Molecular epidemiology of hepatitis B in the Netherlands
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CHAPTER 1

GENERAL INTRODUCTION
Introduction

Epidemiology

Hepatitis B virus (HBV) infections are of major public health importance, due to their high burden of disease. Worldwide, an estimated 2 billion people have been infected at some time or another, with 4 to 5 million new infections occurring each year [1,2]. World-wide, over 350 million people are estimated to be chronically infected with HBV and each year 600,000 people die from HBV-related liver disease or hepatocellular carcinoma. The prevalence of chronic infections is globally differentiated in high endemic areas (> 7%), intermediate endemic areas (2-7%), and low endemic areas (<2%) (figure 1) [3]. High prevalence areas are South-East Asia and sub-Saharan Africa, where 8 to 10% of the population are chronically infected with HBV. Western-Europe, North America, and Australia have the lowest prevalence (0.1-1%).

In high and intermediate endemic countries, most infections are acquired perinatally or during early childhood. Nosocomial infections with HBV are also prevalent in these countries. In low endemic countries, most infections are acquired through adult risk behaviour, such as injecting drug use and unprotected sexual contacts. In these countries, certain population groups are more at risk for HBV infection than others. These risk groups can be indigenous or can be formed by migration from endemic areas. Most infections in low-endemic countries can be found in adults who are involved in risk behaviour, such as injecting drug users, men having sex with men, and commercial sex workers. Besides these ‘behavioural’ risk groups, healthcare workers, children with developmental disabilities, patients undergoing haemodialysis, children of infectious mothers, and close contacts of infected people are also at increased risk for HBV infection.

Figure 1. Worldwide prevalence of chronic hepatitis B; CDC, 2006 [3]
Virology

HBV is a small, protein-coated, partially double-stranded DNA virus, which is a member of the hepadnaviridae. The HBV genome is about 3200 nucleotides long, and is dependent on a reverse-transcriptional step for its replication. The virus is organized into four overlapping open reading frames (C, P, S, and X) that encode 7 viral proteins (Figure 2). A single core protein, three envelope glycoproteins, and the viral DNA polymerase are incorporated into mature virus particles. A sixth protein, the so-called HBV X protein, is a transactivator of viral replication that is not incorporated into the virion. The protein, known as the HBV e antigen, contains the full amino acid sequence of the core protein, and is not incorporated into the virus particle, but is secreted in a soluble form by an infected hepatocyte.

Four HBV serotypes have been identified based on peptide differences in the hepatitis B surface antigen (HBsAg). The common determinant a and two pairs of mutually exclusive determinants, d/y and w/r, enable the distinction of four major subtypes: adr, adw, ayr, and ayw [4;5]. Additional sub-determinants of w (w1-w4) have allowed the definition of six more serotypes [6]. Currently, the introduction of molecular typing techniques has made this classification obsolete. HBV strains are classified into eight main genomic groups that have distinct geographical distributions [7-11]. Genotype A is widespread around the world, but is most prevalent in Northern Europe, North America, and South Africa. Genotypes B and C are most common in Asia and the Pacific. Genotype D has the broadest distribution around the world, with the highest prevalence in the Mediterranean area, the Middle East, and India. Genotype E is mainly found in (West) Africa. Genotype F and H are most frequent in South and Central America. The exact epidemiology of genotype G has yet to be determined [12].

![Figure 2. Schematic representation of the hepatitis B virus genome](image)

Transmission

HBV has been found in all body secretions and is transmitted by blood, semen and other genital fluids. The viral load in serum is about 1000-fold greater than in other body fluids. The viral load in body fluids, such as sweat, tears, urine, and faeces is so low that
transmission via these body secretions occurs rarely. HBV is transmitted by percutaneous or percutaneous or permucosal exposure to blood or body fluids from infected persons. Transmission usually takes place either through sexual contact, blood contact or perinatally. Perinatal transmission is highly efficient and is most common in areas where HBV is highly endemic, like South-East Asia. Parenteral transmission is through percutaneous and permucosal exposure to blood or fluids from an infected individual. Transfusion of blood or blood products, sharing needles for intravenous drug use, haemodialysis, acupuncture, tattooing, and injuries from contaminated sharp medical instruments are examples of parenteral transmission. Sexual transmission usually results from mucous membrane exposure (vaginal, anal, oral) to genital fluids or blood from an infected individual. Parenteral and sexual transmission are most common in low HBV endemic areas. HBV transmission can also take place within households through close non-sexual contact. This transmission occurs via cutaneous scratches, abrasions, or via mucosal surfaces; or through inanimate objects like toothbrushes, baby bottles, toys, and razors. The predominant mode of transmission in the Netherlands is (unprotected) sexual contact, especially between men [13;14].

Clinical manifestations and diagnostic features
Two types of HBV infection can be distinguished: acute and chronic HBV infection. The acute phase occurs after an incubation period ranging from 4 to 12 weeks, and can last up to 6 months depending on the initial dose of virus, the mode of transmission, and the host factors [15]. Acute HBV infection is usually self-limiting, and most adult patients recover without significant consequences. These patients are then immune for any type of hepatitis B. However, 5-10% of the adults with an acute HBV infection will become chronically infected. In neonates, acute infections are often asymptomatic, but the majority become chronically infected [16;17]. Chronic infections cause the burden of disease, due to its sequelae cirrhosis and hepatocellular carcinoma. The first serologic markers to become detectable in persons with an acute HBV infection are HBsAg, HBeAg, and antibodies to hepatitis B core antigen (anti-HBc IgM) (figure 3a) [18]. The prodromal symptoms of an acute HBV infection are systemic and variable. Constitutional symptoms of anorexia, fatigue, nausea and vomiting, malaise, arthralgias, myalgias, headache, pharyngitis, cough, and coryza may precede the onset of jaundice by 1 to 2 weeks. In some cases, specific symptoms like dark urine followed by pale stools and jaundice appear within 10 days of this initial phase, which is accompanied by hepatomegaly and splenomegaly. About 4-12 weeks thereafter, the jaundice disappears and the illness resolves with the development of natural, protective antibodies (anti-HBs). During recovery, the anti-HBc IgM converts to IgG, and the levels of HBsAg, HBeAg and viral DNA decline. Chronic hepatitis B is a prolonged (>6 months) infection with persistent serum levels of HBsAg and anti-HBc IgG and the absence of an HBsAg antibody response, and anti-HBc IgM (figure 3b) [18]. HBV DNA and HBeAg are often detectable at high concentrations. Chronic hepatitis B can be divided into two phases based on the relative level of HBV
replication. The replicative phase is characterized by the presence of HBeAg and HBV DNA in the serum and HBcAg in the liver, by high infectivity, and by accompanying liver damage. In contrast, the non-replicative phase is characterized by the absence of conventional markers of HBV replication, but there is an association with anti-HBe. Furthermore, chronically infected patients in the non-replicative phase have low infectivity, and limited liver damage. The associated inflammatory liver disease varies in severity. Chronic HBV can last for decades with no or little inflammation and damage, but it may also proceed to cirrhosis in up to 20% of the cases. Chronic HBV is associated with a 100-fold increase in the risk of developing a hepatocellular carcinoma. Globally, HBV causes 60-80% of the world’s primary liver cancers [19]. It is estimated that men who develop cirrhosis and/or hepatocellular carcinoma have a lifetime risk of death of between 40 and 50%. In women, the risk is about 15%, placing chronic hepatitis B infections among the 10 leading causes of death in men [1]. In the Netherlands, an estimated yearly average of 26 persons died due to hepatitis B infection in the period 2000 to 2006 [20].

![Diagram](image.png)

**Figure 3.** Serology of a hepatitis B infection; The body, the HIV/AIDS resource [18]

**Treatment**

Most icteric patients (99%) with an acute HBV infection resolve their infection and do not require treatment, since the rate of recovery is not likely to be improved. Fulminant hepatitis is a rare but severe form of an acute infection, and is complicated by encephalopathy,
bleeding, and liver failure. Patients with fulminant hepatitis should be considered for liver transplantation if appropriate [21].

Management of chronic hepatitis B depends on the level of viral replication. Although progression to cirrhosis is more likely in severe than in mild or moderate chronic hepatitis B, all forms of chronic HBV infection can be progressive. Treatment of a chronic infection is indicated if there is active viral replication (HBV DNA >10^5 IU/ml), combined with signs of disturbance of the liver function (elevated ALAT), or presence of liver inflammation or fibrosis.

HBsAg loss or seroconversion after therapy is considered the ultimate therapeutic goal of anti-HBV therapy, since it is associated with positive long-term clinical outcomes. Present treatment options include immune modulation with interferon-α (IFNα) or pegylated(PEG)-IFNα and direct antiviral treatment with nucleoside analogues. Interferons bind to cellular receptors and activate secondary messengers to initiate the production of multiple proteins that are pivotal in the defence of the cell against viruses. The main advantages of interferons over nucleoside analogues are the absence of resistance and the possibility of immune-mediated clearance of HBV [22]. However, inconvenient administration (subcutaneous) and frequently occurring side-effects hamper wider use. The recommended duration of treatment for PEG-IFNα is 12 months. Nucleoside analogues have structures similar to the natural nucleotides and compete at the HBV polymerase catalytic site during the synthesis of viral DNA. Nucleoside analogues can be given for prolonged periods without any side-effects. One year of treatment leads to a reduction in the viral load, a decrease in the transaminases, and improvement of the liver histology in almost all patients. However, the major disadvantage of long-term use of nucleoside analogues is the high rate of resistance, observed in both HBeAg and anti-HBe positive patients. This is especially true of lamivudine, but also of adefovir. Resistance against the newer entecavir and tenofovir is at present limited, but the follow-up period is still short. After discontinuation of antiviral therapy, the viral load might increase to pre-treatment values, making it important to develop new antiviral agents with low sensitivity to resistance. The concept of a combination therapy for viral infections is not new, but is rapidly emerging as an important issue to avoid and overcome the problem of the selection of HBV drug-resistant mutants.

Prevention

Hepatitis B is preventable with a safe and effective vaccine (>95% effective). In 1992, the WHO advised worldwide universal vaccination in countries where HBV is highly endemic by 1995 and in low-endemic countries by 1997. Vaccination is the most effective tool to prevent the transmission of HBV. After completion of a series of three vaccinations, protective antibody levels are induced in 90% of adults and 95% of children. After revaccinating the non-responders, 30 to 50% in that group will achieve protective levels. The immunized persons are protected for at least 25 years, even if anti-HBs levels decline below detectable levels [23]. Universal vaccination in high and medium endemic countries has led to a
considerable reduction in prevalence, morbidity, and mortality. However, financial problems are the major barrier to vaccine introduction in developing countries. Besides vaccination, notification of hepatitis B is of great importance in preventing HBV transmission. In this way, sources and contacts can be traced and vaccinated if still susceptible. Furthermore, the most probable mode of transmission can be retrieved and trends in HBV transmission can be monitored. These are important data on which new policies are based. Increasing the awareness of the chance and severity of an HBV infection is also essential for prevention of HBV in and outside healthcare settings. Screening blood products and organs, using sterile instruments, and needle exchange programmes for injecting drug users are just a few measures that can be taken to avoid HBV transmission.

Prevention in the Netherlands
The Netherlands is a low endemic country with an estimated HBsAg prevalence of 0.3-0.5% and a mean reported incidence between 1.4 and 2.0 per 100,000 inhabitants for the past decade (figure 4) [24;25]. Furthermore, HBV transmission is mainly restricted to risk groups, especially to behavioural risk groups. Therefore the Netherlands, like Britain, and the Scandinavian countries, has adopted a policy of vaccination targeted at behavioural risk groups rather than universal vaccination [26]. This targeted programme was implemented in November 2002, after a two-year pilot conducted in several regions of the Netherlands to investigate its feasibility [27]. Within the auspices of the programme, commercial sex workers, hard drug users, and men having sex with men are currently 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. Before implementation of this targeted vaccination programme, HBV prevention in the Netherlands consisted mainly of the prenatal screening and vaccination of newborns with a chronically infected mother, vaccination of certain patient groups (e.g., haemophiliacs), and healthcare workers. These preventive measures are still ongoing and, were extended with the targeted vaccination programme and, as of 2003, with the vaccination of newborns with at least one parent born in a country with an HBV prevalence of over 2%.
Molecular epidemiology
Phylogenetic analysis estimates the relationship between genes or gene fragments by inferring the common history of the genes or gene fragments. To do this, it is essential that homologous sites are compared [28]. Consequently, the homologous sequences under investigation are aligned so that homologous sites form columns in the alignment. A phylogenetic tree can illustrate evolutionary relationships between genes and organisms, which is comparable to a pedigree showing which genes or organisms are most closely related. Phylogenetic trees are described as such, because the various diagrams used for depicting these relationships resemble the structure of a tree and the terms referring to the various parts of these diagrams (e.g., root, stem, branch, node and leaf) are also reminiscent of trees. The branches between the sequences can be of a different length. These differences in length denote a difference in genetic distance between two sequences, meaning that they are not similar. The greater the genetic distance, the greater the difference between two sequences. The genetic distance is deduced from the percentage of differences in nucleotides between sequences, taking into account the likelihood of occurring mutations and the locus of a mutation. Groups of genetically highly related virus strains form clusters in a phylogenetic tree. Full length sequencing is preferable to reveal evolutionary relationships. However, this is time consuming and, for epidemiological purposes, a less detailed dendrogram 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 was sequenced and used in the phylogenetic analyses.

Molecular epidemiology is a two-sided approach, and combines the phylogenetic analysis in which the genetic variability is characterized by the demographic and behavioural characteristics of the host. After combining these data, transmission networks and risk groups can be distinguished. By making use of phylogeny, sources and contacts can be confirmed or ruled out. The big advantage is that problems are approached from two sides,
combining the power of two different methods. As a result, more information can be collected on the epidemiology of a pathogen. PCR, coupled with nucleotide sequencing, has now become a powerful tool in epidemiologic investigation of new and emerging infectious diseases.

**This thesis**
The studies in this thesis are focused on the transmission of HBV in a low endemic country. These studies use molecular information on HBV, combined with demographic and behavioural data of patients, as well as vaccination numbers. Only acute cases were included in these studies, because they give insight into the evolution of HBV transmission in the Netherlands.

To obtain more insight into HBV transmission, molecular data need to be combined with epidemiological data. One should preferably be able to discriminate between acute and chronic cases, as acute cases give insight into the virus strains circulating at that time.

Chapter 2 provides an outline of the molecular epidemiology of hepatitis B in the Netherlands by using data of all reported acute cases in the Netherlands in 2004.

Little is still known about the HBV incidence, risk factors, and changes in the circulating genotypes among risk groups over time. The first study in chapter 3 describes the HBV and HIV incidence and the circulation of a single genotype A strain among men having sex with men over time. The second study describes the changes in HBV incidence, risk factors and genotypes over time in respect of injecting and non-injecting drug users and debates whether hard-drug users are still a risk group for HBV infection. Both these studies were performed among participants in the Amsterdam Cohort Studies.

The Netherlands is one of the few countries that has adopted a vaccination programme targeted at behavioural risk groups and not a universal vaccination programme. It is important to monitor the effect of this current strategy. Such an evaluation may have implications for countries with a comparable vaccination strategy and for countries with a universal vaccination programme, as they have the same risk groups. Chapter 4 includes two studies on the impact of this current targeted vaccination strategy using molecular epidemiology, one performed in Amsterdam and one nation-wide survey.

Chapter 5 demonstrates that, besides conventional case registration, molecular sequence data provide a powerful additional monitoring instrument for assessing the impact of vaccination when closely examining sequence data from HBV strains found among acutely infected patients in Amsterdam.

The general discussion in chapter 6 considers the molecular epidemiology and transmission of HBV in the Netherlands. Furthermore, the impact of the current vaccination strategy targeted at behavioural risk groups is also discussed, while the implementation of universal vaccination in the Netherlands is also taken into consideration. The implications for prevention are examined and recommendations are presented for future research.
References


Chapter 1


