Trends in hepatitis C virus infections among MSM attending a sexually transmitted infection clinic; 1995–2010

Anouk T. Urbanus[a,e,f], Thijs J.W. Van De Laar[b], Ronald Geskus[a,c], Joost W. Vanhommerig[a], Martijn S. Van Rooijen[a], Janke Schinkel[d], Titia Heijman[a,f], Roel A. Coutinho[e,g] and Maria Prins[a,f]

Background: Since 2000, there is growing evidence that hepatitis C virus (HCV) infection has emerged as a sexually transmitted infection (STI) among HIV-positive MSM. Here, we present a 15-year overview of the HCV epidemic among MSM visiting a large STI-clinic in the Netherlands.

Methods: During biannual cross-sectional anonymous surveys (1995–2010), participants were interviewed and tested for HIV and HCV-antibodies. Additional HCV RNA tests were performed in all HIV-positives. Determinants of HCV infection were analysed using logistic regression. Phylogenetic analysis provided evidence for sexual transmission.

Results: HCV prevalence among HIV-positive MSM increased from 1995 onwards (5.6%) and peaked in 2008 (20.9%). Prevalent HCV infection was more strongly associated with fisting in 2007–2008 [adjusted odds ratio (aOR) 2.85, 95% confidence interval (CI) 1.19–6.82] than in 2009–2010 (aOR 0.92, 95% CI0.42–2.02). In addition, HCV infection was independently associated with Chlamydia, injecting drug use, unprotected anal intercourse and older age. Phylogenetic analysis revealed a high degree of MSM-specific clustering from 2000 onwards. Identification of a new MSM-specific HCV lineage and the finding of recent HCV infections (0–4%) in established HCV clusters during recent years argue for ongoing transmission of HCV among HIV-positive MSM. HCV prevalence among HIV-negative MSM remained low (2007–2010: 0.5%).

Conclusion: HCV prevalence among HIV-positive MSM significantly increased over calendar time but appears to level off in recent years, possibly due to increased awareness, saturation in the population, decreased risk behaviour and earlier HCV screening and treatment. The association with fisting became less strong over time, but our analyses continue to support sexual transmission. Monitoring HIV-positive and HIV-negative MSM for HCV infection remains needed to guide prevention efforts.

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[a]Amsterdam Public Health Service, Cluster of Infectious Diseases, [b]Sanquin Blood Supply Foundation, Department of Bloodborne Infections, [c]Academic Medical Center (AMC), Department of Clinical Epidemiology, Biostatistics and Bioinformatics, [d]AMC, Department of Clinical Virology, Amsterdam, [e]National Institute for Public Health and the Environment, Center for Infectious Disease Control, Bilthoven, [f]AMC, Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS (CINIMA), Amsterdam, and [g]Julius Center for Health Science and Primary Care, University Medical Center, Utrecht, The Netherlands.

Correspondence to Anouk Urbanus, Amsterdam Public Health Service, Cluster of Infectious Diseases, Department of Research, P.O. Box 2200, 1000 CE Amsterdam, The Netherlands.

E-mail: aurbanus@ggd.amsterdam.nl
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Introduction

Infection with hepatitis C virus (HCV) is transmitted primarily by blood and occurs frequently in IDUs [1]. Since 2000, acute HCV infections have increasingly been reported among HIV-positive MSM in Europe, Australia and the United States [2–9]. Phylogenetic analysis has confirmed the existence of MSM-specific HCV transmission networks. The absence of reported parenteral routes of transmission suggests that most of these infections were acquired sexually [2,5,6,9–11].

HCV incidence data from HIV-seroconverter cohorts (CASCADE) and molecular-clock calculations based on circulating MSM-specific HCV strains indicate that the HCV-epidemic among HIV-positive MSM in Europe arose with the introduction of combination antiretroviral therapy (cART) for HIV in 1996, with a substantial increase after 2002 [11–13].

In the Netherlands, an alarming HCV prevalence of 17% among HIV-infected MSM was found in a biannual cross-sectional survey conducted in 2007–2008 at a large city sexually transmitted infection (STI) outpatient clinic in Amsterdam [14]. Phylogenetic analyses revealed multiple MSM-specific HCV clusters of genotypes 1a and 4d. HCV infection was independently associated with HIV infection, IDU, fisting and noninjecting recreational drug use [14].

Adequate longitudinal data on acute and chronic HCV infections among HIV-positive and HIV-negative MSM are needed to determine the past and current state of this epidemic, reveal possible causes and to plan and evaluate prevention and case-finding strategies. In the Netherlands, our first study on HCV spread in HIV-infected MSM [14] led to prevention measures focused on the sexual transmission of HCV. Even before publication, our finding of a high HCV prevalence spurred the Amsterdam STI clinic to start routine HCV-antibody testing for all MSM clients with positive or unknown HIV status [15].

To put our earlier results on the HCV epidemic among HIV-positive MSM (2007–2008) [14] in a larger time-frame, we retrospectively tested stored serum samples of HIV-infected MSM participating in the same biannual cross-sectional survey at the Amsterdam STI clinic during the period 1995–2004 and complemented the dataset with newly recruited HIV-positive and HIV-negative MSM participating in the period 2008–2010. Phylogenetic analysis was used to gain insight into transmission networks and the emergence of MSM-specific HCV clusters.

Materials and methods

Participants

Since 1991, biannual cross-sectional and anonymous HIV surveys have been performed at the STI outpatient clinic in Amsterdam [16]. Each spring and autumn, consecutive clinic visitors (heterosexual, bisexual and homosexual) are asked to participate until 1000 are included, of whom about 22–25% are MSM. Upon informed consent, their blood is drawn, tested and stored. As participation is anonymous, multiple visits of one visitor cannot be linked and therefore the number of possible duplicate visits during the surveys is unknown. Participants are interviewed about risk factors for blood-borne and STI using a standardized questionnaire. Since 2007, the interview has addressed traditional HCV-related risk behaviour (e.g. blood transfusion prior to 1992) and HCV status. Over the years, the participation rate of the clinic population has varied between 65 and 95%, depending on changes in screening procedures. Looking at 2007–2010, characteristics (nationality, age, history of IDU, HIV status) of survey participants and nonparticipants were comparable, except for HIV status: participation was refused more often by HIV-positive MSM than by HIV-negative MSM ($P = 0.005$).

The present study includes HIV-infected MSM who took part in the biannual surveys of 1995–1996, 1999–2000, 2003–2004 and 2007–2010. A total of 831 HIV-positive MSM participated in 19 surveys (the spring survey of 2004 was not performed). We excluded 54 MSM who had no or insufficient serum samples available for HCV testing. Samples from the remaining 777 MSM were tested for the presence of both HCV antibodies and HCV RNA. In addition, we tested all HIV-negative MSM participating in the surveys from 2007 to 2010 ($n = 1513$) for anti-HCV: 358 from 2007, 383 from 2008, 400 from 2009 and 372 from 2010.

The biannual survey and basic data collection (e.g. age, sexual preference) were performed according to the STI clinic's standard protocol, described elsewhere [17,18]. All MSM were tested for *Chlamydia trachomatis*, *Neisseria gonorrhoea* and antibodies to *Treponema pallidum*. Since 2004, they have been tested for hepatitis B virus except when vaccinated or natural immunity has been previously documented. HIV testing was optional through 2006; since 2007, an opt-out system allows testing of all HIV-negative or HIV-unknown clients who do not expressly refuse it.

Laboratory testing

HCV antibody testing is performed using a third-generation commercial microparticle EIA system (AxSym HCV version 3.0; Abbott Laboratories, Abbott Park, Illinois, USA) with Immunoblot confirmation (Chiron RIBA HCV 3.0 SIA; Ortho-Clinical Diagnostics, Raritan, New Jersey, USA). All anti-HCV positive MSM are tested for HCV RNA using transcription-mediated amplification (TMA: VERSANT HCV RNA Qualitative Assay; Siemens Healthcare Diagnostics, Tarrytown, New York, USA), with a detection limit of 5 IU/ml.

Testing procedure and hepatitis C virus definition

As HIV-infected individuals may have prolonged windows of seroconversion [19–21], all HIV-positive participants of our surveys were screened for HCV RNA regardless of their HCV antibody status. Those confirmed positive for HCV antibodies and/or HCV RNA are considered HCV-positive. Detectable HCV RNA in the absence of HCV antibodies, or detectable HCV RNA in the presence of a weak anti-HCV response (Axsym ratio below 5) with negative or indeterminate Immunoblot is indicative for recent HCV infection. This definition of recent HCV infection was also used in our previous article in which a subset of these data has been described [14].

Hepatitis C virus RT-PCR, sequencing and phylogenetic analysis

HCV RNA isolation was performed on 100 μl of serum using the TriPure method (Roche Diagnostics, Indianapolis, Indiana, USA). A 436-nucleotide fragment of the HCV NS5B region was amplified and sequenced [22]. Viral genotype was determined by phylogenetic analysis of NS5B sequences obtained from the study participants, along with GenBank reference sequences [23]. HCV phylogenetic trees were constructed by the maximum-likelihood approach using the Hasegawa–Kishino–Yano substitution model with a γ distribution of among-site rate heterogeneity (HKY-Γ) implemented in PHYML 3.0 software [24]. Bootstrap values ($n = 1000$) were calculated to analyse the stability of the tree topology. Phylogenetic trees were constructed for HCV genotypes 1, 2, 3 and 4 separately.

Statistical analysis

Given two surveys per year, HCV prevalence was calculated per survey and not per year. To evaluate trends in HCV prevalence over time, outcomes were modelled via logistic regression with calendar year as a continuous variable, using restricted cubic splines [25]. The R statistical package, version 2.13.0, was used for these analyses [26].

Determinants of HCV infection were evaluated using logistic regression analysis restricted to data from 2007 to 2010 because data on behaviour before 2007 were not fully available. Correlations between the variables were examined using the Spearman correlation test. Multivariate logistic regression models were built, using backward stepwise techniques and considering variables with a univariate P value of 0.25 or less as potential independent determinants. To examine changes in effects over calendar time, we forced calendar time as a dichotomous variable into the model. In addition, we evaluated interaction between calendar time and the variables within the final model. A P value of less than 0.05 was considered statistically significant. For logistic regression analysis, SPSS 19.0 was used.

Characteristics of HIV-positive MSM

Among the 777 HIV-positive MSM we surveyed, the median age was 40 years [interquartile range (IQR) 34–47 years]; 71% were born in the Netherlands, and only 3.5% reported ever injected drugs. The median number of sex partners per lifetime was 200 (IQR 95–998). Data collected between 2007–2010 showed that 74% (383/517) used recreational noninjecting drugs [e.g. gamma hydroxy butyrate (GHB), XTC, cocaine] in the previous 6 months; questions added in 2008 have shown that 75% (287/383) of these MSM used such drugs shortly before or during sexual activities.

Hepatitis C virus prevalence in HIV-positive MSM

Of the 777 HIV-positive MSM in our study, 91 [11.7%, 95% confidence interval (CI) 9.6–14.2] tested positive for HCV antibodies and/or HCV RNA. Of the HIV/HCV-coinfected MSM, 10 out of 91 (11%) reported having ever injected drugs.

Among HIV-positive MSM, the observed HCV prevalence gradually increased from 0 to 5.6% in the 1995 surveys to 9.4% (3/32) in the second survey of 2003. In 2004, HCV prevalence increased to 13.3% (4/30) and increased further to 20.9% (14/67) in the first survey of 2008. In 2010, our last survey year, HCV prevalence again decreased to 10.3% (15/146) (see Table 1 and Fig. 1).

Overall, HCV prevalence among HIV-positive MSM increased from 1995–1996 until 2007. Prevalence was significantly higher ($P < 0.001$) in 2007 than in the first survey period tested (1995/1996), with an odds ratio (OR) of 9.54 (95% CI 2.65–34.3). Although HCV prevalence seems to decrease after 2008, HCV prevalence in 2010 was not statistically lower than in 2008 ($P = 0.08$; OR 0.57, 95% CI 0.27–1.17).

Hepatitis C virus prevalence in HIV-negative MSM

Of the HIV-negative MSM, 10/1513 (0.6%) tested positive for anti-HCV from 2007 through 2010. In 2007, the biannual surveys yielded a prevalence of 0.5% (1/195) and 0.6% (1/163); in 2008, 0% (0/174) and 0.5% (1/209); in 2009, 1.7% (4/234) and 0.6% (1/166), and in 2010, 1.1% (2/178) and 0% (0/194). There was no significant calendar time effect found in HCV prevalence among HIV-negative MSM ($P = 0.54$).

Recent hepatitis C virus infection among HIV-positive MSM

Of the 91 HCV/HIV-positive MSM, 14 (15.3%) were defined as having a recent HCV infection (i.e. anti-HCV negative and HCV RNA-positive). During the study period, the percentage of HIV-positive MSM with recent infections fluctuated between 0 and 4%, and we did not find a significant increase or decrease in the number of recent infections over time (lower dashed line, Fig. 1)

Table 1. Hepatitis C virus genotype distribution among HIV-positive MSM attending the sexually transmitted infection clinic surveys.

Number tested	1995 ($n=36$)	1996 ($n=41$)	1999 ($n=42$)	2000 ($n=58$)	2003 ($n=53$)	2004[c] ($n=30$)	2007 ($n=90$)	2008 ($n=142$)	2009 ($n=139$)	2010 ($n=146$)	Total ($n=777$)
HCV+[a]	1 (2.8%)	1 (2.4%)	0	3 (5.2%)	3 (5.7%)	4 (13%)	14 (16%)	26 (18%)	24 (17%)	15 (10%)	91 (12%)
RNA+[b]	1 (100%)	1 (100%)	0	2 (67%)	2 (67%)	3 (75%)	10 (71%)	22 (85%)	14 (58%)	9 (60%)	64 (70%)
1a	0	0	0	0	1 (50%)	1 (33%)	8 (80%)	15 (68%)	11 (85%)	5 (63%)	41 (69%)
1b	0	0	0	0	0	0	0	1 (5%)	0	1 (13%)	2 (3%)
2b	0	0	0	0	0	0	0	1 (5%)	0	0	1 (2%)
3a	0	0	0	0	0	0	1 (10%)	2 (9%)	0	0	3 (5%)
4d	0	0	0	1 (100%)	1 (50%)	2 (67%)	1 (10%)	3 (14%)	2 (15%)	2 (25%)	12 (20%)
Undetermined	1	1	0	1	0	0	0	0	1	1	5

HCV, hepatitis C virus.
[a]Positive for anti-HCV and/or HCV RNA, % calculated from the number of HIV-positive MSM.
[b]HCV RNA-positive, % calculated from the number of coinfected MSM.
[c]Only one of the usual two surveys was performed in 2004.

(1995: $n=0$, 1996: $n=1$, 1999: $n=0$, 2000: $n=1$, 2003: $n=1$, 2004: $n=1$, 2007: $n=2$, 2008: $n=5$, 2009: $n=0$, 2010: $n=3$).

It must be noted that due to the anonymous and cross-sectional design of the study, we were unable to identify sequential samples of one participant. A verified diagnosis of recent or acute HCV, meaning anti-HCV seroconversion in two sequential samples drawn 6 months apart, could therefore not be made.

Hepatitis C virus and risk behaviour among HIV-positive MSM

In univariate analysis, older age, number of sexual partners in lifetime, fisting (active and/or passive), ever injecting drugs, using GHB, Chlamydia diagnosis and unprotected anal intercourse (UAI, passive and active) were significantly associated with HCV infection in HIV-positive MSM in the period of 2007–2010 (Table 2).

Because GHB use might be a proxy for unmeasured sexual risk behaviour, we ran two separate multivariate models, one including the interaction between fisting and calendar time ($P=0.087$) and one including GHB use and calendar time ($P=0.025$). In both models, older age, ever-IDU, Chlamydia diagnosis and UAI were independently associated with HCV infection (Table 2). The OR for fisting was 2.85 (95% CI 1.19–6.82) in 2007–2008 but became less strong in 2009–2010 (OR 0.92, 95% CI 0.42–2.02). Likewise, the OR for GHB use declined

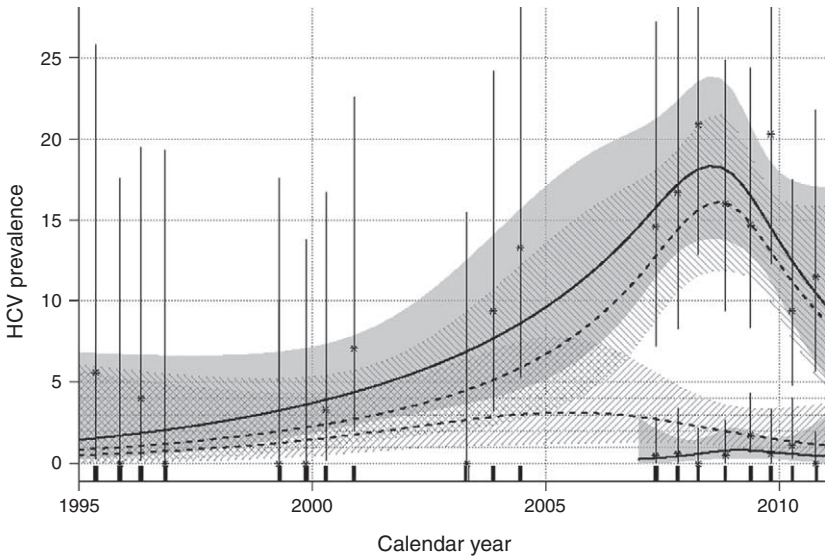

Fig. 1. Modelled and observed hepatitis C virus prevalence among HIV-positive and HIV-negative MSM included in the sexually transmitted infection clinic survey 1995–2005. Upper solid line: modelled HCV prevalence among HIV-positive MSM positive for anti-HCV and/or HCV RNA; gray area 95% confidence interval (CI). Upper dashed line: modelled HCV prevalence among HIV-positive MSM (positive for anti-HCV and negative for HCV RNA); gray stripes: 95% CI. Lower dashed line: modelled HCV prevalence among HIV-positive MSM negative for anti-HCV and positive for HCV RNA; gray stripes: 95% CI. Lower solid line: modelled HCV prevalence among HIV-negative MSM (positive for anti-HCV and/or HCV RNA); gray area 95% CI. Asterisks: observed HCV prevalence among HIV-positive and HIV-negative MSM including 95% CI. Bold bar on x-axis: time points of observed data (longer bars represent a longer survey period).

Table 2. Univariate and multivariate associations between risk behaviour, other characteristics and epatitis C virus infection among 517 HIV-positive MSM participating in the Amsterdam sexually transmitted infection clinic biannual surveys, 2007–2010.

	HCV status		2007–2010 Univariate analysis		2007–2010 Multivariate analysis (with fisting)		2007–2010 Multivariate analysis (with GHB use)	
	Negative ($N=438$)	Positive ($N=79$)	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age								
≤35 years	126 (28.8%)	9 (11.4%)	1	0.009	1	0.009	1	0.006
36–42 years	115 (26.3%)	30 (38.0%)	3.65 (1.66–8.02)		4.17 (1.67–10.4)		3.61 (1.56–8.31)	
43–48 years	100 (22.8%)	17 (24.5%)	2.38 (1.02–5.57)		2.16 (0.80–5.82)		2.08 (0.85–5.11)	
≥49 years	97 (22.1%)	23 (29.1%)	3.32 (1.47–7.50		4.00 (1.55–10.3)		4.17 (1.74–9.97)	
Number of sex partners over lifetime								
0–95	74 (16.9%)	6 (7.6%)	1	0.015				
96–200	61 (13.9%)	17 (21.5%)	3.44 (1.28–9.26)					
201–998	61 (13.9%)	18 (22.8%)	3.64 (1.36–9.74)					
>998	63 (14.4%)	15 (19.0%)	2.94 (1.08–8.02)					
Data missing[a]	179 (40.9%)	23 (29.1%)	1.59 (0.62–4.05)					
Calendar period and fisting[b]								
None reported 2007–2008	117 (30.8%)	18 (25.7%)	1	0.007	1	0.095		
Fisting reported 2007–2008	26 (6.8%)	14 (20.0%)	3.50 (1.55–7.93)		2.85 (1.19–6.82)			
None reported 2009–2010	171 (45.0%)	25 (35.7%)	1		1			
Fisting reported 2009–2010	66 (17.4%)	13 (18.6%)	1.35 (0.65–2.79)		0.92 (0.42–2.02)			
Calendar period and GHB use								
None reported 2007–2008	142 (33.0%)	15 (19.2%)	1	<0.001			1	0.001
GHB use reported 2007–2008	44 (10.1%)	25 (31.1%)	5.38 (2.61–11.09)				4.38 (2.03–9.48)	
None reported 2009–2010	176 (40.9%)	23 (29.5%)	1				1	
GHB use reported 2009–2010	68 (15.8%)	15 (19.2%)	1.69 (0.83–3.42)				1.18 (0.55–2.55)	
Ever IDU								
No	427 (97.5%)	70 (88.6%)	1	0.001	1	0.003	1	0.003
Yes	11 (2.5%)	9 (11.4%)	4.99 (1.99–12.48)		5.21 (1.72–15.73)		4.93 (1.73–14.05)	
Chlamydia found at visit								
No	369 (84.2%)	56 (70.9%)	1	0.05	1	0.014	1	0.019
Yes	69 (15.8%)	23 (29.1%)	2.20 (1.27–3.80)		2.27 (1.18–4.35)		2.10 (1.13–3.90)	
Unprotected anal intercourse[b]								
No	113 (27.3%)	7 (9.0%)	1	0.001	1	0.003	1	0.024
Yes	301 (72.7%)	71 (91.0%)	3.81 (1.70–8.26)		5.01 (1.74–14.4)		2.63 (1.14–6.10)	

CI, confidence interval; GHB, gamma hydroxy butyrate; HCV, hepatitis C virus; IDU, injection drug user; OR, odds ratio.
[a]Question asked only from October 2007 onwards.
[b]Active and/or passive.

from 4.38 (95% CI 2.03–9.48) to OR 1.18 (95% CI 0.55–2.55) in that period.

Genotyping and phylogenetic analysis

HCV RNA was detected in 64 out of 91 (70%) of HIV-infected MSM defined as HCV-positive. HCV genotyping and sequencing succeeded for 59 out of 64 (92%) of the HCV RNA-positive samples. HCV genotypes 1a (41/59; 69%) and 4d (12/59; 20%) predominated. Only six out of 59 (10%) were infected with genotypes 3a ($n=3$), 1b ($n=2$) and 2b ($n=1$) (Table 2).

Of the HIV-negative MSM, 10 out of 1513 (0.6%) tested positive for anti-HCV, of whom six out of 10 (60%) were positive for HCV RNA. These six were infected with HCV genotypes 1b ($n=4$), 1a ($n=1$) and 4d ($n=1$).

Phylogenetic trees were constructed for HCV genotypes 1, 2, 3 and 4 separately, and included 75 HCV sequences from HIV-positive MSM: 59 MSM from the STI-clinic cohort (see Table 1) and 16 MSM participating in the Amsterdam Cohort Studies (ACS) during the period 1985–2003 [6]. Of the MSM participating in the ACS, seven were diagnosed with acute HCV infection and nine had a prevalent HCV infection. Phylogenetic trees were also supplemented with 12 HCV sequences from HIV-negative MSM (six MSM from the STI-clinic cohort and six from the ACS) and 79 HCV sequences from acute HCV infections (including reinfection and superinfection) among 59 IDUs who seroconverted in the ACS in the period 1985–2005 [27]. ACS MSM samples were added to expand our sample and put our STI-clinic samples in a larger timeframe. ACS sequences of drug users were added to distinguish MSM-specific HCV strains from those circulating in other high-risk groups.

Phylogenetic analysis revealed five strongly supported monophyletic clusters (bootstrap >70) of MSM-specific strains that have no overlap with IDU-clusters containing a total of 53 HIV-positive MSM and one MSM without HIV; two homologous pairs of HCV sequences obtained from HIV-positive MSM and 28 singleton MSM sequences that were more closely related to HCV strains obtained from IDU than to other HCV strains circulating among MSM (Fig. 2). MSM-specific clusters ranged from three to 17 sequences; four clusters were subtype 1a and one subtype 4d.

Among HIV-positive MSM, phylogenetic clustering showed a distinct pattern over time (Table 3). None of the 11 HCV sequences from HIV-positive MSM before 2000, all obtained from the ACS, were part of an MSM-specific cluster. In contrast, eight of 11 (73%) and 45 of 53 (85%) HCV sequences from HIV-positive MSM in the periods 2000–2005 and 2006–2010, respectively, belonged to MSM-specific clusters (Fig. 2). The presence of five phylogenetically robust MSM-specific clusters is typical of independent parallel chains of transmission, each seeded by independent introductions of distinct HCV lineages in the Amsterdam MSM population over time. Among our participants, the earliest representatives of the five MSM-specific clusters were identified in 2000 for Cluster I, 2003 for Cluster II, 2007 for Clusters III and IV, and 2009 for Cluster V.

Interestingly, only one out of 12 (8.3%) HIV-negative MSM was part of an MSM-specific HCV cluster (Cluster II, genotype 4d), compared with 53 out of 75 (71%) HIV-positive MSM. The 11 HIV-negative MSM with non-MSM- specific sequences included eight MSM infected with HCV 1b, the most common genotype among unpaid blood donors in the Dutch population [28]. The three others each harboured a different genotype: 1a, 2b and 4d.

Discussion

This study examined the HCV epidemic among HIV-positive MSM in the period 1995–2010 and among HIV-negative MSM in the period 2007–2010. Among HIV-infected MSM, HCV prevalence significantly increased until 2007. After 2007, no further increase was observed. On the contrary, our data suggest a decrease after 2007, but this was not statistically significant. Previous studies suggest that the current HCV epidemic started between 1996 and 2000, with HCV incidence rising substantially since 2000–2002 [12,13], which is in line with our findings. The most recent estimates of HCV incidence include data from the Swiss HIV cohort study [29] and the MACS (Multicenter AIDS Cohort) in four metropolitan areas of the United States both up to 2011 [30]. These studies still suggest an increasing incidence in contrast to our finding of a levelling off of the epidemic. Larger incidence studies, including recent data among high-risk MSM, are needed to gain more insight into the ongoing HCV epidemic.

Our observation that the HCV prevalence in Amsterdam has levelled off in recent years might reflect an increased awareness among MSM, leading to reduced risk behaviour and increased uptake of early screening and treatment. In November 2007, routine HCV testing was introduced at the STI clinic and also HIV specialists intensified routine HCV screening with elevated ALT levels [31]. Alternatively, but less optimistic, the levelling off of the HCV prevalence might be a result of HCV saturation in the highest risk groups. In that case, the stabilizing epidemic would be explained by a lack of susceptible individuals rather than by decreased sexual risk behaviour among the population at risk. However, the effect of pronounced HCV risk factors such as fisting and GHB use became less strong over time, suggesting behavioural change and possibly increased awareness leading to less risky sexual practices. As our data did not

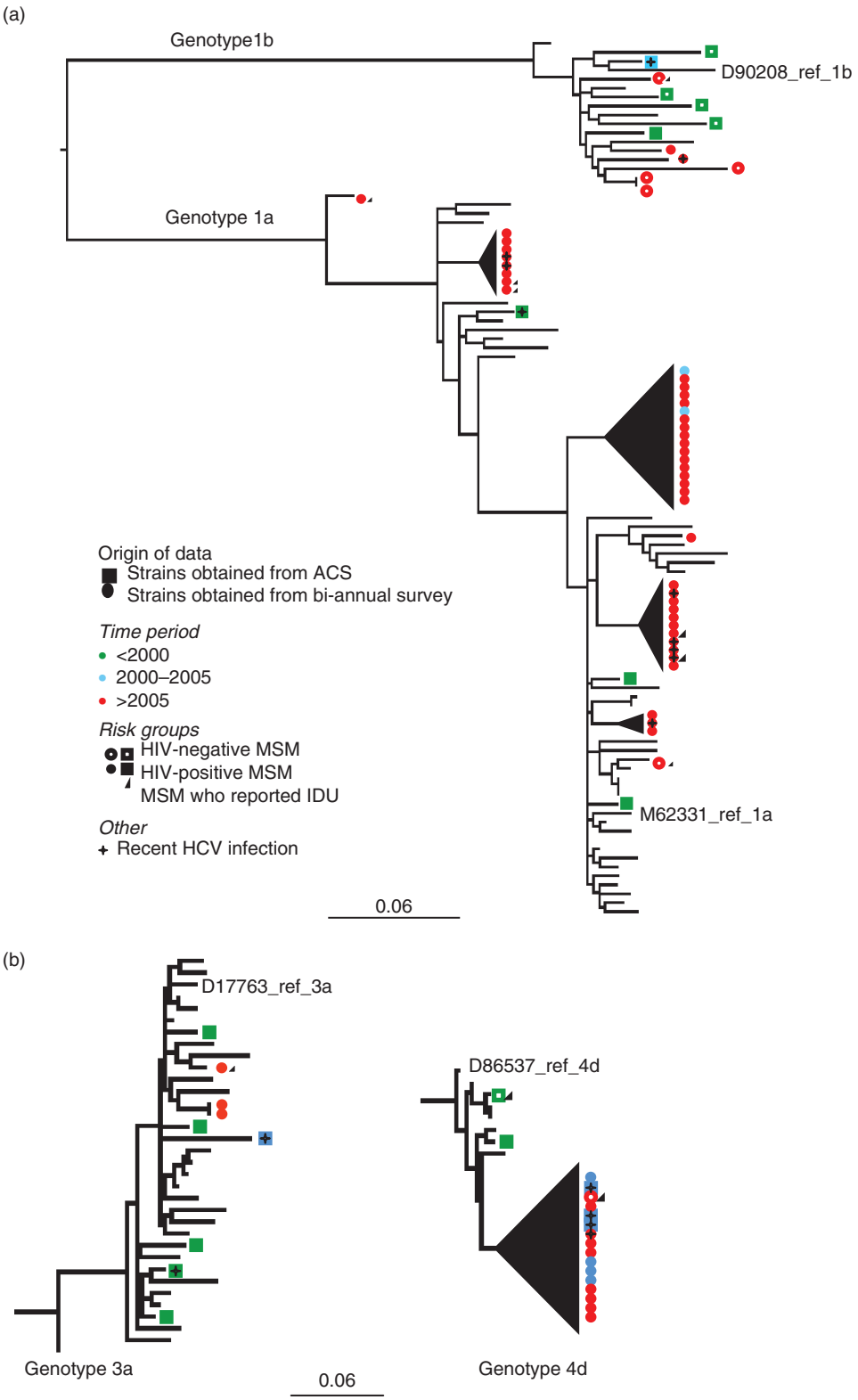

Fig. 2. Hepatitis C virus NS5B phylogenetic tree comparing HCV-infected MSM with and without HIV coinfection visiting the STI clinic in the period 1995–2010 along with HCV-infected MSM and IDU participating in the Amsterdam cohort studies (ACS). (a) Genotypes 1a and 1b. (b) Genotypes 3a and 4d.

Table 3. Distribution over time of MSM-specific hepatitis C virus strains in HIV-positive MSM from both sexually transmitted infection clinic surveys and the Amsterdam Cohort Studies (ACS).

	Number of isolates					
	<2000	2000–2002	2003–2004	2007–2008	2009–2010	Total
Cluster I (4d)	–	3	3	4	4	15[a]
Cluster II (1a)	–	–	2	5	10	17
Cluster III (1a)	–	–	–	10	1	11
Cluster IV (1a)	–	–	–	7	1	8
Cluster V (1a)	–	–	–	–	3	3
Unrelated sequence[b]	11	2	–	6	2	

[a]Total sequences in Cluster I is 15, as it includes one HIV-negative MSM.
[b]None of the sequences in these columns were linked to the MSM-specific clusters.

include the precise moment of infection, our study of risk behaviour was limited. It should preferably be studied in longitudinal studies on HCV incidence.

In an additional analysis, we estimated the annual HCV incidence according to an approach estimating the HIV incidence from data derived in cross-sectional surveys [32]. We found, consistent with our prevalence data, a peak in 2008 (estimated HCV incidence: 14.0/100 person-years; 95% CI 5.0–37.2), and no further increase thereafter, although no statistically significant time trend was found, probably as a result of small numbers (data not shown). It must be noted that the approach used to estimate HCV incidence gives rise to a number of uncertainties that might lead to overestimation as well as underestimation of the true HCV incidence in our population. Unfortunately, the anonymous and cross-sectional design of our study did not allow us to analyse sequential samples of one individual to estimate incidence.

In addition, chronic seronegative HCV infection has been described in HIV-infected individuals with low $CD4^+$ cell counts [19]. However, as HCV chronic antibody negative infection is not a common event [19,33] and our study period largely covers the cART era, we believe it is unlikely that time trends found are seriously biased. In addition, our study population was tested with a third-generation ELISA, which is more sensitive than a second-generation ELISA, and therefore we believe that it is highly unlikely that HCV seronegatives were missed [19,34].

Due to the anonymous character of STI clinic visits, in case MSM might have participated more than once in our surveys, we could not correct for multiple measurements per person in our analysis. However, this will not impact the effect estimates and we expect only minor bias in the estimated standard error and CIs, as we expect that most individuals are included only once.

Using a phylogenetic approach, we confirmed the presence of at least five MSM-specific HCV lineages circulating among HIV/HCV-coinfected MSM in Amsterdam. Over time, multiple independent introductions of HCV genotypes 4d and 1a into a susceptible MSM community have led to ongoing transmission of HCV among HIV-positive MSM. Identification of another new MSM-specific HCV lineage (Cluster V) and the continuous finding of recent HCV infection in established HCV clusters during recent years confirm the ongoing transmission of HCV among HIV-positive MSM. The introduction and ongoing circulation of multiple HCV lineages among HIV-positive MSM suggests that HCV emergence is caused by behavioural change, rather than evolution of one viral strain into a more virulent variant [13].

In contrast to a study from Australia [8], where half of the HIV/HCV coinfected MSM reported injection drug use, we found no overlap between MSM clusters and IDU clusters (data from the Amsterdam Cohort Studies). The percentage of coinfected MSM who reported IDU in the Netherlands was only 11% compared with the 50% in Australia. Moreover, in the Netherlands, the HCV epidemics among IDU and among HIV-positive MSM have occurred in separate timeframes. Drug injection behaviour severely declined as a result of harm reduction campaigns, eventually halting the HCV epidemic among IDU in Amsterdam with sustained low HCV incidence rates since the late 1990s [35]. In contrast, new HCV infections among MSM have occurred mainly after 2000. Australia has an ongoing HCV epidemic among IDUs, with new infections occurring simultaneously in both MSM and IDU. Together with the higher prevalence of injecting drugs reported by Australian MSM, this might explain the discrepant findings as to overlapping clusters [8,14].

Although HIV infection is not a prerequisite for sexually transmission of HCV [15], HIV-negative MSM remain largely unaffected by this outbreak. The HCV prevalence among HIV-negative MSM remained stable over time (0.6%) and was comparable to the HCV prevalence found in the general Amsterdam population [36]. Five out of six HCV strains obtained from HIV-negative MSM were neither closely related to strains circulating among HIV-positive men nor were they closely related to each

other, which is typical for isolated HCV infections acquired through unrelated transmission events. Nevertheless, the fact that one of the HCV-infected MSM without HIV was infected with an MSM-specific HCV strain (cluster I), suggests that some overlap with the sexual transmission networks affecting HIV-positive MSM does occur [15,37], and potential spillover to the HIV-negative community cannot yet be excluded.

In conclusion, HCV prevalence among HIV-positive MSM significantly increased until 2007, but appears to be levelling off, at least in Amsterdam. Reasons are unclear, but several factors may play a role, including reduced risk behaviour, increased HCV awareness among those at risk, enhanced screening and treatment uptake and saturation within the highest risk group. HCV prevalence among HIV-negative MSM remained stable and did not exceed the HCV prevalence among the general population in Amsterdam. The association with pronounced HCV risk factors such as fisting and recreational drug use declined over the years, but both risk factor analysis and phylogenetic analysis continue to support ongoing sexual transmission of HCV among HIV-positive MSM. Monitoring HCV prevalence and incidence remains important in order to follow trends and possible future epidemics. Coinfection with HCV among HIV-positive MSM has serious clinical implications [38] and early detection and treatment of HCV improve treatment outcome [39]. Unfortunately, the costs of HCV RNA screening might hamper adequate diagnosis of HCV in its earliest stage, as often HCV antibody testing is performed, which in HIV-positives might be false-negative for a prolonged period of time [21]. As factors shaping the current epidemic remain unclear, it remains needed to evaluate interventions to halt further transmission to HIV-negative MSM and others with lower risk profiles in the MSM community.

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Conflicts of interest

There are no conficts of interest.

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