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Title Page:

Changing trends in international versus domestic HCV transmission in HIV-positive MSM:

A perspective for the DAA scale-up era

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Brief summary: We used phylogenetics and phylodynamics to classify over time HCV infections in HIV-positive MSM as either domestically or internationally acquired. We found that while international transmission dominated initially and persists, local transmission has established as the main source of infections.

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Footnote Page:

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*LSV and RDK contributed equally to this study.

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Abstract

Background: Scale-up of direct-acting antiviral(DAA) therapy is expected to abate HCV incidence among HIV-positive men-who-have-sex-with-men(MSM). Treatment programs in neighbouring countries may influence each other's outcomes through international transmission.

We aimed at classifying HCV infections in HIV-positive MSM as either domestically or internationally acquired, and at estimating how this classification changed over time.

Methods: HCV subtype 1a (the most frequent subtype among MSM) genomes from 99 persons enrolled in the Swiss-HIV-Cohort-Study(SHCS) and diagnosed with replicating HCV infections between 1999 and 2016, were sequenced. Sixty-six of these sequences were from MSM. We inferred maximum-likelihood phylogenetic-trees and time-trees containing a fragment of the NS5B region of these and other 374 circulating strains retrieved from national and international databases. We inferred transmission clusters from these trees and used the country composition of such clusters to attribute infections to domestic or international transmission.

Results: Fifty to 80% of HCV transmissions were classified as domestic depending on the classification criterion. Between 2000 and 2007, the fraction attributable to domestic transmission was 54%[range:0%-75%]. It increased to 85%[range:67%-100%] between 2008 and 2016.

Conclusions: International and domestic transmission have played major roles in the epidemic. While international transmission persists, local transmission has established as the main source of infections.

Keywords: Men who have sex with men; Hepatitis C virus; HIV; Direct-acting antivirals; transmission.

Introduction

HIV-positive men who have sex with men (MSM) have become a main transmission group for hepatitis C virus (HCV) infection in Switzerland and other industrialized countries. Increased HCV transmission among HIV-positive men who have sex with men started to be reported in the early 2000's[1-4]. Molecular epidemiological analyses demonstrated international networks of sexual transmission of HCV [5, 6]. The spread of HCV among HIV-positive MSM has continued ever since [4, 7-9].

Rapid scale-up of early direct-acting antiviral (DAA) therapy for HCV is ongoing in many European and non-European countries [10, 11] as reimbursement restrictions are progressively relieved. Projections from mathematical models for several Western countries [12-15] and early surveillance data from the Netherlands and Switzerland support the claim that DAA scale-up may abate the high incidence of HCV in HIV-positive MSM [15-19].

The relative magnitude of domestic versus international HCV transmission could be a key parameter for the success of isolated national treatment strategies: national micro-elimination and prevention programs can only be successful if a significant fraction of infections are acquired domestically, while international coordination becomes more important with frequent international transmission. Indirect genetic evidence for Switzerland suggests an important role of domestic transmission [20]. Switzerland is ideally placed to study international transmission due to its small population

size, the high proportion of MSM followed within the Swiss HIV Cohort Study (SHCS; www.shcs.ch) [21], and its connections to international networks of sexual transmission [22, 23].

This study aimed at classifying HCV infections in HIV-positive MSM in Switzerland as domestically or internationally acquired, and at estimating how this classification changed over time. We did this by locating infections in transmission clusters inferred from phylogenetic reconstructions and by timing transmission events.

Methods

This study took place in the SHCS. The SHCS is a nationwide prospective cohort that routinely collects clinical and epidemiological data from HIV-positive persons aged ≥ 18 years since 1988. The SHCS has been approved by the ethics committee of the participating centers and written informed consent had been obtained from all participants. The prospectively collected data comprise behavioural and clinical information as well as laboratory measurements including routine HCV antibody screening for incident infections and HCV-RNA to detect reinfections. We estimate that more than 80% of all MSM diagnosed with HIV in Switzerland are followed within the cohort network [24] [25]. The prevalence of HCV in HIV-positive MSM in the SHCS was 4.8% in June-2016, and the fraction of infections with genotypes 1, 2, 3 and 4 were 72%, 1%, 5% and 22%, respectively [17]. This molecular analysis deals with subtype 1a, the most frequent one in our study

population, accounting for 60% of HCV infections. For genome sequencing, we included all MSM with HCV subtype 1a replicating infections who had available plasma sample for sequencing. We refer to these as "Swiss MSM sequences" from now on.

To enable comparative analyses, we also sequenced additional cohort participants (other than MSM) with incident infections, also subject to availability of plasma sample from the SHCS biobank.

Sequencing of hepatitis C virus infections: HCV RNA genome sequences were generated by amplification of almost full length HCV RNA followed by massive parallel sequencing. Viral RNA was extracted from plasma stored in the SHCS biobank (www.shcs.ch) by an automated nucleic acid extractor (EasyMag, BioMerieux) [26] and Nucleospin RNA Virus Kit (Macherey-Nagel). RNA was then amplified by RT-PCR. A MiSeq (Illumina) instrument was utilized for sequencing. Finally, a consensus sequence was generated for each HCV isolate by iterative sequence alignment against an HCV reference sequence with *SmaltAlign* (MIT). The Hepatitis C Virus isolate H77 (GenBank accession number NC_004102) served as reference sequence. Our near full length sequencing spanned nucleotides 62 to 9'376 of this reference. To allow comparison with international sequences we focused on the 436 base-pair fragment coding for the C-terminal part of the NS5B region, because this segment is relatively abundant in public databases [27] and the basis of previous studies on HCV transmission among MSM [5, 8, 9, 27].

Maximum likelihood phylogenies: We compared 66 Swiss MSM sequences with a set of background sequences, which included 33 derived from incident infections in persons enrolled in the SHCS with HIV transmission risk other than MSM, 81 from chronic infections in persons enrolled in the SHCS and 293 sequences retrieved from public databases [28] using the Basic Local Alignment Search Tool (BLAST) [29] to identify relevant background sequences by means of similarity criteria with respect to the sequences obtained for this study. We used RAxML [30] to infer Maximum Likelihood (ML) phylogenetic trees assuming a general time reversible with invariant sites (GTRI) model with gamma-distributed substitution rates with four categories. One hundred replicates resulted in an estimation of the best tree and bootstrap supporting values for each bipartition. **Transmission Clusters:** A subtree with a bootstrap support value $\geq$70% was defined as a transmission cluster (3 members or more) or a transmission pair (2 members). We only analyzed transmission clusters or pairs that included study sequences because only those are relevant for the study question.

Likely geographic origin of infections: We classified our study sequences as corresponding to transmissions likely to have occurred by contact with MSM living either in Switzerland or abroad, and termed them domestic or international transmissions, respectively. We classified a study sequence as corresponding to domestic transmission if it was located in a Swiss transmission cluster, and as corresponding to international transmission if it was located in an international transmission cluster. A transmission

cluster was defined as Swiss when the fraction of its sequences that were Swiss equaled or exceeded a given threshold named "Swiss dominance criterion". We studied Swiss dominance criteria ranging between 50% and 90%. Likely geographic origin of sequences found to belong to more than one cluster was determined based on the most internal cluster they belonged to.

International linkages of the Swiss epidemic: We defined and computed a measure of the strength of the linkage between the Swiss epidemic and those in other countries whose sequences could be included in our analyses. This measure consisted of assigning weights to each region according to its frequency of appearance in clusters containing Swiss MSM sequences. We defined the measure as follows: $\theta_g = \frac{1}{\Gamma}\sum_i \rho_g^i \gamma^i$ where $\Gamma = \sum_i \gamma^i$ is the total number of Swiss MSM sequences, i indexes international clusters, γ^i is the number of Swiss MSM sequences in cluster i, g indexes countries of origin others than Switzerland, and ρ_g^i is the fraction of sequences in the international cluster i which country of origin is g.

Time origin of Swiss clusters: We estimated the origin of Swiss transmission clusters identified from the ML phylogenetic tree. These origins can be interpreted as proxies for introduction times of HCV into Switzerland, which resulted in domestic sub-epidemics.

For this purpose, we re-inferred Swiss transmission clusters with five or more members using BEAST [31], which resulted in time trees for each of these clusters. Our estimations of a cluster's origin corresponded to the

heights of such the time trees. BEAST analyses utilized the general time-reversible substitution model with invariant sites (GTRI) with gamma-distributed rate variation (in accordance with the ML estimation depicted above) and an uncorrelated lognormal relaxed molecular clock model [32]. The Birth-Death Skyline Serial model (BDSKY) implemented in BEAST provided a surrogate HCV transmission model [33]. **Table S1** provides further details on the parameterization of this analysis. The results reported in this manuscript correspond to runs that reached markov chain montecarlo (MCMC) chain lengths of 5 x 10^7. The minimum effective sample sizes (ESS) across estimated parameters was> 800.

Trends in geographic origin of HCV infections: We identified time changes in the fraction of infections attributable to domestic (versus international) transmission by timing the transmission events associated with our study sequences. As proxy for transmission time, we used branching times involving our study sequences in a time tree. This time tree was constructed using BEAST by the same method as above and included the same set of sequences the ML tree did. For this estimation, we only considered branching times corresponding to nodes with posterior probabilities ≥70%. BEAST analyses only included sequences with available sampling date.

Sensitivity Analysis: In order to test the robustness of the *trends in geographic origin of HCV infections*, we performed a sensitivity analysis on the time-tree. This sensitivity analysis accounted for potential imbalance of

sampling rates (Swiss versus international sequences) over time. Such imbalance may arise from the fact that while the sampling of international sequences slowed down towards the end of the study period, the SHCS continued to perform HCV sequencing (see Text S1).

Results

Sequencing of hepatitis C virus infections: Overall, by means of Illumina technology we obtained 99 HCV subtype 1a genomes from persons who were diagnosed with HCV infections between 1999 and 2016. Sixty-six were from 63 MSM (3 reinfections) and constitute our study sequences: "the Swiss MSM sequences". The Swiss MSM sequences account for 66% of infections with this genotype diagnosed in MSM enrolled in the SHCS during the study period. **Table 1** shows the characteristics of the MSM participants. Patients' median age was 45 years [IQR: 41 - 51] and 75% (47/63) ever reported inconsistent condom use with occasional partners. Fifty four percent (34/63) and 14% (9/63) reported the use of any or intravenous recreational drugs respectively. Previous publications have described in detail HIV/HCV co-infected MSM enrolled in the SHCS [17, 18, 34] Sampling dates of Swiss MSM sequences ranged between June-2002 and May-2016. The Swiss MSM sequences have been submitted to GenBank, accession numbers are MK314737 to MK314802.

Transmission Clusters: Ninety percent (60/66) of the Swiss MSM sequences were located within 14 MSM transmission clusters (7 nested, i.e., embedded in larger clusters) and 12 transmission pairs (8 nested) (**Table 2, Figure 1**). Within-cluster patristic distance was (mean[range]) 0.27%[0%-0.68%]. This value fulfils commonly used criteria for clustering (e.g. 1-10%), and therefore provides further support for the adopted cluster definition based on

bootstrap values. Only two study sequences clustered or paired with sequences from men enrolled in the SHCS who reported intravenous drug use as the most likely mode of HIV transmission. **Figure 1** displays all transmission clusters and pairs containing study sequences; The median number of identifiable countries involved in not Swiss-only clusters was 4 [range 2-9]. **Figure S1** shows the full phylogenetic tree.

The aforementioned transmission clusters were identified as MSM clusters based on cohort data in the case of Swiss sequences and by inspection of the associated papers in the case of international sequences. **Table 2** depicts the clusters identified in this analysis.

Likely geographic origin of infections: Fifty percent (30/60, **Figure 1**) of Swiss MSM sequences were located in cluster C1 consisting predominantly of Swiss and German sequences. This cluster contains sub-transmission clusters C4, C8 and tree transmission pairs, all consisting only of Swiss sequences and including 13 Swiss MSM sequences in total. Eighteen percent of study sequences (11/60) were located in cluster C2 consisting predominantly of Swiss and British sequences. The Swiss part of this cluster is a Swiss-only subtree, but the bootstrap support value for this subtree (60%) does not fulfil the criteria for a transmission cluster as defined for this study. Three sequences could not be linked to any transmission cluster. Overall, 50% (33/66) of Swiss MSM sequences were located in Swiss only clusters or transmission pairs.

Panel A of **Figure 2** shows the fractions of study sequences classified as domestic, international and unknown transmission for a range of Swiss

dominance criteria (minimum percentage of sequences in the clusters that are required to classify a cluster as Swiss). When this criterion was set to 50% or 60%, 80% and 79% of sequences were classified as domestic transmission respectively. When the criterion was raised to 90%, half of the sequences were classified as domestic transmission.

International linkages of the Swiss epidemic: Panel B of **Figure 2** shows a measure of the strength of the linkage between the HCV epidemic in Switzerland and those in other countries as suggested by the ML phylogenetic tree. This measure suggests that the Swiss epidemic is closely linked to those in the UK, The Netherlands and Germany.

Time origin of Swiss clusters: We next investigated the time origin of the best-supported Swiss dominated clusters. For these estimates, monophyletic trees containing five or more members and fulfilling a Swiss dominance criterion of 80% (see **Table 2**) defined a best-supported Swiss cluster. Phylodynamic re-inference of these clusters estimated that their origins ranged between (median) October-1999 and June-2007 (**Table 2; Supplementary Figure S2**).

Trends in geographic origin of HCV infections: The time tree constructed including all sequences with available sampling dates suggests that until 2004 most transmissions occurred by contact with MSM not living in Switzerland (**Figure 3**). Of note, none of MSM whose transmissions were estimated to have occurred before 2002 ever reported drug injection. Neither did they cluster with Swiss sequences from persons reporting drug injection.

Between 2000 and 2007, the fraction attributable to domestic transmission was 54%[range: 0%-75%]. It increased to 85%[range: 67%-100%] from 2008 to the end of the study period (**Figure 3**).

Sensitivity Analysis: The sensitivity analysis (**Figure S3**) supports the observation, derived from **Figure 3**: that the contribution of domestic transmission increased over time. **Figure S3** suggests that changing sampling rates do not explain the increasing trend in domestic transmission displayed in **Figure 3**. The simulated fraction attributable to domestic transmission (for 80% dominance criterion) increased from 25% (95%CI: 11%-25%) between 2000 and 2007 to 82% (51%-85%) between 2008 and 2016 (versus 54% and 85% for the same time periods in the main analysis). These results suggest that our main findings were robust to variations in sampling density.

Discussion

This molecular epidemiology study suggests that both Swiss-domestic and international HCV transmission contribute substantially to the HCV epidemic among HIV-positive MSM in Switzerland. The relative contribution of international transmission declined with time, and gave place to domestic transmission as the major source of infections. We found no significant trace of transmission bridging from persons who became infected with HIV through drug injection and MSM within Switzerland, suggesting that the HCV epidemics in these two HIV transmission groups are likely

disconnected. This is in line with a former phylogenetic study of HIV transmission in Switzerland were only 2% of HIV sequences from persons who inject drugs clustered with those from MSM [23]. Due to lack of behavioural data on concurrence of sexual practices associated with HCV transmission and the use of intravenous drugs [35, 36], our study could not assess frequencies of HCV transmission routes (i.e., traumatic sex versus intravenous drug use).

Our analyses suggest that the initial cases of sexually transmitted HCV in MSM in Switzerland are mainly attributable to international transmissions among MSM, and not to spillovers between persons who inject drugs and MSM within Switzerland. Swiss-domestic HCV transmission among MSM has grown over time. This implies that intensive test-and-treat interventions at the national level are likely to succeed. This notion is supported by data from Switzerland, where the Swiss HCVree Trial, a test-treat-and-counsel study [14, 16-18] has been followed by a decline in the number of cases of acute HCV infections among MSM in the SHCS. Importantly, early data from the Netherlands also indicates declines in incident cases in this population after introduction of universal access to DAA therapy [10]. Our finding of persistent international transmissions may imply that coordinated HCV treatment scale-up in different countries could amplify the decrease in HCV transmissions.

The estimated time origins of Swiss transmission clusters can be interpreted as illustrations of critical time-points when treatment and/or behavioural interventions could have had prevented new transmission chains. We

estimated origins of Swiss clusters ranging between 1999 and 2007, which overlaps with a sharp rise in HCV incidence among HIV-positive MSM in Switzerland [34]. This may have occurred, at least partially, due to such introduced cases spreading in Switzerland.

Limitations: This analysis relies on public databases to obtain background international sequences. While a large number of those were included, the fact that European countries may be unevenly represented in Genbank, is a limitation of this study. Our estimations on linkages of the Swiss epidemic to specific countries were therefore likely biased. The segment of the HCV genome we chose for these analyses was the one that resulted in the most suitable set of background sequences, in particular because it has been the basis of other phylogenetic analyses across Europe [5, 37]. However, countries such as France and Italy were likely under-represented in our analyses (only 8 and 3 sequences respectively were included). Evener sampling rates across geographic regions as well as increasing sequencing of longer regions of the HCV genome, should improve estimates of transmission patterns in the future [38].

The fact that the number of international HCV sequences available from public databases declined towards the end of the study period, is also a potential limitation of this study. It could potentially have led to overestimations of the contribution of domestic transmission towards the end of the study period. However, the contribution of domestic transmission started to increase early on, before the aforementioned disparity could have had any effect. Moreover, the estimated time trend in domestic versus

international transmission held true when confronted with a sensitivity analysis that assumed a set of hypothetical international sequences iteratively attaching to the transmission clusters, which deliberately decreased the likelihood of domestic transmission. Finally, potential under-reporting of intravenous drug injection may have prevented us from recognizing more transmission bridges between MSM and persons who inject drugs.

To our knowledge, this is the first study to quantify the contribution over time of international transmission to a national HCV epidemic in MSM. We did this by combining phylogenetic and phylodynamic analyses of a representative sample of HCV genome sequences from infections observed in Switzerland.

Previous studies have demonstrated the emergence of epidemics of HCV transmitted through sexual practices and depicted its separation from transmission networks formed by persons who inject drugs [5, 37]. These molecular epidemiological studies revealed international networks of HCV transmission among HIV-positive MSM. Our study evidences high levels and increases of domestic transmission within Switzerland. It also indicates that the contribution of international HCV transmission networks persists although its importance declined over time.

Implications of findings:

Previous modelling studies and empiric data indicate that DAA scale-up has the potential to curb the HCV epidemic among HIV-positive MSM [12-15]. Our results suggest that for a successful treatment-as-prevention approach and a correct interpretation of its effects, international transmission should be considered; and that targeted joint European scale-up schemes may increase the efficiency of such interventions.

Time-updated HCV phylogenies for Europe could help monitoring the impact of national DAA scale-up programs, and they would allow measuring the effect of treatment-as-prevention on large-scale transmission networks. This requires international coordination to sequence the same or overlapping regions of the HCV genome.

Conclusion

This molecular epidemiological study suggests that both international and local transmission have played major roles in the Swiss epidemic of hepatitis C among HIV-positive MSM, and that, while international transmission persists, Swiss domestic transmission has gained importance over time.

Authors contributions: LSV, RDK and AR designed the study. LSV and RDK formulated the analyses. LSV performed the analyses. KM, KCC, CS and JB performed HCV sequencing. JF, DB, EB, MH, NL, MC, MR, HG and OK contributed cohort data and/or contributed to the analyses. LSV, RDK, and AR drafted the first version of the manuscript which was then revised by all the other authors. All authors contributed to the interpretation of the results.

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Figure legends

Figure 1. Phylogenetic tree showing the clusters that contain the study sequences (Swiss MSM sequences). Clusters were defined as monophyletic trees with ≥70% bootstrap support value. The full tree (from which this one is derived **Figure S1**) was computed using RAxML with 100 replicates and a general time reversible model with invariant sites (GTRI) and gamma-distributed substitution rates.

Figure 2. Likely geographic origin of infection (A) and international linkages of the Swiss MSM sequences (B). This classification was derived from the clusters identified in the ML phylogenetic tree.
Swiss dominance criteria: minimum percentage of sequences in the clusters that are Swiss necessary to classify a cluster as Swiss.

Figure 3. Time trends in the fraction of HCV infections attributable to domestic and international transmission. This classification assumed an 80% dominance criteria and was derived from the time tree inferred using BEAST 2.0. Branching times involving our study sequences (Swiss MSM sequences) served as proxy for transmission times.

Figure 1

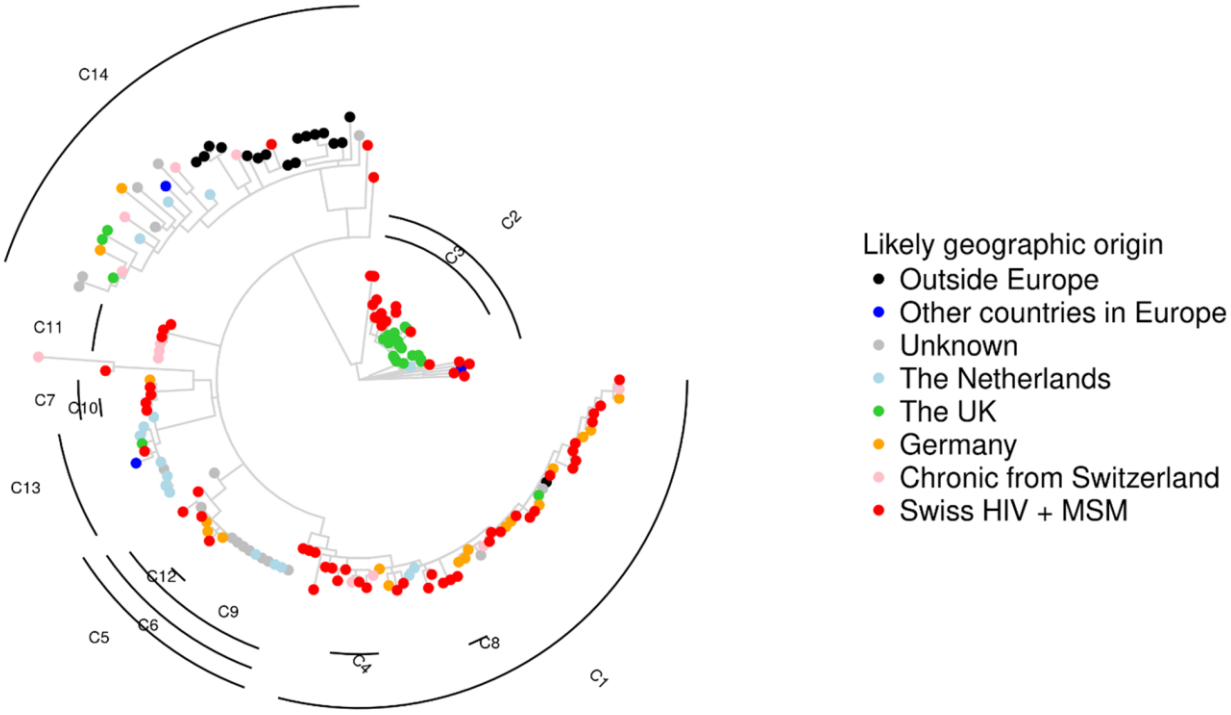

Figure 2

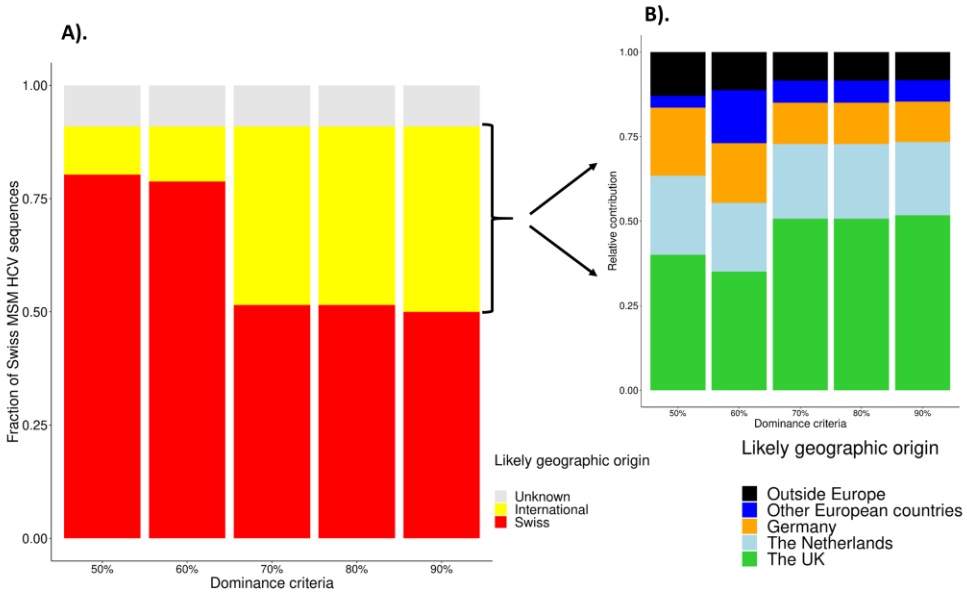

Figure 3

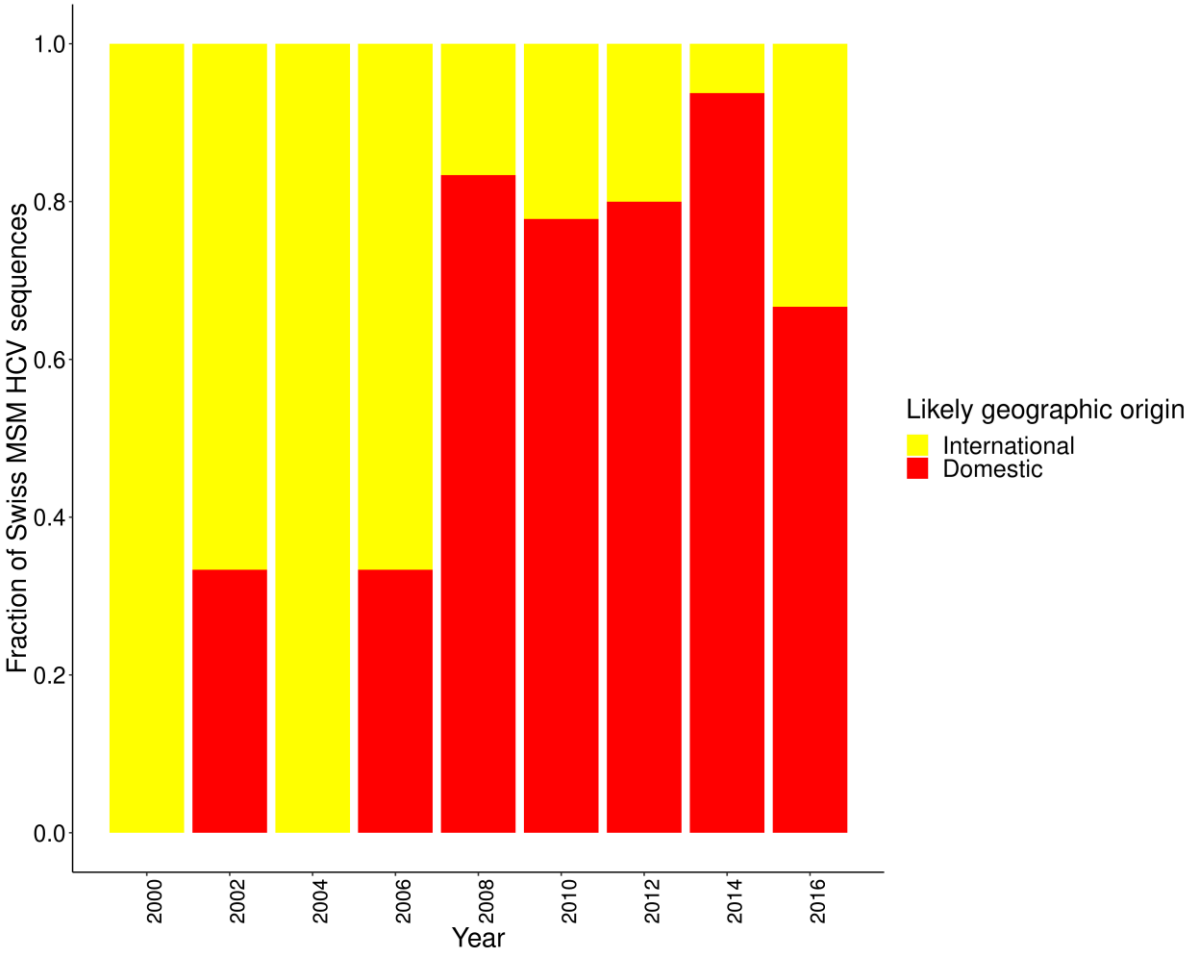

Table 1. Characteristics of men who have sex with men included in the study. Sixty-six sequences (including three reinfections) were obtained from 63 men who have sex with men.

Number of patients	63
Age at sampling time (years, median[IQR])	45 [41 - 51]
ART ever started (N [%])	62 [94]
Drug use during follow-up	
Ever - all modes of administration (N [%])	34 [54]
Ever - intravenous (N [%])	9 [14]
Sex with occasional partners	
Ever	63 [100]
Condom use with ocassional partners	
Consistent	12 [19]
Inconsistent	47 [75]
Refuse to answer	0
Missing	4 [6]
Ethnicity	
Caucasian	55 [87]
Education*	
Lower	6 [10]
Intermediate	44 [70]
High	11 [17]

	Unknown	2 [3]

*Lower: Mandatory school or lower; High: University

Table 2. Description of transmission clusters in Figure 1 and estimated origin. The order of cluster indices reflects the number of studied sequences located in the respective cluster; "Swissness" refers to the fraction of sequences of Swiss origin in the cluster. The table reports time of origin for clusters with five or more members and swissness equal or larger than 80%. Time trees are shown in Figure S2.

Primary	Nested	Size	Study sequences (n)	Swissness	Estimated time origin
C1		52	30	65%	-
	C4	6	4	100%	Jun 2007
	C8	3	3	100%	-
C2		33	11	33%	-
	C3	27	11	41%	-
C5		19	4	21%	-
	C6	18	4	22%	-
	C9	17	3	18%	-
	C12	3	1	33%	-
C7		5	4	80%	Nov 2000
	C10	3	3	100%	-
C11		6	3	100%	Oct 1999
C13		11	1	9%	-

C14	36	1	14%	-