Virus genomic epidemiology to inform public health policy

Understanding hepatitis C virus and SARS-CoV-2 transmission in elimination and outbreak settings

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Chapter 8

General Discussion

The research presented in this thesis has provided insights into HCV transmission among MSM, as well as SARS-CoV-2 transmission patterns in Amsterdam. The results of Part 1 of this thesis can be used to improve HCV prevention efforts among MSM. The outcomes of Part 2 of this thesis have been used to guide SARS-CoV-2 hospital policy, as well as to inform public health policy regarding local SARS-CoV-2 transmission risks. Infectious diseases will continue to (re)emerge and the burden on global health will be determined by our ability to respond to these threats. Combined, this body of research provides a framework for using genomic epidemiology to inform public health decision-making in different epidemic settings. In this chapter, some key questions about HCV and SARS-CoV-2 (genomic) epidemiology are discussed in the context of the findings presented in this thesis.

HCV epidemiology among MSM

Are we on track to eliminate HCV among MSM?

The discovery of DAAs for HCV treatment in the early 2010s has resulted in a tremendous breakthrough in HCV care, eliminating the need for prolonged treatment regimens with frequent side effects and increasing cure rates to over 95%. It has thereby greatly changed HCV epidemiology and the public health response: The WHO envisions eliminating HCV as a public health threat by 2030 [1]. The elimination targets have been endorsed by 194 countries but only a few countries have funding-backed national programmes for HCV elimination [2]. In fact, most countries have failed to meet the intermediate 2020 targets [3]. Among MSM, there have been mixed results: While some studies have reported a decline in HCV prevalence after unrestricted DAA availability [4–6], reports on a lack of decline in HCV incidence have also been published in several countries [7–10]. This indicates that upscaling and/or optimization of elimination efforts are probably required to reach elimination targets in the MSM population.

Is further scaling up of DAA treatment without additional prevention efforts sufficient for the micro-elimination of HCV among MSM?

Over the last few years, evidence has emerged that solely relying on diagnosis and treatment in routine clinical care may not be sufficient for the elimination of HCV among MSM in certain settings [11]. In addition, behavioural changes required for elimination are unlikely to occur, as evidenced by the recent decline in condom

use [11]. This leaves enhanced or improved diagnosis and treatment as the only approach for achieving elimination. One of the central questions in HCV public health is whether current DAA treatment practices in routine clinical care are sufficient for eliminating HCV among MSM without additional prevention. In this thesis, we provide a framework of how HCV genomic epidemiology can guide public health policy by evaluating such questions. Part 1 of this thesis provides some insights into why additional prevention efforts are probably needed. Firstly, chapter 2 provides evidence that external introductions are becoming more frequent among MSM in the Amsterdam region. While this suggests that local clusters are successfully treated and local transmission seems to be declining, these introductions can keep incidence levels high. In addition, they have the potential to cause outbreaks and onward transmission if the infection is undiagnosed or when viraemia is prolonged. Secondly, chapter 3 highlights that international transmission networks are still active in the DAA era and that international transmission has not been halted by current treatment practices.

In chapter 4, we highlighted that there is overlap in active HCV transmission networks in Amsterdam, Berlin, and Paris but that certain local clusters also remain active, suggesting current diagnosis and treatment practices do not sufficiently prevent HCV transmission in this population. While there is some evidence that the upscaling of DAA treatment is sufficient for significantly lowering HCV incidence among MSM [4], these effects are often short-term [5, 12], not reproducible in all settings, and were usually only observed for primary infections rather than reinfections [7, 8]. Passive HCV screening in routine clinical care will likely not be sufficient for eliminating HCV from the MSM population. Combined, these studies highlight that additional active screening (linked to treatment) and case finding are probably necessary for micro-elimination among MSM. Screening should be informed by the characteristics of active transmission clusters. Understanding transmission dynamics will provide guidance for the design of these additional prevention efforts.

HCV transmission cluster dynamics among MSM

What is driving new HCV infections among MSM?

Within the current HCV epidemic, MSM and people who inject drugs disproportionately contribute to endemic growth [13]. The transmission of HIV within these populations varies between subgroups and clusters, with active transmission disproportionately contributing to new infections [14, 15]. This is likely to also be true

for HCV. Identifying these growing transmission clusters, as well as characterising them, may provide tools for efficiently directing the public health response required to control transmission. Enriching these clusters with epidemiological and demographic metadata allows for their characterisation and discerning between local and external transmission, thereby increasing our understanding of what is driving new infections.

In chapter 2, we showed that external introductions are an increasingly important driver of the HCV epidemic among MSM in Amsterdam. This has been substantiated by previous research [16] and by findings in chapters 3 and 4, where we identified several active international transmission clusters.

What are the characteristics of HCV transmission clusters?

In chapters 2-4, we aimed to better understand the characteristics of the different HCV transmission clusters. We used genomic epidemiology to provide evidence of active transmission clusters involving both MSM with and without HIV, adding to the evidence that MSM without HIV have emerged as a novel population at risk for HCV infection. MSM without HIV were previously considered as at very low risk of HCV infection [17]. The increased risk of HCV infection for MSM with HIV may be partly explained by the association of HIV infection with increased susceptibility to HCV infection by the disruption of mucosal epithelial junctions, as well as a higher semen HCV viral load [18–20]. However, it appears that a change in sexual network structure has impacted the HCV risk for MSM without HIV: Pre-exposure prophylaxis (PrEP) for HIV (which protects against HIV infection, but, importantly, not against HCV infection) has changed network dynamics. In a PrEP demonstration project in Amsterdam, 62% of MSM without HIV using PrEP reported sexual contact with partners with HIV [21]. In addition, sex parties that were restricted to MSM with the same HIV status now also welcome MSM on PrEP [21]. Phylogenetic analyses have previously shown that MSM with and without HIV have overlapping networks [22]. This suggests that a public health response to these clusters should involve people with and without HIV. Prospective monitoring of these clusters is key for understanding the changing dynamics. Chapters 3 and 4 also highlight that injecting drug use (IDU) remains an important risk factor for HCV infection. In chapter 3, we described active clusters where both IDU and sexual exposure were reported as self-reported risk factors, but also clusters where only IDU or sexual exposure was reported. This adds to the evidence that HCV transmission networks are diverse in terms of risk behaviour [23, 24] and that there is likely no one-size-fits-all prevention strategy. In Europe, IDU remains the most prevalent risk factor associated with acute

HCV infections [13]. However, this differs between countries and in the Netherlands, HCV infections are mostly detected among MSM [25].

How is a phylogenetic transmission cluster defined for HCV?

There is no consensus about what degree of phylogenetic relatedness can be considered a transmission cluster [26]. The lack of such a consensus, as well as an incomplete understanding of the HCV epidemiological dynamics, have led to varying cluster definitions based on often arbitrary thresholds of genetic relatedness. Multiple different thresholds have been used in prior research [16, 27] and depend on the genomic region used for analysis [28]. Throughout chapters 2, 3, and 4, we used an algorithm to infer transmission clusters that negates the need for an arbitrary threshold for relatedness. Instead, Phydelity uses the overall distribution of patristic distances to statically infer clusters of sequences more related to each other than to be expected from the overall distribution [29]. This means that a research question-informed background set of sequences is important for the analysis, as they will make up the overall distribution of sequence distances. For example, if the research question is whether MSM-specific transmission clusters exist, the background sequence set should contain a random selection of sequences derived from non-MSM. In chapter 2, such a background set was used to define these MSM-specific clusters and to show that recent infections were not part of these existing MSM-specific clusters. However, in chapter 4, only MSM-specific sequences were used to define more closely related sequences with more recent common ancestors to zoom in on the characteristics of recent transmission. The most recent common ancestor of an inferred cluster is informative for the recency of putative transmission events and should also be considered for a targeted public health response.

The risk of emerging treatment-resistant HCV variants

Are treatment-resistant variants currently a threat for micro-elimination?

HCV variants that are resistant to current DAA treatment options are frequently detected after first-line treatment failure. Due to the increasing number of people treated with DAAs globally, the risk of emergence and spread of variants resistant to current treatment is also increasing. Treatment uptake of DAAs among MSM has been generally high [30] and the transmission of certain resistance-associated substitutions (RASs) has been described among MSM, albeit with low clinical signif-

icance [31, 32]. Clinically relevant RAS has been detected in high-risk populations where the risk of onward transmission was high [33]. In chapter 4, the presence of RAS among European MSM was discussed and while transmission of RAS was detected, its clinical significance was low. Infections with baseline RAS are currently still mostly successfully treated [34], and additional treatment options still exist if first-line treatment fails [35]. Currently, RAS is not a barrier to HCV elimination among MSM, but this could change in the future. The public health community should be prepared if such variants arise. Genomic surveillance of HCV among MSM enables the early detection of such variants, differentiation between outbreaks and multiple independent introductions of variants, and the epidemiological characterization of RAS-enriched clusters. Genomic surveillance can thereby direct public health efforts towards high-risk, RAS-enriched clusters if or when they emerge.

SARS-CoV-2 epidemiology in Amsterdam

In stark contrast to HCV, the digital infrastructure for global SARS-CoV-2 surveillance has been set up in record time. SARS-CoV-2 has rapidly become the most sequenced organism in history due to the combination of the exceedingly low cost per sequence, an increase in computational power, and the global awareness of the potential of genomic epidemiology. Within weeks of the first detection of the virus, the first SARS-CoV-2 genomes were being shared publicly [36] and annotated with important metadata such as the time and location of sampling. Over 12.5 million sequences are currently publicly available, which has enabled global research on the sources and scale of transmission, factors contributing to transmission, and outbreak investigations. In chapters 5-7, we discussed these topics and several applications of genomic epidemiology that were deployed to investigate transmission in the Amsterdam region, as well as among healthcare workers in the Amsterdam University Medical Centres.

What is the role of superspreading events in SARS-CoV-2 transmission?

Genomic epidemiology has the potential to guide local public health policy by elucidating sources of elevated spread. It has previously been described that very local, situational sources of transmission are an important driver of respiratory disease spread [37]. Early on in the SARS-CoV-2 pandemic, it was demonstrated that transmission has a highly-overdispersed offspring distribution [38, 39], indicating that a small percentage of the infected population causes a large proportion of

secondary transmissions. Identifying these sources can greatly support the public health response. In chapter 5, we performed a case-study showing that superspreading events in nightclubs were likely important in the rapid increase of SARS-CoV-2 cases in the Amsterdam region. Our study supported health policy by indicating that the measures in place to prevent rapid transmission at the time (a mandatory negative test < 40 hours before attendance or a completed vaccination series) was not sufficient for preventing superspreading. Internationally, superspreading has also been demonstrated to be a key contributor to the propagation of SARS-CoV-2 in local communities. Several studies have highlighted the benefit of genomic epidemiology for determining the sources of spread. In Georgia, USA, researchers identified that young individuals tend to be the main driver of superspreading events in local communities [40]. In Ho Chi Minh, Vietnam, researchers elucidated that a local bar was the source for a superspreading event [41]. In Boston, USA, researchers identified superspreading events in a nursing facility and an international business conference [42], and in Hong Kong, a fitness centre was determined to be the source location of superspreading [43]. Our work in chapter 5, combined with examples from around the world, highlights that genomic epidemiology can identify local sources of elevated transmission. To inform control measures during the outbreak, sources need to be identified rapidly enough such that interventions can halt transmission. However, the generation time of SARS-CoV-2 is often shorter than the time it takes for genomic results to be generated. SARS-CoV-2 genomic data is therefore often only used to gain retrospective insights into an outbreak and to improve future prevention efforts. For viruses with a longer generation time (such as HCV), halting transmission chains guided by genomic data is more likely.

How can SARS-CoV-2 genomic epidemiology support hospital policy?

Healthcare worker (HCW)-associated SARS-CoV-2 transmission is of special concern due to the risk of nosocomial spread and significant staff shortages. In addition, contacts between HCWs and patients are numerous and patients are often highly vulnerable. Hospital-associated infections make up a significant proportion of hospital-diagnosed infections (in the UK, 15% [44]). It remains difficult to differentiate between nosocomial and community transmission, as the precise timing of the infection is rarely known. Genomic epidemiology can aid in elucidating these transmission dynamics as nosocomial infections are more likely to be genetically similar than infections acquired in the community. It can guide hospital policy by identifying ward-specific outbreaks, elucidating nosocomial transmission dynamics, and determining risk factors [45]. In chapter 6, we used genomic epidemiology to investigate an outbreak at an in-patient orthopaedic ward at the Amsterdam

UMC with the then recently emerged Alpha variant. At the time, many factors of the newly emerged Alpha variant were unknown. In chapter 6, we described an outbreak of highly similar strains and demonstrated that rapid reinfection with an Alpha variant was possible. In addition, we showed that the reinforcement of current guidelines was able to control the outbreak. Hospital policy was therefore not altered, but adherence to applicable guidelines was reinforced. Secondly, in chapter 7, genomic analyses of SARS-CoV-2 infections obtained from healthcare workers returning to work after COVID-19 symptom resolution allowed us to evaluate the hospital prevention policies at the time. At the time, the hospital guidelines dictated that unvaccinated HCWs were allowed to return to work 24 hours after symptom resolution. We showed that almost half of the HCWs had a positive PCR test upon return to work, but that there was no genomic evidence for onward spread. Two epidemiologically linked cases were found to be unrelated infections based on the genetic differences between the virus genomes. Additional genomic epidemiology studies at the Amsterdam UMC determined that outbreaks at COVID wards were more likely to be due to HCW-HCW transmission than patient-HCW transmission [46].

Virus genomic epidemiology for public health action

Advances in whole genome sequencing technology in the last few decades have revolutionized virus epidemiology. Genomic epidemiology has become more powerful with rapid increases in computational power, decreasing sequencing costs, more and better statistical models, and new sample collection protocols [47]. Combined, this has led to genomic epidemiology becoming an important component of virus outbreak response, as demonstrated in this thesis. For viruses with a high diversity such as HCV, transmission cluster identification based on genomic relatedness outperforms traditional methods such as partner naming or survey-based investigations [48]. Genomic analysis provides an independent metric of relatedness whereas traditional surveillance methods have significant barriers to identifying clusters. In chapter 4, we showed that surveys regarding where and how sexual partners met are not able to untangle transmission patterns. The improved computational power and better analysis models have allowed genomic analyses, such as those presented in this thesis, to be performed in real-time [48, 49]. This means that genomic epidemiology can shift from retrospective research, to (near) real-time genomic surveillance (as demonstrated in chapter 4). Consequently, this means that historical outbreaks can be studied to inform future outbreaks and that control and prevention measures of ongoing outbreaks may be informed by genomic surveillance [50, 51]. The goal of

genomic surveillance is therefore to inform public health action, based on real-time genomic data. In this section, we discuss the current status as well as the challenges and future perspectives of HCV and SARS-CoV-2 genomic surveillance.

Current status of HCV and SARS-CoV-2 genomic surveillance

HCV surveillance is important for evaluating progress towards HCV elimination, as well as for designing public health programs. Unfortunately, HCV surveillance data is often incomplete and heterogeneity of the data prevents effective comparisons and interpretation [13]. Currently, no coordinated HCV genomic surveillance program exists. In chapter 4, we presented a framework for an international genomic surveillance platform called MS-TRACE. This is the first framework for HCV surveillance among MSM. While other outbreak and surveillance tools exist for HCV [52], MS-TRACE is the only tool that also incorporates a dataset of HCV sequences derived from MSM. Chapters 2, 3, and 4 have highlighted that the epidemiology of HCV among MSM is changing: Transmission networks are often international, different risk profiles for clusters exist, and MSM without HIV have emerged as a novel group at risk for HCV. This warrants the continuous surveillance of such factors, for which genomic surveillance would be optimal. Integrating genomic data with key epidemiological parameters creates a clearer picture of the epidemic. A real-time genomic HCV surveillance system would provide opportunities for a more proactive response to new infections and outbreaks. These opportunities are not without barriers and successful genomic surveillance still faces challenges, which are discussed in Challenges in HCV and SARS-CoV-2 genomic surveillance. One of the main limitations of MS-TRACE is the current lack of sequences for many European countries, and subsequently, only a partial snapshot of the true transmission clusters in Europe is available. The results from chapter 3 suggest that especially European countries could benefit from improved, coordinated genomic surveillance. International collaboration between sentinel sites in Europe is needed to monitor these European networks. With MS-TRACE, we hope to encourage and invite researchers and public health officials to collaborate on the genomic surveillance of HCV.

SARS-CoV-2 genomic surveillance has rapidly become the most elaborate surveillance system for any pathogen. In stark contrast to HCV, more than 12.5 million annotated SARS-CoV-2 genomic sequences are available from >185 countries (www.gisaid.org), providing an unprecedented geographical resolution for sequence contextualization. Due to platforms such as GISAID and GenBank, SARS-CoV-2 genomic data has become readily available at a previously unthinkable speed. Surveil-

lance systems that incorporate these platforms have been used for understanding the characteristics of different variants such as transmissibility, disease severity, vaccine effectiveness, and many others. Locally, national surveillance systems have been set up to allow for local and regional surveillance. In the Netherlands, this surveillance system has allowed for a plethora of research such as identifying importations [53], local vaccine effectiveness [54], the risk of infection per variant [55], and reinfections (Chapter 5). Chapters 5-7 of this thesis have all relied on national and local genomic surveillance frameworks, highlighting their importance.

Challenges in HCV and SARS-CoV-2 genomic surveillance

The interpretation of genomic epidemiology requires virus-specific consideration

In addition to sequences, the utility of the genomic epidemiology of a virus depends on several factors, including the evolutionary characteristics of the virus (e.g., substitution rate), the epidemiological features of an outbreak or epidemic (e.g., incubation period), and data collection capabilities (e.g., testing and sampling density). HCV and SARS-CoV-2 differ significantly in all these characteristics and need to be considered when interpreting the results. As presented in chapters 2, 3, and 4 of this thesis, the genomic epidemiology of HCV is mostly used to reconstruct epidemic histories on a timescale of months and years. Uncertainties around introduction dates are of a similar order of magnitude. This data lacks the precision to unravel specific transmission chains but remains important for providing the general characteristics of transmission. In addition, the incubation period of HCV is longer than SARS-CoV-2 (months [56, 57] versus a few days), and an HCV infection can be asymptomatic for decades. This complicates the reconstruction of precise transmission pathways, as many (unsampled) intermediate transmission events may be missed. Unlike HCV, SARS-CoV-2 accumulates mutations at a slower rate than transmission events, resulting in highly similar or identical sequences that complicate phylogenetic reconstruction: A low genetic resolution results in high uncertainty about the phylogeny [58]. It is therefore important to stress that the unrelatedness of strains can rule out transmission, but that infections with identical viruses do not prove direct transmission. It is imperative to consider all epidemiological evidence to infer transmission dynamics. For instance, epidemiological, human mobility, and climatic data were used to infer that a Zika outbreak was likely imported from Brazil [59], where genomic evidence would not have been sufficient. In chapters 5-7, we combined detailed epidemiological data such as contact tracing, presence in the hospital, and detailed symptom onset data to understand SARS-CoV-

2 transmission at the Amsterdam UMC and in a nightclub, highlighting the need for strong epidemiological and other supporting data to prevent misinterpretations.

Suboptimal sampling frameworks can prevent robust conclusions

One of the key challenges in virus genomic surveillance is how to develop sampling frameworks that enable robust conclusions. Phylogenetic clusters only include persons with a diagnosed infection that have had their viral genome sequenced for analysis. A phylogenetic cluster therefore represents a (small) subset of the underlying true transmission cluster, which may also include undiagnosed and/or unsequenced HCV infections. In chapter 4, we detected many sequences without closely related sequences, suggesting many infections were unsampled. This has prevented the contextualisation of these sequences. Because opportunistic sampling remains the standard for HCV, a key challenge is the development of an unbiased sampling framework. We suggest a surveillance framework in chapter 4 of this thesis. Secondly, biased sampling frameworks can impact the reliability of the conclusions. For SARS-CoV-2, this has been problematic, as some countries dominate the global dataset of SARS-CoV-2 sequences (e.g., the UK and USA). Oversampling countries can lead to overestimating their contribution to seeding when using discrete analyses. Inversely, the contribution of undersampled countries will be underestimated [47]. These challenges are also true for other discrete spatial scales such as regions or cities when conducting regional research. A suboptimal sampling of HCV (oversampling of the Netherlands, short period of sampling, and undersampling of non-European countries) prevented such discrete phylogeographic reconstruction in chapter 3. In addition, phylogenies are hypotheses that can be challenged and thus changed as more data become available. Coordinated sampling frameworks for genomic surveillance are required to improve the reliability of the conclusions. This means continued investment in nationally or regionally coordinated genomic surveillance is needed, as called for in reference [60].

The translation of genomic surveillance results into public health action is complex

Identifying active transmission networks can guide the public health response. While for HCV there is little precedent for such responses, its merit has been shown in HIV responses. The Center for Disease Control (CDC) in the United States uses genomic epidemiology to define and characterise HIV transmission clusters [61]. Using HIV-TRACE [50], a clustering algorithm for HIV sequences, clusters are defined

and appropriate health responses are implemented. If clusters with rapid and recent transmission are detected, efforts are implemented with high priority to interrupt ongoing spread. Other clusters where transmission occurred in the distant past were not investigated as thoroughly, due to limited public health resources. However, several barriers prevent efficient action by public health stakeholders.

Firstly, genomic epidemiology results are usually tailored for specially trained scientists. This thesis has highlighted that the interpretation of the genomic epidemiology of HCV and SARS-CoV-2 is markedly different and detailed knowledge of the virus is required for correct interpretation. In chapter 5, we demonstrated a collaboration between genomic epidemiology specialists and local public health officials to elucidate the sources of elevated transmission during the July 2021 peak of cases in the Amsterdam region. By gathering a multidisciplinary research group (called the ARGOS consortium) of evolutionary biologists, mathematicians, bioinformaticians, clinical doctors, and public health officials, we were able to efficiently communicate the findings and take public health action. Secondly, specific barriers exist in hospitals that prevent efficient translation. There are many opportunities for future research, which should focus on actionability, but also specific topics such as liability, cost, payment models, and assay validation [45]. Lastly, public health action will be stronger when robust predictions can be made from genomic data, as action can then be taken proactively rather than reactively. The predictive value of genomic surveillance has not yet been evaluated, but some examples of forecasting approaches have been demonstrated for SARS-CoV-2 [62, 63].

8.1 References

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