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HCV in Democratic Republic of the Congo: The 2013-2014 Demographic and Health Survey

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Abstract

Background: The burden of HCV in sub-Saharan Africa has been poorly studied, despite estimates indicating that the region harbors some of the highest viral hepatitis rates in the world. The 2013-2014 Demographic and Health Survey (DHS) of the Democratic Republic of the Congo (DRC) offered a unique opportunity to delineate the extent and characteristics of HCV infection in the country, allowing us to estimate the national burden of infection in the DRC.

Methods: We extracted RNA from a subset of dried blood spots (DBS) collected during the DHS, including 1,309 adults ≥40 years of age, and performed high-throughput HCV viremia testing using the Abbott m2000 instrument with the RealTime HCV assay (Abbott Molecular, Des Plaines, IL). The lower limit of quantification (LLOQ) for the RealTime HCV 0.2 ml assay is 30 IU/ml in plasma and serum specimens. We tested 6mm punches of control DBS made from whole blood with known HCV viral loads to estimate difference in viral load results between the two sample types. HCV RNA positive samples underwent targeted sequencing, phylogenetic analyses and genotyping.

Results: Viral load results obtained from the control DBS were 1.8-2.0 $\log_{10}$ IU/ml less than known RNA concentrations in whole blood. Amongst DBS collected in the DRC, fifteen infections were identified; the weighted country-wide prevalence of HCV viremia was 0.9% (95% CI 0.3-1.6%) among adults ≥ 40 years of age and 0.7% (95% CI 0.6-0.8%) among the 21 HIV-positive subjects tested. All successfully genotyped cases were due to genotype 4 infection, including two cases that may represent a novel subtype.

Conclusions: HCV viremia is common among adults in the DRC. 100,000-200,000 adults ≥40 years of age may have active infection and be eligible for treatment. DBS-based HCV testing represents a useful tool for defining the burden of HCV viremia and can be easily incorporated into population-based surveys.

Background

- Hepatitis C virus infections frequently go undiagnosed and untreated in developing countries like the DRC.
- DBS are commonly collected in sub-Saharan Africa during large-scale health surveys, such as the 2013-2014 DRC Demographic and Health Survey (DHS).
- As part of the survey, DBS were collected from a nationally representative sample of over 26,000 subjects for the purpose of HIV and vaccine-related serological testing and malaria molecular testing.
- These DBS also afforded the opportunity to delineate the extent and characteristics of HCV infection throughout the country.

Methods

- Random sampling stratified by province
  - 25 % adults ≥40 years old from each of 25 DRCs provinces
  - Additional testing of subjects from the Kasai province with the highest HCV viremia
- 12mm DBS containing 50-70 µl of whole blood were collected from the subjects
- 6-mm punches were tested for HCV viral load using the Abbott RealTi*me* HCV assay on the Abbott m2000 system
  - 6mm DBS were incubated in 0.5 ml of the *m*2000 Wash-1 buffer at 55^{0}C for 30 minutes
  - Eluate was processed as a sample on the Abbott m2000 system
  - Control DBS made from whole blood with target RNA concentration 5.4 and 4.4 $\log_{10}$ IU/ml were tested in 12 replicates
- RT-PCR targeting HCV 5' UTR, core/E1 and NS5b was performed on the HCV-positive samples (1, 2).
- RNA was extracted from HCV positive DBS (11 samples) and DBS eluates (4 samples) using Trizol/chloroform phase separation.
- Amplicons underwent bidirectional Sanger sequencing
- Phylogenetic analysis was performed for subtype determination using MEGA 7.0 or PHYLIP software (v3.5c, J. Felsenstein, University of Washington, Seattle, WA)
- Prevalence was calculated based on sample weights to account for complex sampling design using SAS 9.4 (SAS Institute, Cary, NC)

Results

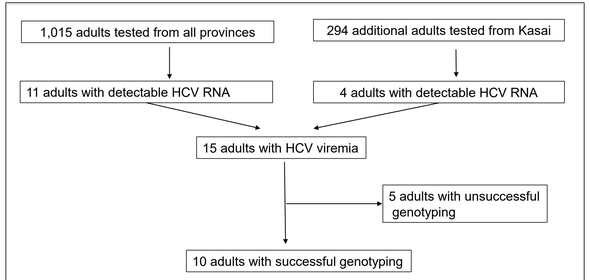


Figure 1. Study population and HCV testing results

Including testing of additional samples selected from Kasai Orientale and Kasai Occidental after initial testing

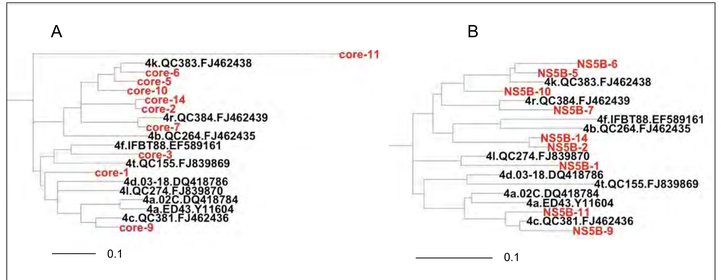


Figure 2. Phylogenetic trees for HCV genotype 4

Samples from the current study (red) clustered with reference sequences (black) from known genotype 4 subtypes for both the HCV core/E1 (panel A) and NS5B (panel B) genomic regions. Samples 2 and 14 formed a separate cluster.

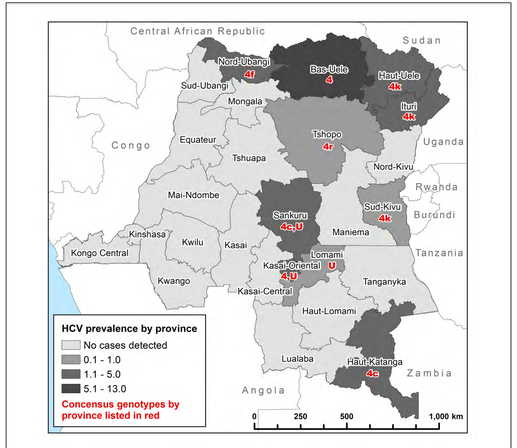


Figure 3. HCV viremia prevalence by province.

Prevalence is expressed as a percent of the population
U indicates unsuccessful genotyping

Table 1. Characteristics of adults with HCV viremia.

Characteristic*	All subjects	HCV RNA +	HCV RNA -
N	1,309	15	1294
Prevalence, % (95% CI)	--	0.9 (0.3 - 1.6)	99.1 (98.4 - 99.7)
Age in years, median (IQR)	44.9 (41.9 – 55.7)	49.0 (43.9 - 55.7)	44.9 (41.9 - 48.5)
Male sex, n (%)	798 (60.4)	12 (74.6)	786 (60.2)
HIV co-infection, n (% [95% CI])	21 (1.5)	1 (1.1 [0.8 - 1.4])	20 (1.5 [0.6 - 2.5])

* All summary statistics are weighted to account for complex survey design. Sample sizes are unweighted.
Abbreviations: CI, confidence interval; IQR, interquartile range.

Table 2. Characteristics of individual subjects and subtypes

		Genotype assignment			
Sample ID	HCV RNA,* $\log_{10}$ IU/ml	5'UTR	core/E1	NS5B	Consensus
1	4.02	4a	4	4l	4
2	3.68	4	4	4	4
3	2.91	4	4f	-	4f
4	2.76	-	-	-	-
5	3.26	4k	4k	4k	4k
6	3.54	4k	4k	4k	4k
7	4.57	4r	4r	4r	4r
8	< 1.48	-	-	-	-
9	4.1	4	4c	4c	4c
10	4.16	4k	4k	4k	4k
11	2.22	4	4	4c	4c
12	2.04	-	-	-	-
13	1.71	-	-	-	-
14	4.34	4	4	4	4
15	< 1.48	-	-	-	-

*Viral load in an eluate from a single 6mm DBS punch.
Testing of 6mm DBS punches made with 70 µL whole blood with known target RNA concentrations suggested a 1.8-2 $\log_{10}$ U/ml difference between viral loads in DBS and known RNA concentration in whole blood. This data confirmed most recent published findings (3).

Conclusions

- The first national HCV viremia prevalence estimates based on DBS sampling revealed that 0.9% of adults ages 40-55 are actively viremic.
- HCV genotype 4 infections predominate in the DRC.
- Diverse genotype 4 strains were identified including subtypes 4c, 4f, 4k and 4r.
- In a resource limited setting, DBS-based HCV RNA testing is a useful tool for defining the prevalence of HCV viremia and can be incorporated into population-based surveys.

References:

1. *Hogan CA, Iles J, Frost EH, Giroux G, Cassar O, Gessain A, Dion MJ, Ilunga V, Rambaut A, Yengo-Ki-Ngimbi AÉ, Behets F, Pybus OG, Pépin J. Epidemic History and Iatrogenic Transmission of Blood-borne Viruses in Mid-20th Century Kinshasa. J Infect Dis. 2016 Aug 1;214(3):353-60. doi: 10.1093/infdis/jiw009.*
2. *Murphy, D. G., Willems, B., Deschênes, M., Hilzenrat, N., Mousseau, R., & Sabbah, S. (2007). Use of Sequence Analysis of the NS5B Region for Routine Genotyping of Hepatitis C Virus with Reference to C/E1 and 5' Untranslated Region Sequences . Journal of Clinical Microbiology, 45(4), 1102–1112. http://doi.org/10.1128/JCM.02366-06*
3. *Soulier A, Poiteau L, Rosa I, Hezode C, Roudot-Thoraval F, Pawlotsky JM, Chevaliez S. 2016. Dried Blood Spots: A Tool to Ensure Broad Access to Hepatitis C Screening, Diagnosis, and Treatment Monitoring. J Infect Dis 213:1087-95.*