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Review

The increasing prevalence of HIV-1 subtype C in Southern Brazil and its dispersion through the continent

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ABSTRACT

The HIV-1 has evolved swiftly and the scenario of HIV-1 genetic diversity is constantly changing. In South America, recombinant forms of subtypes B, F1, and BF1 have historically driven the HIV-1 epidemic. In recent years, however, infection with subtype C has gained prominence as its prevalence increased in Southern Brazil as well as neighboring countries. Current studies point to a single introduction of closely related strains as the beginning of the Brazilian subtype C epidemic. However, the place of origin of these strains, date, and route of introduction are under continuous debate as well as the clinical outcomes of the emergence of subtype C. Therefore, this paper reviews the history of the HIV-1 subtype C in Brazil, particularly in the Southern region, covering its demographic and evolutionary history and the possible implications to the Brazilian AIDS epidemic as well as to neighboring countries.

Contents

Introduction

The first AIDS case was diagnosed in Brazil in 1982 and since then 608,230 cases had been reported throughout the country (Brazilian Ministry of Health, 2011). The prevalence of AIDS in the adult population is 0.6% and the incidence of new cases was 17.9/100,000 inhabitants in 2010. With a population exceeding 192 million, Brazil has the largest number of HIV infected people among countries from Central and South America, and according to UNAIDS accounts for one third of all people living with HIV in this region (Brazilian Institute of Geography and Statistics [IBGE], 2012; World Health Organization [WHO], 2010).

Brazil is a country of continental dimensions, spread over an area of 8,514,877 km^2 which occupies roughly half of South America, divided into 26 states and a Federal District, which are grouped into five geopolitical regions. The states of Rio Grande do Sul (RS), Santa Catarina (SC), and Paraná (PR) compose the Southern region, which is the smallest region of the country with a population of 27 million,

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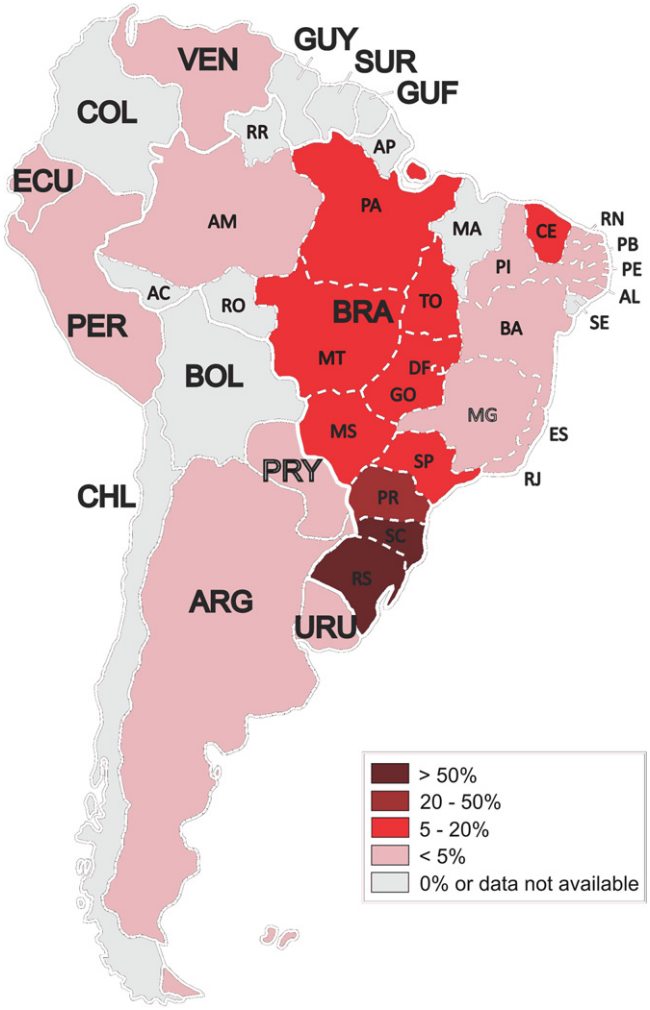

Fig. 1. Prevalence of HIV-1 subtype C and C-containing sequences in Brazilian states and South American countries. Acronyms for Brazilian states: RS (Rio Grande do Sul), SC (Santa Catarina), PR (Paraná), SP (São Paulo), RJ (Rio de Janeiro), MG (Minas Gerais), ES (Espírito Santo), BA (Bahia), SE (Sergipe), AL (Alagoas), PE (Pernambuco), PB (Paraíba), RN (Rio Grande do Norte), CE (Ceará), PI (Piauí), MA (Maranhão), PA (Pará), AP (Amapá), AM (Amazonas), RR (Roraima), AC (Acre), RO (Rondônia), MT (Mato Grosso), TO (Tocantins), GO (Goiás), MS (Mato Grosso do Sul), and DF (Distrito Federal).

14% of its total population (Brazilian Institute of Geography and Statistics [IBGE], 2012). This region shares borders with Uruguay, Argentina, and Paraguay, as well as with the Brazilian states of São Paulo and Mato Grosso do Sul (Fig. 1).

The epidemiological situation in Southern Brazil regarding AIDS is concerning. The AIDS-related mortality is steadily increasing and ever since the beginning of the 2000s is highest among Brazilian regions (Brazilian Ministry of Health, 2011). The number of newly diagnosed cases is also the highest in the country, and in 2010 the incidence rates in the states of Rio Grande do Sul and Santa Catarina were 37.6 and 30.2 new cases per 100,000 persons, respectively, whereas in the state of Paraná the incidence rate was 19 new cases per 100,000 persons. Moreover, the top ten Brazilian cities with the highest incidence of AIDS in 2010 were from the Southern region, and Porto Alegre, the capital city of Rio Grande do Sul, had an incidence of 99,8 new cases per 100,000 persons, the highest incidence of AIDS in the country (Brazilian Ministry of Health, 2011).

High molecular variability is a striking feature of HIV-1 in the global AIDS pandemic. Among four HIV-1 groups (M, N, O, and P), HIV-1 group M is globally distributed, being subdivided into nine subtypes (A–D, F–H, J, and K) and, 51 circulating recombinant forms (CRFs) identified so far (Ng et al., 2012; Plantier et al., 2009; Tebit and Arts, 2011). The AIDS pandemic in Brazil is mostly caused by the HIV-1 subtype B, while subtypes C, F1, and BF1 recombinants are observed in lower frequencies (Gadelha et al., 2003; Machado et al., 2009; Pedroso et al., 2007; Sa Filho et al., 2005; Stefani et al., 2007; Teixeira et al., 2004). However, the distribution of HIV-1 variants is heterogeneous in the inner parts of the country, while subtype C is highly prevalent in Southern Brazil (Gräf et al., 2011; Medeiros et al., 2011; Silva et al., 2010).

Currently, subtype C and C-containing forms are responsible for more than 51% of all worldwide HIV-1 infections (Tebit and Arts, 2011). Dominant in India, Southern African countries, and with increasing presence in China and Eastern Africa, subtype C and C-containing recombinant forms appear to spread more rapidly than other group M subtypes (Ariën et al., 2007; Tebit and Arts, 2011). Moreover, increasing subtype C prevalence has been reported in some countries from Western Europe, the Americas, and Australia (Abecasis et al., 2011; Chibo and Birch, 2012; Dougan et al., 2005; Tatt et al., 2004; Tebit and Arts, 2011). Due to the high prevalence of HIV-1 subtype B in developed countries, research on the natural history and health technology improvements have largely focused on this variant. However, the actual global epidemiological profile requires further studies evaluating the impact of HIV-1 diversity, especially the dominant subtype C on disease progression and treatment outcomes. Thus, molecular epidemiology studies are pivotal for evaluating phenotypic relevance and providing underlying data concerning viral diversity and evolution.

In Brazil, studies published over a decade have investigated the molecular features of the local HIV epidemic, producing valuable data regarding the importance of subtype C and C-containing recombinant forms in the Southern region and its implications to neighboring countries as well other Brazilian states. Given the extensive bibliography available and the actual global relevance of HIV-1 subtype C, this article aims to review the history of subtype C in Brazil, from the early cases in the South to the increasing prevalence in the North.

Origin of Brazilian HIV-1 C clade

The history of how HIV-1 subtype C was introduced in Brazil is being revealed as new epidemiological data are published and more Brazilian subtype C sequences become available. The first phylogenetic analyses indicated that subtype C may have entered Brazil with a single introduction of closely related strains, since Brazilian subtype C sequences (C_{BR}) formed a monophyletic cluster when compared to sequences of the same subtype from India and some African countries (Monteiro et al., 2007; Sanabani et al., 2006; M.A. Soares et al., 2003). Furthermore, amino acid signatures absent in the subtype C world consensus sequence were identified in C_{BR}, reinforcing a monophyletic introduction hypothesis (M.A. Soares et al., 2003). Early studies also reported a smaller genetic distance between sequences of C_{BR} compared with Brazilian subtype B sequences, suggesting a more recent introduction of HIV-1 C than subtype B in Brazil (Monteiro et al., 2007; Sanabani et al., 2006; Soares et al., 2005).

The strong evidence supporting a monophyletic origin of the Brazilian HIV-1C epidemic prompted questions regarding the geographical origin and migration route of the founder strains. In 2008, two studies began elucidating this issue, reporting that C_{BR} sequences were closely related to subtype C sequences from Middle East African countries (Bello et al., 2008; Fontella et al., 2008). More specifically, sequences from Burundi at the root of the Burundi–Brazilian clade showed that C_{BR} originated from a strain related to this country (Bello et al., 2008). Due to scarce social, cultural, and economic relationships between Brazil and

Burundi, a direct introduction of HIV-1C from Burundi or neighboring countries (Democratic Republic of Congo, Rwanda, or Tanzania) into Brazil was further evaluated (Brígido, 2009). Regarding cultural, social, and historical determinants, Mozambique was a putative source for the Brazilian HIV-1C epidemic; however, phylogeny estimations lacked evidence for a Mozambican ancestry (Brígido, 2009; Fontella et al., 2009). A new hypothesis for the introduction of HIV-1C in Brazil emerged recently. Phylogenic analysis revealed that subtype C sequences from the United Kingdom (UK), Brazil, and East Africa grouped together in a monophyletic cluster, where some UK sequences appeared at the base of the Brazilian HIV-1C clade (Oliveira et al., 2010). This suggests that the UK may have played a key role in the introduction of HIV-1C into Brazil. A more recent phylogeographic analysis, however, found no evidence of a direct viral flow from the UK to Brazil, only from both East Africa and Brazil to the UK (Véras et al., 2011), which may be expected since the UK is home to large Brazilian and East African immigrant populations. Thus, the precise migration route of HIV-1C from East Africa to Brazil remains unclear.

The exact time when the HIV-1 C_{BR} clade was introduced in Brazil is also controversial. The onset date of subtype C epidemic in Brazil was first estimated by Salemi et al. (2005), which reported the early 1990s as the most likely period for HIV-1C introduction, about 30 years after subtype B was introduced. Some years later, Bello et al., 2008 reestimated the onset year for the HIV-1 subtype C epidemic in Brazil, citing the early 1980s as the probable time of introduction. Subtype C sequences from patients infected in the 1980s were included in this study, supporting the hypothesis of an introduction prior to 1990. More recently, Véras et al. (2011) reevaluated the dates, suggesting an introduction between the 1960s and the 1970s. The latter study included sequences from the three states that compose Southern Brazil plus the state of São Paulo, while Bello et al. only included sequences from the state of Rio Grande do Sul, which may explain differences between the two estimates.

Recognition of the HIV-1 C epidemic in Rio Grande do Sul

Despite the idea that C_{BR} clade probably circulated in Brazil before the early 1980s, the existence of an established subtype C epidemic in the country was only recognized in the 1990s. The WHO Network for HIV Isolation and Characterization (1994) reported for the first time the existence of HIV-1C in Latin America. This subtype was found in 14 clinical samples obtained in Porto Alegre (capital city of Rio Grande do Sul) between 1992 and 1993. Shortly after that, the same group identified subtype C in three other Brazilian patients, two from Porto Alegre and one from São Paulo (capital city of São Paulo) (Osmanov et al., 1994). At the time only subtypes B and F1 had been identified in South America, and the possibility of a subtype C epidemic in Southern Brazil motivated several other studies focusing on the molecular epidemiology of HIV-1.

The real scenario of the HIV epidemic in Southern Brazil began to be unraveled in 2002 when Martínez et al. found 22% of subtype C among samples collected between 1994 and 1997 in Rio Grande, a port city in the state of Rio Grande do Sul. In 2003 Soares M.A. et al. corroborated these findings reporting a 37% prevalence of HIV-1C in the state of Rio Grande do Sul, reaching as high as 44% when recombinant viruses containing subtype-C related sequences were considered. Also in 2003, the Brazilian Network for HIV Drug Resistance Surveillance reported a high frequency of BC recombinant strains in samples from Rio Grande do Sul, showing the intermixing of subtype B and C epidemics in Southern Brazil (Brindeiro et al., 2003). Table 1 summarizes all studies performed in the state of Rio Grande do Sul before the publication of this review. The co-circulation of multiple HIV-1 clades in a restricted geographic region favors the emergence of recombinant forms, as observed frequently in some sub-Saharan countries (Tebit and Arts, 2011). In Southern Brazil, the epidemiological scenario also proved favorable to the emergence of HIV recombinant forms.

Identification of a new CRF_BC in Rio Grande do Sul

The high variability of HIV is also a consequence of recombination events. Inter-subtype recombination occurs when an individual is co-infected with two different HIV strains at once, or when a previously infected individual acquires a different strain of HIV, which is called superinfection. These two phenomena lead to the emergence of unique recombinant forms (URFs) or circulating recombinant forms (CRFs). In Brazil recombination

Table 1
Summary of studies in HIV molecular epidemiology performed in the state of Rio Grande do Sul (RS), Southern Brazil.

Reference	City	Sampling n (ARV exposure)	Sampling year	HIV-1 diversity	Gene analyzed
Martínez et al. (2002)	Rio Grande	69 (naïve and treated)	1994–1997	22% C, 75% B, 3% F	*env*
M.A. Soares et al. (2003)	Porto Alegre	57 (naïve)	2001	37% C, 7% CB, 56% B+F1	*pol* (PR/RT)
Brindeiro et al. (2003)	Not available	139 (naïve)	2001	45% C, 30% B, 3% F1, 22% recombinants	*pol* (PR/RT)
E.A. Soares et al. (2003)	Porto Alegre	77 (naïve and treated)	2002	31% C, 45% B, 12% F1, 9% CRF31[a], 1,5% BF1, 1,5% BD	*pol* (PR/RT)
E.A. Soares et al. (2005)	Rio Grande	85 (naïve and treated)	2002	41% C, 42% B, 5% F1, 4% CRF31[a], 2% D, 6% URF	*pol* (PR/RT)
Rodrigues et al. (2006)	Porto Alegre	108 (naïve)	2004	33% C, 32% B, 3% F1, 25% CRF31[a], 7% others	*pol* (PR/RT)
Santos et al. (2006)	Porto Alegre and Rio Grande	152 (unknown)	2002–2003	31% C, 46% B, 4% F1, 10% CRF31, 9% others	*pol* (PR/RT)
Monteiro et al. (2007)	Porto Alegre	22 (unknown)	2003	27% C, 37% B, 4.5% F1, 27% BC, 4.5% BF1	*env* and *gag*
Brígido et al. (2007)	Porto Alegre	122 (naïve)	2004–2006	27% C, 30% B, 10% F1, 21% CRF31, 8% URF_BC, 4% URF	*env* and *pol* (PR/RT)
Dias et al. (2009)	Porto Alegre	128 (naïve)	2004–2005	32% C, 25% B, 11% F1, 26% CRF31, 6% URF	*pol* (PR/RT)
Simon et al. (2010)	Canoas	80 (naïve and treated)	2008–2009	44% C, 19% B, 2% F1, 35% CRF31	*pol* (PR/RT)
Medeiros et al. (2011)	Porto Alegre	99 (naïve)	2006–2007	40% C, 26% B, 1% F1, 19% CRF31, 12% URF_BC, 2% URF_BF1	*pol* (PR/RT)
Silveira et al. (2012)	Rio Grande	245 (naïve)	2005–2008	56% C, 21% B, 5% F1, 10.5% URF_BC, 0.5% CRF31, 6% URF_BF1, 1% others	*pol* (PR/RT, CN and RH)

PR/RT to protease/reverse transcriptase, CN to connection, RH to RNase H.

[a] Values reported by Santos et al. (2007).

events were reported between subtypes B and F1, with the BF1 mosaic as the most prevalent HIV genetic form after subtype B in most of the country (Guimarães et al., 2008; Sa Filho et al., 2006). The introduction of subtype C in the Southern region, however, gave rise to several BC mosaic strains and a new CRF.

Most of the early studies revealing the high prevalence of subtype C in the state of Rio Grande do Sul also reported frequencies of recombinant BC forms ranging between 6% and 22% (Brindeiro et al., 2003; M.A. Soares et al., 2003, 2005). Rodrigues et al. reported in 2006 a remarkable 25% of BC recombinants in samples from Porto Alegre. Detailed analyses of these BC sequences showed high similar recombinant structure, lower intra-cluster distances, and common amino acid signatures, pointing strongly to a new circulating recombinant variant. Shortly afterward, Santos et al. (2006) characterized this relevant BC recombination form as a new CRF named CRF31_BC. This HIV-1 variant features a predominantly subtype C genome, with a 240bp subtype B fragment inserted in the reverse transcriptase gene.

Previous studies indicate that the CRF31_BC clade likely originated in the late 1980s and rapidly expanded in the city of Porto Alegre (Passaes et al., 2009). The prevalence of the CRF31_BC in this city was estimated to be 10% in samples collected between 2002 and 2003 and 25% in samples from 2004 (Santos et al., 2007) (Table 1). Despite occasional detection of CRF31_BC clade beyond Porto Alegre, the prevalence of this recombinant variant in other cities in Rio Grande do Sul, Santa Catarina, and Paraná seems to be much lower ($<5\%$) (Brígido et al., 2007; Gräf et al., 2011; Raboni et al., 2010; Rodrigues et al., 2010; Silva et al., 2010; Silveira et al., 2012).

The HIV-1 C epidemic in Santa Catarina and Paraná

The unique features of the HIV/AIDS epidemic in the state of Rio Grande do Sul galvanized researchers to better understand HIV-1 molecular epidemiology in the Southern Brazilian region. In 2007, the first cases of subtype C infection were reported in Santa Catarina, a southern state without previous HIV-1 molecular data, in a small group of three female sex workers from the city of Imbituba (Schuelter-Trevisol et al., 2007). Shortly after that, Locateli et al. (2007) reported a 48% frequency of subtype C in samples obtained in 2004 from Florianópolis (capital city of Santa Catarina). Frequencies of subtype C over 60% were reported a few years later in Florianopólis, Camboriú, Itajaí, and Criciúma (Brígido et al., 2007; Gräf et al., 2011; Rodrigues et al., 2010; Pinto et al., 2012), making Santa Catarina the state with the highest prevalence of HIV-1C in Brazil. High prevalence (16%) of URF_BC in Santa Catarina was also reported, reflecting the co-existence of subtypes C and B in the same population (Gräf et al., 2011).

The first molecular epidemiology study in the state of Paraná, conducted by Brindeiro et al. in 2003, reported a subtype C prevalence of 30%, the same frequency subsequently reported by Ferreira et al., 2008 in the capital city (Curitiba). More recently, Toledo et al. (2010), Raboni et al. (2010) and Silva et al. (2010) evaluated patient samples from several cities in Paraná, reporting 21–27% of subtype C in patients with therapeutic failure. Raboni et al. (2010) also observed higher proportion of subtype C (31%) in cities located in the southern region of the state of Paraná, bordering Santa Catarina, while in the northern region of the state, which borders São Paulo state, only 13% of subtype C was observed. Table 2 summarizes the studies performed in the states of Santa Catarina and Paraná.

Temporal trends and spatial dynamics of HIV-1 C expansion and CRF31_BC epidemics in Southern Brazil

Reconstruction of the demographic history of subtype C and CRF31_BC epidemics in Porto Alegre, performed using a Bayesian coalescent-based approach, indicates that both HIV-1 clades experienced a period of fast exponential spread in this Brazilian city during the 1980s and 1990s (Bello et al., 2009). Consistent with this finding, E.A. Soares et al. (2003) observed an increasing frequency of subtype C in the city of Porto Alegre from 0% among patients diagnosed before 1990 to 43% in patients diagnosed between 2001 and 2002. The following studies confirmed the continued increase in HIV-1 subtype C infections in Porto Alegre: 27% reported by Brígido et al. (2007), 32% observed by Dias et al. (2009), and 40% reported by Medeiros et al. (2011). A study conducted in the city of Canoas (Porto Alegre metropolitan area) found 44% of subtype C in 2010 (Simon et al., 2010). In the same reports, the frequency of subtype B sharply decreased since the early 2000s.

In general, the proportion of CRF31_BC also increased over time in Porto Alegre, though not linearly. A wide variation in the frequency of CRF31_BC between published studies is observed and may reflect the difficulties in identifying the recombination pattern, featured by a short subtype B insertion within a whole subtype C genome. In addition, minimal variations in the point of recombination of CRF31_BC get in the way of the classification, which can reclassify the strain into a second generation recombinant form or a URF_BC (Passaes et al., 2009). Therefore, the differences between CRF31_BC and unique recombination forms may be minimal, explaining why a lower prevalence of CRF31_BC

Table 2
Summary of studies in HIV molecular epidemiology performed in the states of Santa Catarina (SC) and Paraná (PR), Southern Brazil.

Reference	City/State	Sampling n (ARV exposure)	Sampling year	HIV-1 diversity	Gene analyzed
Schuelter-Trevisol et al. (2007)	Imbituba/SC	4 (naïve)	2003–2004	75% C, 25% B	*pol* (PR/RT)
Locateli et al. (2007)	Florianópolis/SC	80 (naïve and treated)	2004	49% C, 22.5% B, 22.5% BC, 6% BF1	*env* and *gag*
Brígido et al. (2007)	Itajaí and Camboriú/ SC	83(naïve)	2004–2006	64% C, 26.5% B, 6% F1, 3.5% CRF31	*env* and *pol* (PR/RT)
Rodrigues et al. (2010)	Criciúma/SC	42 (naïve and treated)	2007	79% C, 7% B, 9.5% F1, 2% CRF31, 2.5% CF1	*pol* (PR/RT)
Gräf et al. (2011)	Florianópolis/SC	82 (naïve)	2008–2009	66% C, 13.5% B, 1% F1, 2.5% CRF31, 16% URF_BC, 1% URF_BCF	*env* and *pol* (PR/RT/INT)
Brindeiro et al. (2003)	Not available/PR	147 (naïve)	2001	30% C, 64% B, 4% F1, 2% rec.	*pol* (PR/RT)
Ferreira et al. (2008)	Curitiba/PR	38 (naïve)	2005–2006	30% C, 52% B, 2% F1, 14% BC, 2% CF1	*env* and *pol* (PR/RT)
Toledo et al. (2010)	several cities/PR	389 (virological failure)	2003–2006	21% C, 61% B, 5% F1, 2.5% BC, 10.5% BF1	*pol* (PR/RT)
Raboni et al. (2010)	several cities/PR	239 (virological failure)	1999–2007	26.5% C, 52% B, 10.5% F1, 8.5% URF	*pol* (PR/RT)
Silva et al. (2010)	Curitiba/PR	191 (virological failure)	1987–2008	21.5% C, 69% B, 5% F1, 4% URF_BF, 0.5% URF_CF	*pol* (PR/RT)

PR/RT to protease/reverse transcriptase, CN to connection, RH to RNase H.

is observed along with a high prevalence of URFs, mostly URF_BC in some studies.

A temporal increase of subtype C was also observed in other cities from Rio Grande do Sul and Santa Catarina, including Rio Grande and Florianópolis, respectively. A subtype C prevalence of 41% was reported in the city of Rio Grande in 2005 and 56% in 2012, over twice as high as the first work performed in the same city in 2002 (Martínez et al., 2002; Silveira et al., 2012; Soares et al., 2005). Evaluation of the HIV-1 genotype distribution at the *env* gene from two studies performed in Florianópolis also revealed a significant increase in the proportion of subtype C in the heterosexual population over time: from 56% in 2004 to 83% in 2008–2009 (Gräf et al., 2011), while the proportion of subtype B in that population decreased from 38% to just 15% in the same period.

No similar temporal increase in the prevalence of subtype C has been detected in Paraná. The prevalence of this HIV-1 subtype has remained relatively stable (20–30%) across different studies performed between 2003 and 2010 (Brindeiro et al., 2003; Ferreira et al., 2008; Raboni et al., 2010; Silva et al., 2010; Toledo et al., 2010). A significant increase in the frequency of URFs_BC over time, however, was reported in Paraná by Ferreira et al. (2008), probably reflecting the frequent and ongoing co-infections of patients living in this region with HIV-1 subtypes C and B clades.

Regarding the spatial dynamics of the HIV-1C epidemic in Brazil, two recent reports using phylogeography inference point to the state of Paraná as the most likely source of subtype C in the southern region (Bello et al., 2012a; Véras et al., 2011). The study of Véras et al. (2011) indicates that subtype C expanded trough Southern Brazil with an asymmetrical net viral flow following a north to south axis, from Paraná to both Santa Catarina and Rio Grande do Sul. A recent study performed by our group with a large number of sequences collected from the three southern capitals reveals a much more complex scenario (Bello et al., 2012a). According to our analyses, Florianópolis (capital of Santa Catarina) acted as an important staging post between Curitiba (capital of Paraná) and Porto Alegre (capital of Rio Grande do Sul), both receiving and sending viral lineages to the neighboring cities. Meanwhile, the direct viral migration flow between Curitiba and Porto Alegre seems to be much lower. Such estimated subtype C migration flows could be partially explained by the spatial distance and routine migration of people between Southern Brazilian states.

HIV-1C expansion throughout the country

In general, subtype C seems to be slowly expanding north in the Brazilian territory as can be seen in Fig. 1. In 2010 a slight increase in the prevalence of HIV-1C was observed in the state of São Paulo, ranging from 0.3% in samples from 2005 to 2.9% in 2008, reaching 8.3% if only newly diagnosed patients from 2008 are considered (Brígido et al., 2011). These findings were recently confirmed by a study reporting 7.3% of subtype C and C-containing sequences in the city of São Paulo (Alcalde et al., 2012). No evidence of fast expansion of subtype C has been reported in other Southeast states, including Rio de Janeiro and Minas Gerais (Castro et al., 2010; Westin et al., 2011). Thus, despite the close geographical proximity and the frequent routine movement of people from the Southern region, subtype B continues to be the widely dominant HIV-1 clade in Southeast Brazil.

Nonetheless, a relatively high prevalence of HIV-1C infections has been reported recently in some states from the Central-West, North and Northeast regions. In 2009, a study from the Brazilian Network for HIV Drug Resistance Surveillance found 6% of subtype C in the state of Pará and in the national capital Brasília (Inocencio et al., 2009). In 2011, two studies reported, respectively, 11.7% and 9.6% of subtype C and C-containing forms in the inland cities of Cuiabá, capital of Mato Grosso (MT), and Palmas, capital of Tocantins (TO) (Ferreira et al., 2011; Carvalho et al., 2011) (Fig. 2). Also in 2011, a frequency of subtype C and C-containing forms of close to 20% was reported by a study performed in inmates from Central-West Brazil (Cardoso et al., 2011). Due to job opportunities in agriculture and livestock, Brazilian Central-West region attracts a large number of immigrants, mainly from the Southern region. Therefore, it is possible that this migration route is responsible for the dissemination of subtype C to this region in recent years. Moreover, a prevalence of 5.4% HIV-1C was recently reported in the state of Ceará (CE), the highest prevalence ever recorded in that state (Arruda et al., 2011).

HIV-1C in South American countries

The HIV epidemic in South American countries is characterized by a predominant circulation of subtypes B and BF recombinants, with the latter being observed with higher prevalence in Southern Cone countries (Dilernia et al., 2007; Montano et al., 2005; Ruchansky et al., 2009). Carrion et al., 2004 were the first to report HIV-1 subtype C infections in South America after analyzing 1629 samples from Ecuador, Colombia, Bolivia, Venezuela, Peru, Chile, Argentina, Paraguay, and Uruguay. Seven subtype C and one BC strains were identified in Argentina, Paraguay, and Uruguay, countries that share borders with the Brazilian Southern region and compose the so-called Southern Cone. Corroborating these findings, subsequent reports also observed prevalence of less than 1% in subtype C or C-containing forms in patients from Ecuador, Peru, and Southern Cone countries (Aulicino et al., 2005a, 2005b; Montano et al., 2005; Pando et al., 2006). In these reports, patients infected with HIV-1C or C-containing forms frequently reported some epidemiologic link to Brazil, such as living in border towns or having had sexual partners from Brazil.

Lately several studies have detected the circulation of subtype C in Paraguay, Uruguay, and Argentina, although still at low frequencies, accounting for less than 5% of the HIV infections in those countries (Aguayo et al., 2008; Pando et al., 2011; Ruchansky et al., 2009). In addition, Jones et al. described in 2009 two CRF31_BC sequences in

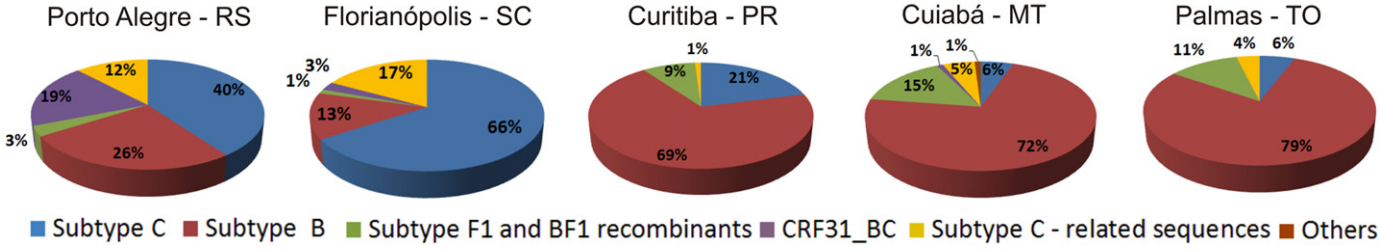

Fig. 2. HIV-1 diversity in the Brazilian state capitals mainly affected by subtype C epidemic according to the most recent studies. Data according to Medeiros et al. (2011), Gräf et al. (2011), Silva et al. (2010), Ferreira et al. (2011), and Carvalho et al. (2011).

Argentina. These studies point to a pattern of very slow spread of HIV-1C and related BC sequences from Brazil to neighboring countries from the Southern Cone. In fact, HIV-1C sequences from Argentina and Uruguay were found to be closely related to Brazilian strains in several phylogenetic studies, supporting the hypothesis of a single introduction of subtype C in Southern Brazil which later spread to other South American countries (Dilernia et al., 2007; Fontella et al., 2008; Jones et al., 2009; Monteiro et al., 2007; Véras et al., 2011). Nevertheless, studies performed in Venezuela in 2005 and 2012 found subtype C sequences that grouped outside the Brazilian HIV-1C cluster (Castro et al., 2005; Rangel et al., 2012). Epidemiologic data showed that two of these sequences were originated from patients most likely infected in South Africa, while the other case was identified in an Amerindian woman who lacked epidemiological information. Though these studies raised the possibility that new HIV-1C lineages may have been introduced in South America, more specifically in Venezuela, a more comprehensive study performed in 2009 did not detect subtype C infections in 425 Venezuelan patients (Rangel et al., 2009). The HIV-1 epidemic in Venezuela is predominantly driven by subtype B and the few reported cases of HIV-1C infections might not mean that subtype C is circulating in the Venezuelan population. In Fig. 1 the overall prevalence of HIV-1C and C-containing sequences in South American countries is shown.

The subtype C epidemic and its relevance

The rapid emergence of HIV-1 subtype C globally warrants special attention to the pathogen itself. However, little is known about genotypic and phenotypic differences that could explain how or why HIV-1 subtype C is associated with the disastrous epidemic in Southern Africa and its rapid emergence in other countries. Salemi et al. (2005) investigated differences in epidemic behavior between subtypes B and C in Brazil. Using a Maximum Likelihood coalescent-based approach, Salemi et al. estimated that the C_{BR} epidemic expanded twice as fast as Brazilian subtype B or even South African subtype C. Subsequent studies using Bayesian coalescent-based methods also indicate that the mean growth rate of the C_{BR} epidemic (0.70–0.87 $year^{-1}$) was higher than that estimated for the B_{BR} (0.46–0.56 $year^{-1}$) and $F1_{BR}$ (0.59–0.69 $year^{-1}$) epidemics (Bello et al., 2008, 2009, 2012b). Despite such differences, the confidence intervals of those estimates displayed a great overlap making it unclear whether the initial rate of dissemination of different Brazilian HIV-1 subtypes was or in fact significantly different (Bello et al., 2011).

As reviewed by Ariën et al. (2007), the successful worldwide dissemination of subtype C may be due to this strain being less virulent in comparison with other HIV-1 group M subtypes while maintaining the same transmission efficiency. Subtype C strains predominantly use CCR5 co-receptor and rarely switch to CXCR4 or dual tropic use (Vasan et al., 2006). A switch to CXCR4 usage has been associated with acceleration of disease progression. Furthermore, Abraha et al. (2009) showed in *ex vivo* experiments that subtype C HIV-1 isolates might have a reduced replicative fitness in peripheral blood mononuclear cells. This HIV-1C feature may reflect in a decrease in CD4 cell count, which supports the findings reported by Silveira et al. (2012) with higher CD4 cell counts in subtype C infected patients compared with patients infected with other HIV-1 subtypes. A lower pathogenicity of HIV-1C would lead to a long asymptomatic period, which would increase opportunity for transmission and favor the epidemic expansion. Although the lower pathogenicity of HIV-1C may have contributed to a rapid dissemination of this subtype in Southern Brazil, the relatively slow rate of expansion of subtype C in other Brazilian regions and South American countries that share borders with Southern Brazil suggests that factors other than intrinsic viral transmissibility have also shaped the dissemination pattern of HIV subtypes in the Southern Cone.

Among these factors, the viral transmission networks and the availability of host individuals may have had a central role in the forging of the current epidemiological scenario. Several epidemiological studies in Southern Brazil observed a relevant association between HIV-1C and heterosexual individuals of both genders, while HIV-1B was found to be more frequent in men who have sex with men (MSM) (Brindeiro et al., 2003; Gräf et al., 2011; Raboni et al., 2010; Silva et al., 2010; Silveira et al., 2012; Soares et al., 2005; Toledo et al., 2010). The number of HIV infected people in the heterosexual exposure category has been increasing rapidly in Brazil due to a process of feminization of the AIDS epidemic: in 1990 the man/woman new AIDS cases ratio was 5.4 while in 2009 it had decreased to 1.6 (Brazilian Ministry of Health, 2011). It is conceivable that an efficient introduction of subtype C in the heterosexual group allowed for a rapid spread through a large and expanding network of host individuals. Therefore, segregation of exposure categories and HIV-1 variants may be due to differences in the transmission networks where subtypes were introduced and disseminated at early times, rather than to differences in viral transmissibility. A study assessing the temporal dynamics of HIV-1 subtypes in different exposure categories in Porto Alegre observed a significant increase in subtype C in both heterosexual and MSM individuals over time, resulting in a progressive loss of the association between HIV subtypes and exposure categories (Almeida et al., 2011). A progressive intermixing of the epidemics and the consequent loss of associations between HIV variants and exposure categories appears to be a natural tendency as seen in other countries (Tovanabutra et al., 2004). On the other hand, a study performed in Porto Alegre by Dias et al. (2009) found evidence of a different viral transmission pattern. This study, although based on a small sample size, linked anal sex to clade B transmission in both genders – and not only in the MSM exposure category. These findings suggest that though transmission networks might justify most clade segregation, clade C could be less dependent on high-risk anal sex behavior, a factor that could favor its heterosexual transmission. However, very little is known about differences in pathogenic potential and transmissibility of HIV-1 subtypes, and further studies are needed to explain this issue.

Another important issue that needs to be addressed is whether HIV-1 subtypes may differ in susceptibility to antiretroviral (ARV) treatment and the rate of fixation of mutations conferring drug resistance. Again, little data is available on the impact of antiretroviral therapy on subtype C and other non-subtype B strains, since HIV-1B are dominant in developed countries and most ARVs were designed for subtype B. Nevertheless, some studies have reported that under drug selective pressure subtype C may acquire drug resistance faster than other strains. K65R mutation confers partial resistance to most nucleoside reverse transcriptase inhibitors (NRTIs) and is selected by NRTIs such as tenofovir (TDF), abacavir (ABC), and didanosine (ddI) (Brenner and Coutsinos, 2009). The overall incidence of K65R is very low due to high genetic barriers and fitness constraints, but K65R was observed in 23–30% of subtype C patients failing therapy in Africa who were genotyped (Doualla-Bell et al., 2006; Hosseinipour et al., 2009). *In vitro* studies, thereafter, demonstrated that two subtype C polymorphisms in 64/65 RT codons accelerate K65R selection leading to low susceptibility to NRTIs (Brenner et al., 2006; Invernizzi et al., 2009). The lower genetic barrier of subtype C, combined with suboptimal treatment regimens in most poor resource countries, may favor the acquisition of K65R, leading to limitations in treatment.

In contrast, another report suggested a lower rate of accumulation of drug resistance mutations in subtype C (Soares et al.,

2007). In this study, patient samples from Porto Alegre were collected and grouped by HIV-1 clade, ARV class, and time of exposure. The results showed a significantly higher frequency of resistance mutations in subtype B infected patients compared with subtype C. These findings were observed for protease inhibitors (PI) and NRTI, but not for non-NRTI, and were later supported by Munerato et al. (2010) who found less resistance mutations to PI and NRTI in subtype C infected patients. Such observations were somehow unexpected, undermining concerns that non-subtype B infected individuals are at a disadvantage because ARV drugs have been designed primarily for subtype B infections. Reinforcing this point, Scherrer et al. (2011) observed an improved virological outcome in non-subtype B infected patients on ARV therapy from the Swiss HIV Cohort Study. Although subtype C infected patients only accounted for 12% of the non-B subtype individuals in the Swiss Cohort, these findings are consistent with a lower acquisition of drug resistance mutations in subtype C. However, follow-up studies with larger number of individuals are required to elucidate the real impact of ARV therapy on non-subtype B strains (Soares et al., 2012).

Naturally occurring mutations and polymorphism that may impair the effectiveness of entry inhibitors, a new class of ARV drugs, have also been investigated (Araújo et al., 2012; Gonzalez et al., 2010). These studies indicated that, compared with subtype B, HIV-1C has a higher number of natural accumulated polymorphisms that may cause resistance to entry inhibitors. So far, the Food and Drug Administration have approved only two entry inhibitors: Maraviroc, a negative allosteric modulator of the CCR5 cell receptor, and Efuvirtide, which blocks gp41 protein conformational changes. In Brazil, entry inhibitors are still seldom used except in cases of multidrug virological failure, even though the genetic background of HIV-1C may reduce susceptibility to this class of antiretroviral drug. *In vitro* and *in vivo* studies are still required to confirm such findings, and careful planning is needed before employing this class of ARV as rescue therapy.

Concluding remarks

The global HIV pandemic is characterized by dynamism and complexity, and the Brazilian experience with the introduction of a new variant to a pre-defined epidemic can yield valuable data regarding HIV expansion and interactions between different strains. Presently, subtype C is responsible for more than half of all global HIV infections, mainly in South and East Africa, and India. Therefore, Southern Brazil represents an important site for epidemiological studies, where HIV-1C (the most prevalent) and HIV-1B (the most studied) co-exist in similar frequencies and infected individuals who have access to antiretroviral treatment and receive follow-up in a reasonable health care system from an HIV care stand point. Despite some obscure issues, studies have shown that HIV-1 subtype C was introduced in Brazil between 1960 and 1980 somewhere in the Southern region, with evidence pointing to the state of Paraná, and originated from an East African country. This strain was very successful in spreading among heterosexual individuals, but not limited to them, becoming highly prevalent in the Brazilian region that is most heavily affected by AIDS. Whether HIV-1C is the cause of the most disturbing scenario of AIDS epidemic in Brazil, or whether it just benefited from the conditions present in the site where it was introduced, is still up for debate. The recent emergence of subtype C infections in other Brazilian regions and South American countries signals the possibility of continental expansion, although it seems that HIV-1C is not spreading as quickly in these regions as in Southern Brazil. The understanding of the dynamics and diversity of the HIV-1 epidemic in Southern Brazil is of utmost importance for designing new public health policies, affecting prevention, treatment, and vaccine development. Therefore, continued epidemiologic surveillance should be maintained in these regions as well as further evolutionary studies and a close clinical monitoring of infected patients to ensure a better understanding of dissemination within a population, differences in virulence, and transmissibility of the different HIV circulating strains.

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