The epidemic history of hepatitis C among injecting drug users in Flanders, Belgium

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SUMMARY. We employed recently developed statistical methods to explore the epidemic behaviour of hepatitis C subtype 1a and subtype 3a among injecting drug users (IDUs) in Flanders, Belgium, using new gene sequence data sampled among two geographically distinct populations of IDUs. First the extent of hepatitis C transmission across regions/countries was studied through calculation of association indices. It was shown that viral exchange had occurred between both populations in Flanders as well as across international borders. Furthermore, evidence was found suggestive of subtypes 1a and 3a predominantly circulating in subpopulations of Flemish IDUs, exhibiting different degrees of travelling/migration behaviour. Secondly, through coalescent-based analysis the viral epidemic history of the hepatitis C subtype 1a and 3a epidemics was inferred. Evidence was found for different dynamic forces driving both epidemics. Moreover, results suggested that the hepatitis C subtype 3a epidemic has reached a steady state, while the hepatitis C 1a epidemic has not, which therefore might become the predominant subtype among IDUs.

INTRODUCTION

Soon after the discovery of the hepatitis C virus (HCV) in 1989, preventive measures have been implemented to prevent HCV transmission [1]. These measures have been proven very effective except with respect to the epidemic among injecting drug users (IDUs) [2,3]. Hepatitis C continues to spread rapidly among IDUs [4–8]. Preventive measures such as information, education, needle-exchange programmes, etc. have been proven to be successful in containing the human immunodeficiency virus (HIV) epidemic among IDUs; however, they seem quite ineffective for HCV, at most slowing the epidemic [9–18].

A good understanding of the dynamics of the HCV epidemic is the cornerstone for the development of an effective prevention policy. The rate of spread of HCV is often derived from seroprevalence data collected over time course of the epidemic. Such data are difficult to obtain and to interpret for the HCV epidemic for a number of reasons. First, the beginning of the epidemic pre-dated the discovery of the virus and there is lack of archived specimens enabling retrospective seroprevalence measurements. Secondly, because hepatitis C is seldom diagnosed during the acute stage, studies often have to estimate the date of infection based on assumptions regarding risk factors or exposures [19]. It is customary to estimate the date of infection with hepatitis C for IDUs in the year that he/she started to inject. However, it has been shown that this assumption is not correct for about 50% of the cases [20]. Thirdly, infection with HCV does not confer to life-long immunity. Recent studies have showed that re-infection with HCV after spontaneous clearance of the virus and sur-infection are quite common events among IDUs [21–23]. Therefore seroprevalence data can be considered as a measure for seroconversion but they underestimate the incidence or the rate of spread of HCV among IDUs.

Several recent studies have demonstrated how coalescent theory can be used to infer the past epidemic growth of HCV infection [24–27]. A fundamental result of coalescent theory is the finding of a relationship between coalescent time and population size. For any two sequences drawn from a population, the probability that they coalesce at a given point in history is a function of population size. Thus, a change in the estimated number of HCV infections over time can then be...
used to infer the growth rate and the basic reproductive number \( R_0 \). The latter is defined as the number of secondary infections caused by an infectious person in an entirely susceptible population.

In previous studies it has been shown that HCV exchange of European IDU populations has occurred on a large scale, although regional differences were observed, with for example strains from London being the most phylogenetically dispersed [28,29]. The phenomenon of international travelling and international contacts between IDUs should be kept in mind when developing prevention programmes.

In the present study new sequence data were obtained from two geographically distinct populations of IDUs in Flanders, Belgium. These data were used to infer the epidemic and migration history of HCV subtypes 1a and 3a among IDUs in Flanders, Belgium. First, the degree of transmission of HCV among IDUs between two regions in Flanders and across international borders was investigated. Secondly, the epidemic history of HCV among IDUs in Flanders was estimated.

**METHODS**

**Study subjects and samples**

For this study, 155 serum samples, stored at −80 °C from IDUs infected with HCV were available. Eighty of them were genotyped by the use of the line probe assay (LIPA HCV; Innogenetics, Zwijndrecht, Belgium) with genotype 3 and 75 strains. All genotype 3 strains were found to belong to subtype 3a. Sixty-eight of the 75 genotype 1 strains were subtyped 1a.

RNA isolation

RNA was extracted from 500 or 1000 µL of serum by using the QIAamp Ultrasens Virus Kit (Qiagen, Westburg, Leusden, the Netherlands) in accordance with the manufacturer’s instructions. All RNA samples were stored at −80 °C until amplification.

RT-PCR

Techniques described by Cochrane *et al.* were used [29]. In brief, two overlapping fragments in the NS5B region of the HCV genome were reverse-transcribed and amplified by means of the ACCESS reverse transcriptase-polymerase chain reaction (RT-PCR) kit (Promega, Benelux BV, Leiden, the Netherlands) from 10 µL of RNA according to the manufacturer’s instructions. Thermocycling conditions were one cycle of 48 °C for 45 min; one cycle of 94 °C for 2 min; 40 cycles of 94 °C for 30 s, 45 °C for 1 min and 68 °C for 2 min; and final extension at 68 °C for 6 min. A second nested PCR was performed with Taq polymerase (Promega).

Thermocycling conditions were 25 cycles of 94 °C for 36 s, 50 °C for 21 s and 72 °C for 1.5 min and final extension at 72 °C for 6 min. Sequences from nucleotide (nt) 8293 to nt 8997 (genotype 1a) and from nt 8295 to nt 8986 (genotype 3a) were used for construction of phylogenetic trees (positions numbered as for HPCPLYPRE, GenBank accession no. M62321).

**Sequencing**

Purified PCR products were obtained by use of the QIAquick purification Kit (Qiagen). Both sense and antisense strands were cycle-sequenced by use of the BigDye Terminator system version 3.1 (Applied Biosystems, Lennik, Belgium) with the same primers that were used for the nested PCR. Analysis of the sequencing product was performed using the ABI Prism 3100 (Applied Biosystems, Lennik, Belgium). GenBank accession numbers for the sequences presented are DQ363039–DQ363130.

**Phylogenetic analysis**

The obtained sequences were aligned by using ClustalW multiple alignment, manually edited and gap-stripped [31]. The model of substitution, base frequencies, gamma distribution, invariant sites, transition/transversion ratio and rate matrix values were determined by use of Modeltest 3.06 [32]. Maximum likelihood (ML) phylogenetic trees were constructed by using Paup 4.0. The general time-reversible model was used with and without the molecular clock enforced. The genealogies estimated with the molecular clock enforced served as the input for the estimation of epidemic histories and demographic parameters. Bootstrap analysis (1000 replicates) was performed on each ML tree. Trees were visualized with use of Treeview software. Subtyping of the HCV strains was performed by phylogenetic analysis with a broad panel of reference strains. All genotype 3 strains were found to belong to subtype 3a. Sixty-eight of the 75 genotype 1 strains were subtyped 1a.

**Analysis of isolation and migration of HCV sequences**

The degree to which HCV circulating in one Flemish region was phylogenetically distinct from HCV circulating in the other Flemish region or in other European regions was measured in terms of the association index (AI), as described elsewhere [29,33]. In brief, the degree of phylogenetic mixing of defined groups (in this case, sequences from different geographic regions) is scored in a phylogenetic tree. This value is then compared with the score from a tree with the same topology, but with the geographic regions randomly allocated to the tips of the tree. The ratio from the observed score to the control score produces the AI. AI values approaching zero represent almost complete segregation of
sequences, whereas values equal to or above 1 suggest no more segregation of sequences between the regions than would be expected by chance.

**Estimation of epidemic history and demographic parameters**

The estimation of the epidemic history of HCV among IDUs involved two steps. First, several models of demographic history including constant population size, exponential growth, logistic growth, expansion growth infections, piecewise expansion growth and piecewise logistic growth, were compared to select the best-fit model. The demographic models were evaluated by the likelihood ratio test from likelihoods calculated by the program GENIE 3.1, as described elsewhere [24]. Secondly, the demographic and evolutionary parameters of the epidemic, together with their confidence intervals, were estimated using the Bayesian skyline plot as implemented in the program Beast 1.3 [34]. The Bayesian skyline plot uses a Markov chain Monte Carlo (MCMC) procedure to sample the distribution of generalized skyline plots, given a set of sampled gene sequences, and then combine these plots to generate a posterior distribution of effective number of infections through time. Each MCMC was run for 10,000,000 states and sampled every 1000 states. A graphical representation of the effective number of infections through time was generated using the program TRACER (http://evolve.zoo.ox.ac.uk/software.html?id=tracer). To generate results on a timescale of years, evolutionary distance was converted into time (years) using the evolutionary rate estimated by Pybus et al. [24,25]. The estimated rate was $4 \times 10^{-4}$ substitutions per site per year (95% confidence intervals $3 \times 10^{-4}$ to $5 \times 10^{-4}$), $R_0$ was calculated from $r$ using the equation $R_0 = r \times D + 1$, where $D$ represents the duration of infectiousness, as described by Pybus et al. [24,25]. For the latter two analyses, sequences obtained from IDUs residing in France and the UK were included (GenBank accession nos AF516368–AF516395, AY100123–AY100176, AY100178–AY100187, AY100037–AY100082, AY100084–AY100102, AY100104, AY100107–AY100113).

**RESULTS**

**Subject characteristics**

The mean age of the Belgian IDUs included in this study was 33.5 years (±6.8). The mean age at initiation of injecting drug use was 21.7 years (±6.9). The mean duration of injecting was 12.4 years (±7.2). The success rate of amplification of the NS5B region was 66% (45 of 68 samples) for genotype 1a. Amplification success rate for genotype 3a amounted to 62% (47 of 80 samples). Samples for which amplification failed tended to be those with a low viral load.

In general a viral load of at least 500 IU/mL was required for successful amplification.

**Analysis of isolation and migration and isolation of HCV sequences among regions**

Figure 1 shows the phylogenies of the HCV subtype 1a and 3a HCV sequences obtained in the present study. The HCV subtypes 1a and 3a phylogenetic trees were constructed by means of the general time reversible model with correction for rate variation across sites and for invariant sites. For both subtypes clusters containing sequences only from one region as well as clusters containing sequences from Antwerp and Limburg can be found. This finding indicates that the HCV population in Limburg IDUs is not completely distinct from that among Antwerp IDUs and that either transmission between regions or independent introduction of the same virus in both regions did occur.

The degree of phylogenetic segregation was further explored by calculation of AI values (Table 1). Sequences obtained from IDUs from elsewhere in Europe (France and the UK) were included in this analysis in order to investigate, in addition to transmission between regions in Flanders, the extent of transmission across international borders. All of the AI values were lower than 1. An association index of 1 or more correlates with complete mixing or no more segregation than could be observed by chance. The null hypothesis of no difference between observed and control values compared was rejected for both HCV subtypes (Wilcoxon signed rank test, $P < 0.0001$). A moderate degree of segregation was observed for subtype 3a between strains from Limburg and Antwerp and for the other pairs of regions containing Limburg. Less transmission of HCV 3a strains seemed to have occurred between IDUs in Antwerp and IDUs in France and the UK. The degree of phylogenetic mixing observed for subtype 1a was in general less than that for subtype 3a.

The relationship between HCV subtype and the phylogenetic segregation of HCV between regions was studied by plotting of the AI of subtype 1a against the AI of subtype 3a for each pair of regions. No linear relation could be found (Spearman’s rank $\rho = -0.314; P = 0.5$), indicating that transmission patterns across regions among IDUs differ for both subtypes. A trend was observed towards greater segregation in genotype 1a, however this trend was not statistically significant (Wilcoxon signed rank test, $P = 0.25$).

**Analysis of the epidemic history of HCV in Flanders**

The epidemic history of HCV was first inferred based on strains sampled among Belgian drug users only. As it was shown in the previous section that the epidemic of HCV among IDUs in Flanders is not isolated from the epidemic among IDUs elsewhere in Europe, this analysis was repeated in a second instance including HCV strains sampled among drug users in the UK and France. Estimates of the effective
Fig. 1 Unrooted maximum likelihood trees for HCV NS5B sequences derived from IDUs in Flanders, Belgium. The positions of sequences of IDUs from Limburg are indicated with an asterisk.

Table 1 Association values and indices between different geographic regions for hepatitis C subtypes 1a and 3a

<table>
<thead>
<tr>
<th>Regions compared</th>
<th>Median association value</th>
<th>Observed trees</th>
<th>Control trees</th>
<th>Association index</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antwerp and Limburg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV1a</td>
<td>1.30</td>
<td>4.59</td>
<td>0.29</td>
<td>0.175–0.403</td>
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</tr>
<tr>
<td>HCV3a</td>
<td>1.13</td>
<td>2.29</td>
<td>0.49</td>
<td>0.131–0.777</td>
<td></td>
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<tr>
<td>Antwerp and UK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV1a</td>
<td>2.63</td>
<td>10.9</td>
<td>0.25</td>
<td>0.112–0.891</td>
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</tr>
<tr>
<td>HCV3a</td>
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<td>0.16</td>
<td>0.067–0.251</td>
<td></td>
</tr>
<tr>
<td>Antwerp and France</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV1a</td>
<td>2.20</td>
<td>6.54</td>
<td>0.33</td>
<td>0.148–0.470</td>
<td></td>
</tr>
<tr>
<td>HCV3a</td>
<td>1.64</td>
<td>5.98</td>
<td>0.28</td>
<td>0.144–0.405</td>
<td></td>
</tr>
<tr>
<td>Limburg and UK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV1a</td>
<td>2.00</td>
<td>4.93</td>
<td>0.21</td>
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<tr>
<td>HCV3a</td>
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<td></td>
</tr>
<tr>
<td>Limburg and France</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HCV1a</td>
<td>0.47</td>
<td>3.95</td>
<td>0.14</td>
<td>0.043–0.289</td>
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<td>HCV3a</td>
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<td>1.80</td>
<td>0.48</td>
<td>0.144–0.731</td>
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</tr>
<tr>
<td>UK and France</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV1a</td>
<td>2.70</td>
<td>7.41</td>
<td>0.37</td>
<td>0.136–0.598</td>
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<tr>
<td>HCV3a</td>
<td>2.59</td>
<td>6.68</td>
<td>0.38</td>
<td>0.218–0.513</td>
<td></td>
</tr>
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</table>
numbers \( (N_t) \) of HCV infections are shown in Fig. 2 (Belgian data set) and Fig. 3 (extended data set). The estimates represent the time of divergence of the sampled viruses to the time of the sampling year (2000).

The HCV 1a Belgian data set seems to best fit an expansion growth model. However, a simpler model of exponential growth model cannot be rejected \( (LRS = 0) \). HCV 1a strains sampled from IDUs in Flanders, Belgium have a common ancestor around 1850. The extended data set best fits an expansion growth model \( (LRS = 5.65, \ P < 0.25) \). The most recent common ancestor for the extended data set is situated around 1880.

Both the HCV 3a data sets best fit a logistic growth model \( (LRS = 19.2, \ P < 0.01 \) for the Belgian data set and \( LRS = 32.4, \ P < 0.005 \) for the extended data set). The divergence time of the most recent common ancestor for HCV 3a strains was estimated around 1890 and 1825 for the Belgian and extended data set respectively. Estimates of \( r \) which is the growth rate achieved during the exponential growth phase and the basic reproductive numbers \( (R_0) \) are given in Table 2.

DISCUSSION

In this study new subtype 1a and 3a HCV strains sampled in 2000 from two geographic distinct populations of IDUs in Flanders were used to infer information about the epidemiology of HCV in space and over time among IDUs in Flanders. It should be noticed that the results of this study describing the epidemic behaviour of HCV in these two populations of IDUs might not be representative of all populations of IDUs in Belgium.

It has been shown in this study that transmission of HCV between different IDU populations within Flanders and across the Flemish borders does occur on a moderate scale. This confirms the results of studies by Cochrane et al. and van Asten et al. \[28,29\]. Both studies demonstrated the existence of virus exchange between populations of IDUs within and across national borders. However, whereas Cochrane et al. \[29\] found similar patterns of geographic spread among populations for both subtypes 1a and 3a, this was not the case in this study. No positive linear correlation between the AIs for both subtypes and a trend towards more segregation for genotype 1a were found. This could reflect the existence of subpopulations of IDUs in Flanders with different degrees of contact with other populations of IDUs, with subtype 3a being predominant among the less isolated subgroups. This is in accordance with that found in an earlier study by Matheï et al. where the distributions of genotypes among IDUs in Limburg and Antwerp were compared in relation to behavioural and demographic characteristics of the IDs \[35\]. In this study it was
demonstrated that genotype 1 was predominant in the mixed urban–rural Limburg population of IDUs and genotype 3 in the urban Antwerp population. The latter was characterized by more pronounced drug-related risk behaviour, more social problems and more foreign nationalities than the Limburg population [30].

Following previous studies the epidemic history for HCV subtype 1a and 3a circulating among Flemish IDUs was estimated [24–26,36,37]. Both subtypes showed an exponential growth during the 20th century.

Based on the Belgian data set a steady state of the HCV 3a epidemic was observed in the middle of the century, preceding the onset of injecting drug use in Belgium between 1960 and 1970 [38]. The same was observed for the extended database; however in this case the steady state was reached only in the early 1970s. The epidemic curve previously estimated by Pybus et al. based on strains from IDUs was also suggestive of a decrease in growth rate however of much more recent date and this finding was not statistically significant [25]. Their analysis included the same strains from IDUs residing in France and the UK used in this study plus strains from IDUs from Australia. It seems therefore that the exponential growth phase of the HCV type 3a epidemic among IDUs occurred mainly in populations outside Flanders and that at the start of the habit of injecting drug use in Belgium HCV subtype 3a was introduced on multiple occasions among the Flemish IDUs, where after soon an endemic state was reached.

The estimated epidemic curves of both the Belgian and extended database show that the HCV 1a epidemic has not
reached a steady state yet and that the number of HCV 1a infections among IDUs is still increasing. This in contrast to HCV 3a for which a steady state seems to have been reached. One can thus expect, based on these observations, that the HCV 1 genotype will become the predominant HCV subtype among IDUs. As, HCV genotype 3 infections are characterized by a significantly better therapeutic response when compared with HCV genotype 1 infections, this trend might be harmful in the long term with respect to the disease burden due to hepatitis C among IDUs.

Estimates of the growth rates of the exponential phase of the epidemics were found to be significantly different for both subtypes, representing a doubling of the number of HCV 1a infections approximately every 15 and 10 years and a doubling rate of HCV 3a infections approximately every 4 and 5.5 years for the Belgian and extended database respectively. This finding is in sharp contrast with what was demonstrated by Pybus et al. [25]. They found similar growth rates for both subtypes with a doubling time of the number of infections of about 7 years. There exists no evidence so far to the effect that subtype 3a might be more infectious than subtype 1a. These findings therefore rather suggest that among IDUs the subtypes might have been predominantly circulating in different subgroups with different risk behavioural profiles, subtype 3a being predominant among IDUs with high-risk behaviour. However, this does not explain the observed decrease of growth rate of subtype 3a in the second part of the last century. The reduction in growth rate of subtype 3a might in part be explained by reduction in risk behaviour through a growing awareness of the risks and the implementation of harm-reduction measures, e.g. needle-exchange. However, one would expect a similar decrease of the growth rate of subtype 1a. Another hypothesis relies on a recent publication by Herring et al. reporting frequent super-infection (20%) with HCV in young IDUs [22]. HCV super-infection was observed between viral strains belonging to the same, as well as different, genotypes. Subtype-specific differences in viral fitness might exist favouring in case of a co- or super-infection involving subtype 1a and subtype 3a, the survival of subtype 1a at the expense of subtype 3a. The latter could be an explanation for the observation that the number of HCV 1a infections is still rising while HCV 3a infections are not. The study of Laskus et al. supports part of this hypothesis [39]. They followed up patients co-infected with different HCV genotypes through several transfusions administered in a single day over time. Their observations were compatible with the presence of competition among infecting HCV strains, resulting in the dominance of one strain and exclusion or suppression of other strains. Furthermore, for subtype 1a a significantly lower growth rate was observed considering the Belgian database when compared with the extended database, indicating that HCV subtype 1a has been spread less rapidly among Belgian IDUs than among IDUs in France and the UK. This confirms the existence of a variation of transmission dynamics of hepatitis C among IDUs.

The values of $R_0$ presented in this paper vary depending on the value of the duration of infectiousness (10–25 years) and the database considered for subtype 1a around 2 and for subtype 3a around 3–4. From $R_0$ one can estimate the equilibrium prevalence lying between 33.3% and 75% for values of the $R_0$ varying between 2 and 4. These rather low

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### Table 2

<table>
<thead>
<tr>
<th>HCV subtype</th>
<th>Number of sequences</th>
<th>Exponential growth rate (95% CI)</th>
<th>D</th>
<th>$R_0$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgian data set</td>
<td>45</td>
<td>0.045 (0.025–0.068)</td>
<td>10</td>
<td>1.45 (1.25–1.68)</td>
</tr>
<tr>
<td></td>
<td>Extended data set</td>
<td>122</td>
<td>0.061 (0.043–0.081)</td>
<td>10</td>
</tr>
<tr>
<td>3a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgian data set</td>
<td>47</td>
<td>0.191 (0.118–0.315)</td>
<td>10</td>
<td>2.91 (2.18–4.15)</td>
</tr>
<tr>
<td></td>
<td>Extended data set</td>
<td>134</td>
<td>0.126 (0.105–0.148)</td>
<td>10</td>
</tr>
</tbody>
</table>

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estimates do not seem to be in accordance with the empirical observations of HCV prevalence among IDUs [25]. Many populations of IDUs exhibit HCV seroprevalence rates as high as 80–90% [40]. However, a slightly different picture appears when taking into account the following. First, it is possible to recover spontaneously from an HCV infection. Whereas it was for long time accepted that spontaneous recovery of an hepatitis C infection only occurred in about 15–20% of the cases, recent publications show that up to 40–50% of the acute HCV infections evolve to spontaneous clearance of the virus [23,41–43]. Secondly, by definition in all analysis in this paper only chronic HCV infections are considered except perhaps for a few rare acute infections, as in the case of resolved infections no HCV-RNA is available. Therefore, the estimates of growth rate and $R_0$ only concern HCV infections resulting in a chronic infection, whereas prevalence rates represent a measure for all infections, the ones resulting in a chronic infections as well as the ones spontaneously cleared. This might, at least in part, explain why the estimated values of $R_0$ seem lower than one intuitively expects considering the observed high HCV prevalence rates. It has been demonstrated that infection with HCV does not confer absolute immunity against subsequent HCV infections either with heterologous genotypes or with homologous genotypes. Moreover, it has been demonstrated in two recent studies that re-infections with HCV after recovery of an earlier infection and super-infections are common events among IDUs [21,22]. The possibility of the occurrence of secondary infections would not change $R_0$ but one would expect the endemic prevalence of chronic infections to be even higher than in the case of lifelong immunity [44].

Another empirical observation involves the fact that infection with HCV seems to be acquired soon after onset with injecting drug use [20,45]. Again in this situation one would expect higher values of $R_0$ and the endemic state in a static population to be reached very rapidly. Yet, it has been hypothesized that young inexperienced IDUs exhibit more high-risk behaviour than do the more experienced elder ones. This was confirmed by a study by Matheı¨et al. who estimated the force of infection, defined as the per capita rate at which one becomes infected, based on seroprevalence data derived from Belgian IDUs [20]. They found the force of infection to be very high during the first year after onset of injecting drug use where after it rapidly decreased but never reached zero. The existence of subgroups of IDUs exhibiting high-risk behaviour and consequently high values of $R_0$ for HCV, however, does not exclude low to moderate overall values of $R_0$ for the total population.

Our $R_0$ estimates are somewhat lower for HCV subtype 1a and somewhat higher for HCV 3a than those estimated by Pybus et al. They argued that transmission of HCV among IDUs has probably been constrained by IDU population size, such that the $R_0$ estimates reflect the growth of the IDU population rather than the transmission potential of the virus. This hypothesis is not contradictory to the one presented in this paper and both might even be complementary.

To conclude, this study showed that virus exchange between distinct populations of IDUs within Flanders as well as across national borders has occurred. The dissimilarity of the pattern and extent of segregation between subtypes 1a and 3a suggest that both subtypes have been predominantly circulating in separate transmission networks exhibiting different degrees of travelling/migration. Furthermore, investigation of the viral history of HCV subtypes 1a and 3a among IDUs in Flanders gave evidence of different dynamic forces driving both epidemics. It seems that the exponential growth of the HCV subtype 3a epidemic among IDUs mainly occurred in populations of IDUs outside Flanders, Belgium. In contrast to the HCV 1a epidemic, the HCV 3a epidemic among IDUs seems to have reached a steady state, indicating that HCV 1a might become the dominant subtype among IDUs. Finally, it was showed that the rather low estimates of $R_0$ using coalescent theory are not inconsistent with the epidemiological reality of hepatitis C among IDUs, if one takes into account that a substantial part of the HCV infections among IDUs do resolve spontaneously and that re-infections are possible. The $R_0$ estimates in this study thus represent the $R_0$ for HCV infections leading to a chronic infection rather than for all HCV infections among IDUs.

**REFERENCES**


