Here, we report the rapid expansion of a recently originated HIV-1 subtype F cluster among MSM in the region of Galicia, Northwest Spain.

To the Editors:
The prevalence of HIV-1 infections among men who have sex with men (MSM) has experienced a notable increase in recent years in many countries, frequently associated with the expansion of local transmission clusters. In Western Europe, these clusters are predominantly of subtype B, but occasional clusters of non–subtype B clades have also been reported among MSM. Here, we report the rapid expansion of a recently originated HIV-1 subtype F cluster among MSM in the region of Galicia, Northwest Spain.

Since 1999, we regularly process plasma samples from HIV-1–infected individuals, including new HIV-1 diagnoses, attended at public hospitals of Galicia in the cities of Vigo, Pontevedra, Ourense, Lugo, Ferrol, and A Coruña. These samples are routinely analyzed in HIV-1 protease-reverse transcriptase (PR-RT) sequences for detection of antiretroviral drug resistance–associated mutations, subtyping, and determination of phylogenetic relationships. Our previous studies have shown that in this region, as in the rest of Spain and Western Europe, subtype B is the predominant HIV-1 clade, but multiple other variants are also detected. Among non–subtype B genetic forms, from 1999 through 2009, the most prevalent were subtype G and BG recombinant viruses [including circulating recombinant form (CRF) 14_BG], which circulate among the local population. Subtype F viruses represented a very minor proportion of infections, 1.8%, among new HIV-1 diagnoses from 2001 through 2009. However, since 2010, subtype F infections have increased dramatically in Galicia, representing, among new HIV-1 diagnoses, 27.4% in 2010, and 30% in the first 4 months of 2011 (other genetic forms detected in Galicia during this period among new HIV-1 diagnoses were, in 2010, subtype B 57.5%, CRFs 02_AG, 14_BG, and 47_BF 1.9% each, subtypes D and G and CRF01_AE 0.9% each, and unique recombinant forms 5.7%; and in the first 4 months of 2011, subtype B 53.3%, subtype C and CRFs 01_AE, 02_AG and 47_BF 3.3% each, and BC recombinants 6.7%).

In phylogenetic trees of PR-RT (HXB2 positions 2253–3452), all but 2 HIV-1 subtype F infections newly diagnosed in Galicia in 2010 and the first 4 months of 2011, together with 4 diagnosed in this region in 2009 and 1 in Bilbao (Basque country, North Spain) in 2010, form a strongly supported monophyletic cluster of 41 viruses of F1 subtype with a mean intracluster genetic distance of 0.4% (Fig. 1). Through BLAST searches in the Los Alamos HIV Sequence Database and subsequent inclusion in phylogenetic analyses of the highest scoring database sequences, we found 4 viruses closely related to the Galician F1 cluster, 3 of them (PHI_2, PHI_3 and PHI_22) collected in Geneva, Switzerland, in 2005 or 2006, and 1 (BRGO3010) collected in Goiania, Central West Brazil, in 2007 (Fig. 1). An additional subtype F virus (433 odsesy) from France collected in 2006, sequenced only in RT, is also closely related to the Galician F1 cluster (data not shown). Among subtype F viruses sequenced in full-length genomes deposited in databases, the one most closely related to the Galician F1 cluster is 06BR579, collected in the Brazilian city of Sao Paulo (Fig. 1).

All 41 individuals in the Galician F1 cluster are men, 38 of them native Spanish, and, among 40 with known transmission modes, all reported sexual risk exposures, 28 homosexual, 2 bisexual, 3 heterosexual, and 7 nonspecified sexual. At least 6 infections correspond to recent transmissions (less than 6 months since seroconversion). The epicentre of this outbreak is in the province of A Coruña, where 30 (73.2%) individuals reside [20 (48.8%) diagnosed in the city of A Coruña and 10 (24.4%) in Ferrol], and where the 5 earliest cases were diagnosed, although cases were also diagnosed in the 3 other Galician provinces. The spread of this cluster to other areas of Spain seems to be currently limited because among 94 HIV-1 infections newly diagnosed in 2010 in another Spanish region, the Basque Country, only 1 (also a sexually infected man) belonged to the Galician F1 cluster.

Among the 5 viruses closely related to the Galician F1 cluster collected outside of Spain, risk exposure data are available for the 3 collected in Switzerland, 2 of which correspond to MSM, and 1 to a heterosexual man. To estimate the date of the most recent common ancestor (MRCA) and to analyze the demographic growth of the Galician F1 cluster, we used a Bayesian Markov Chain Monte Carlo (MCMC) method as implemented in BEAST v1.6.1. Because the evolutionary rate could not be inferred directly from the sequences of the cluster, due to the narrow time span of sample collection, it was estimated using 68 subtype F1 PR-RT sequences retrieved from the Los Alamos HIV Sequence Database or obtained by us from viruses sampled between 1993 and 2011 and lacking drug resistance–associated mutations. In a subsequent analysis, the estimated posterior distribution of the substitution rate was incorporated as a prior distribution to estimate the time of the MRCA of the F1 cluster. The substitution model used...
for the BEAST analyses was HKY with gamma-distributed rate heterogeneity among sites and 2 partitions in the codon positions (first + second and third); other priors used in the analyses were an uncorrelated lognormal relaxed clock model and a Bayesian skyline plot demographic model. Each MCMC chain was run for 40 million generations and sampled every 10,000 generations, with the first 20% discarded as burn-in. MCMC convergence and effective samples sizes were checked using the program Tracer v1.5 (available at: http://tree.bio.ed.ac.uk/software/tracer/).

The estimated mean evolutionary rate was $1.783 \times 10^{-3}$ nucleotide substitutions per site per year [95% highest posterior density (HPD) interval $1.076 \times 10^{-3} - 2.439 \times 10^{-3}$], the estimated date of the MRCA of the Galician F1 cluster was 2007.9 (95% HPD interval 2007.0 to 2008.8) and that of the cluster comprising the Galician F1 viruses and the closely related viruses from Switzerland and Brazil was 2003.0 (95% HPD interval 2000.4 to 2004.7). The Bayesian skyline plot of the Galician F1 cluster shows a rapid increase in the effective number of infections beginning in late 2009 and continuing in the first half of 2010, followed by a period of stable population size (see Figure, Supplemental Digital Content 1, http://links.lww.com/QAI/A237).

The analysis of near full-length genome sequences of 5 in vitro–cultured isolates of the Galician cluster showed that they were uniformly of subsubtype F1 along their genomes (results not shown).

In summary, we report the rapid expansion of a recently originated HIV-1 subtype F cluster among MSM in the region of Galicia, Northwest Spain, which includes at least 1 virus from another geographically distant Spanish region, the Basque Country. Phylogenetic and molecular clock analyses allowed to trace the ancestry of this cluster in Brazil and showed that viruses closely related to it were circulating in Western Europe (at least in Switzerland and France) several years before the beginning of the expansion of the F1 cluster in Galicia. The rapid expansion of this cluster is reminiscent of the high rate of initial population

**FIGURE 1.** Maximum likelihood tree of PR-RT sequences of the F1 cluster of Galicia. The tree was constructed with RAxML using the general time reversible substitution model with CAT approximation for among-site rate heterogeneity (GTR + CAT). Names of viruses sampled in Galicia begin with X; P2563 is a virus from Basque Country, Spain, branching within the Galician F1 cluster, which is bracketed on the right. (3 viruses, X2890, X2902, and X2955, were excluded from this analysis because they could not be polymerase chain reaction-amplified in PR-RT; however, they were amplified and sequenced in protease, where in a phylogenetic tree they branched within the Galician F1 cluster). For the analysis, all subtype F1 viruses whose full-length genome sequences are available at the Los Alamos HIV Sequence Database, and 4 partially sequenced database viruses with high similarity scores with the Galician F1 cluster, as determined with the BLAST algorithm, were used. The countries of sample collection of the database F1 viruses are indicated with the 2-letter ISO code: AO, Angola; AR, Argentina; BE, Belgium; BR, Brazil; CH, Switzerland; CY, Cyprus; DE, Germany; ES, Spain; FI, Finland; FR, France; RO, Romania; RU, Russia. Only bootstrap values \( \geq 70\% \) of relevant clades are shown. Sequences are deposited in GenBank under accessions JN010216-JN010254, JN859590, and JN859591.
growth estimated for BF recombinant viruses circulating in Argentina, whose largely subtype F genomes are also of Brazilian ancestry. Although other HIV-1 non-subtype B clusters have been described among MSM in Western Europe, this is the largest and most rapidly expanding one reported to date. It will be important to monitor its propagation to determine whether it causes a major sustained change in the genetic composition of the HIV-1 epidemic in Galicia and whether it spreads to other geographical areas. The rapid expansion of this cluster reflects the existence of transmission networks among MSM unaware of their HIV status engaging in high-risk behavior in Galicia, which is also inferred from the frequent clustering of HIV-1 sequences among new diagnoses in MSM in this region, coincident with reported observations among MSM elsewhere in Spain and in other countries. The recent emergence and expansion of HIV-1 transmission clusters among MSM in developed countries implies that, contrary to a common belief, universal access to effective antiretroviral therapies does not guarantee the control of the HIV-1 epidemic and that prevention of high-risk behavior, particularly among MSM, should remain one of the highest public health priorities in these countries.

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