Predominance of hepatitis C virus genotype 4 infection and rapid transmission between 1935 and 1965 in the Central African Republic

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The molecular epidemiology of hepatitis C virus (HCV) in the Central African Republic (CAR) is poorly documented. Thus, we conducted phylogenetic analyses of NS5B gene sequences from 58 HCV-infected inhabitants of a remote area of south-west CAR, which indicated that 48 (82.8 %) were infected with genotype 4 (HCV-4), five (8.6 %) with genotype 2 and five (8.6 %) with genotype 1. HCV-4 strains were highly heterogeneous, containing previously described subtypes 4k (48 %), 4c (27 %), 4r (4 %), 4f (4 %) and unclassified subtypes (17 %). To estimate the epidemic history of these HCV-4 strains, an evolutionary analysis using the coalescent approach was used. The estimated date of the most recent common ancestor of the CAR HCV-4 strains was 1539 (95 % confidence intervals, 1317–1697). They exhibited a rapid, exponential spread from 1935 to 1965, simultaneously with what was recently reported in neighbouring Cameroon and Gabon. The hypothesis of a massive iatrogenic transmission during interventions for the control of endemic tropical diseases is discussed.

Hepatitis C virus (HCV) infection is a major public-health problem worldwide. The WHO estimates that about 3 % of the world’s population (nearly 170 million people) is infected chronically with HCV (WHO, 1999). Sub-Saharan Africa has the highest regional HCV prevalence (5.3 %) (Madhava et al., 2002). Within this area, HCV prevalence is highest among adults of central Africa (Madhava et al., 2002). Recently, a Bayesian coalescent approach was used to estimate the dates of origin and rates of virus spread through time for the HCV genotypes circulating in Cameroon (Njouom et al., 2007) andGabon (Ndong-Atome et al., 2008). By analysing HCV genotype 4 (HCV-4) strains circulating in these two countries, the authors suggested an endemic origin for HCV-4 infection and a period of accelerated transmission during the early 20th century. In the Central African Republic (CAR), the molecular epidemiology of HCV infection is poorly documented (Fretz et al., 1995). In order to delineate mechanisms for transmission of blood-borne viruses, including HCV, we have conducted a cross-sectional survey of individuals aged ≥55 years living in a remote area of south-west CAR (Fig. 1), which used to be hyperendemic for sleeping sickness, a disease that was always treated with injectable drugs. Here, phylogenetic analyses of HCV NS5B gene sequences sampled in this population were undertaken to describe the genetic diversity of HCV in this region. Furthermore, a Bayesian coalescent approach was used to estimate the age of HCV-4 strains and the historical rates at which these strains spread within these populations. By shedding light on the history of HCV transmission, these results can guide HCV-control initiatives and help to understand the future burden of HCV-related disease in Africa.

In April 2006, dried blood spots (DBSs) were obtained from 905 inhabitants aged ≥55 years living in Nola town or in one of 46 villages situated between Nola and the Cameroonian border. DBSs were first tested for HCV antibodies by using Detect-HCV v. 3 (Adaltis). Non-reactive samples were considered HCV-negative (n=724). Reactive samples were tested further with Ortho HCV 3.0...
sequences were aligned initially by using CLUSTAL_X 1.81 (2007). For phylogenetic analysis, NS5B nucleotide sequencing as described previously (Plamondon et al., 2007). Among the 95 HCV-seropositive participants, HCV RNA in the NS5B region could be amplified, sequenced and analysed phylogenetically in 58 individuals (61%). Fig. 2 shows the estimated phylogeny of these and previously published HCV NS5B sequences. Among these 58 HCV strains, 48 (82.8%) were genotype 4 (HCV-4), five (8.6%) were genotype 2 and five (8.6%) were genotype 1. Among genotype 4, a cluster regrouped almost half of all sequences, i.e. 23 (48%), with reference 4k subtype sequences. Thirteen (27%) strains were associated with 4c reference sequences, two (4%) with 4r and two (4%) with 4f. The remaining eight (17%) sequences did not cluster with currently subtyped HCV-4 sequences. Among genotype 1, a cluster of three sequences (60%) corresponded to subtype 1e. One sequence was subtyped as 1b and one was an unclassified HCV-1 subtype. The majority (four, 80%) of HCV genotype 2 sequences were unclassified and one was subtype 2b.

In order to investigate the origin and spread of HCV-4 in this population more carefully, we estimated the divergence date and the epidemic history by using a Bayesian coalescent approach. The date of the most recent common ancestor (MRCA) of the south-west CAR HCV-4 strains was estimated to be 1539 [95% confidence intervals (CI95%), 1317–1697]. The date of the 4k MRCA is estimated to be 1761 (CI95%, 1652–1841), whilst the 4c MRCA seems more recent (1875; CI95%, 1824–1916). The Bayesian skyline plot (Fig. 3) depicts the estimated change in the effective number of infected individuals through time. This figure shows the history from the HCV-4 MRCA to the year of sampling (2006). The CAR HCV-4 genotype went through roughly three phases of population growth: a long initial period of relatively constant population size, a period of exponential growth during the first part of the 20th century (1935–1965) and, finally, slower exponential growth.

This is the first large study on HCV molecular epidemiology in CAR. Our phylogenetic analysis indicates the circulation of three different HCV genotypes (1, 2 and 4), with a predominance of genotype 4. In a previous study in CAR, only HCV genotype 4 was found (Fretz et al., 1995), (uncorrelated log-normal model) was used, thereby taking into account the variation in evolutionary rate among lineages (Drummond et al., 2006). Because the sequences were sampled over a very short period of time, they contained no information to co-estimate the mean mutation rate. As our isolates were considered as being representative of the sequence, we used prior information obtained from two independent analyses of HCV NS5B evolutionary rates (Pybus et al., 2001; Tanaka et al., 2002). We used an informative normal prior distribution with a mean of 5.0 × 10⁻⁴ and SD of 1.7 × 10⁻⁵. For all other priors, an uninformative distribution as proposed by default by the BEAST 1.4.2 package was used. A final Bayesian skyline plot was obtained by using TRACER 1.3 (http://tree.bio.ed.ac.uk/software/tracer/).

ELISA Test (Ortho Clinical Diagnostics) and Monolisa Anti-HCV Plus Version 2 (Bio-Rad); dually non-reactive samples were considered HCV-negative (n=70), samples reactive with all three ELISAs were considered HCV-positive (n=87), whilst samples with discordant results (n=24) were tested by INNO-LIA HCV Score (Innogenetics), whose result was definitive unless only indeterminate bands were present (n=5), in which case PCR was performed. Ninety-five (10.5%) individuals were found to be HCV-seropositive. Prevalence of HCV infection increased with age: it was 8.5% (42 of 496), 12.8% (30 of 234) and 13.8% (23 of 167) among those aged respectively 55–64, 65–74 and ≥75 years.

HCV RNA was extracted from DBS samples from HCV-positive subjects and the NS5B gene was amplified and sequenced as described previously (Plamondon et al., 2007). For phylogenetic analysis, NS5B nucleotide sequences were aligned initially by using CLUSTAL_X 1.81 (Thompson et al., 1997) and subsequently adjusted by hand. Sequences were compared with reference sequences from the European HCV database (http://euhcvdb.ibcp.fr/euHCVdb/) and the Los Alamos database (http://hcv.lanl.gov/). Phylogenetic trees were estimated and assessed by using the bootstrapping and neighbour-joining methods under the Kimura two-parameter substitution model, as implemented in MEGA version 4.0 (Tamura et al., 2007). Bootstrapping was performed with 1000 replicates.

The past population dynamics of HCV strains were investigated by using the Bayesian skyline plot method, as implemented in the BEAST 1.4.2 software (Drummond & Rambaut, 2007). As analysed in our previous studies (Njouom et al., 2007; Pouillot et al., 2008), the selected substitution model (GTR + invariant sites + gamma rate heterogeneity) was used during the BEAST analysis. As recommended, a relaxed molecular clock approach

Fig. 1. Map showing the location of the Central African Republic in Africa and the areas where the sampling was performed in the country.

http://vir.sgmjournals.org
but only five HCV strains were sequenced. The current study documented that, within genotype 4, there is a high diversity and many unsubtyped sequences. We (Pasquier et al., 2005) and others (Ndjomou et al., 2003) have also reported the circulation of three different HCV genotypes (1, 2 and 4), with a greater genetic diversity within genotype 4, in neighbouring Cameroon. HCV genotype 4 also predominates and exhibits a great genetic diversity in nearby Gabon (Ndong-Atome et al., 2008). An interesting finding is the difference in the predominant HCV-4 subtype in these three countries: subtype 4f in Cameroon (Pasquier et al., 2005), subtype 4e in Gabon (Ndong-Atome et al., 2008) and subtype 4k in south-west CAR (this study). These three different HCV-4 subtypes thus represent the signature of HCV infection in the corresponding country. As we recently reported with HCV subtype 4f (Hmaied et al., 2007), characterization of the full genomes of these specific subtypes is needed for correct classification and further studies.

The date of the MRCA of the south-west CAR HCV-4 strains was estimated to be 1539 (CI95 %, 1317–1697), suggesting long-term endemic HCV-4 transmission in CAR, in accordance with previous findings in Cameroon (MRCA, 1541) (Njouom et al., 2007). The routes of long-term endemic HCV transmission in tropical regions are not known; current plausible hypotheses include exposure to blood through cultural practices or mechanical insect transmission (Pybus et al., 2007). Vertical transmission of HCV is rare in central Africa (Njouom et al., 2005). The great genetic diversity of HCV-4 plus its high relative prevalence compared with genotypes 1 and 2 indicates that the former has probably been present within the human population of south-west CAR for a long time. The MRCA of HCV-4 in CAR and Cameroon are much older than that of the genotype 4 strains found in Europe (MRCA, approx. 1900) (Cantaloube et al., 2008) and Egypt (MRCA existed approx. 100 years ago) (Pybus et al., 2003; Tanaka et al., 2004), reinforcing our previous suggestion that HCV-4 originated in central Africa, diverged and then spread to the rest of the world.

Analysis of the population genetic history of HCV-4 circulating in south-west CAR indicates a period of exponential growth between 1935 and 1965, in the same range as the periods identified in Cameroon (Njouom et al., 2007) and Gabon (Ndong-Atome et al., 2008), reinforcing the hypothesis of massive iatrogenic transmission through improperly sterilized syringes and needles suggested previously (Gisselquist, 2003; Pépin & Labbé, 2008). Until 1960, CAR (then known as Oubangui-Chari) and Gabon were part of the Afrique Equatoriale Française federation, whereas Cameroon was administered under a mandate from the League of Nations. However, disease-control interventions were the same in these three territories (Pépin & Labbé, 2008), implemented through a highly hierarchal system of military doctors with a clear goal of controlling these diseases at the population level (rather than just treating affected individuals who showed up in hospitals), to a large extent by active case-finding village by village, where most patients were treated in situ by mobile nurses. Most drugs for the treatment of common tropical diseases were then administered through injections, many of them intravenously, at a time when the very existence of blood-borne viruses was largely unknown. The iatrogenic transmission of HCV would be expected to be much more effective with intravenous injections compared with the intramuscular (IM) or subcutaneous routes. African trypanosomiasis was the first disease for which huge numbers of intravenous injections were given immediately after World War I, soon to be followed by syphilis and yaws, treponemal diseases treated with arsenical drugs or bismuth-containing agents. As reviewed
by Pépin & Labbé (2008), yaws was by far the most common endemic disease (in some parts of southern Cameroon, >20% of the population was treated for yaws each year) and the number of patients treated increased dramatically from the mid-1930s until the more effective drug penicillin superseded arsenical drugs at the end of the 1950s. The widespread use of IM penicillin, including for asymptomatic contacts, led to a dramatic reduction in the incidence of yaws and syphilis around 1960, and the opportunities for the parenteral transmission of blood-borne viruses were reduced drastically (Pépin & Labbé, 2008).

The similarities in population history of HCV-4 between CAR, Cameroon and Gabon, despite differences in the predominant subtype, suggest that these medical interventions amplified whatever HCV-4 subtype happened to be introduced into the cohorts of patients treated with intravenous drugs by mobile teams at a given health centre or in a village. Less common subtypes presumably correspond to those for which this amplification did not occur, or occurred only at the end of this era of massive use of intravenous drugs, with fewer cycles of amplification.

In conclusion, HCV in south-west CAR is characterized by a great genetic diversity, with the presence of three different genotypes (1, 2 and 4) and the predominance of genotype 4. There is also a great genetic diversity within HCV-4 genotypes, with many subtypes different from those reported in Western countries. By using the coalescent method, we confirm that the HCV-4 genotype is endemic in central Africa and we also estimate the exponential spread this genotype in south-west CAR to have occurred between 1935 and 1965.

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References


