MAJOR ARTICLE

Risk Factors for Hepatitis C Virus Transmission in Colonial Cameroon

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(See the article by Pépin et al, on pages 777–784, and the editorial commentary by Strickland, on pages 785–787.)

Background. In southern Cameroon, where SIV_{cpz}, the source of human immunodeficiency virus 1 (HIV-1) group M, is prevalent among wild chimpanzees, ~50% of some human birth cohorts have been infected with hepatitis C virus (HCV) through unclear mechanisms.

Methods. To evaluate indirectly the hypothesis that medical interventions contributed to the early emergence of HIV-1, we conducted a cross-sectional study of 451 inhabitants of Ebolowa in southern Cameroon aged ⩾60 years, using HCV as a marker of parenteral transmission of blood-borne viruses. We administered a questionnaire and tested serum for antibodies against HCV. Viral gene sequences were obtained from HCV-positive sera. Molecular clock analyses provided an independent source of information on epidemic history.

Results. A total of 252 participants (56%) were HCV seropositive. HCV sequences were amplified and genotyped from 171 individuals. Independent risk factors for HCV seropositivity were older age, having received intravenous treatment against malaria, and having attended an ethnic school (women only), whereas having been circumcised by a traditional practitioner (men only) tended to be associated with HCV. In addition, transfusions were associated with HCV genotype 1 transmission. Molecular clock analyses of HCV genotypes 1, 2, and 4 revealed that each independently underwent exponential growth during the first half of the 20th century.

Conclusions. Medical interventions (intravenous antimalarial drugs, transfusions) and to a lesser extent traditional practices (circumcision) were associated with the massive transmission of HCV among this population decades ago. This finding supports the hypothesis that medical interventions contributed to the transmission of blood-borne viruses, perhaps including SIV_{cpz} and HIV-1, in the same region during the early 20th century.

In middle 20th-century Egypt, iatrogenic transmission of the hepatitis C virus (HCV) occurred during the treatment of schistosomiasis with tartar emetic administered intravenously using hastily sterilized reusable syringes and needles [1]. Millions were infected, especially in Lower and Middle Egypt, where ~50% of individuals aged ⩾40 years are HCV seropositive [1]. Central Africa has the second highest HCV prevalence: 6.0% of adults overall and 13.8% in Cameroon [2]. In Yaoundé and forested areas of southern Cameroon, a cohort effect has been demonstrated, with HCV prevalence reaching 40%–50% among people born before 1945, ~15% for those born around 1960, and ~3% for younger individuals [3, 4]. High prevalences (32%–50%) have also been documented among elderly Gabonese [5].

Molecular clock analyses have revealed that the number of HCV infections in Cameroon, Gabon, and the Central African Republic started increasing exponentially from 1920 to 1940, continuing for 2 or 3 decades and coinciding with the implementation of large-scale interventions for the control of tropical diseases, whose treatment often required intravenous (IV) drugs [5–8]. Could such interventions have contributed to the early amplification of human immunodeficiency virus 1 (HIV-1) into human populations, shortly after the cross-species transmission to humans of SIV_{cpz} from the *Pan troglodytes troglodytes* chimpanzee, the source of HIV-1 group M, somewhere within the ape's natural habitat in equatorial Africa [9, 10]? HCV is a useful

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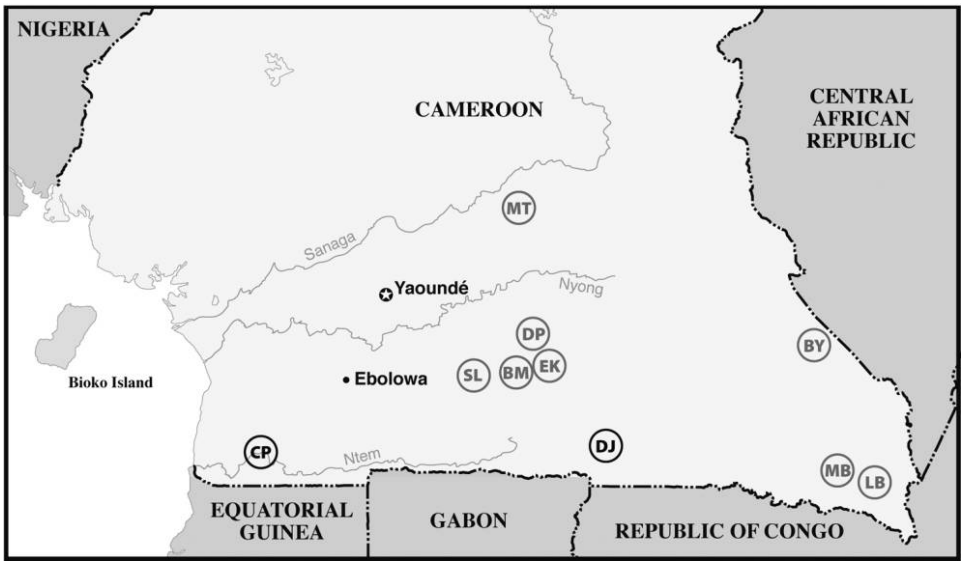

Figure 1. Map of southern Cameroon showing the location of Ebolowa and the sites where SIV_{cpz} infection was documented among free-living chimpanzees (*pale circles*) and SIV_{gor} among gorillas (*dark circles*) [10, 11]. The map was obtained from the United Nations Cartographic Section, and the location of the circles derives from [10].

marker for past parenteral transmission of viruses because sexual or other routes of infection are rather ineffective, and chronic infection can persist for many decades.

To delineate risk factors for HCV infection within the crucible of the HIV-1 pandemic, we conducted a cross-sectional study of elderly Cameroonians in Ebolowa, Southwest Cameroon. Ebolowa was selected because HCV is highly prevalent [3], its population is large (~80,000) and readily accessible, and it experienced a high incidence of yaws during the colonial era. We previously postulated that HCV transmission might have occurred through treatment of this endemic nonvenereal disease caused by *Treponema pallidum* subspecies *pertenue* [8]. Furthermore, Ebolowa lies near sites where SIV_{cpz} and SIV_{gor} strains closely related to the 4 groups of HIV-1 are prevalent among wild chimpanzees and gorillas (Figure 1) [10, 11].

METHODS

Study population. The study was approved by the ethics committees of the Cameroonian Ministry of Health and the Centre Hospitalier Universitaire de Sherbrooke in Canada. Inclusion criteria were age of ⩾60 years and willingness to consent. Exclusion criteria were dementia or inability to speak a language known to interviewers. In March 2009, a convenience sample of compounds was visited to identify individuals aged ⩾60 years. Those who consented were interviewed and a venous blood sample was obtained. Questionnaires and samples were identified only by a study number. Interviewers gathered sociodemographic information, history of blood transfusion, IV treatment for any disease, past treatment of tropical diseases, scarifications, circumcision, or excision. Vaccine scars were documented. For participants unaware of their age, this was estimated on the basis of widely known historical events. Participants were invited to attend the local hospital a few weeks later for their results. HCV viremic participants were examined for cirrhosis, in which case they were referred to a local physician.

Serologic assays. Analyses were performed at the Centre Pasteur du Cameroun in Yaoundé. Samples were tested for anti-HCV antibodies using 2 third-generation enzyme immunoassays: AxSYM HCV version 3 (Abbott) and Monolisa anti-HCV Plus version 2 (BioRad). A sample was considered reactive if its optical density/cutoff ratio was ⩾2.0 with both assays. We considered as indeterminate those samples for which both enzyme immunoassay results were weakly positive (ratio, 1.0–1.99) or for which 1 enzyme immunoassay result was >2.0 whereas the other was <1.0. Treponemal antibodies were detected using Trep-Sure (Phoenix). Serum specimens reactive with Trep-Sure were tested with the rapid plasma reagin (Becton-Dickinson). Patients with a positive rapid plasma reagin result at a dilution of 1:4 who reported a history of genital ulcers were treated with 3 weekly injections of benzathine penicillin. Others were considered to be serofast from a distant infection, presumably yaws (serologic assays do not discriminate among subspecies of *T. pallidum*).

HCV quantification and genotyping. Because HCV RNA is detected only when the Monolisa ratio is ⩾6 or the AxSym ratio is ⩾20 [12], only such samples were selected for HCV quantification and genotyping. Viral RNA was extracted using the Qiamp Viral RNA kit (QIAGEN). HCV RNA detection was performed by in-house qualitative real-time reverse-transcrip-

Table 1. Prevalence of Past Hepatitis C Virus (HCV) Infection, According to Various Characteristics

Characteristic	Proportion (%) of HCV-positive patients	Crude OR (95% CI)	*P*
Age, years			
60–64	48/119 (40.3)	1.00	
65–69	55/92 (59.8)	2.20 (1.26–3.83)	.005
70–74	67/99 (67.7)	3.10 (1.77–5.41)	<.001
⩾75	82/120 (68.3)	3.19 (1.88–5.43)	<.001
Sex			
Male	88/170 (51.8)	1.00	
Female	164/260 (63.1)	1.59 (1.07–2.36)	.02
Education			
Men			
None	6/10 (60.0)	0.98 (0.25–3.79)	.97
Boulou school only	0/2 (0)	0.00	
Primary	40/66 (60.6)	1.00	
Secondary or more	42/92 (45.7)	0.55 (0.29–1.04)	.065
Women			
None	24/43 (55.8)	0.88 (0.44–1.77)	.72
Boulou school only	48/63 (76.2)	2.23 (1.13–4.39)	.02
Primary	76/129 (58.9)	1.00	
Secondary or more	16/25 (64.0)	1.24 (0.51–3.02)	.64
Marital status			
Married, monogamy	67/139 (48.2)	1.00	
Married, polygamy	28/45 (62.2)	1.77 (0.89–3.52)	.1
Never married	9/16 (56.3)	1.38 (0.49–3.92)	.54
Divorced	18/27 (66.7)	2.15 (0.90–5.11)	.08
Widowed	130/203 (64.0)	1.91 (1.23–2.97)	.004
Circumcision, males only			
Medical	27/66 (40.9)	1.00	
Traditional	61/104 (58.7)	2.05 (1.09–3.84)	.025
Past intravenous treatment			
Never	41/79 (51.9)	1.00	
For disease other than malaria	63/125 (50.4)	0.94 (0.54–1.65)	.84
For malaria only	97/146 (66.4)	1.83 (1.05–3.21)	.03
For malaria and other disease	51/80 (63.8)	1.63 (0.86–3.07)	.13
No. of past intravenous treatments			
None	41/79 (51.9)	1.00	
1–3	108/202 (53.5)	1.06 (0.63–1.79)	.81
4 or more	77/111 (69.4)	2.10 (1.15–3.82)	.015
No. unknown	26/38 (69.1)	2.01 (0.89–4.53)	.09
Treponemal antibodies			
Absent	69/115 (60.0)	1.00	
Present	183/315 (58.1)	0.92 (0.60–1.43)	.72
Past diagnosis of yaws			
Never	132/226 (58.4)	1.00	
Ever	120/204 (58.8)	1.02 (0.68–1.53)	.93
Injections for yaws			
Never	167/283 (59.0)	1.00	
Ever	85/147 (57.8)	0.95 (0.64–1.43)	.81
Injections for syphilis			
Never	243/418 (58.1)	1.00	
Ever	9/12 (75.0)	2.16 (0.58–8.10)	.25
Injection for leprosy			
Never	243/417 (58.3)	1.00	
Ever	9/13 (69.2)	1.61 (0.49–5.32)	.43

Table 1. ***(Continued.)***

Characteristic	Proportion (%) of HCV-positive patients	Crude OR (95% CI)	*P*
Transfusion			
Never	224/388 (57.7)	1.00	
Ever	28/42 (66.7)	1.46 (0.75–2.87)	.27
Injections for prevention of sleeping sickness			
Never	237/407 (58.2)	1.00	
Ever	15/23 (65.2)	1.34 (0.56–3.24)	.51
Scarifications			
Never	86/155 (55.5)	1.00	
Ever	166/275 (60.4)	1.22 (0.82–1.82)	.32
Vaccine scar, left arm			
Absent	27/46 (58.7)	1.00	
Present	224/383 (58.5)	0.99 (0.53–1.84)	.98
Vaccine scar, right arm			
Absent	104/154 (67.5)	1.00	
Present	147/274 (53.6)	0.56 (0.37–0.84)	.005

NOTE. Twenty-one participants with an indeterminate HCV serologic samples were excluded. CI, confidence interval; NS, not significant; OR, odds ratio.

tase polymerase chain reaction (RT-PCR) and genotyping by nested RT-PCR followed by phylogenetic analysis of a 340-nt fragment of the *NS5B* gene [13, 14]. To reconstruct epidemic histories, Bayesian skyline plots were calculated using BEAST, version 1.4 (http://.beast.bio.ed.ac.uk), as described elsewhere [7].

Statistical analyses. We estimated that 450 participants were required to assess risk factors present in 10% of the population, assuming a power of 90%, an α of .05, and a prevalence of HCV infection of 50% among the exposed and 25% among the unexposed. Data were analyzed with Stata statistical software, version 10.0 (Stata). Proportions were compared using the χ^2 or Fisher exact test. Variables associated with HCV infection in univariate analysis were tested in logistic regression models built-up sequentially, starting with the variable most strongly associated with the outcome and continuing until no other variable reached significance. Each variable was then dropped to assess its effect using likelihood ratio tests. We kept in the final model variables that enhanced the fit at the $P<.05$ level. Analyses were performed for all past HCV infections (regardless of viremia), as well as for each genotype. Because infection with 1 genotype hampers detection of superinfection with a second genotype and provides some immunity against reinfection with a different genotype once the initial one has

Table 2. Risk Factors for Past Hepatitis C Virus (HCV) Infection in Multivariate Analysis

Risk factor	Adjusted OR (95% CI)	*P*
Age, years		
60–64	1.00	
65–69	2.33 (1.31–4.13)	.004
70–74	2.82 (1.59–4.99)	<.001
⩾75	2.73 (1.55–4.80)	.001
Past intravenous treatment		
Never	1.00	
For disease other than malaria	1.06 (0.58–1.91)	.84
For malaria (with or without other indication)	1.96 (1.13–3.40)	.02
Sex-related factors		
Men, medical circumcision	1.00	
Men, traditional circumcision	1.83 (0.95–3.54)	.07
Women, not Boulou school	1.85 (1.02–3.35)	.04
Women, Boulou school	3.45 (1.54–7.75)	.003

NOTE. CI, confidence interval; NS, not significant; OR, odds ratio.

Table 3. Distribution of Hepatitis C Virus (HCV) Infection with Each Genotype, According to Various Characteristics

Characteristic	Proportion (%) of HCV genotype 1–positive patients[a]	Proportion (%) of HCV genotype 2–positive patients[a]	Proportion (%) of HCV genotype 4–positive patients[a]
Age, years			
60–64	1/72 (1.4)	10/81 (12.3)	22/93 (23.7)
65–69	8/45 (17.8)	14/51 (27.5)	18/55 (32.7)
70–74	10/42 (23.8)	10/42 (23.8)	24/56 (42.9)
⩾75	12/50 (24.0)[b]	13/51 (25.5)	29/67 (43.3)[c]
Education, women only			
None	5/24 (20.8)	5/24 (20.8)	4/23 (17.4)
Boulou school only	4/19 (21.1)	12/27 (44.4)	18/33 (54.5)
Primary	11/64 (17.2)	12/65 (18.5)	26/79 (32.9)
Secondary or more	2/11 (18.2)	1/10 (10.0)[c]	9/18 (50.0)[c]
Circumcision, males only			
Medical	3/42 (7.1)	6/45 (13.3)	10/49 (20.4)
Traditional	6/49 (12.2)	11/54 (20.4)	26/69 (37.7)
Past intravenous treatment			
Never	4/42 (9.5)	6/44 (13.6)	16/54 (29.6)
For disease other than malaria	6/68 (8.8)	14/76 (18.4)	25/87 (28.7)
For malaria (with or without other indication)	21/99 (21.2)[c]	27/105 (25.7)	52/130 (40.0)
Transfusion			
Never	25/189 (13.2)	43/207 (20.8)	83/247 (33.6)
Ever	6/20 (30.0)	4/18 (22.2)	10/24 (41.7)
Vaccine scar, right arm			
Absent	9/59 (15.3)	28/78 (35.9)	43/93 (46.2)
Present	22/149 (14.8)	19/146 (13.0)[b]	50/177 (28.2)[d]

NOTE. HCV, hepatitis C virus.

[a] Excluding those infected with the other genotypes, the HCV-seropositive samples not amplifiable, and those with indeterminate HCV serologic samples.

[b] $P \leqslant .001$.

[c] $P < .05$.

[d] $P < .01$.

cleared [15], genotype-specific analyses considered only seronegative patients plus those infected with the genotype of interest (excluding those infected with the 2 other genotypes or with an unknown genotype).

RESULTS

Characteristics of participants. We recruited 451 participants who were 60–102 years of age (median age, 70 years). One hundred thirty (29%) were born in Ebolowa; the others migrated at a median age of 39 years (interquartile range, 22–59 years), mainly from villages nearby. Treponemal antibodies were found in 332 patients (73.6%); 315 had a positive undiluted rapid plasma reagin result but only 43 a titer ⩾1:4. HCV seropositivity was found in 252 participants (55.9%); 21 (4.7%) with indeterminate results were excluded from further analyses.

Risk factors for HCV seropositivity. HCV prevalence increased with age; two-thirds of those aged ⩾70 years were HCV seropositive (Table 1). Women and widows were more likely to be HCV seropositive, especially those who had attended an ethnic school. All men had been circumcised, and HCV seropositivity was more common among those circumcised during a traditional ceremony. No woman had been excised.

HCV prevalence was higher among those who had ever received IV treatment against malaria and those who underwent ⩾4 episodes of IV treatment (whatever the indication) (Table 1). IV antimalarial treatment was strongly correlated with the total number of IV treatments in one's lifetime, which was a median of 1 among individuals treated for other indications, 2 among those treated for malaria only, and 5 if treated for malaria plus some other indication ($P < .001$). HCV prevalence being similar, the groups treated for malaria only and malaria plus some other indication were merged for further analyses. Median age at the time of IV malaria treatment was 35 years (range, 4–78 years).

HCV seropositivity was not associated with treponemal antibodies, past diagnosis of yaws, or parenteral treatment against yaws. Of 146 patients who recalled having received injections against yaws, only 16 (11%) had been treated intravenously.

Table 4. Risk Factors for Hepatitis C Virus (HCV) Infection with Each Genotype

Risk factor	Adjusted OR (95% CI)		
	HCV genotype 1[a]	HCV genotype 2[a]	HCV genotype 4[a]
Age[b]	1.10 (1.04–1.16)[c]	…	1.04 (1.00–1.08)[d]
Sex-related factors			
Men, medical circumcision	…	1.00	1.00
Men, traditional circumcision	…	1.78 (0.58–5.44)	2.05 (0.86–4.90)
Women, not Boulou school	…	1.13 (0.40–3.18)	1.62 (0.72–3.65)
Women, Boulou school	…	4.43 (1.33–14.74)[d]	3.45 (1.23–9.63)[d]
Past intravenous treatment			
Never	1.00	…	…
For disease other than malaria	0.85 (0.21–3.42)	…	…
For malaria (with or without for other indication)	2.99 (0.91–9.80)	…	…
Transfusion			
Never	1.00	…	…
Ever	3.19 (1.01–10.06)[d]	…	…
Vaccine scar, right arm			
Absent	…	1.00	1.00
Present	…	0.27 (0.13–0.55)[c]	0.54 (0.31–0.94)[d]

NOTE. CI, confidence interval; OR, odds ratio.

[a] Excluding those infected with the other genotypes, the HCV-seropositive samples not amplifiable, and those with indeterminate HCV serologic samples.

[b] Age treated as a continuous variable; the adjusted ORs apply to each additional year of age.

[c] $P \leqslant .001$.

[d] $P < .05$.

Of 217 participants who recalled an episode of yaws, 209 (96%) carried treponemal antibodies versus 123 (53%) of 234 of those who did not ($P < .001$). Median age at yaws diagnosis was 9 years (interquartile range, 6–10 years).

HCV seropositivity was more common, but not significantly so, among patients who had received injections against syphilis or leprosy or for prophylaxis of sleeping sickness, who had received transfusions, or who had undergone scarifications. Unexpectedly, HCV seropositivity was less common among those with a vaccine scar on their right arm. Only 6 patients reported having received parenteral treatment against tuberculosis, 2 against schistosomiasis and 1 against trypanosomiasis.

In multivariate analysis, HCV seropositivity was independently associated with older age, with past IV treatment against malaria (both sexes combined), and with having attended Boulou school among women, and it tended to be more frequent among men who had undergone traditional circumcision (Table 2). Total number of IV treatments could not be fitted in the same model because of colinearity with IV antimalarial drugs. If the former was used instead of the latter, the odds ratios for other variables changed little (data not shown) and having received $\geqslant 4$ IV treatments was associated with HCV seropositivity (adjusted odds ratio, 2.26; 95% confidence interval, 1.20–4.23; $P = .01$).

HCV genotyping. Among 193 samples selected for amplification, HCV RNA was detectable in 179. Seven samples could be amplified by real-time RT-PCR but not by *NS5B* analysis, whereas 1 was *NS5B* amplifiable but could not be sequenced. Finally, HCV could be genotyped from 171 specimens: 31 corresponded to genotype 1, 47 to genotype 2, and 93 to genotype 4. Although genotype 4 predominated among all age groups, genotype 1 was almost absent in those aged 60–64 years (Table 3). Having attended a Boulou school was associated with genotypes 2 and 4, traditional circumcision with genotype 4, and IV antimalarial treatment with genotype 1. Genotypes 2 and 4 were less common among participants with a vaccine scar on their right arm (no such difference was seen for those with a scar on the left arm). Other risk factors were not associated with any genotype (data not shown).

Results of multivariate analyses are given in Table 4. Age was associated with genotypes 1 and 4 but not 2. Genotype 1 was associated with transfusions. Genotypes 2 and 4 were associated with having attended a Boulou school and were less common in those with a vaccine scar on their right arm. Although not significant for each genotype individually, traditional circumcision and past IV antimalarial treatment had similar relative risks for each genotype (data not shown). In the case of genotype 1, the latter variable was kept in the model because it was globally significant.

Epidemic history. Figure 2 displays the Bayesian skyline plot for each genotype, estimated directly from viral *NS5B* gene sequences. For genotypes 1 and 2, exponential growth started

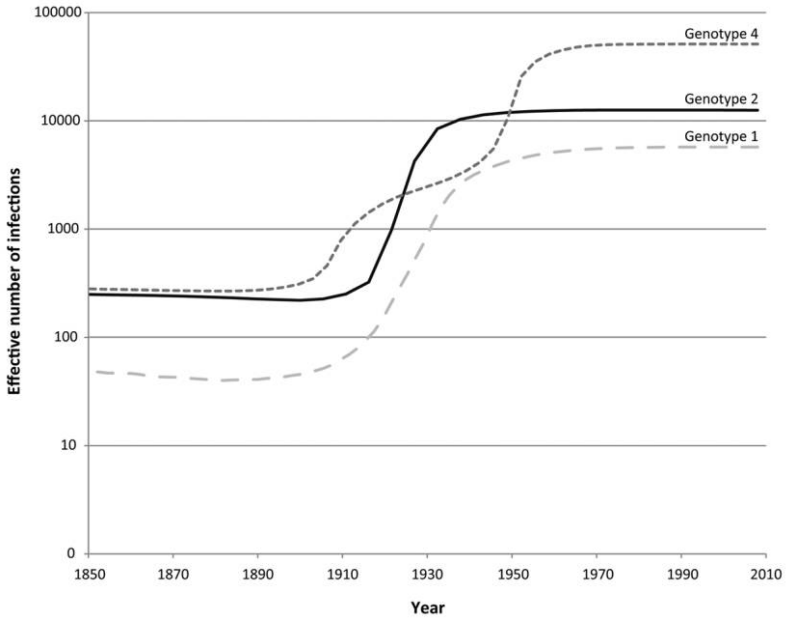

Figure 2. Bayesian skyline plots showing effective number of infections with genotypes 1, 2, and 4.

around 1920, continuing until their effective population sizes stabilized during the 1940s. The effective population size of genotype 4 increased exponentially from ~1910.

DISCUSSION

The prevalence of past HCV infection within this population is among the highest in the world and comparable to that documented in the schistosomiasis-endemic areas of Egypt [1]. Considering the excess mortality associated with HCV infection [16] and that some of the indeterminate serologic samples may reflect antibodies levels that had decreased after a long period, it seems likely that more than three-quarters of the older birth cohorts had been infected with HCV at some point in their lives.

After a review of interventions for tropical disease control during the colonial era, we had hypothesized that the Cameroonian HCV epidemic might have been driven by campaigns against yaws because of is high incidence in southern regions, its age distribution, and its peak after 1935 [8]. The current study did not support this hypothesis. Although the prevalence of a past treponemal infection was extraordinary (74%), HCV was not associated with parenteral treatment of yaws or with treponemal antibodies, presumably because few participants with yaws had been treated with IV arsenical agents. Not all injections carry the same risk: health care workers occupationally exposed to HCV have a 100-fold higher risk of infection if injured with a needle placed in the index patient's vein or artery or if they sustain a deep injury [17], reflecting the number of viral particles inoculated. Extrapolating to the iatrogenic transmission of HCV, the risk must have been higher with IV than with intramuscular, subcutaneous, or intradermal injections and commensurate with the total number of injections received over a lifetime.

However, we documented an independent association between past HCV infection and having received IV treatments against malaria (presumably, quinine). With hindsight, the same arguments developed for yaws also apply to malaria: a marked north-south gradient, a high incidence resulting in a large fraction of the population receiving IV quinine at least once during lifetime (especially during childhood), and a time frame for exposure that concords with that obtained from independent molecular clock data. The frequency of IV antimalarial treatments likely increased in parallel with the development of fixed health services in Cameroon, from the 1930s onward. Malaria occurs throughout tropical Africa but its distribution is heterogeneous, following that of its vectors. In the rainy areas of southern Cameroon, the average number of infective bites is among the highest in the world [18]. Use of antimalarials varies accordingly.

Our review of health care systems of colonial equatorial Africa did not quantify the extent of IV antimalarial treatments [8]. Historically, childhood diseases were given a low priority and malaria was so prevalent that it never became a primary target of health systems: fatalism prevailed. Its treatment was left to local health institutions and no statistics were tabulated. Injectable quinine, available since the early 20th century, was generally administered intravenously rather than intramuscularly because the latter route caused abscesses at injection sites [19]. IV quinine was recommended when a quick effect was necessary (cerebral malaria, severe anemia) or for patients vom-

iting. Injections became popular because many patients believed this route to be more powerful than oral medication, and indications for the IV treatment of malaria were broader than those found in textbooks. Thus, malaria represented a large fraction of the IV treatment episodes during our participants' lifetimes.

IV antimalarial treatment accounted for an etiologic fraction of 33% of cases of HCV infection, but this is an underestimate for 2 reasons. First, exposure to IV antimalarials was underreported because episodes occurring during infancy or childhood would have been forgotten, as suggested by the participants' high median age of receiving IV antimalarials. Second, during several decades, the excess mortality associated with HCV preferentially removed from the population those who were exposed and infected, biasing downward measures of relative risk.

Traditional practices contributed to HCV transmission in these communities. All men were circumcised, but those who had undergone traditional (rather than medical) circumcision tended to be more likely to have been HCV infected. A description of the procedure in the middle 1930s is corroborated by our data [20]. Circumcision, thought to enhance virility, was performed around the age of 7–10 years (median age among our participants, 9 years), generally on several boys at the same time (among our patients, median, 4 boys; range, 1–24 boys). The operator, a specialist who traveled among villages for that purpose, used a small knife, a razor blade, or a broken bottle. In a high-prevalence community of Egypt, male circumcision by an informal practitioner was also associated with HCV [21].

It remains unclear why having attended a Boulou school was associated with HCV seropositivity among women. These schools, operated by the Presbyterian Church and attended mostly by children born into Protestant families, corresponded to the first 3 years of primary education given in Boulou language. One was located in Ebolowa, the others in surrounding villages. Their pupils may have had a better access to medical and dental care facilities where HCV was transmitted or may have been coerced to participate in public health interventions where injections were given [22].

Transfusion was associated with transmission of genotype 1. Genotypes 2 and 4 were undoubtedly transmitted through this route as well, but our statistical power was limited by the small number of transfused participants. In Africa, transfusions became available in district hospitals decades after injectable antimicrobial drugs, limiting their potential for transmission of viruses [23].

We found no evidence that HCV was transmitted during immunizations. The lower prevalence of HCV infection, mostly of genotypes 2 and 4, among individuals with a vaccine scar on their right arm is intriguing. Bacille Calmette-Guérin vaccine was generally given on the right arm, and the smallpox vaccine on the left side [24]. We reexamined a database from Guinea-Bissau and found that a vaccine scar was associated with a lower prevalence of HCV genotype 2 [25]. This needs to be explored in further studies.

The risk factors for HCV infection that we identified were certainly not the only ones that played a role in transmission in this area decades ago but rather were those that could be recognized given our sample size and the history of the population studied. Only 3% of our participants had been treated for syphilis or leprosy. Elsewhere in Africa, Brazil, and Asia, patients with leprosy treated in the remote past are more likely than control subjects to be infected with HCV, the hepatitis B virus, and human T lymphotropic virus 1, presumably through IV injections of chaulmoogra, methylene blue, and *Caloncoba* [26–29]. In the Central African Republic, HCV genotype 4 was associated with treatment of trypanosomiasis before 1951 and human T lymphotropic virus 1 infection with trypanosomiasis chemoprophylaxis [30]. In Guinea-Bissau, HIV-2 infection was associated with treatment of trypanosomiasis and tuberculosis and with ritual clitoridectomy [31].

Genetic analyses showed that the epidemic histories of all 3 genotypes are highly similar, taking into consideration the uncertainty associated with each curve (Figure 2). Each genotype independently underwent roughly the same epidemiologic expansion; hence, viral genetic factors were not important. Genotypes 1, 2, and 4 were already present in Cameroon at the start of the 20th century and therefore ready to be amplified [6, 12, 32]. These epidemic histories indicate that most of our infected participants probably acquired their HCV infection during the colonial era, before 1960. Among our study population, the age distribution varied considerably among genotypes. Genotype 1 became uncommon among those born after 1945, perhaps because the near saturation with other genotypes affected its transmission.

In conclusion, malaria treatments and transfusions—and probably traditional circumcision as well—contributed to the transmission of HCV during the early 20th century in an area of southern Cameroon located only 150 km from sites where SIV_{cpz} was documented among free-living *P. troglodytes troglodytes* chimpanzees [10]. HCV is transmitted mainly parenterally and is compatible with a prolonged survival in many patients, factors that allowed such associations to be documented. Our results support the hypothesis that medical interventions may have contributed to the initial phase of the emergence of SIV_{cpz} into HIV-1 during the first decades of the 20th century.

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