High Frequency of Active HCV Infection Among Seropositive Cases in West Africa and Evidence for Multiple Transmission Pathways

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Background. Sub-Saharan Africa (SSA) has one of the highest global hepatitis C virus (HCV) prevalence estimates. However, reports that suggest high rates of serologic false positives and low levels of viremia have led to uncertainty regarding the burden of active infection in this region. Additionally, little is known about the predominant transmission risk factors in SSA.

Methods. We prospectively recalled 363 past blood donors (180 who were rapid screen assay [RSA] positive and 183 who were RSA negative at time of donation) to identify the level of active infection and risk factors for infection at a teaching hospital in Kumasi, Ghana. Participants had repeat blood testing and were administered a questionnaire on risk factors.

Results. The frequency of HCV active infection ranged from 74.4% to 88% depending on the criteria used to define serologically positive cases. Individuals with active disease had biochemical evidence of liver inflammation and median viral loads of 5.7 log copies/mL. Individuals from the northern and upper regions of Ghana had greater risks of infection compared with participants from other areas. Additional risk factors included traditional circumcision, home birth, tribal scarring, and hepatitis B virus coinfection.

Conclusions. Viremic infection was common among serologically confirmed cases. Attention to testing algorithms is needed in order to define the true HCV burden in SSA. These data also suggest that several transmission modes are likely contributing to the current HCV epidemic in Ghana and that the distribution of these practices may result in substantial regional variation in prevalence.

Keywords. HCV; Africa; prevalence; transmission.

More than 185 million individuals worldwide are seropositive for hepatitis C virus (HCV) [1]. HCV is a leading cause of cirrhosis and cancer, and the disease burden will increase in coming years [2–7]. The global spread is disproportionate, with most infected individuals living in low-income countries. Sub-Saharan Africa (SSA) collectively accounts for 20% of the global burden [1], with the West African region having some of the highest estimates (2.8%; 95% confidence interval [CI], 2.4–3.3). However, there is a wide variation across recent studies [8–17].

The population-level epidemiology of HCV and transmission mechanisms in SSA are poorly defined, with little data detailing individuals with active disease and incomplete data on infection risk factors. Progress toward development of a more comprehensive knowledge base has been hampered by conflicting reports that suggest poor specificity of serologic tests in SSA, high rates of presumed false positivity, and low rates

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of viremia [18–23], leading some to question the true disease burden. Prior studies, though, have used varied assays, with few including virologic assessment; thus, the true disease burden is not well characterized.

Lack of clarity on appropriate serologic testing methods also undermines efforts to understand the transmission pathways of infection and to characterize actively infected individuals. While unsterile needles and transfusion of blood products have played a role [24–27], the varied genotypes, high diversity seen in circulating strains, and lack of age cohort effects do not support transmission via a singular mode in most regions of SSA [19, 20, 28–36]. Several studies have suggested that intrafamilial, scarification, and circumcision are potential transmission modes relevant to this region, [24, 25, 27, 29, 37]; further work needs to confirm such findings.

In this study, we recruited previous blood donors based on their HCV screening results at the time of blood donation at a large tertiary hospital in Kumasi, Ghana. Located in the middle-belt of Ghana, the hospital serves as a primary referral center and receives patients from throughout Ghana and with multiple tribal backgrounds. The primary objectives of this study were to determine the frequency of active infection and identify risk factors for HCV infection.

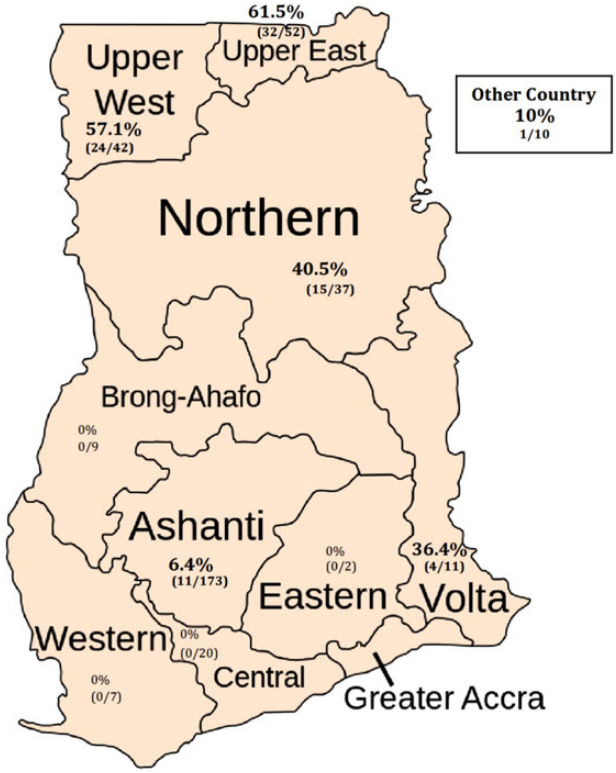

Figure 1. Hepatitis C virus positivity frequency by region of Ghana.

METHODS

Study Population and Recruitment

The study population was derived from individuals who had donated blood at the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana, from 2008 through 2013. Prior to donation, individuals are screened for viral hepatitis with a rapid screen assay (RSA). We recalled all past HCV RSA-positive donors as well as HCV-negative donors who had donated blood at the time closest to each individual who had an HCV positive screen. Individuals were contacted between May 2013 and January 2014 and asked to return to KATH for participation in this study. Upon consent, individuals were administered a questionnaire to assess demographic characteristics and risk factors for HCV infection. Individuals were asked about the region of birth in Ghana, as well as current region they reside. Regions of Ghana were defined by the established 10 administrative regions, which are outlined in Figure 1. Ethical approval was obtained at KATH and at Loyola University (Chicago, Illinois). A nonresearch determination was obtained at the Centers for Disease Control and Prevention (CDC) to test anonymized, unlinked samples for HCV.

Sample Processing

Blood samples were immediately processed and aliquoted. One aliquot was used to run alanine transaminase (ALT) and aspartate transaminase (AST) in the KATH reference laboratories; the remaining samples were immediately stored in a generator-supported −80°C freezer at KATH. Samples were then shipped to Loyola for further assessment under strict temperature-controlled conditions. No more than 1 freeze–thaw cycle was allowed prior to sample testing.

Laboratory and Serologic Assessment

Assays were run in Loyola University Medical Center's reference laboratory, using the hepatitis B virus (HBV) surface antigen Abbott (Abbott Park, Illinois) architect chemiluminescennt immunoassay (CIA), human immunodeficiency virus (HIV) fourth generation Ab/Ag test and the Bayer HCV ADVIA Centaur HCV CIA. The CIA was performed on an automated ADVIA Centaur Immunoassay system (Siemens, Tarrytown, New York). The RSA used by the blood bank was the Accu-Tell HCV (AccuBio Tech Co., Beijing, China).

Virologic Assessment

A real-time polymerase chain reaction (RT-PCR) assay (Abbott) was used to determine HCV RNA quantitative levels on all individuals with sufficient sample volume and with a positive RSA, regardless of the CIA result. Additionally, aliquots from all participants were sent to the molecular branch laboratory of the CDC Division of Viral Hepatitis for HCV genotyping.

Total nucleic acid was extracted and cDNA was generated. Amplification of the NS5b gene region was completed and sequenced, and HCV genotypes were determined by the generated NS5b sequences.

Hepatitis C Virus Outcome Definitions

The lack of availability of the recombinant immunoblot assay (RIBA) hinders the ability to confirm HCV infection and distinguish between true-seropositive and false-positive serologic results. Consequently, the CDC established criteria using signal-to-cutoff ratios (S/C ratios) from available assays to help identify likely confirmed seropositive cases when an RIBA is not available [38]. We used these definitions to define HCV infection states. For the Bayer ADVIA anit-HCV CIA, the S/C ratio is ≥11. Thus, for this study, HCV infection was defined as individuals with both a positive RSA and CIA positive result with S/C ≥11, or detectable viremia. Active infection was defined as individuals with detectable viremia. Cleared infection applied to individuals who were RSA positive, CIA S/C ≥11, but with a negative viral load. Individuals who were negative on RSA and CIA, or RSA positive but CIA S/C <11, and who had no detectable viremia were considered to be free of infection (no prior/current infection). We sued these definitions to define HCV infection and noninfection for the risk factor analyses. With the lack of RIBA testing to confirm serologic testing, we also duplicated all analyses using an alternative definition for serologic confirmation (S/C >1 per manufacturer's report, as opposed to previous CDC published work). Importantly, neither the direction nor magnitude of the results changed when this definition was applied.

Statistical Approach

Standard descriptive statistics were used to analyze demographic variables, risk factors, and clinical parameters by HCV infection status. Bivariate analyses were conducted using *t* test for continuous variables and Pearson χ^2 test or Fisher exact test for categorical variables depending on satisfaction of assumptions. Multivariable logistic regression was used to determine risk factors associated with HCV seropositivity based on bivariate significance. Two-way statistical interactions were examined between all variables. Covariate adjusted odds ratios (OR) and their respective 95% CIs are reported.

RESULTS

Hepatitis C Virus Infection Status Based on Additional Serologic/Virologic Testing

A total of 363 prior blood donors were enrolled. Of those, 180 had a prior positive HCV RSA result at the time of blood donation, and 183 were HCV RSA negative. Based on study definitions applied after the additional serologic and virologic testing (see "Methods" section for study outcome definitions), 87 were determined to have evidence for HCV infection (active or cleared) and 276 were noninfected (Table 1). A total of 82 individuals were both RSA positive and had CIA S/C ratio ≥11. Of these 82 individuals, 66 had detectable viremia, 9 were viral load negative, and 7 had inadequate sample volume for viral load testing. An additional 5 persons were defined as HCV infected due to detectable viremia. Of these, 3 were RSA positive but CIA negative, 1 was RSA positive but CIA S/C <11, and 1 was both RSA and CIA negative. Therefore, 276 individuals were classified as noninfected. Importantly, all individuals with a negative RSA at the time of blood donation also had a negative CIA result (N = 183).

Risk Factors for Hepatitis C Virus Infection Status

Comparison of demographics and risk factors between HCV-infected and HCV-noninfected participants is presented in Table 2. The study participants were predominantly young men, which was consistent with the blood donor population in general. However, neither age nor gender significantly varied across HCV disease state. Those in the HCV-infected group were more often born at home (OR, 2.0; 95% CI, 1.2–3.4), more frequently reported tribal scarring (OR, 2.2; 95% CI, 1.3–3.6), and more likely to have traditional circumcision as opposed to hospital-based circumcision (OR, 3.8; 95% CI,

Table 1. Serologic and Virologic Testing Results of Study Population

RSA Results From Time of Blood Donation. New Blood Draw at Time of Consent for Following Tests: CIA, Quantitative Viral Load, and Sequencing	RSA Positive (N = 180)		RSA Negative (N = 183)	
	CIA Positive per Manufacturer Value and S/C Ratio ≥11 (N = 82)	CIA Positive per Manufacturer Value and S/C Ratio <11 (N = 15)	CIA Negative (N = 83)	CIA Negative (N = 183)
With detectable, quantifiable viremia	66 (88%)[a]	1 (6.7%)[a]	3 (3.9%)[a]	1 (1.0%)[a]
No detectable viremia	9 (12%)[b]	14 (92.3%)	73 (96.1%)	96 (99.0%)
Quantitative RNA viral load not assessed[c]	7[b]	0	7	86

Abbreviations: CIA, chemiluminescennt immunoassay; RSA, rapid screen assay; S/C, signal to cutoff.

[a] Defined as active infection.

[b] Defined as cleared hepatitis C virus (HCV) infection.

[c] Quantitative viral load not assessed due to insufficient sample volume size.

Table 2. Demographic and Risk Factor Analyses According to Hepatitis C Virus Infection Status

Demographic Variable	HCV Infection (N = 87)	No HCV Infect (N = 276)	Odds Ratio (95% Confidence Interval)[a]	*P* Value
Gender, n (% male)	70 (80.5)	231 (83.7)	0.8 (.4–1.5)	*.48*
Age (yr), mean (standard deviation)	32.3 (7.3)	30.6 (8.1)	1.02 (.99–1.05)	*.07*
Birth place, n (%)				***.005***
Home	59 (67.8)	140 (50.7)	2.0 (1.2–3.4)	
Clinic/Hospital	28 (32.2)	136 (49.3)		
Marital status, n (%)				***.03***[b]
Single	24 (27.6)	95 (34.4)	REF	
Married/Cohabiting	57 (65.5)	177 (64.1)	1.3 (.74–2.2)	*.08*
Separated/Widowed/Divorced	6 (6.9)	4 (1.5)	5.9 (1.6–22.7)	*.01*
Education level, n (%)				***<.001***[b]
None/Primary	29 (33.3)	32 (11.6)	1.7 (.8–3.5)	*.16*
JHS/MSLC/SHS/Tech	37 (42.5)	205 (74.3)	0.3 (.2–.6)	*<.001*
Tertiary	21 (24.1)	39 (14.1)	REF	
Religion, n (%)				***.053***[b]
Christian	61 (70.1)	218 (78.9)	REF	
Muslim	24 (27.6)	45 (16.3)	1.9 (1.1–3.4)	*.04*
None	2 (2.3)	13 (4.7)	0.6 (.1–2.5)	*.23*
Tribal scarring, n (%)	34 (39.1)	63 (22.8)	2.2 (1.3–3.6)	**.003**
Acupuncture, n (%)	2 (2.3)	6 (2.2)	1.1 (.2–5.3)	.99[c]
Female circumcision, n (%)	2 (11.8)	1 (2.2)	5.9 (.5–69.4)	.18[c]
Male circumcision, n (%)	68 (97.1)	229 (99.1)	0.3 (.04–2.1)	.23[c]
Location where circumcision was performed, n (%)				**<.001**
Traditional (non-clinic/hospital procedure)	54 (62.1)	108 (39.1)	3.8 (2.1–7.1)	.002
Hospital	16 (18.4)	123 (44.6)	REF	
None	17 (19.5)	45 (16.3)	2.9 (1.4–6.2)	.006
Intravenous drug use, n (%)	0 (0)	7 (2.5)	N/A	.20[c]
History of transfusion, n (%)	1 (1.2)	0 (0)	N/A	.24[c]
History of hospitalization, n (%)	9 (10.3)	18 (6.5)	1.7 (.7–3.8)	.24
History of surgery/Dental procedure, n (%)	5 (5.8)	11 (4.0)	1.5 (.5–4.4)	.55[c]
Alcohol use, n (%)	22 (25.3)	54 (19.6)	1.4 (.8–2.5)	.25
Human immunodeficiency virus positive, n (%)	5 (5.8)	5 (1.8)	3.3 (.9–11.7)	.06[c]
Hepatitis B virus surface antigen positive, n (%)	8 (9.2)	10 (3.6)	2.7 (1.2–7.8)	**.03**
Region of origin[e], n (%)				**<.001**
Ashanti	11 (12.6)	162 (58.7)	REF	
Upper	56 (64.4)	38 (13.7)	21.7 (10.4–45.3)	<.001
Northern	15 (17.2)	22 (7.9)	9.4 (3.8–23.2)	<.001
Other[d]	4 (4.6)	43 (15.6)	1.3 (.4–4.3)	.66
Different country	1 (1.1)	9 (3.3)	1.6 (.2–14.1)	.65

Bold/italic was used for significant ($P < .05$) results.

Abbreviations: HCV, hepatitis C virus; JHS/MSLC/SHS/Tech, Junior High School, Middle School, Senior High School, Technical School; N/A, not applicable; REF, reference.

[a] Odds ratio with 95% confidence Intervals.

[b] Indicates simple logistic regression; otherwise, Pearson χ^2 test.

[c] Indicates Fisher exact test.

[d] Other = (western, central, greater Accra, Easter, Volta, and Brong Ahafo).

[e] Defined by 10 administrative regions of Ghana, as shown in Figure 1.

2.1–7.1) or no circumcision (OR, 2.9; 95% CI, 1.4–6.2). Those in the HCV-infected group were also more frequently coinfected with HBV (OR, 2.7; 95% CI, 1.2–7.8); HIV coinfection neared significance. There was an increased frequency of HCV-positive individuals reporting minimal education, but there was a higher rate of higher education in this group as well. Overall, marital

status was significant but was driven by a higher frequency of HCV infection among individuals who were separated, widowed, or divorced. There was a higher frequency of individuals of Muslim faith compared with Christian faith (OR, 1.9; 95% CI, 1.1–3.4).

A highly significant association with region of tribal origin was observed with HCV infection. Individuals whose tribal origin was in the upper and northern parts of Ghana had an increased risk of HCV infection compared with individuals reporting Ashanti tribal origin (OR, 21.7; 95% CI, 10.4–45.3; $P < .001$ and OR, 9.4; 95% CI, 3.8–23.2; $P < .001$, respectively; Table 2). In Figure 1, the frequency of positive HCV infection among individuals born in different regions is depicted. We examined the association of HCV infection with risk factors across regions (Table 3) and observed substantial confounding, that is, the frequency of numerous HCV exposures varied across tribal region. For example, tribal scarring was reported in 45% (42/94) and 50% (18/36) of individuals from upper and northern regions compared with 16.2% (28/173) for individuals from Ashanti land. Similar significant differences in frequency of risk factors were observed for traditional circumcision and home birth. There were no statistical interactions noted between risk factors and region of origin.

In multivariable analyses, tribal region of origin was the most significant characteristic associated with HCV infection. Compared with Ashanti participants, individuals from the northern and upper regions had, respectively, 6.6 (95% CI, 2.4–18.2) and 18.7 (95% CI, 8.3–42.2) times the odd's to be infected with HCV ($P < .001$). Accounting for this major structural element in the data, traditional circumcision (OR, 3.3; 95% CI, 1.5–7.2;

Table 3. Hepatitis C Virus Risk Factor Variation Across Regions of Origin

	Region of Origin[c]						
Risk Factor	Upper (N = 94)	Northern (N = 36)	Other[a] (N = 40)	Brong Ahafo (N = 9)	Ashanti (N = 173)	Different Country (N = 10)	*P* Value[b]
History of tribal scarring							
Yes (%)	42 (45)	18 (50)	3 (8)	3 (33)	28 (16)	2 (20)	<.001
No (%)	52 (55)	18 (50)	37 (93)	6 (67)	145 (84)	8 (80)	
History of circumcision							
Yes (%)	82 (87)	27 (75)	32 (80)	8 (89)	141 (82)	9 (90)	.48
No (%)	12 (13)	9 (25)	8 (20)	1 (11)	32 (19)	1 (10)	
Traditional circumcision							
Yes (%)	58 (62)	21 (58)	15 (38)	4 (44)	58 (34)	5 (50)	<.001
No (%)	24 (26)	6 (17)	17 (43)	4 (44)	84 (49)	4 (40)	
N/A (%)	12 (13)	9 (25)	8 (20)	1 (11)	31 (18)	1 (10)	
Born at home							
Yes (%)	66 (70)	26 (72)	20 (50)	5 (56)	76 (44)	5 (50)	<.001
No (%)	28 (30)	10 (28)	20 (50)	4 (44)	97 (56)	5 (50)	
History of hospitalization							
Yes (%)	6 (6)	6 (17)	2 (5)	0 (0)	11 (6)	2 (20)	.28
No (%)	88 (94)	30 (83)	38 (95)	9 (100)	162 (94)	8 (80)	
History of surgery							
Yes (%)	5 (5)	4 (11)	1 (3)	0 (0)	6 (3)	0 (0)	.33
No (%)	89 (95)	32 (89)	39 (98)	9 (100)	167 (97)	10 (100)	
Alcohol use							
Yes (%)	20 (21)	3 (8)	8 (20)	1 (11)	42 (24)	2 (20)	.27
No (%)	74 (79)	33 (92)	32 (80)	8 (89%)	131 (76)	8 (80)	
Hepatitis B virus surface antigen positive							
Yes (%)	6 (6)	2 (6)	1 (3)	1 (11)	6 (3)	2 (20)	.46
No (%)	88 (94)	34 (94)	39 (97)	8 (89)	167 (97)	8 (80)	
Family member with liver disease							
Yes (%)	3 (3)	1 (3)	0 (0)	0 (0)	2 (1)	1 (10)	.52
No (%)	91 (97)	35 (97)	40 (100)	9 (100)	171 (99)	9 (90)	

Abbreviation: N/A, not applicable.

[a] Other = (western, central, greater Accra; Easter; and Volta).

[b] Fisher exact test used; "different country" category excluded for all analyses.

[c] Regions defined as shown in Figure 1.

Table 4. Multivariable Logistic Regression Model for Hepatitis C Virus Seropositivity

Variable	Odds Ratio (95% Confidence Interval)	*P* Value
Region of origin		**<.001**
Northern vs Ashanti	6.6 (2.4–18.2)	<.001
Other vs Ashanti	1.2 (.3–3.9)	.83
Upper vs Ashanti	18.7 (8.3–42.2)	<.001
Education level		**.01**
None/Primary vs tertiary	0.4 (.2–1.1)	.08
JHS/MSLC/SHS/Tech vs tertiary	0.3 (.1–.7)	.003
Marital status	2.3 (.8–6.7)	**.06**
Married vs single	0.9 (.5–1.9)	.83
Other vs single	8.0 (1.23–50.4)	.03
Hepatitis B virus surface antigen positive	3.8 (1.1–13.5)	**.04**
Circumcision		**.01**
None vs hospital	3.5 (1.3–9.1)	.01
Traditional vs hospital	3.3 (1.5–7.2)	.004

Bold was used for significant ($P < .05$) results.

Abbreviation: JHS/MSLC/SHS/Tech, Junior High School, Middle School, Senior High School, Technical School.

$P = .004$), no circumcision (OR, 3.5; 95% CI, 1.3–9.1; $P = .01$), and HBV seropositivity (OR, 3.8; 95% CI, 1.1–13.5; $P = .04$) remained significantly associated with HCV (Table 4).

Frequency of Active Hepatitis C Virus Infection

The frequency of active infection varied based on the definition of serologically confirmed HCV infection. Among the individuals with a positive RSA and CIA S/C≥11 who we were able to assess for viremia (7 individuals with inadequate sample size to test viral load), 88% (66/75) had active viremia (Table 1). Of note, if we used less stringent criteria to define HCV infection (RSA positive and CIA S/C >1, as opposed to S/C >11), the result of active viremia was also high, 74.4% (67/90). Based solely on a positive RSA, regardless of CIA, only 42.2% had active infection.

Hepatitis C Virus Active Infection

When we compared actively infected individuals with those in the group who had cleared the infection, there was no difference in age, gender, or HIV/HBV status (Table 5). The median ALT and AST values of viremic individuals were 54 (interquartile range [IQR], 29–75) and 38 (IQR, 27–60), which were both significantly higher than for individuals who had cleared infection. Among those who cleared infections, median ALT and AST values were 32 (IQR, 28–41) and 28 (IQR, 26–33), respectively. The median viral load was 5.75 log copies/mL, with an overall range of 1.08–6.88 log copies/mL. Genotype 2 was predominant (84.7%), with genotype 1 making up the remainder (15.3%; Figure 2).

DISCUSSION

Here, we describe a group of 363 persons recalled from a large teaching hospital's blood bank registry in Ghana, with similar proportions of participants who screened HCV positive and negative when they attempted to donate blood. The subsequent results highlight the importance of stringent diagnostic strategies for future studies of HCV in SSA. Past studies from SSA have suggested a high rate of false-positive serologic results and low levels of detectable viremia [18, 19, 21, 22]. In our study, we collected new samples of blood, which were immediately frozen and kept in a continuous cold chain to avoid degradation by freeze–thaw cycles. We subsequently performed an automated CIA, quantitative RT-PCR viral load assessment, and viral sequencing. In participants with a positive CIA per

Table 5. Comparison of Cleared and Active Infection

Variable	HCV Seropositive/Cleared Virus (N = 9)	HCV Active Infection (N = 71)	*P* Value
Median age, yr (IQR)	36.0 (31–38)	30 (28–36)	.26
Gender (% male)	8 (88.9)	56 (78.9)	.68
Median AST, U/L (IQR)	28 (26–33)	38 (27–60)	.04
Median ALT, U/L (IQR)	32 (28–41)	54 (29–75)	.05
Median AST/ALT ratio (IQR)	0.81 (0.80–0.89)	0.78 (0.62–0.93)	.23
		5.75 (4.9–6.3)	
Viral load, log IU / mL (IQR)	NA	Overall range: 1.08–6.88	. . .
Alcohol use (% Yes)	1 (11.1)	21 (29.6)	.43
Hepatitis B virus surface antigen positive (%)	1 (11.1)	7 (9.4)	.99
Human immunodeficiency virus positive (%)	1 (11.1)	4 (5.6)	.46

Fisher exact test used for categorical comparisons; Wilcoxon rank sum test used for continuous comparisons.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; HCV, hepatitis C virus; IQR, interquartile range; NA, not applicable.

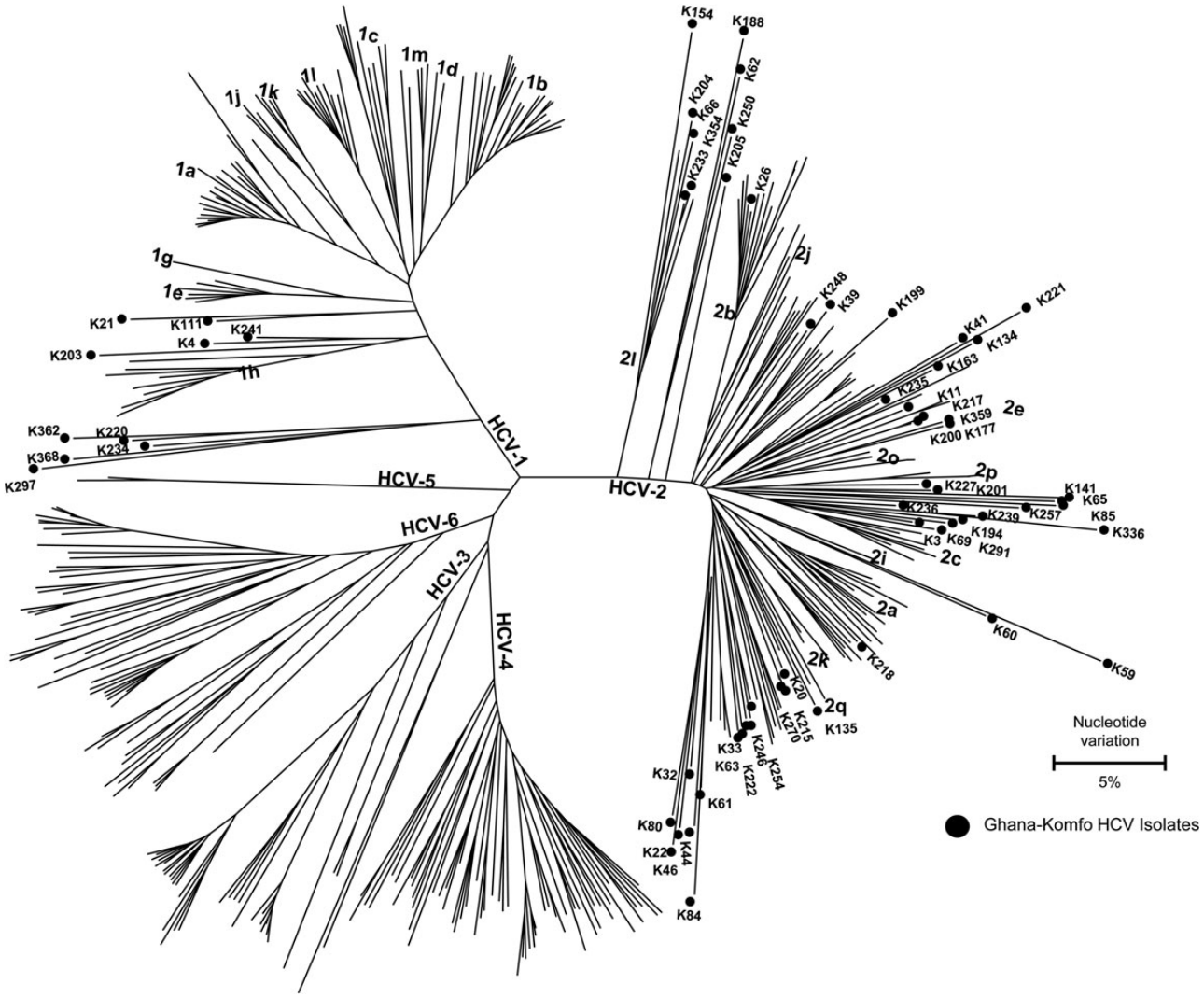

Figure 2. Hepatitis C virus (HCV) genotype distribution. K followed by number are study isolated. Rest are genbank isolates.

manufacturer's guidelines (S/C >1), the rate of active viremia was 74.4% and increased to 88% among individuals with a S/C ≥11, based on CDC-derived S/C ratios.

These rates of active infection fall within the range reported in the United States [7], especially for blacks who have a higher rate of chronic infection than whites and Hispanics [39, 40]. However, the rates are in sharp contrast to those from prior studies in SSA. Mullis et al found no detectable viremia in the 7.6% of the cohort who were positive by a third generation EIA [21]. Seremba et al found detectable RNA in only 29% of individuals with a positive RSA or third generation CIA [18]. Candotti et al conducted a large-scale screening of blood donors in Ghana and also found low rates of viremic disease, with results suggesting a high rate of natural acute clearance [19]. In contrast, a study in Nigeria found a 75.3% rate of viremia in seropositive individuals [29]. One likely factor that contributed to the widely discrepant outcomes is the large variation in assays and testing algorithms implemented. Variation in age of specimens and storage and shipping conditions prior to serologic and virologic testing may also be factors, as RNA is susceptible to degradation with time and temperature changes [41].

It is essential to highlight that while the HCV RSA had high rates of false positivity, it had excellent specificity. Of those with a negative RSA, all had negative CIA serologic results. One individual was negative for both RSA and CIA and had detectable but low viral load (2.05 log copies/mL). It is unclear if this was due to false-negative tests results for both RSA and CAI or if the patient had developed acute HCV infection between the time of blood donation and proximal to the time of recall.

This study enabled the characterization of a group of individuals with active infection. Individuals in Ghana were predominantly infected with genotype 2 vs genotype 1. They had high serum HCV viral loads and evidence of biochemical liver disease, as evidenced by elevated ALT and AST levels. With the advent of new, highly effective antivirals, this information illustrates that active infection is occurring. However, as this and other studies have shown, genotype 2 appears to be the predominant genotype, thus giving hope that favorable outcomes would be observed with appropriate treatment.

Detailed questionnaires allowed for identification of HCV risk factors in this population. The most significant risk factor for HCV infection was the region of tribal origin, with the risk of HCV infection being substantially higher in individuals from tribes of northern or upper regions of Ghana (Figure 1, Table 4). In multivariable analyses, HBV coinfection; traditional circumcision or no circumcision, as opposed to hospital-based circumcision; and education level also remained significant. The association with HBV suggests either the possibility of common sources of transmission or that HBV infection serves as a marker for individuals who may engage in practices that put them at risk for acquiring HCV. In SSA, HBV infection is thought to be a result predominately of horizontal transmission early in life (ages 0–5 years) [42, 43]. This finding raises the possibility that HCV may also be transmitted within families, as has been suggested by other studies [25, 29]. Whether the associations of circumcision and education are causal risk factors or markers for other practices remains unknown.

The data presented here suggest that substantial geographic variation in exposure risks for HCV infection exists in Ghana (Table 3), and this may be one reason why a wide distribution in seroprevalence rates have been reported in SSA [1]. Well-described variation in HIV infection rates has previously been reported within African countries, which in some instances reflects region of origin, tribe, or specific practices [44–47]. A similar phenomenon may be contributing to variation in HCV infections.

Some limitations to this study should be noted. Information about risk factors was obtained by questionnaire of persons with a mean age of 30 years and thus may not capture risk factors for all age groups. Individuals who donated blood in Kumasi, Ghana, may not be representative of the general population. Given that individuals had been informed of the HCV status after blood donation, it is likewise possible that they asked family members about potential events that could have resulted in infection. Further studies that incorporate sample size calculations into their design need to be conducted in order to look at risk factors.

Finally, this study was limited by the lack of availability of the RIBA confirmatory test. This limitation is of most importance when a low level but positive CIA S/C ratio is observed. In our study, of the individuals with a positive CIA but S/C <11, only 1/15 (6.7%) had detectable virus. In these instances, the ability to distinguish cleared from false-positive results is difficult due to higher rates of false positives [38]. Therefore, in our study we used the CDC-derived definitions to define HCV infection as we felt it was not possible to rule out false-positive results in these instances. Another approach would be to define seropositives as individuals positive on 2 serologic tests, regardless of S/C ratios. Of note, we reran all analyses using such a definition (RSA positive and CIA positive, regardless of S/C) and found no differences in our risk factor analyses. The largest difference in these 2 approaches was the frequency of active infection (88% vs 74.4%); both frequencies, however, were higher than what has been reported previously. The appropriate testing strategy may largely depend on the purpose of testing. For example, in blood bank screening, identification of all positives is of critical importance; for these purposes, the RSA appears to be an excellent choice.

In summary, we examined the rates of active HCV infection in a sample of potential blood bank donors from a West African country and we identified a high frequency of active HCV in confirmed seropositive cases, which has significant public health implications. Important risk factors for HCV infection, specifically variation across tribal region of origin, HBV coinfection, and circumcision practices, were identified. Future studies are warranted to characterize the population-level epidemiology of HCV across SSA using standardized diagnostic criteria and to refine our knowledge of region-specific HCV risk factors.

Notes

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