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HEPATITIS C VIRUS AND HUMAN PEGIVIRUS 2 SURVEILLANCE IN A LARGE CAMEROONIAN COHORT

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Abstract

Background Estimates of the burden of chronic hepatitis C virus (HCV) require HCV RNA prevalence measurements in diverse populations. HCV infection is a risk factor for infection with human pegivirus 2 (HPgV-2), a novel virus for which the global distribution and genetic diversity are unknown. To characterize the prevalence of HCV viremia and presence of HPgV-2 in South Cameroon, archived samples primarily collected from patients seeking healthcare were screened for HCV RNA, followed by HPgV-2 antibody and RNA testing amongst the positives.

Methods Well characterized retrospective plasma specimens from N=12,369 consenting subjects participating in surveillance studies in South Cameroon from 2013 – 2016 were included in the study. All specimens were screened for HCV in pools of 5 in an off-label use of the Abbott HCV RealTime viral load assay. HCV RNA positive specimens with remaining volume were also screened for HPgV-2 antibodies on the ARCHITECT instrument, followed by molecular characterization. All viral sequences were classified by phylogenetic analysis.

Results Overall, HCV RNA was detected in 305 (2.47%; 95% CI: 2.21-2.75%) specimens. Notably, the median age of HCV RNA negative individuals was 31 years while the median for positive individuals was 52.5, with the prevalence of HCV RNA being (9.09%, 95% CI: 7.86-10.49%) in participants over age 40. Phylogenetic classification of sequences from N=103 specimens identified HCV genotypes 1 (19.4%), 2 (15.5%), and 4 (65.1%). HPgV-2 antibodies were detected in N=39 (14.8%; 95% CI: 11-19.6%) of the HCV RNA positive specimens. Of these, N=5 viremic HPgV-2 infections were confirmed by sequencing. Phylogenetic inference indicates they share more than 90% nucleotide identity with strains identified previously on other continents.

Conclusions In the first large scale HCV RNA surveillance study to date in Cameroon, we found the overall prevalence of viremic HCV infections to be lower than previous seroprevalence reports and provide new HCV sequences. The discovery of HPgV-2 in South Cameroon expands the geography of this virus to the African continent, indicating a more widespread distribution exists.

Methods

Figure 1: Testing Algorithm

The number of specimens tested for each assay is shown in black adjacent to the appropriate arrow and the number of positives are indicated in the boxes for each test.Samples were tested for HCV viral load in pools of 5 in an off label use of the Abbott RealTi*m*e assay, followed by individual testing for specimens in positive pools. Anti-HPgV-2 positive specimens include both grayzone and reactive test results. Sequences were obtained by Sanger or next generation metagenomic sequencing methods.

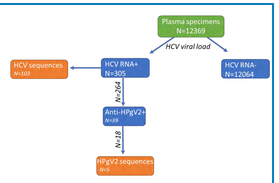
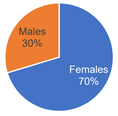
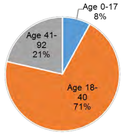


Figure 2: Study Population



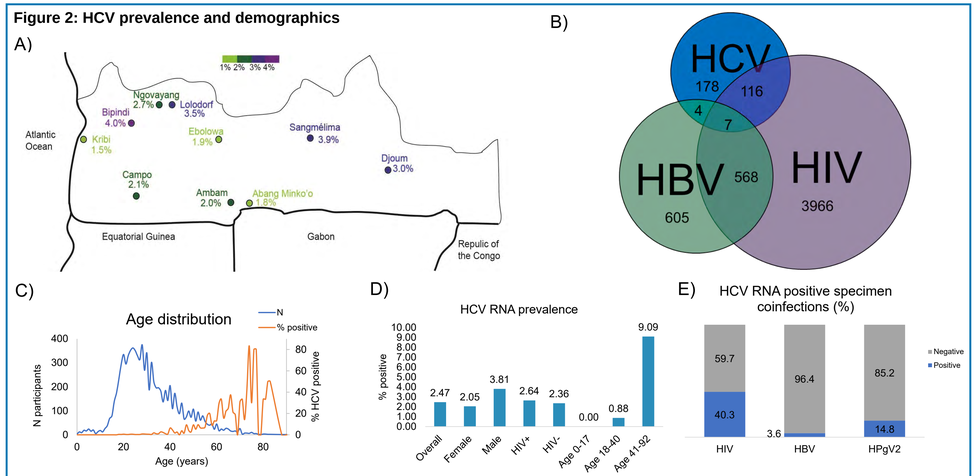
Recruitment category	N	%
Door to door	119	1
Voluntary testing campaign	235	1.9
Ill patient in clinic/hospital	12015	97.1
Total	12369	



The sex (A), recruitment category (B), and age (C) of the N=12,369 participants of the study are shown

Results - HCV Prevalence

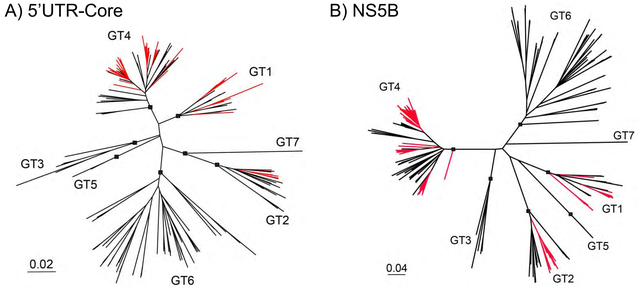
Figure 2: HCV prevalence and demographics



A) The 10 study sites with at least 100 participants are shown by dots colored to represent HCV prevalence as indicated by color key on a map of the South Region of Cameroon with the prevalence of HCV RNA at each site indicated as a percentage of the total samples at each site. B) The number of single, dual, and triple infections identified in the study cohort are indicated by a venn diagram C) The distribution of ages of all study participants is shown in orange and the prevalence of HCV RNA for each age is shown in blue as a percentage of total samples for each age D) the prevalence of HCV RNA in each cohort is indicated as a percentage E) The portion of the 305 HCV RNA positive specimens with each co-infection are indicated as a percentage

Results - HCV diversity

Figure 3: HCV classifications

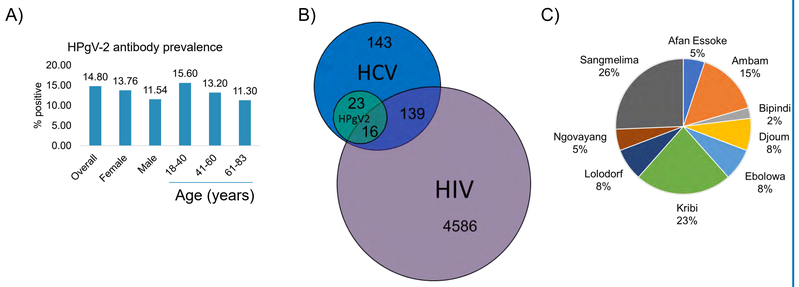


Classification	N	%
1c	1	0.97
1e	8	7.77
1g	4	3.88
1h	6	5.83
1l	1	0.97
2	14	13.59
2a	1	0.97
2q	1	0.97
4	5	4.85
4f	37	35.92
4p	11	10.68
4t	14	13.59
Total	103	

Neighbor joining phylogenetic trees for A) 5'UTR-core and B) NS5B are shown with specimen sequences in red and references in black. Bootstrap values >90 for relevant nodes are indicated by a black box. A summary of the classifications for all N=103 sequences is shown in the table on the right.

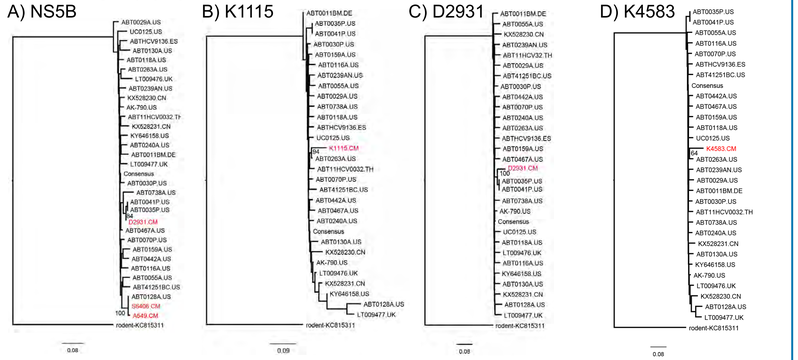
Results - HPgV-2 prevalence and phylogeny

Figure 4: HPgV-2 prevalence and demographics



A) The prevalence of HPgV-2 antibody positive and grayzone samples amongst HCV positive specimens tested in the indicated populations is shown as a percentage. B) The number of HPgV-2 antibody and grayzone samples co-infected with HIV are indicated by a Venn diagram and C) The distribution of HPgV-2 antibody positive and grayzone specimens across the 9 sites where they were identified are shown as a percentage

Figure 5: HPgV-2 phylogenetic trees



Neighbor joining phylogenetic trees for A) NS5B Sanger sequences and NGS sequences for B) K1115 C) D2931 and D) K4583 are shown with specimen sequences in red and references in black. Both NGS and NS5B Sanger sequences were available for D2931. Bootstrap values are indicated for branches containing specimen sequence. All HPgV-2 sequences from Cameroon share 90.9-99.5% nucleotide identity with closest relative strains.

Conclusions

- In the first large scale HCV RNA surveillance study to date in Cameroon, we find the prevalence of viremic HCV infections to be 2.47%, with higher prevalence rates amongst participants over age 40 and males.
- Since the majority of the study population included people seeking healthcare, these rates suggest that targeted outreach to people over age 40 and males seeking healthcare in Cameroon could have an impact on reducing the local burden of viremic HCV infections.
- New HCV and HPgV-2 sequences from this study document the circulating strains in Cameroon and confirm the first known viremic cases of HPgV-2 infections in Africa.