Increasing prevalence and local transmission of non-B HIV-1 subtypes in the French Antilles and French Guiana between 1995 and 2018

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Abstract

The Caribbean and South American French Overseas Territories (CSAFOT) are the regions most heavily affected by the Human Immunodeficiency Virus type 1 (HIV-1) epidemic in France. Although dominated by HIV-1 subtype B, the detection of non-B subtypes and the great proportion of HIV-positive persons born abroad demonstrated the potential for local spread of non-B subtype strains in CSAFOT. To reconstruct the epidemiologic dynamics of major non-B subtype clusters spreading in CSAFOT, we conducted phylogenetic and evolutionary analyses of 2,523 HIV-1 pol sequences collected from patients...
living in Martinique, Guadeloupe, and French Guiana from 1995 to 2018. A large variety of HIV-1 non-B subtype strains (eight subtypes, twelve CRFs, and multiple URFs) have been introduced in CSAFOT and their prevalence significantly increases over time in Martinique and Guadeloupe. We identified twelve major transmission networks of non-B subtypes (CRF02_AG and subtypes A3, C, D, and F1) that probably arose in Guadeloupe, Martinique, French Guiana, and mainland France between the late 1970s and the middle 2000s. Phylogeographic analyses support frequent non-B subtype viral transmissions within CSAFOT as well as transatlantic transmission between CSAFOT and mainland France. Domestic transmission networks of non-B subtype variants in CSAFOT comprise both men having sex with men and heterosexual individuals from different age groups. Different HIV-1 non-B subtype variants were sequentially introduced in CSAFOT between the late 1970s and the middle 2000s and are currently spreading through domestic, regional, and/or transatlantic networks of individuals from different age and risk groups.

Key words: HIV-1; non-B subtype; HIV cluster; Phyldynamics; French Guiana; Guadeloupe; Martinique.

1. Introduction

The Caribbean and South American French Overseas Territories (CSAFOT), Guadeloupe and Martinique (Caribbean Islands), French Guiana (northern coast of South America), are the regions of France the most heavily affected by the Human Immunodeficiency Virus type 1 (HIV-1) epidemic. In 2015, the rate per million inhabitants of new HIV diagnoses in Martinique (214), Guadeloupe (342), and French Guiana (743) was well above the mean rate in France (89) (Six and Quet 2016). Although in CSAFOT, HIV has primarily spread through heterosexual contact and the epidemic is often considered to be ‘generalized,’ (particularly in French Guiana where prevalence in pregnant women exceeds 1%) (Cabié, Georger-Sow and Nacher 2005; Pan American Health Organization 2012), HIV reaches a high prevalence in some vulnerable groups such as migrants, crack cocaine users, and/or men who have sex with men (MSM) (Nacher et al. 2010; Klingelschmidt et al. 2017; Parraut et al. 2017).

HIV-1 subtype B is the predominant lineage in mainland France, but the prevalence of non-B subtypes is increasing (Chaix et al. 2013; Visseaux et al. 2020). This changing molecular epidemiologic pattern was attributed to both increased migration of HIV-infected individuals from non-European countries and to local transmission of non-B subtypes, particularly among MSM (Brand et al. 2014, 2017; Chaillon et al. 2017). While the HIV-1 epidemic in CSAFOT seems to be also mostly driven by subtype B, the previous identification of non-B subtypes in Martinique and French Guiana (Desgranges et al. 1996; Ouka et al. 1998; Kazanjii et al. 2001; Darciassac et al. 2016) and the great proportion of HIV-positive persons born abroad among those living in CSAFOT (up to 80%, French Guiana) (Cabié, Georger-Sow and Nacher 2005; Nacher et al. 2018), demonstrated the potential for introduction and local dissemination of non-B subtype strains in these French American regions.

The aim of the present study was to explore the origin and dissemination dynamics of HIV-1 non-B subtype variants circulating in CSAFOT. To this end, we analysed 2,543 HIV-1 pol sequences collected from patients living in CSAFOT from 1995 to 2018. Socio-demographic, geographic, and viral sequence data were combined with phylogenetic and molecular clock methods to identify non-B transmission networks and reconstruct their spatiotemporal dynamics.

2. Materials and Methods

2.1. Study population

HIV-1 pol sequences covering the complete protease and part of the reverse transcriptase genes (nucleotides 2,253–3,275 of reference strain HXB2) were obtained from adult patients accessing clinical care at the University Hospital of Martinique (Fort-de-France, Martinique) between 1995 and 2018, at the University Hospital of Guadeloupe (Pointe à Pitre, Guadeloupe) between 1999 and 2014, or at the Institut Pasteur de la Guyane (Cayenne, French Guiana) between 2006 and 2017. In the three locations all HIV-1 genotyping tests were prescribed according the same French national guidelines that didn’t recommend test prior entry to care until 2006 (Yeni 2006). Drug resistance sequences were established either by the Virology laboratory of the University Hospital of Martinique (Guadeloupe, Martinique) or by the Institut Pasteur de la Guyane (French Guiana). Only one sequence per subject was selected and if several sequences were available for a patient the first collected was selected. The epidemiologic data were extracted from eNadis/Dat’AIDS®, a computerized medical record.

2.2. Ethical statement

All phylogenetic and statistical analyses were performed on de-identified database to protect patient’s anonymity. The Dat’AIDS cohort is approved by the French ‘Commission Nationale Informatique et Liberté’ (Registration number: 2001/762876/nadiscnil.doc). The study was based on routine patient follow-up biological data and did not involve any additional sampling. Ethical approval was not needed for this study in accordance with French laws and regulations.

2.3. HIV-1 subtyping

HIV-1 pol sequences were aligned with reference sequences representative of HIV-1 group M subtypes and circulating recombinant forms (CRFs) available in the Los Alamos HIV Sequence Database (http://hiv-web.lanl.gov; accessed 15 Jul 2018) using the ClustalW program (Thompson et al. 1997). Codons associated with major antiretroviral drug resistance positions in protease (n = 12) and reverse transcriptase (n = 21) were excluded. Subtyping of HIV-1 sequences was first performed using the REGA HIV subtyping tool v2 (de Oliveira et al. 2005) and confirmed by bootscanning using SimPlot software v3.5.1 (Lole et al. 1999) Maximum Likelihood (ML) phylogenetic analyses using the PhyML v3 program (Guindon et al. 2010) (see Supplementary Data).

2.4. Identification of non-B subtype transmission clusters

HIV-1 sequences from the most prevalent clades circulating in CSAFOT were aligned with reference sequences of the same
subtypes/CRFs that were isolated in mainland France, the Caribbean, Latin America, and other geographic regions where these HIV-1 variants reach a high (>5%) prevalence. All available other CRF02_AG sequences from mainland France previously described (Chaix et al. 2013; Visseaux et al. 2020) were also added as this clade is regularly increasing and present strong clustering patterns in France. Sequences were subjected to ML phylogenetic analyses as described above and highly supported (approximate likelihood-ratio test aLR > 0.85) monophyletic clusters comprising more than five sequences from CSAFOT were selected for subsequent analyses.

2.5. Spatiotemporal reconstructions

Major CSAFOT non-B subtype lineages and a subset of closely related HIV-1 reference sequences identified in previous ML analyses were analyzed using a Bayesian phylogeographic approach. The evolutionary rate, the age of the most common ancestor (TMRCA; years) and the spatial diffusion pattern were jointly estimated using the Bayesian Markov Chain Monte Carlo (MCMC) approach as implemented in BEAST v1.10 (Drummond and Rambaut 2007) with BEAGLE (Suchard and Rambaut 2009) to improve run-time (Supplementary Fig. S1). MCMC chains were run for 100–200 × 10⁶ generations and convergence (Effective Sample Size > 200) and uncertainty (95% Highest Probability Density [HPD] values) in parameter estimates were assessed using the TRACER v1.7 program (Rambaut et al. 2018). Maximum clade credibility (MCC) trees were summarized with TreeAnnotator v1.10.

2.6. Statistical analysis

Epidemiological and demographic characteristics of the cohort included in the present study were cross-tabulated with phylogenetic clusters using Fisher’s exact test or chi². Data were analyzed with Stata©13.0 (Statacorp, College Station, TX). To search for temporal trends, the trend-chi² was calculated. Statistical significance was defined as P < 0.05.

3. Results

3.1. Viral diversity in the French Antilles

In this study, we analyzed 1,432 HIV-1 pol sequences from Martinique and 1,025 HIV-1 pol sequences from Guadeloupe (31%) that correspond to 51% and 31% of the total number of HIV-1 positive individuals diagnosed since the beginning of the epidemic and up to the end of the sampling interval in Martinique (n = 2,828) and Guadeloupe (n = 3,360), respectively. In each location, 87% of the HIV-1 pol sequences were classified as subtype B. The most common non-B subtype variants were CRF02_AG (45.1%), D (8.6%), C (6.7%), A (3.2%), and F1 (3.2%) (Table 1, Supplementary Fig. S2). Two other pure subtypes (G, H), eleven other CRFs, and numerous unique recombinants forms (URFs) were also identified (Table 1). The most common mosaic structures among URFs were B/F1 (30/68) and B/D (19/68).

3.2. Increasing prevalence of HIV-1 non-B Subtypes in the French Antilles

HIV-1 subtype B was the most prevalent variant at all time-intervals but the frequency of non-B subtype variants significantly increased over time in both Martinique (Chi² for linear trend = 30.9, P < 0.001) and Guadeloupe (Chi² for linear trend = 31.5, P = 0.07) (Fig. 1; Supplementary Table S1). The prevalence of non-B subtype-infected patients quadrupled in Martinique from 6% (1995–2002) to 24% (2015–2018) and doubled in Guadeloupe from 8% (1999–2005) to 17% (2012–2014).

3.3. Identification of major non-B subtype clades circulating in CSAFOT

The most prevalent HIV-1 non-B clades (CRF02_AG and subtypes A, C, D, and F1) detected in Martinique (n = 136) and Guadeloupe (n = 74) were aligned with sequences of the same subtype/CRF from French Guiana (n = 86), mainland France (n = 786), neighboring American countries and other geographic regions where these HIV-1 variants are quite frequent (>5%, Supplementary Table S2). ML phylogenetic analyses revealed multiple introductions of major non-B strains into CSAFOT (Fig. 2 and Supplementary Fig. S3). Most CSAFOT non-B subtype sequences analyzed (59%) were distributed among 12 clusters of medium/large size (n ≥ 5 sequences) within clade CRF02_AG and subtypes A3, C, D, and F1 (Fig. 2; Supplementary Table S3). These clusters comprised a significant fraction of the non-B subtype sequences from Guadeloupe (66%), Martinique (63%), and French Guiana (46%), as well as a small fraction of sequences from mainland France (7%). Clusters CRF02_I, CRF02_V, A3, Cn, Dn, comprised only sequences from CSAFOT and were thus defined as French American clusters. The other clusters comprise sequences from CSAFOT and mainland France (CRF02_V, CRF02_n, CRF02_V, F1n, and F1v) or from CSAFOT and Brazil (F1 and F1b). Clusters CRF02_II, A3, F1b, and F1v were detected in a single French territory, while others comprised sequences from two (CRF02_v, Cn, and F1v), three (CRF02_n, CRF02_v, and Dn) and four (CRF02_v and F1n) different French territories. The remaining sequences from CSAFOT were distributed in local clusters of small size (n < 5 sequences, 13%) or appeared as non-clustered infections (26%, Supplementary Table S4). A few CRF02_AG French Caribbean sequences (3%) branched within large French

| Table 1. Distribution of non-B subtypes in Martinique and Guadeloupe (n = 315). |
|---------------------------------|--------|--------|--------|
| Subtype            | Martinique (n = 185) | Guadeloupe (n = 130) | Total (n = 315) |
| Pure subtype | n | % | n | % | % |
| A1 | 3 | 1.6 | 1 | 0.8 | 1.3 |
| A2 | 2 | 1.1 | – | – | 0.6 |
| A3 | 2 | 1.1 | 2 | 1.5 | 1.3 |
| C  | 11 | 5.9 | 10 | 7.7 | 6.7 |
| D  | 13 | 7.0 | 14 | 10.8 | 8.6 |
| F1  | 8 | 4.3 | 2 | 1.5 | 3.2 |
| G  | 4 | 2.2 | 4 | 3.1 | 2.5 |
| H  | 1 | 0.5 | – | – | <0.5 |
| CRFs | | | | | |
| CRF02_AG | 97 | 52.4 | 45 | 34.6 | 45.1 |
| CRF01_AE | 6 | 3.2 | 2 | 1.5 | 2.5 |
| Others* | 10 | 5.4 | 10 | 7.7 | 6.4 |
| Unassigned URFs | 28 | 15.1 | 40 | 30.8 | 21.6 |

CRF, circulating recombinant form; URF, unique recombinant form; *Other CRFs (number reported: Martinique - Guadeloupe): CRF06_cpx (1-0), CRF09_cpx (0-1), CRF11_cpx (3-0), CRF12_cpx (1-0), CRF13_cpx (0-1), CRF18_cpx (1-0), CRF19_cpx (0-4), CRF24_cpx (1-0), CRF25_cpx (0-3), CRF37_cpx (3-1).
clusters mostly restricted to mainland France (Supplementary Table S4).

### 3.4. Origin and dissemination of major non-B subtype clades circulating in CSAFOT

To reconstruct the most probable source location and subsequent dispersion pattern of major non-B subtype lineages here identified in CSAFOT, HIV-1 French sequences from those clades and a subset of non-French HIV-1 reference sequences closely related in previous ML analyses (Supplementary Table S5) were analyzed using Bayesian phylogeographic reconstructions. All major non-B subtypes CSAFOT clusters displayed a high clade support in Bayesian analyses (PP > 0.93), thus confirming the ML tree topology (Supplementary Table S6). The most probable origin was traced to West Africa for clusters CRF02a, CRF02b, CRF02c, CRF02d, and A3, Cameroon for cluster CRF02d, Central Africa for clusters D1, F1d, and F1v, Burundi for clade C, and Brazil for clades F1 and F1h (Fig. 3 and Table 2). The clade root was most probably placed in Martinique for clusters CRF02a, CRF02b, CRF02c, CRF02d, and A3, and in mainland France for clusters CRF02a and CRF02d (Table 2). The root of clusters F1 and F1h was placed in French Guiana or Brazil and that of clade F1d in Martinique or Guadeloupe with a similar probability (Table 2). The median TMRCA was traced to around the late 1970s for clade D1, during the 1980s for clades F1d, F1d, and F1v, during the 1990s for clades CRF02a, CRF02b, CRF02c, A3, and F1h, and during the 2000s for clades CRF02a, CRF02c, and C (Table 2). Our analyses support the occurrence of bidirectional disseminations of non-B strains between Martinique and Guadeloupe (CRF02a, CRF02c, C, D1, and F1v), from Martinique to French Guiana (D1), and from Guadeloupe to French Guiana (F1v) as well as frequent transatlantic disseminations of non-B strains from French Caribbean islands to mainland France (CRF02a, CRF02b, F1v, and F1v) and from mainland France to French Caribbean islands (CRF02a, CRF02c, and CRF02v) and French Guiana (CRF02a) (Fig. 4).

### 3.5. Epidemiological characteristics of individuals belonging to major non-B subtype clades circulating in CSAFOT

The cross-tabulation of epidemiological and demographic characteristics showed significant differences between clusters and sex, mode of transmission, age, country of origin, and stage of disease (Table 3). Clusters CRF02a and CRF02b were mainly linked (>75%) to males with MSM-transmission; while clusters CRF02a, CRF02b, A3, C, D1, F1v, and F1v were mainly found (>65%) among individuals with heterosexual transmission. When looking at age, clusters CRF02a and CRF02b mostly (>80%) comprised young individuals (<30 years old), cluster C mostly (57%) comprised individuals >50 years old, whereas other clusters were more evenly distributed across individuals with different ages. Most individuals (>69%) from clusters CRF02a to CRF02c, C, and F1v were from Martinique/Guadeloupe, cluster...
A3I was found in French Guianese patients from the border area with Suriname, as well as in individuals from Suriname. Cluster D1 comprised a large fraction (18%) of individuals born outside of France (Congo, Dominica, and Suriname), and most individuals (10 of 12 for whom origin was known) from clusters FI and FII were from Brazil and one had a Brazilian partner. Clusters CRF02V and DI comprised a large proportion (>60%) of immunosuppressed individuals (<200 CD4+ T cells per mm3), clusters CRF02I and CRF02II, F1I and F1II were the most frequent (>80%) in patients without advanced HIV disease (>200 CD4+ T cells/mm3), whereas remaining clusters comprised roughly similar proportions of individuals with and without advanced HIV disease. There was no significant link between clusters and viral load categorized by log10 values (P = 0.148).

4. Discussion

This study reveals that a large variety of HIV-1 non-B subtype strains, comprising eight subtypes, twelve CRFs, and various URFs have been introduced at multiple times in CSAFOT. Excepting Cuba (Pérez, 2006) such diversity has not been observed previously in the Caribbean region. Prevalence of non-B subtypes has significantly increased over time in Martinique and Guadeloupe, reaching about 15–25% of HIV-infected subjects enrolled after 2010. This pattern resembles the growing complexity of the HIV-1 molecular epidemiologic scenario observed in mainland France, where prevalence of non-B subtypes has also increased over time (Chaix et al. 2013; Brand et al. 2014; Chaillon et al. 2017; Visseaux et al. 2020). Thus, despite the small size of the HIV epidemic in CSAFOT, this region exhibits a complex and changing HIV-1 molecular epidemiologic profile.

In CSAFOT, the most prevalent HIV-1 non-B clades were successfully spread by multiple active local and regional transmission chains and 12 major transmission networks drove most of CRF02_AG, A3, C, D, and F1 infections. Some of the major non-B subtype CSAFOT transmission clusters comprised sequences from a single territory, while others comprised sequences from two or three different CSAFOT. Our analyses support frequent viral exchanges between Martinique and Guadeloupe and more sporadic dissemination of non-B subtypes between the two French Caribbean islands and French Guiana, which is fully consistent with the overall population mobility in CSAFOT (Marie and Temporal 2011).

Phylogeographic analyses support frequent non-B subtype viral transmissions between CSAFOT and mainland France. Two CRF02 clusters most probably arose in mainland France and were independently disseminated to Martinique and Guadeloupe, while three clusters most probably arose in French Caribbean islands and were disseminated to mainland France. The most notable example was cluster CRF02I for which we estimate a total of 13 possible transatlantic dissemination events. This cluster, comprising only men, most probably arose in Guadeloupe and from there it was disseminated to Martinique and mainland France multiple times. We also detected multiple viral exchanges of CRF02 between Martinique and mainland France and at least one dissemination event from mainland France.
France to French Guiana. Despite its recent origin (around the mid-2000s), cluster CRF02, was able to disseminate and establish local transmissions in all French territories.

The CRF02_AG was the most prevalent and successfully disseminated HIV-1 non-B subtype in Martinique and Guadeloupe, accounting for 45% of non-B subtype infections in the region. Previous studies have demonstrated that CRF02_AG is the most prevalent non-B subtype in France, representing about 60% of non-B infections, and further showed that MSM individuals infected in France are involved in the local transmission of this non-B subtype (Brand et al. 2014, 2017; Chaillon et al. 2017). This study revealed that CRF02_AG is spreading among both MSM and heterosexual local transmission networks in Martinique and Guadeloupe. Three CRF02 clusters probably arose in Guadeloupe and mainland France and seem to be more actively spreading among MSM individuals. By contrast, two other clusters probably arose in Martinique and mainly disseminated among heterosexual individuals.

The subtype A3 established the largest non-B transmission cluster identified in French Guiana and its origin was traced to

| Cluster | n  | Origin (PSP) | Location of MRCA (PSP) | T of MRCA (95% HPD) |
|---------|----|--------------|------------------------|---------------------|
| CRF02I  | 71 | West Africa (1) | Guadeloupe (0.98) | 2004 (2000–2007) |
| CRF02II | 34 | West Africa (1) | Mainland France (0.68) | 1995 (1991–1998) |
| CRF02III| 16 | West Africa (1) | Martinique (0.97) | 1995 (1991–1999) |
| CRF02IV | 13 | Cameroon (1) | Mainland France (0.97) | 2005 (2000–2008) |
| CRF02V  | 8  | West Africa (0.99) | Martinique (0.55) | 1996 (1992–2000) |
| A3I     | 13 | Mali (0.97) | French Guiana (1) | 1998 (1993–2003) |
| C1      | 16 | Burundi (0.99) | Guadeloupe (1) | 2003 (2001–2004) |
| D1      | 17 | Central Africa (0.99) | Guadeloupe (0.91) | 1978 (1973–1983) |
| F1I     | 9  | Brazil (1) | Brazil-North (0.62) | 1997 (1991–2002) |
| F1II    | 7  | Brazil (0.97) | French Guiana (0.58) | 1986 (1981–1991) |
| F1III   | 7  | Central Africa (0.99) | Martinique (0.45) | 1984 (1978–1989) |
| F1IV    | 5  | Central Africa (0.99) | Martinique (0.99) | 1983 (1977–1988) |
the late 1990s. Subtype A3 is a distinct subtype A clade quite frequent (5–30%) among HIV-infected subjects in several West African countries (Meloni et al. 2004). This A3 cluster identified in French Guiana represents the first recognized outbreak of subtype A3 outside West Africa. Notably, we detected five additional introductions of subtype A3 strains in French Guiana, some of them associated with individuals from West Africa (Benin, Guinea Bissau, and Ivory Coast), showing that dissemination of this viral lineage into French Guiana is not a rare phenomenon. Epidemiological data also support a dissemination of this subtype A3 lineage in the border region between French Guiana and Suriname.

West and Central African regions, which concentrate most of the French-speaking African countries, appear to be the most probable source of all CRF02_AG clades as well as of A3, D, F1III, and F1IV clusters circulating in CSAFOT. Remarkably, the clade C1 mostly comprising heterosexual individuals > 50 years old, was probably introduced into Guadeloupe around the early 2000s from East Africa (Burundi), as the other two largest subtype C epidemics in the Americas (Brazilian and Cuban). The origin of the Brazilian subtype C epidemic was traced to Burundi around the middle 1970s (Delatorre et al. 2013) and that of the Cuban subtype C epidemic to Ethiopia around the middle 1990s (Delatorre and Bello 2013). The historical links of CSAFOT with

Table 3. Epidemiological characteristics of subjects in major HIV-1 non-B subtype clusters circulating in CSAFOT.

| CRF02I | CRF02II | CRF02III | CRF02IV | A3I | CI | DI | F1I | F1II | F1III | F1IV | P |
|--------|---------|----------|---------|-----|----|----|-----|-----|-------|------|---|
| (n = 71) | (n = 34) | (n = 13) | (n = 8) | (n = 13) | (n = 16) | (n = 27) | (n = 9) | (n = 7) | (n = 7) | (n = 5) |

| Sex (%) | F | – | 12 | 56 | 17 | 50 | 46 | 71 | 26 | 11 | 33 | 83 | 75 | <0.0001 |
|---------|---|---|----|----|----|----|----|----|----|----|----|----|----|------|
| M       | 100 | 88 | 44 | 83 | 50 | 54 | 29 | 74 | 89 | 67 | 17 | 25 |      |

| Mode of transmission (%) | MSM | 87 | 82 | 6 | na | – | 8 | – | 30 | na | na | – | – | <0.0001 |
|--------------------------|-----|----|----|---|----|---|---|---|----|----|----|---|---|------|
| Hetero. | 13 | 18 | 88 | na | 100 | 92 | 100 | 65 | na | na | 100 | 75 |      |
| Others | – | – | 6 | na | – | – | – | 4 | na | na | – | 25 |      |

| Age group, years (%) | ≤30 | 85 | 59 | 81 | 50 | 33 | 46 | – | 46 | 56 | – | 60 | 25 | <0.0001 |
|----------------------|-----|----|----|----|----|----|----|---|----|----|---|----|----|------|
| 31–40 | 7 | 35 | 19 | 25 | 33 | 31 | – | 31 | 11 | 50 | 20 | 50 |      |
| 41–50 | 7 | 6 | – | – | – | 17 | – | 43 | 15 | 11 | 17 | 20 | – |      |
| ≥51 | – | – | – | – | 17 | 23 | 57 | 8 | 22 | 34 | – | 25 |      |

| CDC stage (%) | 1 | 28 | 29 | 12 | – | – | 36 | 29 | 8 | 33 | 17 | – | – | 0.005 |
|---------------|---|----|----|----|---|---|----|----|---|----|----|---|---|------|
| 2 | 64 | 53 | 44 | – | 17 | 18 | 43 | 31 | 56 | 67 | 60 | 50 |      |
| 3 | 8 | 18 | 44 | – | 83 | 45 | 29 | 61 | 11 | 17 | 40 | 50 |      |

| Country of origin (%) | MQ | 28 | 82 | 69 | 100 | 100 | – | 86 | 18 | – | – | 40 | 50 | <0.0001 |
|----------------------|----|----|----|----|-----|-----|---|----|----|---|----|----|----|------|
| GP | 41 | – | – | – | – | – | – | 48 | – | – | 40 | – |      |
| GF | 8 | – | – | – | – | – | – | 69 | – | 4 | – | – | – |      |
| FR | 5 | – | 19 | – | – | – | – | 14 | 4 | – | 50 | – | – |      |
| BR | 3 | – | – | – | – | – | – | 100 | 50 | – | – | – | – |      |
| SR | – | 6 | – | – | – | 31 | – | 7 | – | – | – | – | – |      |
| Others/na | 16 | 12 | 12 | – | – | – | – | 18 | – | – | 20 | 50 |      |

F, female; M, male; Hetero., heterosexual; MSM, men who have sex with men; na, not available. MQ, Martinique; GP, Guadeloupe; GF, French Guiana; FR, mainland France; BR, Brazil; SR, Suriname.
Burundi are scarce and all individuals within the cluster C7 were of French origin.

All non-B subtype clusters circulating in French Caribbean islands are mostly or exclusively composed of individuals of French origin, with exception of the subtype D1 cluster that comprised a significant proportion (19%) of migrant individuals from Congo, Dominica, and Suriname that probably got infected after migration into the French Caribbean region. The subtype D1 transmission cluster was probably introduced in Guadeloupe from Central Africa around the late 1970s, constituting the oldest non-B subtype lineage detected in CSAFOT, consistent with the high frequency of URFs_BD also detected in that region. This transmission cluster comprises a significant fraction of both heterosexual (65%) and MSM (31%) individuals of different ages, supporting that this lineage is not restricted to a specific population group. Another peculiarity is that most (92%) of subtype D1-infected individuals were symptomatic, which may reflect longstanding chronic infections and/or faster disease progression rate (Amornkul et al. 2013).

The large number of Brazilian immigrants living in French Guiana may provide an epidemiological link for sporadic transmissions of non-B subtype strains from Brazil into French Guiana (Nacher et al. 2018). Consistent with this hypothesis, nearly all subtype F1 sequences and a great proportion of subtype C (29%) sequences detected in French Guiana were nested with Brazilian subtype F1 and subtype C clades. Clusters F11 and F111 detected in French Guiana mostly comprise sequences from Brazilian individuals living in French Guiana and in neighboring Northern Brazilian states. This supports that F111 and F111 were transmission clusters probably driven by Brazilian migrants that frequently travel across the French Guiana–Brazilian border, but these lineages seem to have a very restricted transmission to the native French Guianese population.

A previous study supports that competition of HIV-1 strains at the epidemic level may involve an advantage for the strain that was the first to colonize a population (Ferdinandy et al. 2015). The timing of introductions of different non-B subtype strains in CSAFOT that range from the late 1970s to the early 2000s, however, was clearly not related with the size of current non-B subtype epidemic outbreaks. The non-B subtype strain most successfully disseminated in the CSAFOT (CRF02_AG) started to spread much later than other non-B viral clades. The epidemic expansion of different clusters may have also been influenced by the virus genotype (Bertels et al. 2018). However, we failed to detect significant differences in viral load between different transmission clusters. These observations suggest that the characteristics of the existing contact transmission network are probably the most determinant driving force of the epidemic expansion of different HIV-1 non-B subtype lineages introduced in CSAFOT.

Although this study covers an important fraction of all HIV diagnosed individuals in Martinique (51%) and Guadeloupe (31%) over the study period, one important limitation is the variable sampling density along time which may introduce some bias in our results. Sampling density significantly increased between the mid-1990s and the middle 2000s in Martinique and between the mid-1990s and the late 2000s in Guadeloupe (Supplementary Fig. 4). The increasing prevalence of non-B variants in Martinique between 2006–2008 and 2015–2018, however, could not be explained by differences in sampling density as this remained roughly constant in that period. Another limitation of our study is the limited access to sequences from French Guiana of some specified non-B subtypes and sampled over a narrow time interval (2006–2012) that did not permit us to provide a picture of the overall prevalence and temporal trend of non-B subtypes in this French territory.

The results of this study offer important insights into HIV diversity trends in CSAFOT. Our data highlight that prevalence of HIV-1 non-B subtype variants has significantly increased over time in Martinique and Guadeloupe. The HIV-1 non-B subtype epidemic in CSAFOT resulted from independent introductions of a large variety of subtypes, CRFs, and URFs as well as from the subsequent local and regional expansion of some viral strains that were sequentially introduced between the late 1970s and the middle of the 2000s and spread through MSM and heterosexual contact networks. Our study emphasizes that early detection and treatment of people being part of the largest transmission chains combined with a coordinated regional healthcare response and pre-exposure prophylaxis in high-risk groups should have a significant impact on reducing the local spread of non-B subtypes that are continuously introduced in CSAFOT.

**Supplementary data**

Supplementary data are available at Virus Evolution online.

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**Author contributions**

G.D.S., G.B., V.L., and M.N. conceived and designed the study. G.D.S., C.H.S., E.D.A., and V.L. collected the samples and established HIV sequences from the French American Overseas territories. G.B. and E.D.E. performed the phylogenetic and phylodynamics inferences. A.C., B.T., I.L., O.C., and R.C. contributed with epidemiological data collection from respective region. M.L.C., D.D., and B.V. contributed with sequence data from France. M.N. performed the statistical analyses. G.B., E.D.E., V.L., G.D.S., and M.N. wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

**Conflict of interest**: None declared.

**Data availability**

The GenBank accession numbers for 325 non-B subtype pol sequences from CSAFOT are MT741097–MT741486. A subset of sequences is made available due to the potential for identification of direct transmission among individuals in such large datasets which could have near complete sampling of local HIV cases.
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