Molecular epidemiology of hepatitis B in the Netherlands
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CHAPTER 6

GENERAL DISCUSSION
General discussion

Molecular epidemiology

The occurrence of different serotypes of the hepatitis B surface antigen is well documented. The common determinant $a$ and two pairs of mutually exclusive determinants, $d/y$ and $w/r$, enable the distinction of four major subtypes, $adw$, $adr$, $ayw$, and $ayr$ [1;2]. Additional sub-determinants of $w$ ($w1-w4$) have allowed the definition of six more serotypes [3]. Serotyping has, however, been outdated by the introduction of the much more discriminative molecular typing. Although the hepatitis B virus (HBV) is a highly compact virus with overlapping open reading frames, which constrain possible nucleotide substitutions, the genetic distance is large enough to discriminate between various strains. HBV strains have been classified into eight genomic groups designated A-H, which is based on an intergroup divergence of 8% or more in the complete nucleotide sequence [4-6]. As described in the ‘General Introduction’, these genotypes have distinct geographical distributions. In the Netherlands, HBV genotypes A and D are predominant.

Sequencing parts of the HBV genome can be sufficient to determine the various genotypes. However, full length sequencing is preferable, since the relation between the sequences is less discriminative when only a part of the viral DNA is sequenced. However, full length sequencing is time consuming and, for epidemiological purposes, a less complex phylogenetic analysis can often provide more information on the transmission pathways of certain risk groups. In the studies presented in this thesis, the pre-S2 and S-region (680 nt.) was sequenced and used in the phylogenetic analyses. The S-region codes for the surface protein, including the HBV surface antigen (HBsAg). Mutations associated with vaccine escape and weak HBsAg signals in serological assays are located in this domain [7]. The objective of the studies in this thesis was obtaining more insight into transmission pathways, therefore sequencing the S-gene of the virus was discriminative enough to suit our purpose.

Genetic analyses alone are insufficient to obtain insight into transmission pathways. In order to reveal transmission networks and risk groups, molecular data need to be combined with the demographic and behavioural characteristics of the host. Initially, this combination of two different tools in research was used to identify various genotypes among populations and to explain the infection with a certain genotype according to its geographical origin [8-10]. Subsequently, epidemiological data were increasingly used in molecular epidemiological analyses. Molecular epidemiology was used for source and contact tracing, for example, infections with HBV found all over England could be related to a single outbreak of HBV in a prison [11]. Genotypes could be linked to risk groups, for example, in Denmark, injecting drug users were proven to be infected with an identical genotype D strain [12]. Molecular epidemiology also proved to be a powerful tool in revealing the spread of HBV in Amsterdam [13;14]. The studies in this thesis all used molecular epidemiology to track the spread of HBV in different population groups. Finally, chapter 5 shows that molecular techniques are an important extension of surveillance to obtain better insight into the dynamics of HBV transmission.
**Hepatitis B in the Netherlands**

In the Netherlands, hepatitis B is a notifiable disease. This makes it possible to keep track of the reported HBV incidence and provides the Public Health Services with extra information on the patient’s risk behaviour six months prior to infection. As of January 2004, the viral DNA of the majority of reported acutely infected patients in the Netherlands is sequenced and linked to the accompanying demographic data and data on risk behaviour. This is essential in obtaining more insight into the nation-wide transmission pathways of HBV. The Netherlands is a low endemic country with an HBsAg prevalence between 0.3 and 0.5% [15]. The reported annual incidence in the Netherlands is also low: between 1.4 and 2.0 per 100,000 inhabitants, equal to 200 to 300 individuals reported with an acute HBV infection annually [16;17]. However, this incidence is only based on symptomatic people who are reported to the Public Health Services; the actual incidence will therefore be higher. Sexual contacts, especially between men, are the major source of new infections in the Netherlands (chapter 2). In the Netherlands, injecting drug use plays a minor role in HBV transmission, in contrast to some European countries [12;18]. This is due to a steep decline in injecting drug use among hard-drug users in the Netherlands during the past two decades [19].

HBV genotypes A and D are predominant in the Netherlands (chapter 2). Men having sex with men (MSM) are primarily infected with genotype A. Van Steenbergen et al. and the study in chapter 4.1 found a genotype D strain in Amsterdam which seemed to be specific in respect of Moroccans [13;20]. However, the more elaborate study in chapter 2 demonstrated that this genotype D strain can be found among all people linked to the Mediterranean area by ethnicity or by contact with people originating from that area [16]. There is also a genotype F strain circulating in the Netherlands, probably for a much longer period than was previously assumed. This strain had already been found among MSM in Amsterdam in 1995 and 1998. Although this strain mainly circulates among MSM, it has also been found among men and women infected with HBV through other modes of transmission. This is remarkable, since genotype F is primarily found in South America. More research is therefore needed to pinpoint the source of this strain is and the point at which it was introduced in the Netherlands.

**Men having sex with men**

The anti-HBc prevalence among the general population in the Netherlands is estimated to be 2.1% [21]. In contrast, the overall anti-HBc prevalence among MSM entering the Amsterdam Cohort Studies was 42% (chapter 3.1). The HBV incidence among MSM in this cohort was lower than expected and although HBV is more infectious than HIV, the HBV incidence in this cohort followed the same trend as that of HIV. The majority of MSM in the Netherlands who are infected with HBV, are infected with an identical genotype A strain. This has been the case for at least 25 years. Infections with other genotypes or other genotype A strains seem to be single introductions with no ongoing transmission. This MSM-specific genotype A strain has also been found among MSM in Japan and can probably be found among MSM throughout Europe [22], as this was also observed in respect of other viruses, such as hepatitis A and C, of which the same virus
strains circulate among MSM throughout Europe [23;24]. Kretzschmar et al. calculated the basic reproduction number ($R_0$) of HBV within the MSM population in the Netherlands to be larger than one, implying that HBV circulates endemically among the MSM population [25]. Although this suggests that import from outside the Netherlands plays a minor role, the viral DNA of acutely infected patients from other European countries should nevertheless be sequenced to see whether this strain is also circulating among MSM in the rest of Europe. Analyzing only the S-region might not be conclusive enough, because the HBV genome is too conserved due to its overlapping open reading frames. Furthermore, genotype A is a highly prevalent genotype in North-western Europe [8;12;26]. To identify the moment when this strain was introduced in the MSM population, full-length sequencing is a necessity (chapter 5).

In the Netherlands, only a few women are infected with this MSM specific genotype A strain. In addition, about a quarter of the individuals infected with genotype A are men who maintain that they have only heterosexual contacts. Furthermore, most people infected with genotype A through an unknown mode of transmission were men and their age at the moment of infection was comparable with the age of HBV infection of MSM. This suggests that many men infected with HBV are, in fact, men infected through homosexual contact who do not want to admit this.

**Drug users**

HBV incidence among drug users participating in the Amsterdam Cohort Studies was low and as of 1993, the incidence started to decline to 0 per 100 PY in 2002 (chapter 3.2). The HIV incidence among drug users was lower during the study period and the decline in HIV incidence was already present at the start of the Amsterdam Cohort Studies. This is contrary to the trend in HIV and HBV incidences among MSM, for whom the HBV and HIV incidences were similar in amplitude and trend. Furthermore, the decline in HBV incidence among drug users started almost a decade later than among MSM. This is remarkable, as both groups are involved in high-risk behaviour, and for both groups there is evidence that HIV was introduced in both populations simultaneously and HBV had been circulating for a long time, resulting in a high proportion of carriers. The only difference between the two groups is the route of transmission. For MSM, the mode of transmission is through sexual contact, whereas injecting is the major mode of transmission for drug users. The viral load of HBV is higher in blood, therefore the chance of contracting an HBV infection when sharing needles or paraphernalia is also higher than through sexual contacts. Another explanation for the ongoing transmission might be that there was no evidence of a change in sexual risk behaviour among drug users like the one seen among MSM.

The majority of drug users, both injecting and non-injecting, are infected with the same genotype D strain. This means that drug users infect one another regardless of their risk behaviour. Transmission of this strain among drug users is not restricted to the Netherlands, but can also be found in other countries, not only in Europe, but also in the USA [12;27;28]. In contrast to other countries, the drug-user strain has now disappeared and no new HBV infections were reported among injecting drug users in Amsterdam after 2000 [20].
Furthermore, the spill-over from the injecting drug user population to the general population has also disappeared. Together with the decline in HBV incidence, this suggests that drug users are no longer a high-risk group for HBV infection. However, trends in drug use need to be monitored, because if injecting regains popularity in the Netherlands, HBV incidence among this group might also increase again. This is especially important as chapter 3.2 demonstrated that injecting remains the most important risk factor for HBV infection over time.

Prevention programmes

Prevention of HBV transmission among the general population of the Netherlands started with prenatal screening and subsequently vaccinating newborns with a chronically infected mother. Other risk groups, such as healthcare workers and certain patient groups like haemophiliacs are also vaccinated. The Netherlands is a low-endemic country with HBV transmission mainly restricted to risk groups. Therefore, the Netherlands, like Britain, Ireland, and the Scandinavian countries, adopted a policy of vaccination targeted at behavioural risk groups rather than universal vaccination [29]. This targeted programme was implemented in November 2002, after a two-year pilot had been conducted in several regions of the Netherlands to investigate its feasibility [30]. Within the programme, commercial sex workers, hard drug users, and MSM are vaccinated free of charge at their local Public Health Service and at clinics that treat sexually transmitted infections (STI). Furthermore, various outreach strategies are tailored to these risk groups. Until October 2007, heterosexuals with an indication for an STI examination were also considered a risk group and therefore vaccinated. However, chapter 2 demonstrated that HBV transmission is not sustained in the heterosexual population [16]. Based on these data, heterosexuals visiting STI clinics as of November 2007 were no longer considered a high-risk group for HBV infection to whom HBV vaccination should be offered free of charge. These preventive measures are still ongoing and were extended as of 2003 with the vaccination of newborns with at least one parent born in a country with an HBV prevalence over 2%.

The $R_0$ of HBV within the MSM population in the Netherlands was calculated to be larger than one, implying that HBV circulates endemically among the MSM population and that import plays a minor role [25]. Among the heterosexual population, the $R_0$ of HBV is smaller than one, implying that without immigration of chronic carriers into the population, HBV transmission among heterosexuals will end. This finding is confirmed by the molecular epidemiology in chapter 2, which demonstrated that the infections were single introductions and that, unlike among MSM, there was no ongoing transmission of these strains. Furthermore, a large number of heterosexuals with an acute HBV infection could be linked to the Mediterranean area. These people are excluded from the risk group vaccination programme. To prevent these transmissions, migrants from medium and high endemic countries should also be screened and, if at risk, their household contacts should be vaccinated. This will prevent new infections and allow chronically infected people to be treated. Depending on future improvements in HBV therapy, more migrant populations originating from high endemic countries and living within the Netherlands can be screened.
However, prior to implementing this type of screening, a cost-effectiveness analysis should be performed.

Although a considerable number of people have been reached by the targeted vaccination programme, its coverage was too low to have had a significant impact on the HBV incidence among the various risk groups (chapter 4). The overall coverage was estimated to be only 9-17%. The estimated vaccination coverage among MSM was even lower, 5-9%, while the majority of new infections can be found among this group. The median age of participants in the targeted programme was relatively high at first vaccination. Clearly, in the current vaccination approach, people have already been involved in risk behaviour for several years before they are vaccinated. Furthermore, it is debatable whether the programme reaches the core group of MSM, since the anti-HBc prevalence found among MSM participating in this programme was only 13%, whereas among MSM in the Amsterdam Cohort Studies the overall prevalence was 42%. In other words, a smaller proportion of the MSM reached by the vaccination programme has been infected with HBV at some time or another, suggesting that they are at lower risk. Modelling studies have shown that vaccinating even a limited number of MSM belonging to the core group with high risk behaviour will result in a rapid decline in HBV incidence [31]. On the other hand, the population sizes of the various risk groups in the Netherlands were roughly estimated, and the estimated anti-HBc seroprevalences had a large range. Furthermore, people vaccinated outside the targeted programme were not included in the calculations. The vaccination coverage will therefore be somewhat higher than was estimated in this thesis.

Whether the vaccination programme targeted at behavioural risk groups has contributed to the decline in the total number of reported acute HBV cases, especially among MSM, remains unclear. A possible decline in sexual risk behaviour, reflected by a decline in the proportion of STIs, especially gonorrhoea and syphilis, in the Netherlands [32] might be an additional explanation for the decrease in reported HBV incidence.

Chapters 4 clearly shows that the coverage of the current programme is too low to have had a large impact on the HBV incidence among the various risk groups. As the current programme continues and more effort is put into vaccinating more susceptibles and younger people in the risk groups, the coverage will somewhat increase, but this will not be sufficient to substantially reduce the incidence of HBV. Therefore, low endemic countries such as the Netherlands should introduce universal vaccination against HBV. Mathematical modelling also demonstrated that implementing universal vaccination is the best option to reduce the HBV incidence to almost zero within 25 years [33].

Concluding remarks
Although HBV transmission in the Netherlands is mainly restricted to risk groups, implementing universal vaccination should be introduced, as discussed in chapter 4. With the current strategy targeted at behavioural risk groups, prevention is always one step behind. This is emphasized by the relatively high age at the first vaccination, suggesting that the majority of people reached by the programme are already involved in risk behaviour prior to vaccination. It is also debatable whether the core group at risk is being reached by the
current programme, which is very important for this strategy’s success. Furthermore, after
five years of vaccination, those who are easily reached have now been vaccinated. To reach
the harder to reach individuals, more and new labour-intensive outreach strategies need to
be developed and deployed. And then there is always the group who will not allow
themselves to be vaccinated.

However, even if universal vaccination is introduced in future, The Netherlands has to
continue with the risk group vaccination programme for several years, depending on the
catch-up vaccination of adolescents [33]. Optimizing the current programme is therefore
important, as it will probably last for at least another decade, probably two. Consequently, it
is essential to have a programme that is as effective and cost effective as possible.
Combining molecular epidemiology with mathematical modelling will be a helpful tool in
guiding this prevention programme. Optimizing can also mean redefining high-risk groups
and targeting most effort at the most important risk group, who are the MSM. Based on the
molecular epidemiological studies in this thesis, heterosexuals with multiple sex partners
were no longer considered at risk for HBV infection as of November 2007. Furthermore,
chapter 5 debates whether drug users are still a high-risk group. Although trends in drug use
need to be monitored, less effort should be exerted in vaccinating drug users and it should
even be reconsidered in respect of this group in the long run.

Unfortunately, universal vaccination will not solve all medical problems related to hepatitis B.
Chronic carriers form the burden of disease, since they have a high chance of developing
liver cirrhosis and its sequela hepatocellular carcinoma, and therefore also dominate the
cost side. Although universal vaccination will prevent HBV transmission and the incidence
will decline to almost zero, its effect on the occurrence of chronic carriers will be relatively
small. This is due to most chronic carriers in the Netherlands being migrants from high and
medium endemic countries who are infected at birth and during early childhood [15]. The
vast majority of new HBV infections in the Netherlands can be found among adults and, in
contrast to newborns or young children, adults are quite capable of clearing the virus [34]. It
is estimated that only a maximum of 10% of these acute infections among adults will lead to
chronic infections.

Finally, looking beyond our borders is essential, since HBV infection is a global problem,
especially with the ever increasing rate of immigration and globalisation. Effective
vaccination programmes in high endemic countries might be even more important in the
long run to reduce the burden of disease in low endemic countries than universal
vaccination in the latter. Therefore, a successful HBV prevention strategy does not end with
the introduction of universal vaccination in our country. It is also in our interest to support
HBV vaccination programmes in medium and high endemic countries.
References

Chapter 6


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