



LUND UNIVERSITY

Molecular epidemiology of HIV-1 in Iceland

Early introductions, transmission dynamics and recent outbreaks among injection drug users

Sallam, Malik; Esbjörnsson, Joakim; Baldvinsdóttir, Guðrún; Indriðason, Hlynur; Björnsdóttir, Thora Björg; Widell, Anders; Gottfreðsson, Magnús; Löve, Arthur; Medstrand, Patrik

Published in:

Infection, Genetics and Evolution

DOI:

[10.1016/j.meegid.2017.01.004](https://doi.org/10.1016/j.meegid.2017.01.004)

2017

Document Version:

Peer reviewed version (aka post-print)

[Link to publication](#)

Citation for published version (APA):

Sallam, M., Esbjörnsson, J., Baldvinsdóttir, G., Indriðason, H., Björnsdóttir, T. B., Widell, A., Gottfreðsson, M., Löve, A., & Medstrand, P. (2017). Molecular epidemiology of HIV-1 in Iceland: Early introductions, transmission dynamics and recent outbreaks among injection drug users. *Infection, Genetics and Evolution*, 49, 157-163. <https://doi.org/10.1016/j.meegid.2017.01.004>

Total number of authors:

9

Creative Commons License:

CC BY-NC-ND

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Molecular epidemiology of HIV-1 in Iceland: Early introductions, transmission dynamics and recent outbreaks among injection drug users

Authors:

Malik Sallam ^{a,*}, Joakim Esbjörnsson ^{b,c}, Guðrún Baldvinsdóttir ^d, Hlynur Indriðason ^e, Thora Björg Björnsdóttir ^d, Anders Widell ^a, Magnús Gottfreðsson ^{e,f}, Arthur Löve ^{d,e} and Patrik Medstrand ^a

Authors' affiliations:

^aLund University, Department of Translational Medicine, Malmö, Sweden

^bUniversity of Oxford, Nuffield Department of Medicine, Oxford, UK

^cKarolinska Institute, Microbiology, Tumor and Cell Biology, Stockholm, Sweden

^dLandspítali University Hospital, Department of Virology, Reykjavik, Iceland

^eUniversity of Iceland, Faculty of Medicine, School of Health Sciences, Reykjavik, Iceland

^fLandspítali University Hospital, Department of Infectious Diseases, Reykjavik, Iceland

E-mail addresses of authors:

MS: malik.sallam@med.lu.se

JE: joakim.esbjornsson@med.lu.se

GB: geb@landspitali.is

HI: hlynurind@gmail.com

TBB: thorabbj@landspitali.is

AW: anders.widell@med.lu.se

MG: magnusgo@landspitali.is

AL: arthur@landspitali.is

PM: patrik.medstrand@med.lu.se

***Corresponding author:**

Malik Sallam, MD

Faculty of Medicine, Lund University

Department of Translational Medicine, Clinical Virology

BMC B13, Sölvegatan 19, 223 62 Lund, Sweden

Tel: +46 72 877 6022

E-mail: malik.sallam@med.lu.se

ORCID ID: 0000-0002-0165-9670, ResearcherID: O-5021-2014

Abstract:

The molecular epidemiology of HIV-1 in Iceland has not been described so far. Detailed analyses of the dynamics of HIV-1 can give insights for prevention of virus spread. The objective of the current study was to characterize the genetic diversity and transmission dynamics of HIV-1 in Iceland. Partial HIV-1 *pol* (1020 bp) sequences were generated from 230 Icelandic samples, representing 77% of all HIV-1 infected individuals reported in the country 1985-2012. Maximum likelihood phylogenies were reconstructed for subtype/CRF assignment and determination of transmission clusters. Timing and demographic growth patterns were determined in BEAST. HIV-1 infection in Iceland was dominated by subtype B (63%, n=145) followed by subtype C (10%, n=23), CRF01_AE (10%, n=22), sub-subtype A1 (7%, n=15) and CRF02_AG (7%, n=15). Trend analysis showed an increase in non-B subtypes/CRFs in Iceland over the study period ($p=0.003$). The highest proportion of phylogenetic clustering was found among injection drug users (IDUs; 89%), followed by heterosexuals (70%) and men who have sex with men (35%). The time to the most recent common ancestor of the oldest subtype B cluster dated back to 1978 (median estimate, 95% highest posterior density interval: 1974-1981) suggesting an early introduction of HIV-1 into Iceland. A previously reported increase in HIV-1 incidence among IDUs 2009-2011 was revealed to be due to two separate outbreaks. Our study showed that a variety of HIV-1 subtypes and CRFs were prevalent in Iceland between 1985-2012, with subtype B being the dominant form both in terms of prevalence and domestic spread. The rapid increase of HIV-1 infections among IDUs following a major economic crisis in Iceland raises questions about casual associations between economic factors, drug use and public health.

Keywords:

Phylogeny; subtype B; non-subtype B; MSM; heterosexual; BEAST

Highlights:

- HIV-1 was introduced into Iceland as early as late 1970s.
- Domestic HIV-1 infection in Iceland is dominated by subtype B.
- The genetic diversity of HIV-1 has increased significantly over the study period.
- The recent rise in HIV-1 infection among IDUs was due to two separate introductions.

Abbreviations:

ARV: Anti-retroviral

CRF: Circulating recombinant form

ESS: Effective sample size

FET: Fisher's exact test

HET: Heterosexuals

HPD: Highest posterior density interval

IDU: Injection drug user

IQR: Interquartile range

LBL: Linear-by-linear test for association

MCMC: Markov chain Monte Carlo

ML: Maximum likelihood

MSM: Men who have sex with men

MTCT: Mother-to-child transmission

pol: Polymerase gene

tMRCA: Time to most recent common ancestor

1. Introduction

The molecular epidemiology of HIV-1 has been studied extensively both globally, (Faria et al., 2014; Wertheim et al., 2014) and in limited geographic settings, (Aldous et al., 2012; Bruhn et al., 2014; Ciccozzi et al., 2013; Esbjornsson et al., 2016; Kouyos et al., 2010; Mendoza et al., 2014; Paraskevis et al., 2015) to increase the understanding of the drivers of HIV-1 transmission and to increase the focus of preventive strategies (Brenner et al., 2013). The molecular epidemiology of HIV-1 infection has been shown to differ between countries with low prevalence compared to regions with high prevalence (e.g. Sub-Saharan Africa), in terms of trends, transmission groups and dynamics (Shao and Williamson, 2012; Vermund and Leigh-Brown, 2012). Subtype B continues to be dominant in high-income countries particularly among men who have sex with men (MSM) and injection drug users (IDUs), with non-B subtypes/CRFs rising steadily particularly among heterosexuals (HET) (Chaix et al., 2013; Esbjornsson et al., 2016; Pyne et al., 2013; Ragonnet-Cronin et al., 2016; Vermund and Leigh-Brown, 2012). HIV-1 has been intensely studied in the recent years from a molecular epidemiology perspective which was facilitated by the wide availability of polymerase gene (*pol*) sequences that are generated as a by-product of antiviral drug resistance testing, (Hirsch et al., 2003) along with proving the suitability of this region of HIV-1 genome to resolve epidemiologic linkages (Hue et al., 2004). The advances in sequencing technology and the improved computational power have also augmented the yield of HIV-1 epidemiologic research (Hartfield et al., 2014).

The initial use of phylogenetic analysis on HIV-1 was justified by getting forensic insights into transmission patterns in suspected criminal cases (Albert et al., 1994; de Oliveira et al., 2006). At the present time, the evolutionary and population history of HIV-1 can be traced with reasonable accuracy through a phylodynamic framework (Castro-Nallar et al., 2012), thus resolving the number and timing of viral strain introduction in a specific region and

describing the scope of transmission patterns fuelling the epidemic (Hue et al., 2005; Lewis et al., 2008).

HIV-1 was first reported in Iceland in 1985. By the end of 2012, Iceland had reported a cumulative total of 300 HIV cases, 66 AIDS cases, and 39 deaths due to AIDS (The Directorate of Health in Iceland, 2015). Despite the low prevalence of HIV-1 infection in Iceland, a recent increase in the number of infections warrants vigilant surveillance and monitoring of its spread to prevent future outbreaks. This increase was characterized by frequent use of methylphenidate among IDUs (Briem, 2011; Indridason, 2014).

So far, the molecular epidemiology of HIV-1 in Iceland has not been characterized. The aims of the current study were to analyse the genetic diversity of HIV-1 in Iceland and to unravel characteristics of its spread in different risk groups within the island, including the IDU outbreak that occurred in Iceland in 2009 (Briem, 2011; Indridason, 2014).

2. Materials and methods

2.1. Study population

Plasma samples from 251 HIV-1 infected individuals diagnosed in Iceland during 1985-2012 were included in the study. Demographic and laboratory data were collected for all HIV-1 positive individuals with an available stored plasma sample. Data included date of diagnosis, age, gender, country of birth, self-reported risk factor for HIV acquisition and probable country of infection. The study was approved by the Landspítali Bioethics and biobank committees (refs 45/2012 and SV-08), and registered at the Data Protection Agency of Iceland (ref2012111366HGK/--). As an informed consent from all study subjects in our study was impracticable to obtain, the ethical committees approved the study in accordance with declaration of Helsinki.

2.2. RNA extraction, amplification, sequencing and HIV-1 subtyping

Details on RNA extraction, amplification, sequencing and HIV-1 subtyping are outlined in Supplementary File 1. Briefly, HIV-1 RNA was extracted from plasma using miRNeasy Micro Kit (QIAGEN). Reverse transcription and the first PCR were done using One-Step SuperScript III RT/ Platinum *Taq* High Fidelity Enzyme Mix (ThermoFisher Scientific), with JA269 and JA272 as the primers (*pol*-specific primer pair). For the nested PCR, Platinum *Taq* DNA Polymerase, High Fidelity (ThermoFisher Scientific) was used, with JA270 and JA271 (*pol*-specific primer pair). In total, 230 of the 251 plasma samples resulted in successful amplification. The PCR products were sequenced in both directions (using primers JA270 and JA271) with BigDye terminator kit v 1.1 (Applied Biosystem) followed by sequence analysis on an ABI PRISM 3130xl genetic analyzer (Applied Biosystem). The final length of all the sequences following editing and alignment was 1020 bases; nucleotide positions 2268-3287 of HXB2 (GenBank accession number K03455). The sequences were subtyped through

phylogenetic analysis with group M HIV-1 reference sequences from Los Alamos HIV database (<http://www.hiv.lanl.gov/>).

2.3. Transmission cluster analysis

Details on the identification of Icelandic transmission clusters of all subtypes/CRFs are outlined in Supplementary File 1. Briefly, maximum likelihood (ML) phylogenetic trees for each subtype/CRF were constructed using GARLI v2.0 and branches with aLRT-SH (approximate Likelihood Ratio Test Shimodaira-Hasegawa like) support ≥ 0.9 were considered significant (Bazinet et al., 2014). For each subtype/CRF, transmission clusters were identified by analysis of ML trees from root to tips. Icelandic transmission clusters were defined as clades in the phylogeny with an aLRT SH-like branch support ≥ 0.9 and that were dominated (at least 80%) by sequences from Iceland (Esbjornsson et al., 2016; Kouyos et al., 2010). ML analyses were repeated after removing codons associated with antiretroviral (ARV) resistance, creating alignments of 891 bases (Hue et al., 2004).

An HIV-1 introduction into Iceland was defined as the first appearance of a lineage within the country. Icelandic transmission clusters were classified based on their sizes (number of sequences/cluster), into dyads (two sequences), networks (3-14 sequences) and large clusters (15 or more sequences) (Aldous et al., 2012; Esbjornsson et al., 2016).

2.4. Evolutionary rate estimation and analysis of tMRCAs

For subtype B, the estimation of time to the most recent common ancestors (tMRCAs) of the transmission clusters was obtained using the Bayesian Markov chain Monte Carlo (MCMC) method implemented in BEAST v1.8.2. (Drummond et al., 2012). Details of the evolutionary analysis are outlined in Supplementary File 1.

2.5. Statistical analysis

We used exact two-tailed P value from Fisher's test (FET) through GraphPad Software Inc. available online (<http://graphpad.com/quickcalcs/contingency1/>). The Šidák correction for multiple comparisons was used when appropriate. For trend analysis, we used two-tailed linear-by linear test for association through IBM SPSS Statistics 21.0. Trend analysis was done through dividing the study period into seven groups, each composed of four years.

3. Results

3.1. Population characteristics

From 1985-2012, a total of 300 individuals were diagnosed with HIV-1 in Iceland (The Directorate of Health in Iceland, 2015). Among those, 251 plasma samples were available for analysis (obtained during 1996-2013) and we determined partial *pol* sequences from 230 individuals, representing 77% of all recorded cases in the country during this time period (Figure 1).

Among the 230 individuals with a *pol* sequence available for analysis, 67% were males, the median age at diagnosis was 33 years (IQR: 27-39). The median year of HIV-1 diagnosis was 2004 (IQR: 1998-2010) and the period of sampling was 1996-2013 (median: 2005, interquartile range: 1998-2010). Reported risk factors for HIV-1 infection included 37% HET, 30% MSM, 22% IDU and 12% others (mother-to-child transmission [MTCT], blood transfusion and unknown). Sixty-two percent reported Iceland as the country of birth and 33% reported infection in Iceland (Table 1).

3.2. Subtype distribution and temporal changes in different transmission groups

Phylogenetic subtyping revealed that the majority of infections (n=220, 96%) were caused by five subtypes/CRFs (B, C, CRF01_AE, A1 and CRF02_AG), with subtype B being most predominant (n=145, 63%; Supplementary File 2). The analysis showed that subtype B was first diagnosed in Iceland in 1985, followed by subtype A1 in 1988 and CRF02_AG in 1993, whereas other subtypes/CRFs were diagnosed for the first time between 1995 and 2009. Since the number of individuals with non-B subtypes/CRFs infections was low in comparison with subtype B (each representing $\leq 10\%$ of the total infections), we compared temporal changes and socio-demographic characteristics between subtype B and all minor subtypes/CRFs as a combined group (non-B subtypes/CRFs). Individuals infected with

subtype B were more likely to be male, to report MSM activity and IDU as risk factors for HIV-1 acquisition, to be born in Iceland and to report Iceland as the country of infection, and less likely to report HET as risk factor for HIV-1 infection compared to individuals infected with non-subtype B variants ($p < 0.001$ for all comparisons, FET). Conversely, individuals infected with non-B subtypes/CRFs were more likely to report HET as risk factor for HIV-1 acquisition, to report infection, and to be born outside Iceland compared to individuals infected with subtype B ($p < 0.001$ for all comparisons, FET).

Analysis of the temporal changes of subtype/CRF distribution, showed a significant increase in non-B subtypes/CRFs in Iceland over the study period ($p = 0.003$, two-tailed linear-by-linear test for association). Although there was a significant change over the full study period, the proportions seem to have stabilized in the last 15 years.

3.3. Multiple introductions of both HIV-1 subtype B and non-B subtypes/CRFs in Iceland and association with domestic transmissions

Cluster analysis was performed separately for all subtypes/CRFs. The Icelandic sequences were analysed with GenBank reference sequences obtained through BLAST. Icelandic transmission clusters were defined as statistically supported phylogenetic clusters dominated by Icelandic sequences (see Methods, Supplementary File 3). In total, we estimated that HIV-1 was introduced into Iceland at least 143 times, of which 21 introductions resulted in domestic spread (ten dyads, nine networks and two large clusters). The Icelandic sequences that did not fall in transmission clusters, likely represented introductions with no or limited spread in Iceland. A majority of the Icelandic subtype B sequences (82 of 145, 57%) clustered and formed in total 13 transmission clusters. In contrast, only 19 of 85 (22%) Icelandic non-B subtypes/CRFs sequences were found in clusters (Table 2). The median Icelandic cluster size was three sequences/cluster (range: 2-21).

For subtype B, 19% of introductions (13 out of 70) formed transmission clusters compared with 11% (eight out of 73) of the non-B subtypes/CRFs. Moreover, 12 of 13 subtype B clusters and five of eight non-B subtypes/CRFs clusters, had at least one member that reported Iceland as country of infection. In addition, a larger fraction of individuals with subtype B infection reported Iceland a country of birth compared to individuals infected with non-B subtypes/CRFs (Table 2). Taken together, this indicated that a high proportion of individuals in clusters and with subtype B infection obtained their infection through domestic transmission (68%, 56 of 82 in cluster reported domestic infection), while 32% (six of 19) with non-B subtypes/CRFs infection reported their infection in Iceland ($p=0.004$, FET).

3.4. Clustering according to transmission groups

Three major transmission cluster types were identified as having at least 80% sequences from individuals who reported MSM, IDU or HET activity as a route of infection. A fourth transmission cluster type was identified as mixed since it contained a majority of Icelandic sequences but was associated with different risk groups, most notably MSM and HET. Among the 13 Icelandic subtype B transmission clusters, IDU sequences fell into larger clusters compared to MSM and HET. Notably, the recent outbreak among IDUs was found to be the result of two independent introductions (Table 2). HET sequences were more prevalent in the six mixed transmission clusters ($n=12$) in comparison to one HET cluster with four sequences, whereas sequences obtained from MSM were equally distributed in mixed and pure MSM clusters (13 and 11 sequences, respectively) (Table 2). Overall, 89% (42 out of 47 individuals) of subtype B sequences from IDUs were part of Icelandic transmission clusters which was higher compared to MSM (35%; 24 out of 68 individuals, $p<0.001$, FET) but not different compared to HET (70%; 16 out of 23 individuals, $p=0.147$). Subtype B sequences of HET also had a higher tendency to be part of clusters compared to sequences of MSM

($p=0.020$, FET). Thus, sequences obtained from MSM were found less frequently in clusters in comparison to IDU and HET.

Trend analysis showed a significant increase of IDUs infected with subtype B ($p<0.001$, FET) and HET infected with non-B subtypes/CRFs ($p=0.043$, FET) over the study period, contrary to the trend observed among MSM infected with subtype B, which showed a significant decrease over the study period ($p<0.001$, FET). The proportion of HET infected with subtype B showed no change across the study period ($p=0.177$, FET; Figure 2A, B). To investigate the trends among clustered individuals which assumes epidemiologic linkage and in turn domestic spread, we analyzed the trend of the proportion of clustered individuals stratified by different risk groups. Trend analysis showed a significant increase of clustered IDUs infected with subtype B ($p=0.004$, FET) and a significant decrease of clustered MSM infected with subtype B ($p=0.043$, FET) over the study period. HET in clusters infected with B and non-B subtypes/CRFs showed no changes in trends across the study period ($p=1.000$ and 0.864 respectively; Figure 2C, D).

Of the eight Icelandic non-B subtypes/CRFs clusters, six included sequences isolated exclusively from HET. The remaining two clusters contained sequences obtained from individuals with mixed HET/unknown risk factors (Table 2).

3.5. Dating of subtype B transmission clusters

We next estimated the time to the most recent common ancestor (tMRCA) of each subtype B transmission cluster using BEAST (Table 2). The median estimated evolutionary rate was 1.79×10^{-3} (95% Highest Posterior Density interval [HPD]: 1.49×10^{-3} - 2.08×10^{-3}) substitutions/site/year (Supplementary File 4). The median estimated evolutionary rate of the first IDU cluster (B-IDU-1, Table 2) was 9.71×10^{-4} (95% HPD: 5.78×10^{-4} - 1.44×10^{-3}) substitutions/site/year, which was faster than the rate of whole subtype B data set (see above),

while the median estimated evolutionary rate of the second IDU cluster (B-IDU-2, Table 2) was similar to the subtype B data set (1.53×10^{-3} [95% HPD: 5.12×10^{-4} - 2.82×10^{-3}] substitutions/site/year). The median tMRCA of the first IDU outbreak was dated to 1999 (95% HPD: 1997-1999) and the second to 2006 (95% HPD: 2002-2007) (Supplementary File 4). The Bayesian skyline plot (BSP) for the first IDU cluster showed a rapid exponential increase in the number of effective infections between 2008 and 2010 following a lag phase of approximately nine years. A similar pattern was seen also for the second IDU cluster displaying an exponential increase in effective infections between 2010 and 2011 after a lag phase of approximately four years (Figure 3).

The median tMRCA estimates of the 13 Icelandic transmission clusters ranged from 1978-2008, where the oldest estimate (tMRCA of a transmission cluster with mixed risk groups; MSM/HET) dated back to 1978 (95% HPD: 1974-1981; Table 2). The majority (ten of 13) of median tMRCA of the clusters were estimated before 2000. Only three clusters had an upper 95% HPD after 2000. The lower margin of 95% HPD interval for all clusters was earlier than the time of diagnosis of the first case in each cluster.

4. Discussion

This study represents the first molecular epidemiology study of HIV-1 in Iceland. We analysed sequences representing 77% of all known cases of HIV-1 infection that were diagnosed in Iceland during 1985-2012, which allowed us to identify transmission clusters and spread of HIV-1 infection in Iceland with reasonable accuracy, (Novitsky et al., 2014) (Figure 1). In total, we identified 143 introductions of HIV-1 into Iceland over approximately 35 years. However, the majority of these introductions represented sporadic lineages with limited spread in the country, whereas 21 of the introductions led to domestic spread. Similar to our finding, multiple introductions with concurrent limited domestic dissemination of HIV-1 was reported in Greenland (Bruhn et al., 2014). This number of observed introductions into Iceland represents a minimum of the total since we were not able to obtain samples from all individuals, particularly among the earliest diagnosed. However, our study included samples from patients who were diagnosed as early as 1985 in Iceland, which allowed us to identify early introductions in the country. The first introduction was dated to 1978 which is one of the earliest estimates in Europe (Hue et al., 2005; Tebit and Arts, 2011). Our dating is plausible since it represents a date nine years after the estimated introduction of HIV-1 in the USA and was further supported by our evolutionary rate estimate since it was within the range of previously reported rates of the polymerase gene region in subtype B (Abecasis et al., 2009; Gilbert et al., 2007; Hue et al., 2005).

Subtype B was found to be the major genetic variant of HIV-1 that has fuelled the infections in Iceland since the initial introduction into the country. This result was anticipated since subtype B has been the dominant HIV-1 genetic variant among the long term residents of Europe and based on the findings of an earlier report from Iceland (Love et al., 2000; Paraskevis et al., 2009). Despite the finding that non-B subtypes/CRFs seem to have contributed less than subtype B infections to the domestic spread especially among MSM and

IDU, we found a significant increase in non-B subtypes/CRFs over the study period. Contrary to subtype B infections, the majority of individuals infected with non-B subtypes/CRFs reported infection and country of origin outside Iceland, suggesting that migration have resulted in introduction of novel subtypes/CRFs into the country. Similar observations have been made in other Nordic and Western countries (Chaix et al., 2013; Esbjornsson et al., 2016; Pyne et al., 2013; Ragonnet-Cronin et al., 2016).

Analysis of transmission clusters showed mixing between different risk groups within older clusters with estimated dates before 1990, whereas for younger transmission clusters there was no evidence of HIV-1 lineage movement among risk groups. The early period of HIV-1 infection in Iceland was dominated by MSM but more recently an increase of IDUs and non-B HETs transmission groups has been observed. Although the number of subtype B infected HET has remained low since the first recorded case in Iceland, the proportion of HET in transmission clusters was found to be higher than MSM over time. This observation contrasts findings in other Nordic and Western countries that have epidemics that to a large extent have been shown to be driven by MSM (Esbjornsson et al., 2016; Hughes et al., 2009; Vermund and Leigh-Brown, 2012), while similar low fraction of clustering of MSM has been reported in Switzerland and Greenland in comparison to HET and IDU (Bruhn et al., 2014; Kouyos et al., 2010). A plausible explanation of this discrepancy could be the fact that many Icelandic MSM had their social networks outside the country and that domestic infections dominated among HET. However, both MSM and HET infected with subtype B in Iceland reported similar ratios of infections outside Iceland, making this explanation less likely, although we cannot exclude that our observation might be attributed to misreporting with regard to reported country of infection or risk group.

The majority of HET were part of mixed transmission clusters and 80% of these clusters had an MSM representing the first diagnosed case within cluster and only one pure HET subtype

B cluster was found. Few, smaller HET clusters and a propensity of HET being part of mixed clusters has been observed for HIV subtype B transmissions in studies from Switzerland and the Nordic countries (Esbjornsson et al., 2016; Kouyos et al., 2010), which has been suggested to be associated with a generally lower risk behaviour and less likelihood to sustain transmission networks independent of other risk groups. Taken together, these observations suggest that interaction between MSM/IDU with HET in Iceland has been responsible for HET in transmission clusters.

Epidemiologic reports from Iceland in 2007 and 2008 issued warnings of the possibility of HIV-1 outbreaks among IDUs since the number of HIV-1 infections increased among IDUs during a short period of time (Chief Epidemiologist for Iceland, 2007, 2008). An outbreak among IDUs was realized during 2009-2011 as the number of HIV-1 infections among IDUs accrued rapidly (Briem, 2011; Indridason, 2014). We show here that the rapid increase of new infections among IDUs was in fact due to two separate outbreaks. Both outbreaks showed a lag phase of several years (nine years for the first and four years for the second), before the rapid spread with exponential increase in infections. Many of the IDUs in Iceland have shifted to use methylphenidate as a substance of choice, which requires more frequent injections due to its short half-life (Indridason, 2014). The cause of the increased use of methylphenidate among IDUs in Iceland is not entirely clear (Bjarnadottir et al., 2015). However, it might be ascribed to personal preference and decreased availability of other drugs after the economic crisis. The response by the health care system to the outbreak involved intense contact tracing, early initiation of antiretroviral therapy regardless of CD4 count, along with implementation of a harm reduction unit by the Icelandic Red Cross which has operated a needle exchange program for IDUs since 2009 (Eythorsson et al., 2014; Indridason, 2014). The outbreak has since declined and few infections among IDUs have been recorded during 2013-2015 (The Directorate of Health in Iceland, 2015).

Finally, it is interesting to note that the two outbreaks coincided shortly after the financial crisis that hit Iceland in late 2008. We showed that the two separate introductions of HIV-1 into the IDU community, were spreading slowly for several years before the exponential increase of infections (the real time of the outbreak). Our finding raises a hypothesis that a common factor may have led to social and behavioural changes among IDUs. This observation is not unique to Iceland as recent reports investigated the effects of economic recessions and revealed discernible but variable effects on health of the countries affected (Karanikolos et al., 2013; Tapia Granados and Rodriguez, 2015). Thus, our findings suggest a tentative link between the financial crisis and outbreaks of HIV-1 infection, similar to recent reports from Greece (Paraskevis et al., 2013; Paraskevis et al., 2011).

Incomplete and biased sampling is a caveat in studies using phylogenetic inference to depict the transmission dynamics of HIV-1 epidemics (Grabowski and Redd, 2014). Although our sampling was generally high (77% during the entire study period), the first quarter (1985-1991) was represented only by 40% of the diagnosed patients. Nevertheless, the sampling during this period was unbiased based on the epidemiologic surveillance data which showed a similar distribution per risk group of the study population compared to the diagnosed patients during the same period with the majority represented MSM (Indridason, 2014; The Directorate of Health in Iceland, 2015). Another limitation of our study was the small size of the majority of the identified transmission clusters, which precluded a thorough phylodynamic analysis of the epidemic growth in different risk groups.

5. Conclusions

In conclusion, subtype B was found to be the dominant genetic variant of HIV-1 circulating in Iceland, and contributing to domestic spread among the major risk groups, MSM and IDUs and less among HET, since HIV-1 has been first introduced into the country. The genetic diversity of HIV-1 in Iceland increased over time with the appearance of several subtypes and CRFs. The occurrence of HIV-1 outbreaks despite alertness within the health care system emphasizes the importance of having preventive measures to limit spread of infection. Our analysis of IDU outbreaks points that the time from introduction of HIV-1 to spread within the IDU community was four and nine years for the two outbreaks, indicating a window of opportunity for taking action to prevent spread within the risk group. The close proximity in time between the economic crisis in Iceland and the two outbreaks of HIV-1 infection among IDUs warrants further investigation for causal associations.

Transparency declaration:

The authors declare that no competing interests exist.

Acknowledgments:

The authors would like to thank Eva Friman at Molecular Ecology, Microbial Ecology and Evolutionary Genetics (MEMEG), Institution of Biology, Lund University for performing DNA sequencing and Anna Tomasdóttir at the Department of Infectious Diseases, Landspítali University Hospital for her valuable comments on the manuscript.

Funding:

The study was supported by funding from the Swedish Research Council, Sweden (No. 350-2012-6628 for JE and 321-2012-3274 for PM) and the Faculty of Medicine, Lund University, Sweden, and by the University of Iceland Research Fund. MS was supported by a scholarship from the University of Jordan (Ref: 1/2/5/A/259-2013).

References

- Abecasis, A.B., Vandamme, A.M., Lemey, P., 2009. Quantifying differences in the tempo of human immunodeficiency virus type 1 subtype evolution. *J Virol* 83, 12917-12924.
- Albert, J., Wahlberg, J., Leitner, T., Escanilla, D., Uhlen, M., 1994. Analysis of a rape case by direct sequencing of the human immunodeficiency virus type 1 pol and gag genes. *J Virol* 68, 5918-5924.
- Aldous, J.L., Pond, S.K., Poon, A., Jain, S., Qin, H., Kahn, J.S., et al., 2012. Characterizing HIV transmission networks across the United States. *Clin Infect Dis* 55, 1135-1143.
- Bazinet, A.L., Zwickl, D.J., Cummings, M.P., 2014. A gateway for phylogenetic analysis powered by grid computing featuring GARLI 2.0. *Syst Biol* 63, 812-818.
- Bjarnadottir, G.D., Haraldsson, H.M., Rafnar, B.O., Sigurdsson, E., Steingrímsson, S., Johannsson, M., et al., 2015. Prevalent intravenous abuse of methylphenidate among treatment-seeking patients with substance abuse disorders: a descriptive population-based study. *J Addict Med* 9, 188-194.
- Brenner, B., Wainberg, M.A., Roger, M., 2013. Phylogenetic inferences on HIV-1 transmission: implications for the design of prevention and treatment interventions. *AIDS* 27, 1045-1057.
- Briem, H., 2011. Great increase in the incidence of HIV infection among intravenous drug users. *EPI – ICE* (an electronic newsletter from the Chief Epidemiologist for Iceland, published by the Directorate of Health in English and Icelandic) [Internet] 7, 1-2.
- Bruhn, C.A., Audelin, A.M., Helleberg, M., Bjorn-Mortensen, K., Obel, N., Gerstoft, J., et al., 2014. The origin and emergence of an HIV-1 epidemic: from introduction to endemicity. *AIDS* 28, 1031-1040.
- Castro-Nallar, E., Perez-Losada, M., Burton, G.F., Crandall, K.A., 2012. The evolution of HIV: inferences using phylogenetics. *Mol Phylogenet Evol* 62, 777-792.
- Chaix, M.L., Seng, R., Frange, P., Tran, L., Avettand-Fenoel, V., Ghosn, J., et al., 2013. Increasing HIV-1 non-B subtype primary infections in patients in France and effect of HIV

subtypes on virological and immunological responses to combined antiretroviral therapy. *Clin Infect Dis* 56, 880-887.

Chief Epidemiologist for Iceland, 2007. HIV infection and AIDS in Iceland. A ticking time bomb? EPI – ICE (an electronic newsletter from the Chief Epidemiologist for Iceland, published by the Directorate of Health in English and Icelandic) [Internet] 3, 1.

Chief Epidemiologist for Iceland, 2008. HIV infection and hepatitis B in Iceland in 2007. EPI – ICE (an electronic newsletter from the Chief Epidemiologist for Iceland, published by the Directorate of Health in English and Icelandic) [Internet] 4, 2.

Ciccozzi, M., Madeddu, G., Lo Presti, A., Cella, E., Giovanetti, M., Budroni, C., et al., 2013. HIV type 1 origin and transmission dynamics among different risk groups in sardinia: molecular epidemiology within the close boundaries of an Italian island. *AIDS Res Hum Retroviruses* 29, 404-410.

de Oliveira, T., Pybus, O.G., Rambaut, A., Salemi, M., Cassol, S., Ciccozzi, M., et al., 2006. Molecular epidemiology: HIV-1 and HCV sequences from Libyan outbreak. *Nature* 444, 836-837.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 29, 1969-1973.

Esbjornsson, J., Mild, M., Audelin, A., Fonager, J., Skar, H., Bruun Jorgensen, L., et al., 2016. HIV-1 transmission between MSM and heterosexuals, and increasing proportions of circulating recombinant forms in the Nordic Countries. *Virus Evol* 2, vew010.

Eythorsson, E.S., Asgeirsdottir, T.L., Gottfredsson, M., 2014. [Needle exchange programs are a cost-effective preventative measure against HIV in Iceland]. *Laeknabladid* 100, 379-383.

Faria, N.R., Rambaut, A., Suchard, M.A., Baele, G., Bedford, T., Ward, M.J., et al., 2014. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. *Science* 346, 56-61.

Gilbert, M.T., Rambaut, A., Wlasiuk, G., Spira, T.J., Pitchenik, A.E., Worobey, M., 2007. The emergence of HIV/AIDS in the Americas and beyond. *Proc Natl Acad Sci U S A* 104, 18566-18570.

Grabowski, M.K., Redd, A.D., 2014. Molecular tools for studying HIV transmission in sexual networks. *Curr Opin HIV AIDS* 9, 126-133.

Hartfield, M., Murall, C.L., Alizon, S., 2014. Clinical applications of pathogen phylogenies. *Trends Mol Med* 20, 394-404.

Hirsch, M.S., Brun-Vezinet, F., Clotet, B., Conway, B., Kuritzkes, D.R., D'Aquila, R.T., et al., 2003. Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA Panel. *Clin Infect Dis* 37, 113-128.

Hue, S., Clewley, J.P., Cane, P.A., Pillay, D., 2004. HIV-1 pol gene variation is sufficient for reconstruction of transmissions in the era of antiretroviral therapy. *AIDS* 18, 719-728.

Hue, S., Pillay, D., Clewley, J.P., Pybus, O.G., 2005. Genetic analysis reveals the complex structure of HIV-1 transmission within defined risk groups. *Proc Natl Acad Sci U S A* 102, 4425-4429.

Hughes, G.J., Fearnhill, E., Dunn, D., Lycett, S.J., Rambaut, A., Leigh Brown, A.J., et al., 2009. Molecular phylodynamics of the heterosexual HIV epidemic in the United Kingdom. *PLoS Pathog* 5, e1000590.

Indridason, H., Gudmundsson, S., Karlsdottir, B., Love, A., Briem, H., et al., 2014. Long Term Nationwide Analysis of HIV and AIDS in Iceland, 1983-2012. *J AIDS Clin Res* 5, 387.

Karanikolos, M., Mladovsky, P., Cylus, J., Thomson, S., Basu, S., Stuckler, D., et al., 2013. Financial crisis, austerity, and health in Europe. *Lancet* 381, 1323-1331.

Kouyos, R.D., von Wyl, V., Yerly, S., Boni, J., Taffe, P., Shah, C., et al., 2010. Molecular epidemiology reveals long-term changes in HIV type 1 subtype B transmission in Switzerland. *J Infect Dis* 201, 1488-1497.

Lewis, F., Hughes, G.J., Rambaut, A., Pozniak, A., Leigh Brown, A.J., 2008. Episodic sexual transmission of HIV revealed by molecular phylodynamics. *PLoS Med* 5, e50.

Love, A., Chen, M., Sallberg, M., 2000. Changing profile of HIV-1 serotypes in Iceland during 1989-96. *Scand J Infect Dis* 32, 445-446.

Mendoza, Y., Martinez, A.A., Castillo Mewa, J., Gonzalez, C., Garcia-Morales, C., Avila-Rios, S., et al., 2014. Human immunodeficiency virus type 1 (HIV-1) subtype B epidemic in Panama is mainly driven by dissemination of country-specific clades. *PLoS One* 9, e95360.

Novitsky, V., Moyo, S., Lei, Q., DeGruttola, V., Essex, M., 2014. Impact of sampling density on the extent of HIV clustering. *AIDS Res Hum Retroviruses* 30, 1226-1235.

Paraskevis, D., Nikolopoulos, G., Fotiou, A., Tsiara, C., Paraskeva, D., Sypsa, V., et al., 2013. Economic recession and emergence of an HIV-1 outbreak among drug injectors in Athens metropolitan area: a longitudinal study. *PLoS One* 8, e78941.

Paraskevis, D., Nikolopoulos, G., Tsiara, C., Paraskeva, D., Antoniadou, A., Lazanas, M., et al., 2011. HIV-1 outbreak among injecting drug users in Greece, 2011: a preliminary report. *Euro Surveill* 16.

Paraskevis, D., Paraschiv, S., Sypsa, V., Nikolopoulos, G., Tsiara, C., Magiorkinis, G., et al., 2015. Enhanced HIV-1 surveillance using molecular epidemiology to study and monitor HIV-1 outbreaks among intravenous drug users (IDUs) in Athens and Bucharest. *Infect Genet Evol* 35, 109-121.

Paraskevis, D., Pybus, O., Magiorkinis, G., Hatzakis, A., Wensing, A.M., van de Vijver, D.A., et al., 2009. Tracing the HIV-1 subtype B mobility in Europe: a phylogeographic approach. *Retrovirology* 6, 49.

Pyne, M.T., Hackett, J., Jr., Holzmayer, V., Hillyard, D.R., 2013. Large-scale analysis of the prevalence and geographic distribution of HIV-1 non-B variants in the United States. *J Clin Microbiol* 51, 2662-2669.

Ragonnet-Cronin, M., Lycett, S.J., Hodcroft, E.B., Hue, S., Fearnhill, E., Brown, A.E., et al., 2016. Transmission of Non-B HIV Subtypes in the United Kingdom Is Increasingly Driven by Large Non-Heterosexual Transmission Clusters. *J Infect Dis* 213, 1410-1418.

Shao, Y., Williamson, C., 2012. The HIV-1 epidemic: low- to middle-income countries. *Cold Spring Harb Perspect Med* 2, a007187.

Tapia Granados, J.A., Rodriguez, J.M., 2015. Health, economic crisis, and austerity: A comparison of Greece, Finland and Iceland. *Health Policy* 119, 941-953.

Tebit, D.M., Arts, E.J., 2011. Tracking a century of global expansion and evolution of HIV to drive understanding and to combat disease. *Lancet Infect Dis* 11, 45-56.

The Directorate of Health in Iceland, 2015. Available from: <http://www.landlaeknir.is/> [First published 2015 February 12; cited 2015 September 2].

Vermund, S.H., Leigh-Brown, A.J., 2012. The HIV Epidemic: High-Income Countries. *Cold Spring Harb Perspect Med* 2, a007195.

Wertheim, J.O., Leigh Brown, A.J., Hepler, N.L., Mehta, S.R., Richman, D.D., Smith, D.M., et al., 2014. The global transmission network of HIV-1. *J Infect Dis* 209, 304-313.

Tables

Table 1. Characteristics of 230 individuals included in the study with HIV-1 diagnosis 1985-2012.

Table 2. Characteristics of Icelandic transmission clusters.

Figure Legends

Figure 1. The coverage of HIV-1 partial *pol* sequences included in the study shown as the number of individuals diagnosed with HIV-1 infection in Iceland vs the number of individuals diagnosed and included in the study from 1985-2012. The sequences are assigned to the year of diagnosis.

Figure 2. Trend analysis stratified by risk groups and B vs non-B subtypes/CRFs over the study period. A) Proportion of different risk group categories over the study period. B) Total number of different risk group categories over the study period. C) Proportion of different risk group categories in transmission clusters over the study period. D) Total number of different risk group categories in transmission clusters over the study period.

Figure 3. A) Maximum clade credibility trees of each Icelandic large IDU cluster shown on the same time-scale. B) Bayesian skyline plots of the two large Icelandic IDU clusters (with the line showing the median estimate of effective number of infections over time and blue coloured areas limiting the 95% HPD interval). The two IDU clusters are shown on the same time scale.

Table 1. Characteristics of 230 individuals included in the study with HIV-1 diagnosis 1985-2012.

| Characteristic | N¹ (%) |
|---|--------------------------|
| Total | 230 |
| Sex | |
| <i>Male</i> | 155 (67) |
| <i>Female</i> | 75 (33) |
| Risk factor² | |
| <i>MSM</i> | 69 (30) |
| <i>HET</i> | 84 (37) |
| <i>IDU</i> | 50 (22) |
| <i>Others</i> | 2 (1) |
| <i>Unknown</i> | 25 (11) |
| Country of birth | |
| <i>Iceland</i> | 142 (62) |
| <i>Non-Iceland</i> | 86 (37) |
| <i>Unknown</i> | 2 (1) |
| Country of infection³ | |
| <i>Iceland</i> | 75 (33) |
| <i>Non-Iceland</i> | 99 (43) |
| <i>Unknown</i> | 56 (24) |

¹N: Number; ²Risk factor: Self-reported risk factor for HIV-1 acquisition (MSM: Men who have sex with men; HET: Heterosexual; IDU: Injection drug use; Others: one blood transfusion and one mother to child transmission); ³Country of infection: Self-reported country of infection.

Table 2. Characteristics of Icelandic transmission clusters

| Cluster Name ¹ | Sequences | IS Sequences ² | Sex | | Risk Factor | | | IS Infections ³ | IS Born ⁴ | aLRT-SH ⁵ | Years of Diagnosis | tMRCA ⁶ |
|--------------------------------|----------------|---------------------------|------|--------|------------------|------------------|-------------------|----------------------------|----------------------|----------------------|--------------------|--------------------------------|
| | N ⁷ | % | Male | Female | MSM ⁸ | HET ⁹ | IDU ¹⁰ | % | % | | | |
| B_HET_1 | 4 | 100% | 1 | 3 | 0 | 4 | 0 | 75% | 100% | 0.90 | 2007 | 2005 (2004-2007) |
| B_IDU_1 | 21 | 100% | 13 | 8 | 0 | 0 | 21 | 90% | 95% | 1.00 | 1999-2012 | 1999 (1997-1999) |
| B_IDU_2 | 19 | 90% | 11 | 6 | 0 | 0 | 17 | 94% | 88% | 0.94 | 2007-2012 | 2006 (2002-2007) ¹¹ |
| B_MIX_1 | 2 | 100% | 2 | 0 | 1 | 0 | 1 | 0% | 100% | 0.92 | 1986-1988 | 1987 (1979-1996) |
| B_MIX_2 | 10 | 100% | 7 | 3 | 5 | 5 | 0 | 40% | 90% | 0.93 | 1991-2008 | 1982 (1978-1986) |
| B_MIX_3 | 11 | 82% | 8 | 1 | 5 | 2 | 2 | 33% | 100% | 0.91 | 1985-2001 | 1978 (1974-1981) |
| B_MIX_4 | 3 | 100% | 2 | 1 | 0 | 2 | 1 | 100% | 100% | 0.98 | 1986-1998 | 1985 (1980-1989) |
| B_MIX_5 | 2 | 100% | 2 | 0 | 1 | 1 | 0 | 50% | 100% | 0.95 | 1987-1994 | 1989 (1985-1993) |
| B_MIX_6 | 3 | 100% | 1 | 2 | 1 | 2 | 0 | 33% | 67% | 0.99 | 1994-2002 | 1990 (1986-1994) |
| B_MSM_1 | 4 | 100% | 4 | - | 4 | - | - | 25% | 75% | 0.99 | 1992-2012 | 1991 (1987-1995) |
| B_MSM_2 | 2 | 100% | 2 | - | 2 | - | - | 100% | 100% | 1.00 | 2009-2010 | 2008 (2006-2010) |
| B_MSM_3 | 2 | 100% | 2 | - | 2 | - | - | 50% | 100% | 1.00 | 1987-2003 | 1991 (1987-1994) |
| B_MSM_4 | 3 | 100% | 3 | - | 3 | - | - | 67% | 100% | 0.98 | 1988-1996 | 1985 (1980-1990) |
| 02_HET_1 | 2 | 100% | 1 | 1 | 0 | 2 | 0 | 50% | 100% | 1.00 | 2011 | - |
| 02_HET_2 | 2 | 100% | 1 | 1 | 0 | 2 | 0 | 0% | 50% | 1.00 | 2010 | - |
| 02_HET_3 | 2 | 100% | 1 | 1 | 0 | 2 | 0 | 50% | 50% | 1.00 | 1999-2001 | - |
| 45_HET_1 | 2 | 100% | 1 | 1 | 0 | 2 | 0 | 0% | 0% | 0.98 | 2009 | - |
| A1_HET/U_1¹² | 5 | 80% | 1 | 3 | 0 | 3 | 0 | 50% | 0% | 0.99 | 2010-2012 | - |
| A1_HET_2 | 3 | 100% | 2 | 1 | 0 | 3 | 0 | 33% | 67% | 0.92 | 1988-2012 | - |
| C_HET_1 | 2 | 100% | 1 | 1 | 0 | 2 | 0 | 50% | 50% | 1.00 | 2006 | - |
| C_HET/U_2 | 2 | 100% | 1 | 1 | 0 | 1 | 0 | 0% | 50% | 1.00 | 1999-2010 | - |

¹Cluster Name: The first part corresponds to HIV-1 subtype/CRF with CRFs being referred to, depending on their numbers; the second part corresponds to the risk group dominating the cluster and MIX was used to denote mixed clusters; ²Percentage of Icelandic sequences within the cluster; ³Percentage of infections with Iceland as reported country of infection; ⁴Percentage of individuals who were born in Iceland; ⁵Approximate Likelihood Ratio Test Shimodaira-Hasegawa like; ⁶Median time to the most recent common ancestor of the cluster and 95% highest posterior density interval; ⁷N: Number; ⁸MSM: Men who have sex with men; ⁹HET: Heterosexual; ¹⁰IDU: Injection drug use; ¹¹Time to most recent common ancestor for the pure Icelandic sub-cluster was estimated from the maximum clade credibility tree; ¹²U: unknown (a patient in the cluster with unknown risk factor for acquisition of HIV-1 infection).

Figure 1. The coverage of HIV-1 partial *pol* sequences included in the study shown as the number of individuals diagnosed with HIV-1 infection in Iceland vs the number of individuals diagnosed and included in the study from 1985-2012. The sequences are assigned to the year of diagnosis.

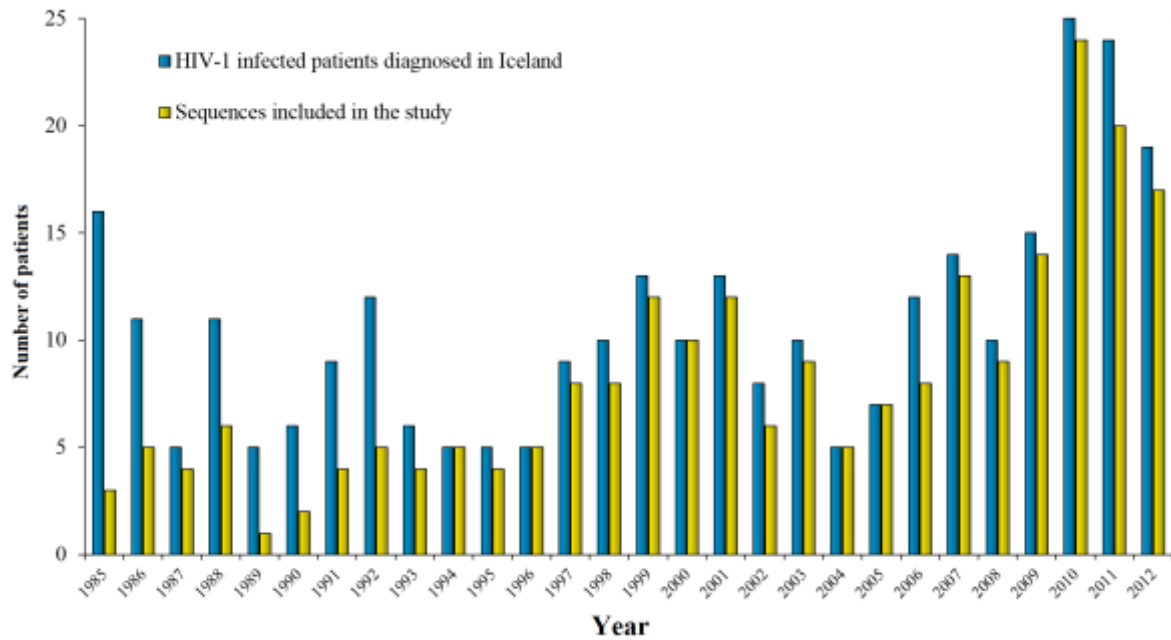


Figure 2. Trend analysis stratified by risk groups and B vs non-B subtypes/CRFs over the study period. A) Proportion of different risk group categories over the study period. B) Total number of different risk group categories over the study period. C) Proportion of different risk group categories in transmission clusters over the study period. D) Total number of different risk group categories in transmission clusters over the study period.

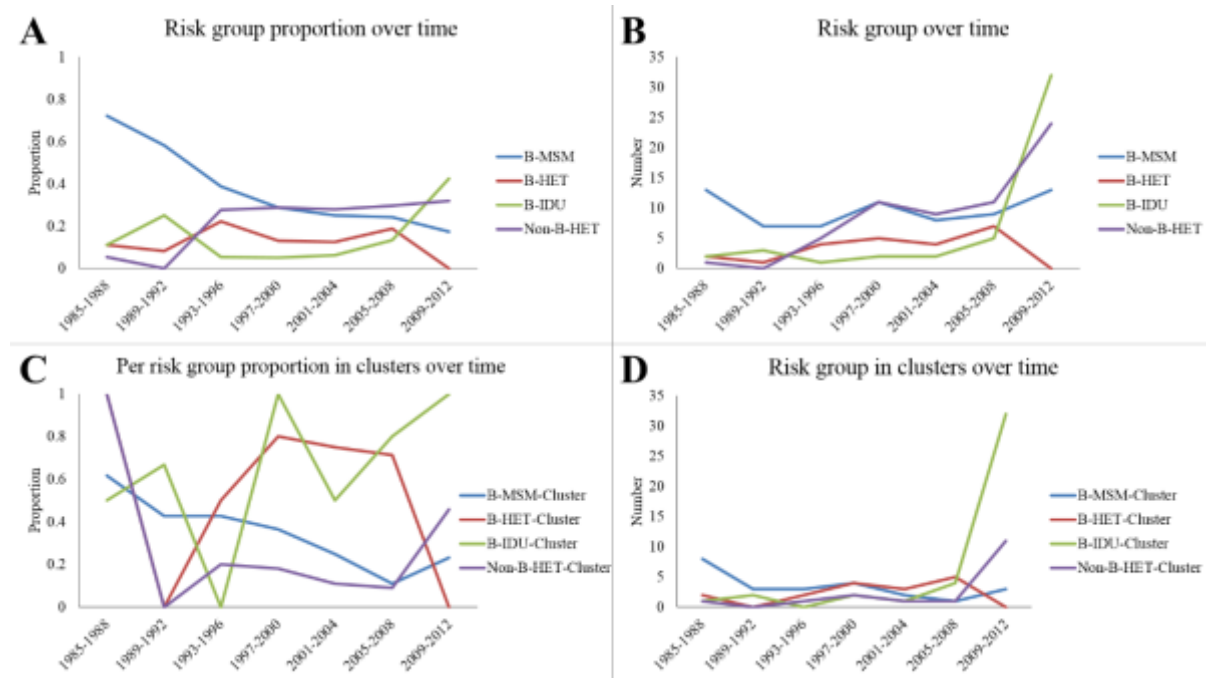


Figure 3. A) Maximum clade credibility trees of each Icelandic large IDU cluster shown on the same time-scale. B) Bayesian skyline plots of the two large Icelandic IDU clusters (with the line showing the median estimate of effective number of infections over time and blue coloured areas limiting the 95% HPD interval). The two IDU clusters are shown on the same time scale.

