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HIV-1 Molecular Epidemiology in the Balkans – A Melting Pot for High Genetic Diversity

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Abstract

The Balkans is a gateway between Europe, Asia, and the African continent, a fact with potential important consequences on the epidemiology of HIV-1 infection in the region. The duration of the HIV-1 epidemics in many countries of the Balkans is similar to the one in the Western European countries. However, striking differences exist in several countries of the region in both the epidemic situation and, even more so, in our knowledge about it. In particular, the molecular epidemiology of HIV in the Balkans is largely unknown. In order to gain some preliminary insight into HIV-1 diversity in the region, we reviewed the available molecular epidemiology data about HIV-1 diversity in 10 countries of the region: Albania, Bulgaria, Croatia, Greece, Montenegro, Romania, Slovenia, Serbia, Turkey, and Hungary, a neighboring country to four Balkan countries. The data were obtained either from published studies or in direct communication with the participating members. The existing molecular epidemiology data revealed a broad diversity in subtype distribution among Balkan countries. In several countries, subtype B is predominant (e.g. Serbia, Slovenia, and Hungary), while in others the proportion of non-B subtypes is much larger (Albania subtype A, Romania subtype F). In some areas, HIV-1 subtype distribution is marked by divergence between different risk groups or transmission routes (e.g. Croatia). Recently, HIV-1/AIDS epidemics in Eastern Europe have been among the fastest growing in the world. Many major contributing factors for the breakout and spread of these epidemics are present in many of the Balkan countries, as reflected through the process of social transition, wars, unemployment, extensive drug use, high sexual risk behavior, as well as other factors. Yet, in the Balkan countries the prevalence rate of HIV-1 infection is low, under 0.1 percent. Concomitantly, the molecular epidemiology of HIV-1 in the Balkans has not been thoroughly studied so far. The review and analysis of the available data indicate a broad diversity of circulating HIV-1 subtypes in the region, with the predominance of non-B clades in some countries, underscoring the need for an ongoing surveillance of HIV-1 diversity. The setup of a collaborative network might provide important information for the better management and control of the HIV-1 epidemic in the area. **(AIDS Rev. 2012;14:28-36)**

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Introduction

Human immunodeficiency virus (HIV) is characterized by extreme genetic variability. The causative agent of the AIDS pandemic, HIV-1, has been phylogenetically classified into four main groups: M (major), O (outlier), N (new), and the recently identified P in Cameroon[1]. Nine pure subtypes of HIV-1 group M are currently known (A-D, F-H, J, and K), with some of them further divided into sub-subtypes, e.g. F1, F2, A1, A2[2]. In addition, numerous circulating recombinant forms (CRF) and a number of unique recombinant forms (URF) have been identified so far[3]. Geographic distribution of different subtypes and recombinants varies, resulting in a complex molecular epidemiology of HIV-1. Subtype B is the predominant clade in the Americas, Western Europe, Japan, and Australia[4,5]. Subtype A prevails in Russia and the former Soviet Union countries, but it is also predominantly present in several African countries such as the Democratic Republic of Congo (DRC), Kenya, and Tanzania[6]. In South Africa, Somalia, Djibouti, Ethiopia, as well as in India, the most abundant subtype is subtype C, while in other regions, such as Uganda, Sudan, and Libya, subtype D is the dominant one. In some geographic areas, recombinants are the most widely distributed forms, as is the case with CRF01_AE in Southeast Asian countries, CRF02_AG in West African countries, CRF07_BC and CRF08_BC in China[6,7]. The overwhelming evolutionary drive of HIV-1, resulting in genotypic heterogeneity, plays a crucial role in the ability of HIV-1 to evade drugs and immune responses and poses formidable challenges for the design of preventive strategies, vaccines, as well as new and effective treatments. Molecular epidemiology has, thus, a profound importance in the ability to act as a link between basic science, clinical medicine, and public health[7-14].

In Europe, the molecular epidemiology of HIV-1 has distinct regional features. In Western Europe, as in North America, subtype B is the most common clade[4-6]. However, throughout Europe numerous non-B subtypes are constantly being introduced and spread locally: in Belgium and Portugal the proportion of subtype B attains ranges around 50 and 40%, respectively[15]. In some European countries, various non-B subtypes constitute significant proportions of the reported known HIV-1 cases, such as subtype A in Greece (21%), Austria (14%), Denmark (8%), and the United Kingdom (8%); subtype C in Denmark (18%), Sweden (30%), and the United Kingdom (17%); subtype G in Portugal (26%)[16-23]. Lately, in many European countries the proportion of non-B subtypes is rising[17,18,20,24,25].

In Eastern and Central Europe, the initial propagation of HIV-1 was much more limited than in Western Europe, with a subsequent outburst of HIV epidemics in the mid-nineties, predominantly in Russia, Ukraine, and the Baltic countries, affecting mainly intravenous drug users (IVDU)[26]. Currently, the HIV-1 epidemic in Eastern Europe is dominated by subtype A, followed, to a lesser extent, by subtype B and CRF03_AB[27-30]. This region, in particular the former Soviet Union countries, is the only region in the world where the particular recombinant CRF03_AB has been found[4,5]. In Central Europe, subtype B strains are the most prevalent[5,6].

Subtype distribution not only varies geographically, but also with regards to transmission route. In Western Europe, subtype B is mainly associated with the HIV-1 epidemic among men who have sex with men (MSM), hemophiliacs, and IVDU, whereas non-B isolates are mainly associated with heterosexual transmission[7,31-38]. In Russia, subtype B is mainly associated with MSM, subtype G is found in nosocomial infected subjects in southern Russia, while subtype A and A/B recombinant predominate among IVDU in Ukraine and Russia[35,36].

The HIV-1 epidemic in the Balkan region is of variable duration and status. Many major contributing factors for the breakout and spread of the HIV/AIDS epidemic are present in a number of the Balkan countries, as reflected through the process of social transition, wars, unemployment, drug use, sexual risk behavior, and other factors[26,37]. Concomitantly, not many in-depth studies of the molecular epidemiology of HIV-1 in the Balkans exist, with the exception of some country specific studies[17,38] and the partial representation of some Balkan countries in wider studies[39]. Some of the existing studies showed inconsistency in classifying Balkan countries in either Eastern or Central Europe[4]. According to the current WHO regional classification, all of the Balkan countries are classified in the Central European region (Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Serbia, FYR Macedonia, Albania, Bulgaria, Romania), except for Greece, which belongs to the Western European region[40].

In order to gain some preliminary insight into HIV-1 diversity in the region, we gathered and compared the available molecular epidemiology information about HIV-1 diversity in 10 countries of the region: Albania, Bulgaria, Croatia, Greece, Montenegro, Romania, Slovenia, Serbia, Turkey, and Hungary, a neighbor of four Balkan countries. Although Hungary does not geographically belong to the Balkan Peninsula, this country is closely

Table 1. Country specific data about HIV infection

Country	Population (thousands)	Total HIV positive*	HIV rate per million 2009†	Predominant transmission route‡
Albania	3,170.0	226	9.0	Heterosexual
Bosnia and Herzegovina	3,843.4	163	2.0	Heterosexual
Bulgaria	7,640.2	1,109	23.0	Heterosexual
Croatia	4,435.4	793	12.0	MSM
Greece	11,215.0	9,933	47.0	MSM
Hungary	10,045.0	1,771	14.0	MSM
FYR Macedonia	2,045.2	32	3.0	Heterosexual
Montenegro	627.5	94	13.0	Heterosexual
Romania	21,528.6	16,162	19.8	Nosocomial
Serbia	7,374.0	2,448	18.0	IDU
Slovenia	2,025.9	454	24.0	MSM
Turkey	69,689.3	2,273	4.6	Heterosexual

*Total number of cases reported by country until December 2009[40].
†HIV incidence rate[40].
‡Predominant transmission route in cumulative number of HIV/AIDS cases until December 2009[40,61].
MSM: men who have sex with men; IDU: intravenous drug users.

associated to and shares many social and historical features, justifying comparison to the neighboring countries. Turkey has a small area in the Balkans but has a strategic position linking Europe and Asia with close relations to the Balkan countries, thus suggesting potential links of the local epidemic with those in the Balkans. For Bosnia and Herzegovina and FYR Macedonia, no HIV molecular epidemiology data were found in peer-reviewed literature.

The HIV-1 epidemiology data used in this study were obtained from the European Centre for Disease Prevention and Control/WHO Regional Office for Europe: HIV/AIDS surveillance in Europe 2009 report[40], from published national data of specific countries, or by direct communication with the participating research laboratories. Country specific population data were obtained from the Eurostat website[41]. To obtain a general view of the subtype distribution in the Balkan region, existing data on country specific HIV-1 subtype proportions were combined with the country specific reported number of people living with HIV, as shown in table 1, similar to what has been described before[4]. The distribution of HIV-1 subtypes and recombinants in the countries of the Balkan region, as described so far, is summarized in figure 1.

Molecular epidemiology of HIV-1 infection in the Balkans

Albania

In Albania, the first case of HIV-1/AIDS was diagnosed in 1992; the cumulative number of HIV-1 cases by the end of 2009 was 226, resulting in an incidence rate of 9.0 per million of population in 2009[40]. In a recent study that included about half of the country's known individuals with HIV-1/AIDS, the genotypic profile of the epidemic was determined, revealing that the majority of the analyzed isolates (56.1%) were of subtype A[42]. The second most abundant was subtype B, covering 34.8% of individuals in the study, while further 6.1% were classified as subtype C, 1.5% as subtype D, and 1.5% as a putative BA recombinant[38].

Bulgaria

In Bulgaria, the HIV-1 epidemic has been present for over 25 years, with an incidence rate of HIV-1 infection of 23 per million and a cumulative number of HIV-1 diagnoses of 1,109 by the end of 2009[40]. The study of the molecular epidemiology of HIV-1 in Bulgaria

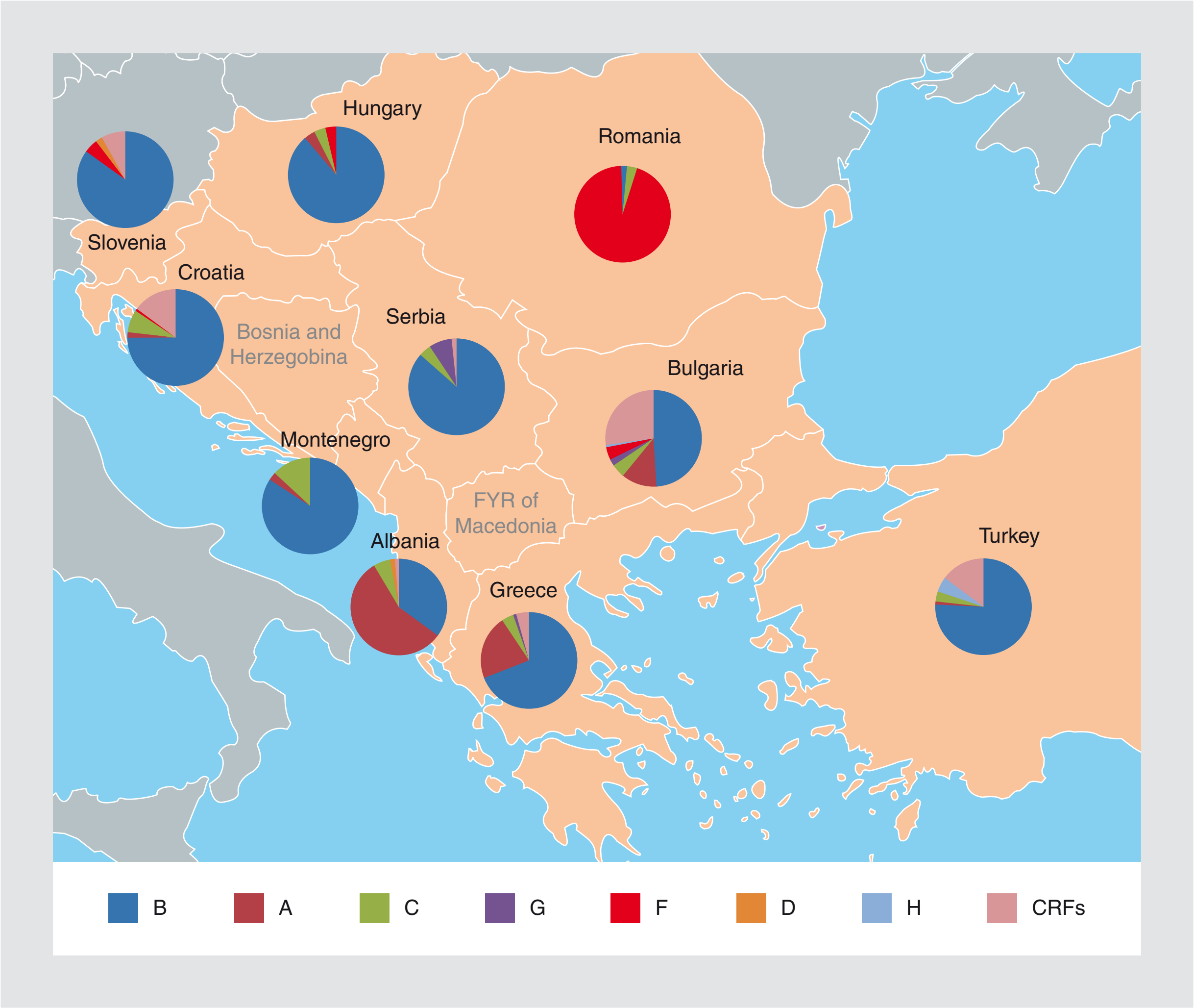

Figure 1. *General distribution of HIV-1 subtypes and recombinants in the Balkan region.*

included individuals diagnosed during 1986-2009 using *pol* gene sequencing and phylogenetic analyses[43-46]. Almost half of the analyzed *pol* sequences (48.8%) were classified as subtype B, 12.6% as subtype A1, an equal prevalence (4.4%) was detected for each subtypes C and F1, and less than 2% for each of the subtypes G or H. Notably, a high prevalence of recombinants was found (26.6%), namely 15.5% CRF01_AE, 6.8% CRF02_AG, and less than 2% was found for each of the CRF05_DF, CRF36_cpx, and CRF04_cpx. The distribution of subtypes differed greatly among the different transmission risk groups. High genetic diversity was found within heterosexuals and injecting drug users comprising subtypes B, A1, C, F1, G, H, and a number of recombinants (CRF02_AG, CRF01_AE, CRF05_DF, CRF36_cpx and CRF04_cpx), while MSM were predominantly infected with subtype B (81.8%)[44,45]. Phylogenetic analyses showed a continuous dispersal of subtype A and B infections between Bulgaria and Western as well as other Eastern European countries[43]. Of note, except for Greece and Cyprus, Bulgaria is the third country in the world where CRF04_cpx virus has been detected[48-50].

Croatia

In Croatia, the HIV-1 epidemic has been registered since 1985, with a cumulative number of known HIV-1 cases of 732 in 2008 and 793 by the end of 2009[51]. The incidence rate of HIV-1 is 12 per million in 2009 (less than 0.05%)[40], while a survey of the HIV-1 prevalence among MSM indicated a much higher prevalence in this risk group, reaching close to 5%[52,53]. In a recent study of the molecular epidemiology of HIV-1 in Croatia, a sharp discrepancy was found between subtype distributions found in seamen and the total of HIV-1-infected patients[53]. Among HIV-1-infected labor migrants (seamen and their female steady sexual partners), only 29% of the patients included in the study were infected with subtype B, compared to 74% subtype B infections in the cumulative number of patients[54]. The most common non-B subtype among seamen was CRF02_AG, while in the total number of patients the second most common subtype was subtype C. In addition, subtypes A and F were found, as well as a number of CRF (CRF01_AE, CRF05_DF, CRF10_CD) but also unique recombinants (URF)[53,54].

Greece

In Greece, AIDS case reporting started in the early 1980's. The cumulative number of HIV-1 infections by the end of 2009 was reported to be 9,933, while the incidence rate in 2009 was 47 per million[40]. According to a published study, in 1,158 HIV-1-infected individuals sampled from 1998 through 2005, the prevalence of subtypes was: subtype B (65.4%), among the non-Bs, A was the most common (20.6%), followed by subtype C (4.4%), G (0.9%), F (0.8%), D (0.7%) and H (0.1%), CRF (3.5%), URF (3.2%), and unclassified strains (0.5%)[17]. Notably, the prevalence of subtype A in Greece significantly increased over time from 6% in 1984 to 42% in 2004. Phylogenetic analysis revealed that most of subtype A sequences isolated in Greece fell within a monophyletic cluster, thus suggesting that subtype A epidemic was the result of a "founder" effect. Moreover, subtype A sequences from Albania appeared as "nested" clades within the A monophyletic Greek clade, suggesting the imported nature of the epidemic in Albania originating in Greece. In a multivariate analysis, subtype A was associated with the year of diagnosis, whereas no significant association was found with any particular transmission group in Greece[38]. Notably, subtype A was also found at a high frequency among Greek individuals, suggesting that it is not associated with immigrants from highly prevalent non-B subtype areas[17]. In addition to subtype A, several non-B types and recombinant forms have been detected in Greece. It is worth mentioning that Greece is the geographic region in which a unique recombinant, initially named as subtype I and later designated CRF04_cpx, has been detected in several cases, in addition to Cyprus and Bulgaria[48-50].

Hungary

In Hungary, the first HIV-1-positive cases were detected in 1985, while the incidence rate of HIV-1 infection is 14 per million for 2009 and the cumulative number of reported cases is 1,771[40]. The distribution of HIV-1 subtype, based on sequencing of *env,* showed a predominance of subtype B (88.45%), while subtypes A, C, and F were found in 3.85%, each[55,56]. A recent study supported the dominance of subtype B among newly HIV-1-infected individuals in Hungary[57].

Montenegro

The first HIV/AIDS positive case in Montenegro was officially registered in 1989[58]. Current data indicate that the incidence rate of HIV-1 infection is 13 per million for 2009, while the cumulative number of HIV/AIDS cases is 94[40]. A recent study of HIV-1 molecular epidemiology in Montenegro, based on an analysis of 32 samples, showed that the predominant subtype is subtype B, with subtypes C and A also being present[59].

Romania

The first AIDS case in Romania was reported in 1985, followed by a dramatic increase in the number of cases, primarily in children (age group 0-14)[60]. Although it is not very clear, the parenteral route is believed to have been the main way of transmission; at the end of 1992, Romania had more than half of the HIV-1 pediatric cases in Europe[61]. According to the nationwide data provided by the National Institute for Infectious Diseases, the reported number of HIV-1/AIDS cases by December 2009 was 16,162, with 428 newly diagnosed cases during that year[62]. This results in an incidence rate of HIV-1 infection of 19.8 per million in 2009. The HIV-1 subtype analysis in Romania revealed that the majority of the HIV-1 strains characterized between 2003 and 2007 belonged to subtype F1, and only a small proportion (5%) of the strains were of different subtypes[63]. More recently, although the F1 subtype continues to be prevalent in Romania, other subtypes, namely C, B, and A, have increasingly been identified, mainly in newly diagnosed patients. For instance, an analysis of HIV-1 strains isolated from newly diagnosed persons in 2007 showed that a higher number of strains belonged to subtypes other than F1 (23%)[64].

Slovenia

In Slovenia, the HIV-1 epidemic started in 1986, and by the end of 2009 a total of 454 HIV-1-infected individuals were cumulatively reported[65]. In 2009, the estimated incidence rate of HIV-1 infection was 24 per million[40], with the HIV-1 prevalence in two risk groups of MSM and IVDU below 5%[65]. In a four-year period (2005-2009), a significant increase in the total number of newly diagnosed HIV-1-infected individuals, as well as the number of newly diagnosed patients with primary/early HIV-1 infection, has been seen in Slovenia[66]. In 2009, the annual incidence rate based on newly diagnosed infections peaked at 25 cases per million. MSM still constitute the most affected population group, as 80% of the newly diagnosed HIV-1 individuals in the last four years belong to that group. The IVDU population in Slovenia has so far largely been spared from HIV-1 infection[67].

A retrospective molecular epidemiological survey was conducted recently on a cohort representing 88% (131/149) of all HIV-1-infected patients diagnosed during 1996-2005[67]. The study revealed that subtype B was the predominant HIV-1 subtype in Slovenia (110/131; 84%), although several non-B subtypes (21/131; 16%) as well as CRF were also found (CRF02_AG, CRF01_AE, subtypes A1, D, and F1). Different unclassified strains were identified in 8/21 non-B subtype samples. Non-B subtype viruses were predominant among heterosexuals (19/21; 90%) and subtype B viruses among MSM (84/110; 76%). Importantly, 86% (18/21) of the patients infected with non-B subtypes were of Slovenian nationality. An analogous, but statistically insignificant increase in the proportion of subtype B infections among the newly diagnosed HIV-1 infected patients was observed in the last decade[67]. The results obtained in this molecular epidemiological survey performed in partial *pol* region was confirmed in another study performed on 20 randomly selected HIV-1-infected individuals and based on analysis of partial *env* (C2V3) and *gag* (p24) regions of the viral genome[68]. In order to obtain a better understanding of HIV-1 transmission networks among the individuals infected with subtype B in Slovenia, 119 *pol* sequences generated from the samples dated from 1996 to 2005 were recently analyzed[69]. The phylogenetic analysis revealed 14 potentially significant transmission clusters (bootstrap value ≥ 98%), comprising 34 HIV-1 strains. The vast majority of clustered individuals were men (91%), and among them, 79% were MSM. Factors significantly associated with the clustering were: recent infection (HIV-1 infection during or after 2003), diagnosis of primary HIV-1 infection, higher CD4 cell count, and acquiring HIV-1 infection in Slovenia[70].

Serbia

The first HIV-1-positive cases in Serbia were identified in 1985[71]. In 2009, the cumulative number of reported HIV cases was 2,448, among them 1,489 (61%) AIDS cases[72]. The incidence rate of HIV-1 infection was 18 per million in 2009[40]. The majority of AIDS cases still belong to the group of IVDU (41.5%), with a similar proportion of sexually transmitted infection (38.2%). However, according to the latest epidemiological data, sexual transmission largely predominates in newly diagnosed patients (81% in 2009, compared to 27% in 1991)[72]. Several studies on HIV-1 subtype distribution in Serbia were conducted, finding predominance of subtype B (in 87.5-91.2%), with other subtypes also appearing, namely G (7.7%), C (4.4%), and CRF (1.8-2.2%)[70]. Non-B subtypes are mostly associated with heterosexual transmission[73].

Turkey

In Turkey, the first HIV-1-positive case was reported in 1985 and the cumulative number of HIV-1/AIDS cases was 3,898 by December 2009[74]. According to national data from the Ministry of Health of Turkey, the incidence rate seems to have increased significantly recently: 2.3 and 4.6 per million in 2000 and 2005, respectively[75]. Although data on the molecular distribution of HIV-1 subtypes in Turkey is scarce, two small studies suggest that subtype B is the predominant subtype in the country, accounting for 70.1% of infections, and is mainly related to heterosexual contact[76,77]. However, a more recent study of samples collected from 2004-2009 suggests that the diversity of subtypes is increasing, with subtype B still being the predominant one (74.5%), but with other subtypes (F and C in 4.1% each, G in 1%) and recombinants (CRF01_AE in 13.3%, CRF02_AG in 1.8%) also present[77].

Discussion

According to the latest data from ECDC/WHO, the HIV-1 epidemic in the Central European region remains at low and stable levels, although there is evidence of an increasing sexual transmission in several countries[40]. The time of the initial identification of the HIV-1 epidemics in the Balkans is comparable to the Western European countries. The first HIV-1/AIDS cases in the majority of countries were diagnosed in the beginning or mid-eighties, with the exception of Albania where the first case was registered in 1992. The distribution of HIV-1 subtypes is marked by significant diversity, with considerable and non-uniform presence of non-B clades.

Specifically, based on the prevalence of different subtypes, the Balkan area can be divided into three major areas, the first one including Serbia, Slovenia, Hungary, Croatia and Montenegro, where the prevalence of subtype B is the highest (> 74%). According to the preliminary data, Turkey probably also belongs to this group of countries. The second area includes Greece, where the epidemic started in the form of subtype B, but the A clade has been increasingly detected over the last few years. The prevalence of subtype B in Greece has been found to be 65%. Finally, the third area includes Romania, Bulgaria, and Albania, where different non-B subtypes were found to circulate at a prevalence higher than 50%. Although in the current review the routes of the epidemic dispersal were not traced, based on the prevalence of the different subtypes it becomes evident that major differences exist with regard to initiation and spread of the epidemic across the Balkans. In the countries where subtype B dominates, different non-B subtypes are circulating, introduced by diverse sources. For example, in Croatia non-B subtypes are mainly associated with seamen who are infected with CRF02_AG at a high prevalence, while in Serbia subtypes G and C are not associated with any particular transmission route. In Slovenia, different non-B clades are circulating linked with heterosexual transmission, thus suggesting that although these three countries are in close proximity and became separated only recently, it seems that the introduction of non-B subtypes occurred from diverse sources. Moreover, the high prevalence of subtype B in these three countries suggests that the epidemic started in the form of subtype B, as in the rest of Western European countries. Given the preliminary nature of the molecular epidemiology studies in Hungary and Turkey, conclusions cannot be drawn about the prevalence of non-B subtypes in these countries.

The recent increase of subtype A in Greece, resulting from a founder effect, indicates a different nature of the epidemic in this country compared to those in the first group. Specifically, the epidemic started in the form of subtype B as in the rest of countries in group 1, but in the last few years subtype A became as prevalent as subtype B among the newly diagnosed individuals. Notably, phylogenetic analyses showed that the subtype A infection in Greece provided a source for the A epidemic in Albania[17].

The subtype distribution in some of the Balkan countries included in the study (Croatia, Slovenia, Montenegro, Hungary, and also Serbia) is similar to that in many of the Western and other European countries, where subtype B prevails (e.g. Italy, Spain, Austria, but also Poland). On the other hand, the molecular epidemiology of HIV-1 in Greece, Albania, and Bulgaria is distinct with regard to particular non-B subtypes circulating at high prevalence in addition to the B clade. Finally, Romania provides an exception as regards the way the epidemic started and the genetic form of the virus. It is the only country in Europe where subtype F1 is the dominant clade. On the other hand, the prevalence of non-F1 subtypes has been increasing recently, suggesting a similar phenomenon as for the non-B subtypes in the rest of the European countries.

Recently, the HIV-1/AIDS epidemics in Eastern Europe have been among the fastest growing in the world. However, in the Balkan countries the prevalence rate of HIV-1 remains relatively low in spite of many social, geographical, and historical connections of some countries in the Balkans to Eastern Europe. However, the explosive HIV-1 epidemic in Russia and the former Soviet Union countries spreads locally mainly through IVDU, thus explaining its limited spread to the Balkan countries, so far. This is reflected also in distinct patterns of the circulating HIV-1 strains between East European and Balkan areas, where in the former subtype A and CRF03_AB predominate[27-30], while in the latter different clades and recombinants were detected[42-77].

Conclusion

This is the first review about HIV-1 subtype diversity in the Balkans. It indicates a highly diverse picture of the subtype distribution in the region, which is different from the patterns found in both Western and Eastern Europe. Molecular epidemiology of HIV-1 in the Balkans is characterized by significant diversity of the circulating HIV-1 subtypes, with the large and non-uniform distribution of non-B clades associated with different risk groups. The high variation in patterns of the circulating HIV-1 strains in South East Europe compared to the rest can be potentially attributed to the differences in socio-economic factors and the particular geographic location of the Balkans at the crossroads connecting different continents, suggesting high population mobility from and to this area.

The particular geographic position of the Balkan peninsula, at the crossroads between continents, the substantial genetic heterogeneity of the epidemic and the prospective significant viral flow to and from the Balkan countries, linked to increased population mobility in the region, have the potential to modify the current HIV-1 epidemiological structure across Europe and highlight the importance of more extensive and continuous monitoring of the epidemic in the Balkans.

The setup of a collaborative network of regional laboratories might provide crucial information for preventive strategies and vaccine design.

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