HCV reference genome using BWA. Primary maximum likelihood phylogenetic analyses were performed using ClustalW and IQTREE to infer the phylogenetic relationships of the sequenced samples in the context of complete genome sequences of the same genotype. NGS sample preparation method of HCV from blood plasma was established. Complete genome sequences of HCV could be obtained with variable coverage depending on the viral load of plasma samples. No significant reduction of host DNA proportion in DNase treated samples in comparison to the controls was observed. The new sequences clustered within the Los Alamos National Laboratory database-deposited HCV subtype 4d samples. The method can be used to obtain full-length sequences of HCV from nucleic acid samples not previously planned for NGS. No improvement was observed when DNase pre-treatment of nucleic acids extracted from blood plasma was performed.

A21 Retrospectively describing hepatitis C virus transmission dynamics and tracking HCV transmission networks in real-time for strategic elimination interventions

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Despite impressive uptake of direct acting antivirals for hepatitis C virus (HCV) in the Netherlands among HIV/HCV co-infected men who have sex with men (MSM), HCV transmission continues, especially among patients previously successfully treated for HCV. The incidence of reinfection occurs at the extremely high rate of 15 per 100 person-years. Clearly, more sophisticated methods are necessary to identify the sources and timing of new HCV infections among MSM. The aim of this research is to phylogenetically characterize HCV transmission dynamics within MSM-specific networks in order to provide a solid base for targeted interventions to monitor, control, and eventually stop the ongoing transmission of HCV among HIV-infected MSM and to prevent further spread of HCV to the community at large. The methodology that will be used is two-fold. Firstly, it concerns setting up a real-time monitoring system to track the HCV epidemic using phylogenetic tools and open-source software from http://nextstrain.org. Secondly, several phylogenetic methods will be used to retrospectively identify transmission clusters in Amsterdam and define epidemiological characteristics, including the directionality of transmission and the size and introduction dates of the clusters. This means that cluster cut-off points will have to be calculated. This research will result in a web-based molecular surveillance tool to monitor the persistence of endemic clades, emergence of new clades, and transmission clusters in 'real time', which, combined with clinical and epidemiological data, will be used for targeted interventions. The surveillance tool will be based on the open-source software from nextstrain.org. Secondly, by retrospectively describing the HCV transmission clusters in terms of introduction dates and subsequent dynamics, we may be able to better predict the future dynamics of the different clusters. High-resolution viral sequencing will allow us to identify the source and timing of (new) HCV infections and follow the trajectory of these MSM-specific lineages through the MSM population. Real-time insight in transmission networks using a web-based molecular surveillance tool will identify key targets for rapid interventions, awareness campaigns, and testing strategies. This can be used to prevent further spread to HIV-negative MSM and to control and eventually eliminate HCV from the MSM population.

A22 Phylogenetic clustering of hepatitis C virus infection among people who inject drugs in Baltimore

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The availability of effective, oral direct acting antivirals for hepatitis C virus (HCV) treatment has fueled optimism for HCV elimination through treatment as prevention (TasP) among people who inject drugs (PWID). Identifying characteristics of individuals in transmission networks would provide critical information for the development and implementation of effective, targeted HCV TasP strategies. The AIDS linked to the IntraVenous Experience (ALIVE) cohort has followed PWID in Baltimore since 1988. Sequencing of the HCV core/E1 region (342 nucleotides) was performed on HCV viremic samples from the most recent study visit attended by ALIVE participants between August, 2005 and December, 2016. Outgroup sequences were retrieved from GenBank through a BLAST search for HCV sequences similar to study sequences to support identification of 'local clusters' and were aligned to study sequences using Clustal O. Phylogenetic trees were inferred for each of HCV subtype 1a and 1b separately through maximum likelihood analysis implemented in the MEGA X software using the Tamura-Nei model with gamma distribution and invariant sites. Nucleotide substitution model selection was based on the corrected Akaike information criterion scores of various models in MEGA. Robustness of the resulting tree was assessed by bootstrapping with 1,000 replicates. Clusters were identified using ClusterPicker software (70% bootstrap threshold and 0.05 maximum genetic distance threshold). Sensitivity analyses were performed by varying the genetic distance threshold between 0.025 and 0.05 to determine the effect on identification of factors associated with clustering. HCV infection clustering was defined as >2 participants with HCV genome sequences satisfying 70 per cent bootstrap and 0.05 genetic threshold distance requirement for sequence similarity. Logistic regression was used to assess sociodemographic factors associated with being in an HCV cluster. Among 512 HCV genotype 1 viremic PWID, HCV subtype prevalence was 83 per cent genotype 1a and 17 per cent genotype 1b. The median age of participants was 54 years, 68 per cent male, 87 per cent Black, and 38 per cent HIV infected. Overall, 9 per cent ($n=44$) were grouped into 21 clusters, consisting of 20 pairs and 1 triad. Of the 425 genotype 1a and 87 genotype 1b samples evaluated, 8 per cent ($n=33$) and 13 per cent ($n=11$) respectively, were in clusters. In unadjusted analyses, membership in a cluster, was associated with younger age (odds ratio (OR) 1.5 [95% confidence interval (CI) 1.1–2.1] per 10 year age decrease); female sex (OR 2.8 [95% CI 1.5–5.3]), HIV infection (OR 4.9 [95% CI 2.5–9.9]), and living in East Baltimore (versus outside East Baltimore, OR 2.0 [95% CI 1.0–3.9]). In adjusted analyses, female sex (OR 2.0 [95% CI 1.0–3.9] and HIV infection (OR 5.4 [95% CI 2.6–11.1] remained independently associated with being in an HCV infection cluster. HIV-infected PWID and their networks should be prioritized for HCV treatment and prevention interventions given an increased likelihood of transmission in these groups.

A23 Population level diversification of hepatitis C viral strains over time among people who inject drugs in Baltimore, MD

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Hepatitis C virus (HCV) infection occurs in 30–90 per cent of people who inject drugs (PWID). Although cure rates can exceed 95 per cent, treatment access is limited and approximately 400,000 people die each year due to complications of chronic infection. A temporal analysis of cluster networks among PWID can be used to inform strategies to interdict transmission. In Baltimore, PWID have been recruited for The AIDS Linked to the IntraVenous Experience (ALIVE) cohort. A demographic questionnaire was administered and recorded for baseline and recent participants. Viral RNA underwent PCR with primers targeting the core and envelope-1 protein (CE1) and sequenced via Sanger sequencing. Sequences with >400 bp reads and Q-scores >370 were used for downstream analysis resulting in 322 ALIVE baseline participants (1988–9) and 548 recently diagnosed subjects enrolled approximately two decades later (2005–16). Cluster networks were rendered with a threshold of 4 per cent in MicrobeTRACE, and statistical analyses were performed in R Studio. Of the 1988–9 subjects, the majority (259/317, 81.7%) were a part of cluster. There were nine clusters and fifty-eight singletons, with two large clusters containing most sequences of genotype 1a (73.5%). Two decades later, a minority of recently diagnosed individuals (235/512, 44.1%) were part of a cluster. There were seventeen clusters with 286 singletons with two large clusters containing 1a genotype