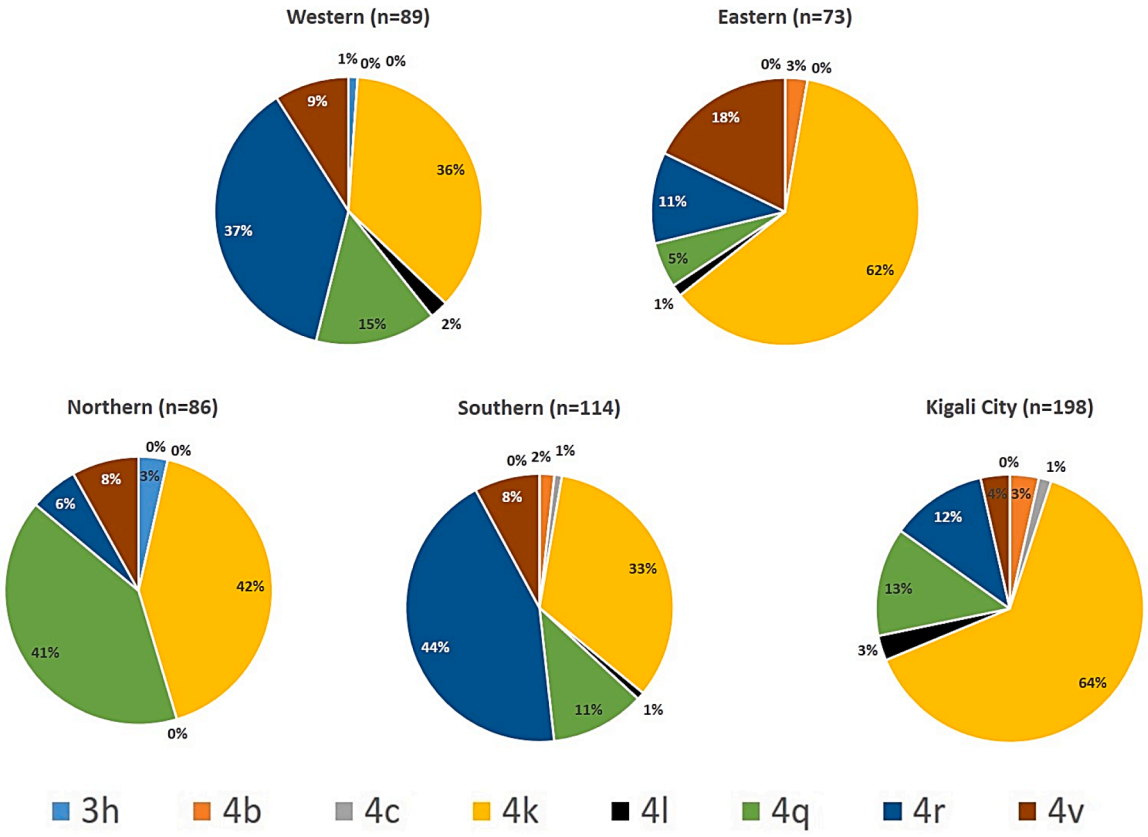

Fig. 3. Geographical distribution (%) of HCV subtypes in Kigali city and in the 4 Rwandan provinces.

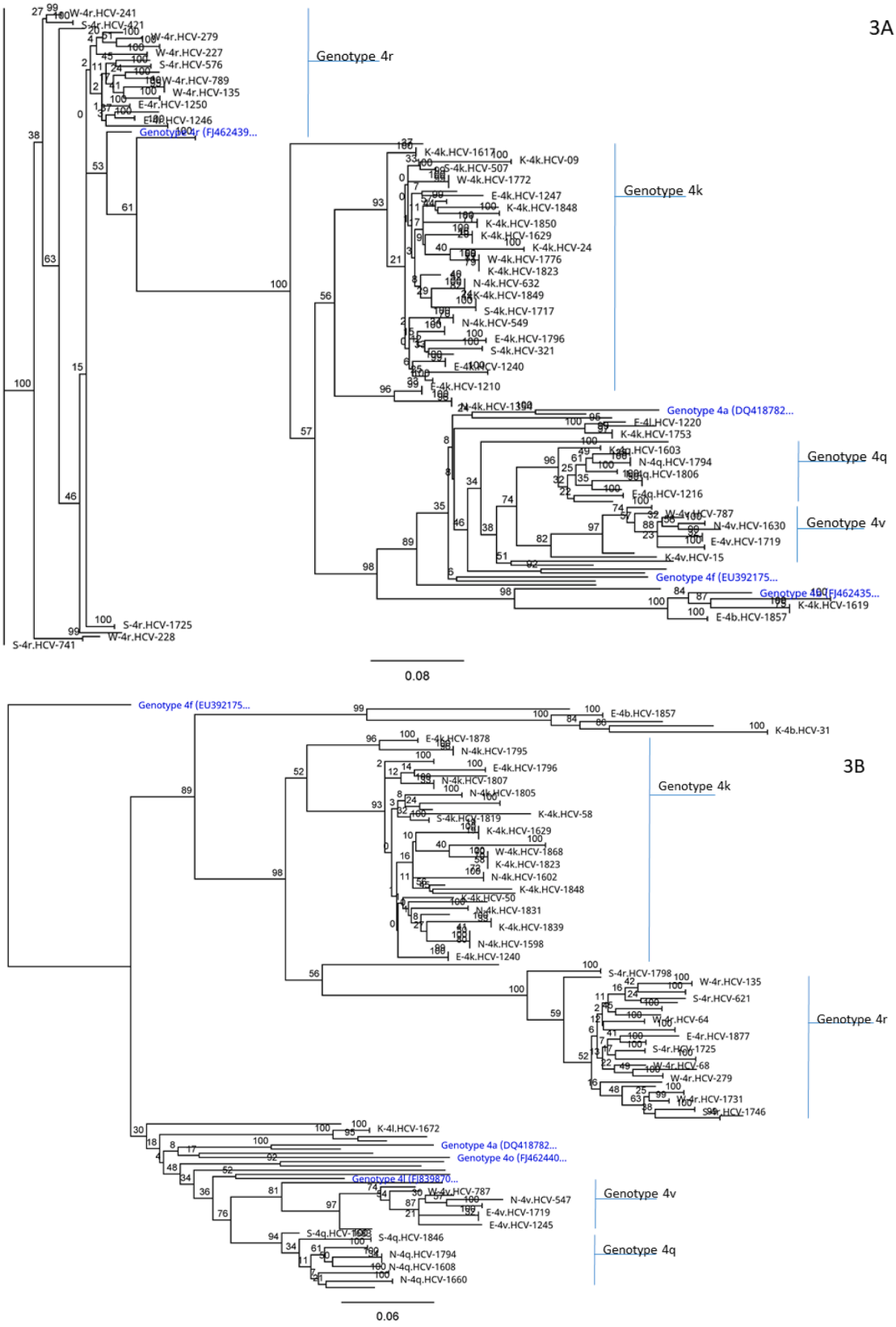

Fig. 4. RAxML trees for some of the HCV genotype 4 sequences identified in the overall population studied (3A) or for the farmers category (3B). References sequences are in blue. The first letter of each individual sequence codes for the location of the sample (E: Eastern province, K: Kigali city N: Northern province, S: Southern province, W: Western province). Bootstraps values and scale bar are indicated.

Table 2
Distribution of HCV subtypes according to clinical and lifestyle factors.

Variables	Total, n(%)	HCV different subtypes by NS5B sequencing								p value
		3h, n(%)	4b, n(%)	4c, n(%)	4k, n(%)	4l, n(%)	4q, n(%)	4r, n(%)	4v, n(%)	
Ever used a needle to inject drugs.										
Yes	7(1.3)	0(0.0)	0(0.0)	0(0.0)	5(71.4)	0(0.0)	0(0.0)	2(28.6)	0(0.0)	0.890
No	553(98.8)	4(0.7)	11(2.0)	4(0.7)	272(49.2)	10(1.8)	91(16.4)	117(21.2)	44(8.0)	
Ever used straws to inhale drugs.										
No	560(100.0)	4(0.7)	11(2.0)	4(0.7)	277(49.5)	10(1.8)	91(16.2)	119(21.2)	44(7.9)	
Ever had experienced tattoos.										
Yes	7(1.3)	0(0.0)	0(0.0)	0(0.0)	4(57.1)	0(0.0)	0(0.0)	2(28.6)	1(14.3)	0.951
No	553(98.8)	4(0.7)	11(2.0)	4(0.7)	273(49.4)	10(1.8)	91(16.5)	117(21.2)	43(7.8)	
Had body piercings										
Yes	48(8.6)	0(0.0)	0(0.0)	0(0.0)	31(64.6)	0(0.0)	6(12.5)	4(8.3)	7(14.6)	0.079
No	512(91.4)	4(0.8)	11(2.1)	4(0.8)	246(48.0)	10(2.0)	85(16.6)	115(22.5)	37(7.2)	
Ever received blood transfusion										
Yes	11(2.0)	0(0.0)	0(0.0)	0(0.0)	7(63.6)	0(0.0)	3(27.3)	1(9.1)	0(0.0)	0.831
No	549(98.0)	4(0.7)	11(2.0)	4(0.7)	270(49.3)	10(1.8)	88(16.0)	118(21.5)	44(8.0)	
Ever had surgery										
Yes	16(2.9)	0(0.0)	0(0.0)	0(0.0)	8(50.0)	0(0.0)	4(25.0)	3(18.8)	1(6.3)	0.973
No	544(97.1)	4(0.7)	11(2.0)	4(0.7)	269(49.5)	10(1.8)	87(16.0)	116(21.3)	43(8.0)	
Ever had traditional practices: Scarification										
Yes	159(28.4)	1(0.6)	2(1.3)	2(1.3)	81(50.9)	5(3.1)	24(15.1)	31(19.5)	13(8.2)	0.729
No	401(71.6)	3(0.8)	9(2.2)	2(0.5)	196(48.9)	5(1.3)	67(16.7)	88(21.9)	31(7.7)	
Ever had hemodialysis										
No	560(100.0)	4(0.7)	11(2.0)	4(0.7)	277(49.5)	10(1.8)	91(16.2)	119(21.2)	44(7.9)	
Born to HCV positive mother										
No	560(100.0)	4(0.7)	11(2.0)	4(0.7)	277(49.5)	10(1.8)	91(16.2)	119(21.2)	44(7.9)	
Household member with HCV										
Yes	27(4.8)	0(0.0)	0(0.0)	0(0.0)	14(51.9)	0(0.0)	7(25.9)	3(11.1)	3(11.1)	0.673
No	533(95.2)	4(0.8)	11(2.0)	4(0.8)	263(49.3)	10(1.9)	84(15.7)	116(21.8)	41(7.7)	
Ever had unprotected sex with different partners.										
Yes	153(27.3)	1(0.7)	1(0.7)	1(0.7)	71(46.4)	4(2.6)	31(20.2)	35(22.8)	9(5.9)	0.487
No	407 (72.7)	3(0.7)	10(2.5)	3(0.7)	206(50.6)	6(1.5)	60(14.8)	84(20.6)	35(8.6)	
Ever been diagnosed with Liver Disease.										
Yes	1(0.2)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0.994
No	559(99.8)	4(0.7)	11(2.0)	4(0.7)	276(49.3)	10(1.8)	91(16.3)	119(21.3)	44(7.9)	

infected participants and the HCV subtypes detected. Consistent with previous findings (Gupta et al., 2019b; Karoney et al., 2013; Rao et al., 2015), the HCV GT4 was highly predominant (99.3%). In countries bordering Rwanda, such as Uganda (70.8%), DRC (96.8%) and Burundi (92.7%), GT4 was also the most common genotype identified (Nankya-Mutyoba et al., 2021; Ntagirabiri et al., 2014; Parr et al., 2018). In Madagascar and West Africa in general, GT4 was less frequently observed than GT1 and GT2. The GT1 dominates in Nigeria (85%), while Burkina Faso shows a mixture of GT2 and GT3. In southern Africa, all genotypes are present, with the exception of GT6. The GT2 prevalence reaches 87% of infections in Ghana and up to 98% in Guinea-Bissau (Sonderup et al., 2017). These data support the extreme diversity of HCV on the African continent.

The most common overall HCV subtype we found was 4k (49.5%), followed by 4r (21.2%) and 4q (16.2%). These findings were comparable to those of a previous study (Gupta et al., 2019a) carried out at the Kigali Military Hospital in Rwanda, where subtype 4k accounted for 45%, 4r for 16%, and 4q for 14%. However, by comparison, our data for the city Kigali indicated a higher percentage of subtype 4k (64.0%) and a slightly lower percentage of subtypes 4r (12.0%) and q (13.0%). We also observed a disparity in the distribution of HCV GT4 subtypes according to geographical location in Rwanda. Rwanda is the most densely populated continental country in Africa, and research suggests that demographic, social and behavioral factors may strongly contribute to disparities in the geographic distribution of different HCV subtypes (Messina et al., 2015). We found that the prevalence of subtype 4q was higher (41.9%) in the northern province than in the other provinces (5% -15%), as opposed to subtype 4r. The majority of participants of our study were women (59,3%) and the vast majority of patients in this study (79.4%) were over 50, which is high compared to the country's age pyramid (Worldometers, 2023). In terms of age, most studies worldwide have shown that the prevalence of HCV increases with age, with the highest rate being reported in individuals older than 40 years (Karoney et al., 2013). Pregnant women are more frequently infected in Africa than in the general population (Bigna et al., 2019). Women's increased exposure to obstetric procedures increases their risk of infection relative to men, which may help explain the gender disparity observed in our HCV-infected cohort.

Among the demographic and economic factors that showed statistically significant variation with HCV subtypes, occupation was the main associated variable (p value = 0.005). Our survey and others reveal also that risky practices and behaviors such as injecting drugs with syringes, tattooing or piercing were rare in Rwanda (Makuza et al., 2019). On the other hand, almost one-third of our patients resorted to traditional medical practices, the majority of whom (50.9%) were infected with subtype 4k. As reported, common traditional practices such as circumcision or scarification rituals using reused instruments are a major route of transmission in Africa (Bigna et al., 2017; Kamali et al., 2022).

Consequently, the differences observed in the distribution of HCV subtypes according to the professional activity and geographical location of patients could be essentially associated with factors linked to the rural environment, such as medical practices, educational or social level. We hypothesize that subtype 4k may be more frequently associated with an urban lifestyle, while subtype 4r may be associated with a generally rural population and lifestyle. Due to the phylogenetic diversity of GT4, subtype 4r appears to have higher rates of substitutions associated with pretreatment resistance than other subtypes (Akiyama et al., 2022), which could justify closer monitoring of populations apparently more susceptible to subtype 4r infection in terms of therapeutic efficacy.

Current initiatives to improve access to DAAs to eradicate HCV face obstacles that involve financial burdens deemed either excessively difficult for governments or totally insurmountable for individuals and

Table 3
Distribution of HCV subtypes according to comorbidities and HIV or HBV coinfections.

Variables	Total, n(%)	HCV different subtypes by NS5B sequencing								p value
		3h, n(%)	4b, n(%)	4c, n(%)	4k, n(%)	4l, n(%)	4q, n(%)	4r, n(%)	4v, n(%)	
Diabetes										
Yes	38(6.8)	0(0.0)	2(5.3)	0(0.0)	21(55.3)	1(2.6)	3(7.9)	9(23.7)	2(5.2)	0.600
No	522(93.2)	4(0.8)	9(1.7)	4(0.8)	256(49.0)	9(1.7)	88(16.9)	110(21.1)	42(8.0)	
Cardiovascular disease										
Yes	1(0.2)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0.994
No	559(99.8)	4(0.7)	11(2.0)	4(0.7)	276(49.4)	10(1.8)	91(16.2)	119(21.3)	44(7.9)	
Cancer										
Yes	5(0.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	3(60.0)	1(20.0)	0.397
No	555(99.1)	4(0.7)	11(2.0)	4(0.7)	277(49.9)	10(1.8)	90(16.2)	116(20.9)	43(7.8)	
Chronic renal failure										
Yes	8(1.4)	0(0.0)	0(0.0)	0(0.0)	3(37.5)	0(0.0)	2(25.0)	2(25.0)	1(12.5)	0.988
No	552(98.6)	4(0.7)	11(2.0)	4(0.7)	274(49.7)	10(1.8)	89(16.1)	117(21.2)	43(7.8)	
AHT*										
Yes	90(16.1)	2(2.2)	3(3.3)	1(1.1)	46(51.1)	2(2.2)	14(15.6)	16(17.8)	6(6.7)	0.578
No	470(83.9)	2(0.4)	8(1.7)	3(0.6)	231(49.1)	8(1.7)	77(16.4)	103(22.0)	38(8.1)	
Tuberculosis										
Yes	1(0.2)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0.994
No	559(99.8)	4(0.7)	11(2.0)	4(0.7)	276(49.4)	10(1.8)	91(16.2)	119(21.3)	44(7.9)	
Asthma										
Yes	4(0.7)	0(0.0)	0(0.0)	0(0.0)	2(50.0)	0(0.0)	0(0.0)	2(50.0)	0(0.0)	0.907
No	556(99.3)	4(0.7)	11(2.0)	4(0.7)	275(49.5)	10(1.8)	91(16.4)	117(21.0)	44(7.9)	
Presence of ascites										
Yes	9(1.6)	0(0.0)	0(0.0)	0(0.0)	6(66.7)	0(0.0)	0(0.0)	3(33.3)	0(0.0)	0.796
No	551(98.4)	4(0.7)	11(2.0)	4(0.7)	271(49.2)	10(1.8)	91(16.5)	116(21.1)	44(8.0)	
HIV										
Yes	93(16.6)	1(1.1)	2(2.2)	0(0.0)	46(49.4)	1(1.1)	17(18.2)	24(25.8)	2(2.2)	0.395
No	467(83.4)	3(0.6)	9(1.9)	4(0.9)	231(49.5)	9(1.9)	74(15.8)	95(20.3)	42(9.0)	
HBV										
Yes	3(0.5)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	2(66.7)	0(0.0)	0(0.0)	0.550
No	557(99.5)	4(0.7)	11(2.0)	4(0.7)	276(49.5)	10(1.8)	89(16.0)	119(21.4)	44(7.9)	
HBV Vaccination										
Fully vaccinated	85(15.2)	0(0.0)	4(4.7)	1(1.2)	45(52.9)	2(2.4)	13(15.3)	13(15.3)	7(8.2)	0.667
Not vaccinated	465(83.0)	4(0.9)	7(1.5)	3(0.6)	224(48.2)	8(1.7)	77(16.5)	105(22.6)	37(8.0)	
Partially vaccinated	10(1.8)	0(0.0)	0(0.0)	0(0.0)	8(80.0)	0(0.0)	1(10.0)	1(10.0)	0(0.0)	

AHT = arterial hypertension

families with modest incomes (Assefa et al., 2017). Consistent with other findings, our analysis revealed that the majority of HCV-infected individuals had a low level of education and perceived themselves to be in the lowest social category; among them, a significant number tended to rely on traditional medicine. This suggests limited access to quality conventional care facilities but also to prevent HCV information.

We did not observe any statistical variation in the distribution of HCV subtypes according to HIV or HBV status. However, it should be noted that the number of participants co-infected with HCV and HBV was low. Furthermore, the prevalence of patients co-infected with HIV and HCV is in line with what has been reported previously for Rwanda (Munyemana et al., 2021).

Our study has some limitations. Firstly, our study is conditional on the voluntary participation of healthcare professionals involved in HCV clinical services, which may lead to selection bias. Moreover, relying on self-reported risk variables presents a new constraint, as the accuracy of these data cannot be verified. Furthermore, our sample size might have been inadequate to identify subtle associations with specific recognized HCV risk factors, including blood transfusion, hemodialysis, drug injection, tattooing or coinfection profiles. In this sense, we also concluded that our questionnaire was not fully adaptable to African populations. Questions such as those concerning injectable drug use, hemodialysis and tattoos are more appropriate for Western populations, underscoring the need for a survey better adapted to African studies.

5. Conclusion

We identified and geographically distributed HCV subtypes in Rwanda in the City of Kigali and in the four different provinces. We also pinpointed several patterns of infection profiles for specific HCV subtypes in Rwanda, providing valuable insights into the history of HCV infection in the region. Identifying at-risk populations, such as farmers in rural areas who make more frequent use of traditional medical practices, could help to improve national priorities for raising awareness of the risks of HCV transmission and for the therapeutic management of these patients. As GT4 is highly prevalent in Rwanda, patients can only be treated with DAAs, which remain costly and, above all, fail to prevent successive reinfections. Until pangenotypic DAA-based therapies become universally effective and affordable, HCV genotyping in Africa remains essential for tailoring treatment in Africa, which must take into account the cost and availability of these treatments.

Abbreviations

DAAs: Direct Antiviral Agents
DRC: Democratic Republic of Congo
GT(s): genotype(s)
HBV: Hepatitis B Virus
HCV: Hepatitis C Virus
HIV: Human Immunodeficiency Virus
ORF: Open Reading Frame
RDT: Rapid Diagnostic Tests
RT-PCR: Reverse Transcription-Polymerase Chain Reaction
UTRs: Untranslated Regions
WHO: World Health Organization

CRediT authorship contribution statement

Jean Claude Uwimbabazi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data

curation, Conceptualization. **Léon Mutesa:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Funding acquisition, Formal analysis, Conceptualization. **Franck JD Mennechet:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Claude Mambo Muvunyi:** Writing – review & editing, Project administration. **Jeanne Françoise Kabanyana:** Writing – review & editing. **Rafiki Madjid Habimana:** Investigation, Data curation. **Jean Baptiste Mazarati:** Project administration. **Isabelle Mukagatare:** Project administration. **Jean de Dieu Iragena:** Writing – review & editing. **Khalid El Moussaoui:** Writing – review & editing. **Pierrette Melin:** Writing – review & editing. **Marie-Pierre Hayette:** Writing – review & editing. **Sébastien Bontems:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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