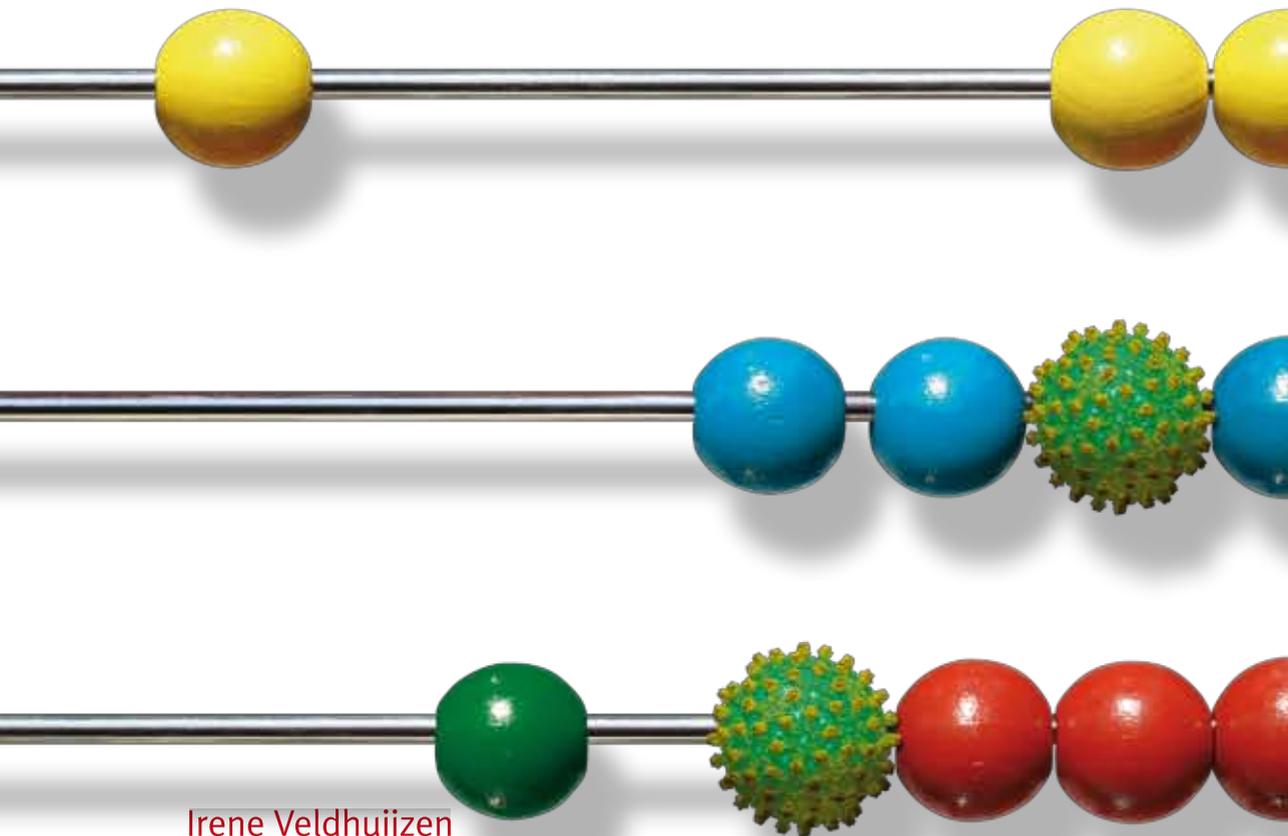# Secondary Prevention of Hepatitis B
in the Netherlands

Irene Veldhuijzen

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Colofon

Secondary Prevention of Hepatitis B
in the Netherlands

Secundaire preventie van hepatitis B
in Nederland

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 4 september 2009 om 13:30 uur

door

Irene Karen Veldhuijzen

geboren te De Bilt

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie

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Contents

Chapter 1

General introduction

Hepatitis B virus infection is widely present worldwide, approximately one third of the world's population has been exposed to the virus and an estimated 350 million people are chronically infected.[1,2] An infection with hepatitis B virus affects the liver and can result in a broad spectrum of disease outcomes. Acute hepatitis B virus infection can resolve and lead to protective immunity, but it can also result in a chronic infection and, in rare cases, cause acute liver failure with a high risk of dying. People with chronic hepatitis B virus infection remain infectious to others and are at risk of serious liver disease such as liver cirrhosis or liver cancer later in life. More than 500,000 people die each year of hepatitis B related diseases.[1,3]

Natural history

Different serological markers can be detected and used to describe the phase of hepatitis B virus infection.[4] The hepatitis B surface antigen (HBsAg) is first to appear after infection and around 8 weeks later antibodies to hepatitis B core antigen (anti-HBc) can be detected. Anti-HBc remains detectable lifelong and indicates a current or past infection. When an acute infection is cleared successfully, HBsAg disappears—usually within 6 months after infection—and antibodies to HBsAg (anti-HBs) are formed. Anti-HBs protects against hepatitis B virus infection and is also produced after vaccination. Acute hepatitis B virus infection becomes chronic when HBsAg remains detectable in serum more than six months after infection.

In the natural history of chronic hepatitis B, four phases of infection can be distinguished.[5-8] The first phase is the immune-tolerant phase, which is mainly observed in patients infected perinatally or in the first years of life. This phase can last for decades, and is characterised by the presence of hepatitis B e-antigen (HBeAg), a surrogate marker for viral replication. A more precise marker is the level of hepatitis B virus DNA (HBV DNA) in serum. Viral replication is high in patients in the immune-tolerant phase, with high levels of HBV DNA, but liver inflammation is absent or mild. The serum level of the liver enzyme alanine aminotransferase (ALT) coincides with the amount of liver inflammation, which can be assessed more accurately with histological analysis of a liver biopsy. Disease progression is generally low in patients in the immune-tolerant phase. However, even when ALT levels were low, fibrosis (scarring) has been observed in childhood, suggesting a degree of progression.[9]

In the second phase, the immune active—or immune clearance—phase, an active immune response is present, which results in liver inflammation and periodically elevated ALT levels, so-called flares. HBeAg is usually present in combination with high or fluctuating levels of HBV DNA. In this phase of active disease progression fibrosis occurs and with

Figure 1   Phases of chronic hepatitis B virus infection
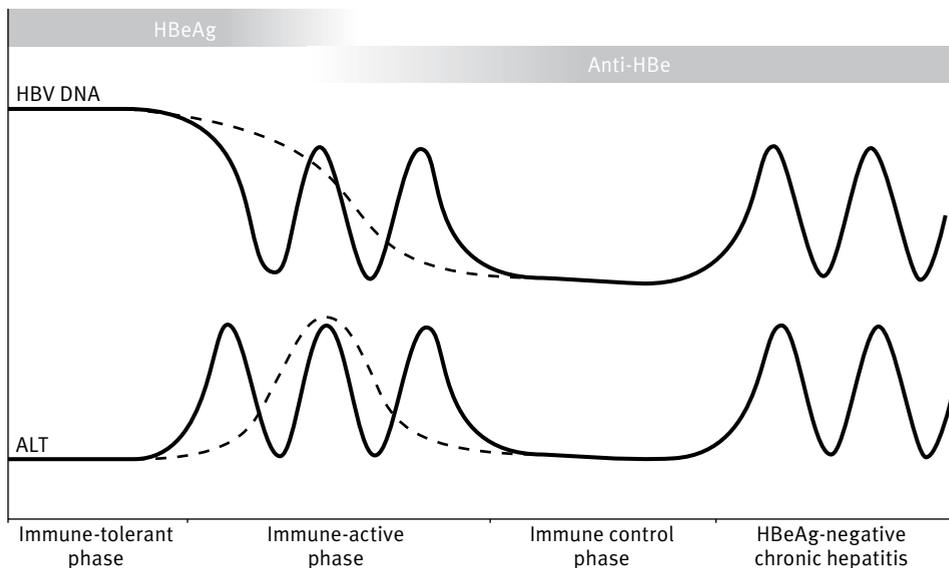
longer duration the risk of developing cirrhosis and hepatocellular carcinoma increases. In this phase, the annual probability of HBeAg clearance is 8-12%.[10]

When antibodies to HBeAg (anti-HBe) appear and HBeAg is cleared (HBeAg seroconversion) patients enter the third phase, the immune control phase, which is also called the inactive carrier phase. In this phase HBV DNA levels drop to low or undetectable levels, which is accompanied by ALT normalization and reduced liver inflammation. Liver disease generally does not progress in this phase.

Unfortunately, a considerable amount of patients, 15–30%, experience disease reactivation.[11-13] In this fourth phase of disease, which is called HBeAg-negative chronic hepatitis, patients experience disease flares. Their disease progression resembles that of the immune active phase. Recently, a fifth phase, the so-called HBsAg-negative phase, also referred to as "occult" or "latent" hepatitis B, was described to categorise patients in whom, after HBsAg loss, low-level HBV replication may persist with detectable HBV DNA in the liver and sometimes in serum.[7] Progression of liver disease is unlikely in this phase.

The 5 year cumulative incidence of cirrhosis in patients with chronic hepatitis ranges from 8-20%.[7] The development of hepatocellular carcinoma depends on the severity of underlying liver disease, in patients in the inactive carrier phase the annual incidence of hepatocellular carcinoma is only 0.1%, it is 1% in patients with active chronic hepatitis B,

and increases to 3-10% in patients with cirrhosis.[14] Follow up studies of chronic hepatitis B patients show that the risk of developing cirrhosis and hepatocellular carcinoma is higher for patients remaining HBeAg positive.[11,13,15,16] A large study from Taiwan found that patients with persistently high HBV DNA levels had the highest risk of developing hepatocellular carcinoma.[17]

Epidemiology

The risk of developing a chronic hepatitis B virus infection is related to the maturity of the immune system and is therefore highly dependent on the age at infection. Of children infected at birth more than 90% develop a chronic infection, this decreases to 25-50% for infections during early childhood (1-5 years of age) and to less than 10% for infections acquired after the age of 5 years.[18] This characteristic of the transmission dynamics of hepatitis B provides a positive feedback mechanism—the higher the prevalence of chronic infection, so the lower the average age at infection, so the higher the prevalence of chronic infection, and so on, which can explain the wide variability in the prevalence of chronic hepatitis B virus infection worldwide.[19] According to the prevalence of HBsAg as an indicator of chronic infection, the World Health Organisation classifies countries in groups where hepatitis B is low (≤2%), intermediate (2-8%) or high (≥8%) endemic.

The hepatitis B virus is transmitted through percutaneous or mucosal contact with infected blood or body fluids.[20] Infection can occur perinatally, i.e. from mother to child during childbirth, through household and sexual contact, sharing of infected needles and through occupational or medical exposure. The risk of perinatal transmission from a positive mother to her child during childbirth is high and varies from 10-30% for HBeAg negative mothers to 70-90% for HBeAg positive mothers.[21,22] Infections in early childhood are mainly caused by household transmission, through extensive contact with HBsAg positive household members.[23,24] The risk of sexually transmitted hepatitis B virus infection is higher for men having sex with men (MSM) compared to heterosexuals and increases with higher number of sexual partners.[25,26] In countries with high hepatitis B prevalence, transmission occurs mainly perinatally and horizontally during early childhood, while in low endemic countries, transmission is largely limited to groups with risk behaviour such as high risk sexual behaviour and intravenous drug use.

The Netherlands is a low endemic country for hepatitis B, the prevalence of chronic infection was 0.2% in a nationwide seroprevalence study.[27] A recent study estimated the prevalence to be between 0.3% and 0.5%.[28] Surveillance data show that the majority of acute infections are sexually transmitted, 34% by MSM and 25% by heterosexual contact.[29] Seventy-seven percent of newly diagnosed chronic HBV patients were born

abroad, almost all in intermediate- or high endemic countries.[29] It was recently calculated that between 58% and 72% of all HBsAg carriers in the Netherlands belong to the group of first generation migrants (FGM).[28]

Molecular epidemiology

The hepatitis B virus genome can be distinguished in eight genotypes, referred to as A–H, based on the level of nucleotide variation in the conserved regions of the viral genome.[30-32] These genotypes have a characteristic geographic distribution[33-36] and are found predominantly in the following regions: Africa, India, Northwest Europe and North America (A); China, Japan, Southeast Asia and the Pacific (B, C); the Mediterranean and the Middle East (D); West Africa (E); Central and South America (F); the United States, Mexico and Europe (G) and Central America (H). Two subgenotypes within genotype A form two major geographical clusters, one European-North American (A2) and one mainly Afro-Asian (A1). The genotype distribution within a country is influenced by the ethnic background and country of birth of the patients with hepatitis B. Besides the association of genotype with geographic areas, genotypes are also found to be associated with the transmission route of HBV infections. In Denmark a large cluster of genotype D viral strains associated with injecting drug use was observed.[37] In Amsterdam, the Netherlands, in the 1990s, a cluster related to MSM was identified in genotype A and a cluster related to injecting drug use in genotype D.[38] In a more recent nationwide survey a large cluster of homosexual men in genotype A was again found but the cluster related to injecting drug use was not observed anymore.[39]

Surveillance

With the surveillance of infectious diseases, changes in disease occurrence can be identified. This information is necessary to evaluate and adjust control policies. The Netherlands has a disease notification system in which the Public Health Law (Wet Publieke Gezondheid) requires that newly diagnosed hepatitis B infections, both acute and chronic, are reported to the Municipal Public Health Service (MPHS). The MPHS contacts the medical professional who requested the laboratory test—often the general practitioner or the midwife—and subsequently the patient. Epidemiological data such as sex, age, country of birth and most likely transmission route are reported by the MPHS to the Center for Infectious Disease Control and collected in OSIRIS, the national infectious diseases surveillance database.

Molecular data can provide insight in the distribution of the various genotypes and can be of added value for the surveillance of hepatitis B.[40] Since 2004, blood samples of patients with acute hepatitis B virus infection in the Netherlands are collected and analysed with molecular techniques.[39] Sequence comparison can give more precise insight into HBV transmission networks as clusters of patients infected by genetically closely related viruses—which potentially infected each other—may be identified.

Beside a disease notification system, serological surveillance studies can be used to monitor the prevalence of hepatitis B in the general population. A large study from the mid 1990s showed that 2.1% of the general population in the Netherlands had evidence of a previous infection with hepatitis B, and 0.2% had a chronic infection.[27] Serological surveillance can also be used to evaluate the performance of hepatitis B vaccination programmes, as shown by the European Sero-Epidemiology Network reporting the comparison of hepatitis B sero-epidemiology in ten European countries.[41,42]

Primary prevention

Primary prevention aims to prevent a disease from occurring. For hepatitis B, primary prevention is possible through vaccination, a safe and effective vaccine is available since the early 1980s. Different approaches to vaccination exist, from selective vaccination of high risk groups, to universal childhood vaccination, the latter often combined with catch-up programmes in adolescents for the first 10-12 years of the universal programme. Many countries have started universal hepatitis B vaccination about a decade ago.[43] The Netherlands has programmes to prevent perinatal infections and infections in early childhood. Since 1989 newborns of chronically infected mothers, identified through the pregnancy screening programme, are vaccinated. Vaccination to prevent perinatal infection is proven effective.[44] Since 2003 all children of which one of the parents is born in a country where hepatitis B is intermediate or high endemic are offered vaccination as well. Furthermore, a vaccination programme targeting behavioural high risk groups, i.e. MSM, hard drug users and commercial sex workers, is in place nationwide since November 2002 after a pilot programme from 1998-2000.[45] Until 2008 heterosexuals with multiple partners were also included in this programme. Last but not least, contacts of persons with chronic hepatitis B who are at risk of infection are offered vaccination. These contacts are identified through source and contact tracing.

Secondary prevention

Despite vaccination, the problem of people already infected with hepatitis B remains. Secondary prevention is aimed at early disease detection to allow interventions to prevent progression of the disease and emergence of symptoms.

Screening for hepatitis B is a form of secondary prevention, and antiviral treatment is an intervention that aims to prevent the occurrence of hepatitis B related liver disease. The goal of antiviral treatment is to suppress viral replication, and thereby slow disease progression. Antiviral treatment is also used as tertiary prevention in patients who already have symptoms of the disease, e.g. to prevent complications in decompensated liver disease.[10] The possibilities for antiviral treatment have greatly improved over the past decade: several registered drug therapies for chronic HBV, that have been shown to be cost-effective, are now available.[46-50] However, as hepatitis B is largely asymptomatic, many patients who might benefit from treatment remain undetected.

In the Netherlands, pregnant women are screened for hepatitis B virus infection and infants of HBsAg positive mothers are vaccinated to prevent perinatal infection. Furthermore, sexual partners and household contacts of HBsAg positive persons are offered screening by the MPHS as they are at risk of infection. Both programmes are aimed at preventing secondary hepatitis B virus infections, and detection of cases is limited to pregnant women and contacts of newly diagnosed patients. A strategy to improve detection of cases is active screening of risk groups. In countries with a low hepatitis B prevalence, migrants from countries with a relatively high hepatitis B endemicity have a higher risk of being infected with HBV.[28,51-53] Hutton et al. recommended screening of Asian and Pacific Islander adults in the United States, which they showed is likely to be cost-effective.[54]

As the health consequences of chronic hepatitis B virus infection can take decades to occur, studying the impact of interventions such as screening and treatment of patients with longitudinal observational studies is very impractical. Mathematical modelling can be used to predict the future impact of such interventions. A mathematical model often used is a Markov model, which describes disease progression of a hypothetical cohort of patients. Based on literature from clinical follow-up studies, the disease process is described in a number of health states with estimates for the annual progression probabilities between health states. The outcome of different models describing natural history or treatment strategies is given in numbers of patients developing a certain disease state and dying over a specified period. A Markov model can also be used for cost effectiveness analysis when the annual costs of liver disease and treatment is added. As cost effectiveness is expressed as costs per quality adjusted life year gained, the utilities of the different disease states are needed to calculate quality adjusted life years.

Research questions and outline of the thesis

In this thesis, we have studied the following research questions.

1 *What are the transmission routes, sources of infection and risk factors for acute hepatitis B virus infection in the Netherlands?*

2 *What is the prevalence of hepatitis B virus infection in different ethnic groups in Rotterdam?*

3 *What is the added value of molecular analysis in hepatitis B source and contact tracing?*

4 *What is the impact of secondary prevention of hepatitis B?*

The studies that address these research questions are described in the following six chapters. *Chapter 2 and 3* are based on a population-based case-control study of acute hepatitis B virus infection in the Netherlands and address the first research question. In *chapter 2* we describe the transmission routes and source of infection of patients with acute hepatitis B virus infection. In *chapter 3* the relative importance of risk factors for acute hepatitis B is studied by comparing patients with acute hepatitis B with a group of healthy controls.

The second research question is addressed in *chapter 4*, which describes the prevalence of previous and current infections with hepatitis A, B, and C found in a multi-ethnic neighbourhood in Rotterdam. We looked at differences between the main ethnicity groups and between first and second generation migrants.

The added value of molecular analysis in hepatitis B source and contact tracing (research question 3) is assessed in *chapter 5*. This study is based on patients with acute and chronic hepatitis B found in Rotterdam. We studied the level of molecular support for epidemiological transmission pairs found as a result of contact tracing.

In *chapter 6, 7, and 8* we address the last research question: What is the impact of secondary prevention of hepatitis B? *Chapter 6* describes the accuracy of a referral guideline for chronic hepatitis B patients in primary care to select patients eligible for evaluation by a specialist. In *chapter 7*, the potential impact of antiviral treatment on the mortality and morbidity of active chronic hepatitis B is estimated. This is done with a mathematical model including progression estimates of chronic hepatitis B patients with and without treatment. In *chapter 8*, the cost-effectiveness of systematic screening of migrants for chronic hepatitis B is calculated.

The general discussion (*chapter 9*) summarizes the main findings and answers the research questions. Finally, recommendations for future implementation of interventions are presented.

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Chapter 2

The importance of imported infections in maintaining hepatitis B in The Netherlands

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Abstract

In The Netherlands, in May 1999 an enhanced surveillance of hepatitis B was begun to collect detailed information of patients with acute hepatitis B virus (HBV) infection. The objective was to gain insight in transmission routes and source of infection of new HBV cases. Through public health services, patients were interviewed on risk factors. It appeared that the majority (59%) acquired the infection through sexual contact; 52% of these by homosexual and 48% by heterosexual contact. In 60% of the heterosexual cases, the source of infection was a partner originating from a hepatitis B-endemic region. Sexual transmission is the most common route of transmission of acute hepatitis B in The Netherlands and introduction of infections from abroad plays a key role in the current epidemiology of HBV. As well as prevention programmes targeted at sexual high-risk groups, prevention efforts should focus more on the heterosexual transmission from HBV carriers.

Introduction

Hepatitis B virus (HBV) infection is an important cause of morbidity and mortality world-wide as it can lead to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.[1] The age at which infection takes place is inversely related to the risk of developing chronic infection.[2] In areas of high and intermediate endemicity for HBV infection (HBsAg carrier rate >2%) the infection is mainly transmitted from mother to child (vertical transmission) and in young children through household contact (horizontal transmission). In low endemic areas (HBsAg carrier rate <2%) most infections occur in adults with risk behaviours such as injecting drug use (IDU) and high-risk sexual activity.[1] To achieve a large reduction in the carrier rate among children, the World Health Organization (WHO) advised all countries to integrate universal hepatitis B vaccination into their national immunization programmes by 1997.[3] A number of western European countries with very low HBsAg carrier rates have been reluctant to do so (e.g. United Kingdom[4], The Netherlands[5]). It is unclear how effective such a universal vaccination programme would be in a situation with low prevalence and a substantial importation of people with HBV infections.

In the Dutch statutory notifications, approximately 50% of acute HBV infections are attributable to sexual contact.[6] Apart from sexual preference, more detailed information on sexual behaviour is not recorded in the notifications. In a substantial percentage of acute HBV infections (37% in 2000) the transmission route is unknown.[6] In The Netherlands, the surveillance was enhanced to gain more insight in the epidemiology of HBV. All patients with acute hepatitis B infection were investigated thoroughly to assess risk factors associated with transmission of HBV and the source of infection.

Methods

Clinicians and microbiologists report patients with acute hepatitis B to their local municipal health services (MHS) as part of the Dutch notification system for communicable diseases. Patients are contacted routinely by the MHS to identify the source of infection and for contact tracing. Patients with acute hepatitis B reported between May 1999 and July 2000 and meeting the case definition were asked to participate in the study. Intake criteria included living in The Netherlands, a positive HBsAg test and elevated aminotransferase levels (>3 times normal). Patients with chronic infection causing this clinical picture were excluded. The MHS was also asked to register the notification number, the date of diagnosis, the sex, and year of birth of the patient. Additional data were collected by means of two questionnaires. During the contact tracing interview the public health nurse recorded details on the medical condition and possible risk factors

in order to identify the most likely mode of transmission and source of infection. The patient supplied additional information on demographic variables and risk exposures on a self administered questionnaire. For participants younger than 12 years of age, parents were asked to complete the questionnaire. Ethical permission was not granted to ask questions on sexual partnership to individuals under 18 years of age. Patients who did not agree to participate in the study were asked to fill out a non-response form. To determine the possible effects of non-response the cases in the study were compared with the notified cases of hepatitis B in the period of data collection. Written informed consent was obtained from all participants. The Medical Ethics Committee of The Netherlands Organization of Applied Scientific Research (TNO) approved the study.

Details on the number and nature of sexual partners in the past 6 months, commercial sexual contacts, drug use and needle sharing were collected in a face-to-face interview with the public health nurse. For each of the three most recent partners the following information was included: type of partner (self-defined as casual or steady), sex, age, nationality and country of birth of partner, condom use (never, sometimes, mostly, always), country where sexual contact took place, intravenous drug use and commercial sex work of partner, other (homo)sexual contacts of partner in the last year. Details on demography (age, ethnicity, education), education, work, (para)medical history (including cosmetic treatment and tattoos/piercings), and international travel were recorded by the patient. In case of international travel, questions regarding (para)-medical and cosmetic treatment while abroad were included. Most questions referred to the period of 6 months prior to diagnosis. Ethnicity was based on the country of birth of the patient and his/her parents; i.e. Dutch if the patient and both parents were born in The Netherlands and non-Dutch if the patient and/or at least one of the parents was born abroad. Educational levels were classified as 'low' (primary school, lower vocational or lower general secondary education), 'intermediate' (intermediate vocational or intermediate general secondary and higher general secondary education) and 'high' (higher vocational secondary education and university education). All variables concerning countries were classified according to the prevalence of HBsAg as defined by the WHO in low (<2%), intermediate (2–8%) and high (>8%) endemic countries. The final classification of the most probable route of transmission was based on the information obtained in the face-to-face interview and the additional questionnaire on risk exposure.

All statistical analyses were done with version 8.1 of the SAS software package (SAS Institute Inc., Cary, NC, USA). For comparison of the age distribution Student's t test was used. The χ^2 test or Fisher's exact test was used for comparing proportions.

Results

Between May 1999 and July 2000 144 patients participated in the study and 20 patients completed a non-response form. In the same period, 289 acute HBV infections were notified. Because these cases were considered eligible for this study, the MHS were contacted to retrieve information on the missing 125 patients. Of those, 27 did not meet the case definition and were excluded. Reasons for non-response of the remaining 98 patients were that the MHS did not inform the patient of the study (e.g. the MHS did not participate at all, could not reach the patient, forgot to ask the patient, did not want to burden the patient) or the patient refused to participate. As a result, 144 (55%) of the total number of 262 eligible patients participated in the study. The sex distribution was similar for the participants (108 men and 36 women) and the non-participants (P=0.32). Also there was no difference in the age distribution between participants and non-participants (t test men, P=0.14; t test women, P=0.18).

The age of the patients ranged from 2 to 75 years and the mean age of men was greater than that of women (*Table 1*). The majority of the patients (92%) were diagnosed because of symptomatic disease. Other reasons for serological testing were medical examination (n=6), new sexual partner (n=2), contact tracing (n=2), pre-vaccination serology (n=1). An HBsAg confirmation test was performed for 108 patients (75%). Twenty patients (14%) were hospitalized due to their HBV infection, women more frequently than men (*Table 1*). The majority of the patients (92%) had Dutch nationality or Dutch ethnicity (77%). Of the patients with non-Dutch ethnicity, 77% (24/31) came from countries with an intermediate or high prevalence of hepatitis B. More women (34%) than men (18%) had non-Dutch ethnicity. For both genders, the educational level was low for 40%, intermediate for 36% and high for 24% of the patients. Intravenous drug use and commercial sex work in the 6 months prior to diagnosis were not reported.

The most likely route of transmission was sexual contact for 85 patients (59%). Other risk exposures were identified for another 38 patients (26%). In 21 cases (15%) the most likely transmission route could not be identified.

Table 1.   Characteristics of patients

	Total		Men		Women		
	n = 144	100%	n = 108	75.0%	n = 36	25.0%	P value
Mean age in years (s.d.)	35		38	(12.5)	28	(13.7)	<0.001
Nationality							**0.47**
Dutch	127	92.0	96	93.2	31	88.6	
Non-Dutch	11	8.0	7	6.8	4	11.4	
Unknown	6	—	5	—	1	—	
Ethnicity							**0.06**
Dutch	105	77.2	82	81.2	23	65.7	
Non-Dutch	31	22.8	19	18.8	12	34.3	
Low endemic	7		5		2		
Intermediate endemic[a]	18		10		8		
High endemic	6		4		2		
Unknown	8	—	7	—	1	—	
Reason for HBsAg test							**0.10**
Symptoms	130	92.2	100	94.3	30	85.7	
Other	11	7.8	6	5.7	5	14.3	
Unknown	3	—	2	—	1	—	
Hospitalised for HBV							**0.008**
Yes	20	14.2	10	9.3	10	29.4	
No	121	85.8	97	90.7	24	70.6	
Unknown	3	—	1	—	2	—	
Transmission route							**n.s.**
Sexual	85	59.0	64	59.3	21	58.3	
Medical/dental treatment	9	6.3	9	8.3	0	0	
Blood contact (first aid, work)	11	7.6	10	9.3	1	2.8	
Contact with HBV carrier	8	5.6	4	3.7	4	11.1	
Percutaneous procedure[b]	6	4.2	3	2.8	3	8.3	
Other[c]	4	2.8	4	3.7	0	0	
Unknown	21	14.6	14	13.0	7	19.4	
Country of infection							**0.14**
The Netherlands	97	82.2	68	79.1	29	90.6	
Abroad	21	17.8	18	20.9	3	9.4	
Unknown	26	—	22	—	4	—	
Sexual contact (last 6 months)[d]							**0.68**
Yes	115	92.7	90	91.8	25	96.2	
No	9	7.3	8	8.2	1	3.8	
Not filled in	6	—	5	—	1	—	

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Of all patients who were probably infected through sexual contact, heterosexual contact was reported by 41 (48%) patients (21 women, 20 men), and men having sex with men (MSM) by 44 men (52%) (*Table 2*). Nineteen patients (22%) had non-Dutch ethnicity, less often for MSM (14%) compared to heterosexual men and women (32%, *P*=0.06), and more often for women (38%) compared to heterosexual men (25%, *P*=0.08). Compared to heterosexuals, MSM more often reported more than one sexual partner in the past 6 months (39% and 82% respectively, *P*<0.0001). Among heterosexuals more men than women reported more than one sex partner in the past 6 months but this difference was not significant. Eighty-nine per cent of MSM reported at least one sexual partner with known other sexual contacts in the previous year vs. 37% of heterosexuals (*P*<0.0001). Consistent condom use with the three most recent partners was reported by only 23% of the MSM (*n*=10 of whom six did not use condoms in oral sex), and by only 20% of the heterosexual men. Three patients had a concurrent sexually transmitted infection at the time of diagnosis (two MSM had HIV and one female had a chlamydial infection).

In all cases of sexual transmission the most probable source of infection was identified. Overall, 59% of the cases became infected through sexual contact with a casual partner. MSM more often (73%) than heterosexuals (44%, *P*=0.01), and heterosexual men (60%) more often than women (29%, *P*=0.01). Women were more likely to become infected by a steady partner. The ethnicity of the source of infection was more often non-Dutch for heterosexual men (65%) and women (57%) than for MSM (30%, *P*=0.01). Almost all non-Dutch partners of heterosexuals, who were identified as the source of infection, originated from countries with a intermediate or high prevalence of HBV (92%); for MSM this was 54% (*P*=0.004). Comparison of the ethnicity of case and source (for heterosexuals only) revealed that a non-Dutch partner infected 16 of the 26 (62%) Dutch cases compared to 9 of the 12 (75%) non-Dutch cases (*P*<0.05). For MSM relatively little information is available on the source of infection. Overall, 15% of the infections were acquired abroad, 25%(5/20) of the heterosexual men and 10% (2/21) of the heterosexual women acquired the infection in countries with an intermediate or high HBV prevalence.

[a] *Countries: Morocco (3), Turkey (3), Surinam/Netherlands Antilles (6), other intermediate endemic countries (6).*

[b] *Including (ear)piercing (2), acupuncture (2), tattoo (1), injection (1).*

[c] *Including shaving at barber's shop (2), living in a high endemic country for 4 months (1), sharing toiletry in students' house (1).*

[d] *Only patients aged 18 years and older.*

Table 2. Characteristics and sources of infection of patients infected through sexual contact

	Total		Homosexual men		Heterosexual men		Women	
	n = 85	100%	n = 44	51.8%	n = 20	23.5%	n = 21	24.7%
Mean age in years (s.d.)	35.9	(12.0)	37.7	(10.6)	37.0	(10.2)	30.3	(14.9)
Mean age in years (partner) (s.d.)	31.7	(9.3)	33.2	(5.9)	29.4	(6.9)	31.9	(13.6)
Ethnicity (patient)								
Dutch	60	70.6	33	75.0	14	70.0	13	61.9
Non-Dutch	19	22.4	6	13.6	5	25.0	8	38.1
Intermediate-high endemic	17		5		5		7	
Unknown	6	7.1	5	11.4	1	5.0	0	0
Number of partners (previous six months)[a]								
1	31	37.8	8	18.2	11	55.0	12	70.6
2	14	17.0	6	13.6	5	25.0	3	17.6
3	11	13.4	8	18.2	2	10.0	1	5.6
4-9	18	22.0	15	34.1	2	10.0	1	5.6
≥10	5	6.1	5	11.4	0	0	0	0
Unknown	3	3.7	2	2.3	0	0	1	0
Did partner have other sexual contacts in previous year								
Yes	54	63.5	39	88.6	8	40.0	7	33.3
No	19	22.4	2	4.6	9	45.0	8	38.1
Unknown	12	14.1	3	6.8	1	15.0	6	28.6
Condom use (6 months)[a]								
Yes (always)	14	17.1	10	22.7	4	20.0	0	0
No (most of the time, sometimes, never)	63	76.8	31	70.5	16	80.0	16	88.9
Unknown	5	6.1	3	6.8	0	0	2	11.1
Source of infection								
Steady partner	35	41.2	12	27.3	8	40.0	15	71.4
Casual partner	50	58.8	32	72.7	12	60.0	6	28.6
Ethnicity of source								
Dutch	38	44.7	24	54.4	6	30.0	8	38.1
Non-Dutch	38	44.7	13	29.5	13	65.0	12	57.1
Intermediate-high endemic	30		7		12		11	
Unknown	9	10.6	7	15.9	1	5.0	1	4.8
Country of infection								
The Netherlands	62	72.9	31	70.5	14	70.0	17	81.0
Abroad	13	15.3	6	13.6	5	25.0	2	9.5
Intermediate-high endemic	9		2		5		2	
Unknown	10	11.7	7	15.9	1	5.0	2	9.5

[a] *Only patients aged 18 years and older (missing data for three women).*

In 38 cases (26% of the total) a variety of risk exposures was identified (see *Table 1*). The source of infection was only identified in eight of the 38 cases (21%) with non-sexual transmission routes. Five cases could be classified as horizontal transmission, of whom four were younger than 15 years of age. All these were non-Dutch and infected through household contact with carriers of the same ethnic group. For 21 cases (15% of total) the mode of transmission was not identified as no risk exposures could be determined. Fourteen cases with non-sexual or unknown transmission routes (24%) reported travel to countries with a middle or high HBV prevalence; six of them (43%) had probably acquired the infection abroad.

Discussion

Sexual contact is the most common route of transmission of acute hepatitis B in The Netherlands. Approximately half of the sexually transmitted HBV infections was attributable to homosexual contact. A non-Dutch partner was often identified as the source of infection for heterosexuals (in 75% of the non-Dutch patients and in 62% of the Dutch patients).

Although the patients in the study were representative of the total group of notified HBV cases regarding sex and age distribution, they may not represent the total group of new infections with HBV. Due to subclinical infections, cases of acute HBV infections may not be recognized and, therefore, be under-reported. As the clinical expression of HBV is age-dependent[2,7], children are more likely to be missed and under-reported. Children in migrant populations with a higher HBsAg carrier rate than the Dutch population are at increased risk of acquiring HBV through horizontal transmission. A study by Franks et al. showed that nearly half of the HBV infections in children born in the United States to Southeast Asian refugees were attributable to horizontal transmission within and between families.[8] In The Netherlands, a screening programme of pregnant women and subsequent immunization of neonates of HBsAg-positive mothers has been in place since 1976. However, this programme is not effective in the prevention of horizontal HBV infections in later childhood in migrant populations. Therefore, the Dutch Ministry of Health has recently decided to offer HBV vaccination to all newborn infants having at least one parent born in a country with an intermediate or high prevalence of hepatitis B.

None of the patients in our study acquired HBV through IDU. In the notification data of 2000, it was found that only 1% of all acute HBV infections could be attributed to IDU.[6] This is different from the United Kingdom, where IDU was the most common exposure for acquisition of acute HBV (21%).[9] In Sweden 38% of all cases were attributed to IDU.[10] A study of IDU in The Netherlands showed that HBsAg prevalence rates in IDU vary between

3 and 7%.[11] Injecting drug users may be possibly under-represented in our study. This could be due to a bias in intake; injecting drug users may not be asked to participate in the study as it is assumed they are less willing to participate. In Amsterdam a large decline in prevalence and initiation of IDU was observed from 1986 to 1998 which might also partially explain the low number of acute HBV infections attributable to IDU.[12]

Of the notified cases of acute HBV in the year 2000, 52% were infected through sexual contact[6], which is similar to the proportion in our study (59%). Within the notification system the proportion has even increased recently to 60% in 2002, probably due to improved data collection.[13] The contribution of sexual transmission may be underestimated as infections with an unknown transmission route may also partially be due to sexual contact. In a case-control study, carried out in the United States among HBV patients who had no known source of infection, an additional 14% of all HBV infections was likely to be attributed to multiple heterosexual partners.[14]

The identification of the most likely route of transmission for acute hepatitis B is complicated. Nevertheless the proportion of cases with unknown transmission route in notification data from The Netherlands declined from 61% in 1993 to 37% in 2000 and 25% in 2002.[6,13,15] This percentage was 46% in laboratory-reported cases in the United Kingdom and approximately 30% in US sentinel surveillance.[9,16] Our study (15% unknown transmission route) demonstrates that enhanced surveillance can further reduce the proportion of unknown routes. This was previously noted by Struve et al.[10] However, it must be emphasized that in case of nonsexual transmission the actual source of infection is only identified in a few cases, e.g. in our study mostly through horizontal transmission.

Although MSM have been recognized as an important risk group for HBV infection for over 30 years, several studies have shown an increase in the number of acute infections due to heterosexual activity during the last two decades.[9,17,18] The MSM in our study reported a large number of casual partners, who also had other numerous contacts, and were mostly of Dutch origin. A total of 60% of the heterosexual cases were infected by partners from medium or high endemic countries. This shows that the importation of new infections plays an important role in the epidemiology of HBV. These results support the findings of the mathematical model of HBV in The Netherlands.[19] The basic reproduction number (Ro) in the MSM population was 4.67, suggesting that HBV can maintain itself in MSM. In the heterosexual population, the Ro is smaller than 1, suggesting that HBV cannot maintain itself without the introduction of new infections into the population.[19] This is also suggested by results from a molecular epidemiological study in Amsterdam.[20]

Our study shows that the prevention of HBV infections in sexual high-risk groups is of the highest importance as more than half of all recognized new infections are attributable

to sexual contact. The feasibility of an enhanced outreach programme of HBV vaccination of risk groups was demonstrated recently.[21] This programme for targeted risk group vaccination is now implemented throughout The Netherlands. The newly implemented vaccination programme for newborn infants having at least one parent born in a HBV-endemic country is aimed at the prevention of HBV in migrant populations. However, individuals with sexual partners from HBV-endemic countries are also at increased risk for HBV infection. Prevention should be focused more on the reduction of sexual transmission from HBV carriers.

Acknowledgements

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Chapter 3

Hepatitis B virus transmission in The Netherlands:
a population-based, hierarchical case-control study in a very low-incidence country

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Abstract

We report the first population-based case-control study on acute hepatitis B in a very low-incidence country. A case was a Netherlands resident, notified between May 1999 and July 2000 with symptoms and serology compatible with acute hepatitis B. Population controls were randomly selected, with oversampling from men and persons aged 20–39 years. Risk factors were studied using logistical regression, distinguishing confounders and mediators through hierarchical analysis. Participants were 120 cases and 3948 controls. The risk of acute hepatitis B was increased in men who have sex with men, with reporting to have had more than two partners in the past 6 months the only significant risk. In children, adult females and heterosexual males, having parents born in a hepatitis B endemic country was a significant risk. For adult females and heterosexual males, this was largely explained by having a foreign partner. For children this was partly explained by parenteral exposures abroad.

Introduction

Transmission of hepatitis B virus (HBV) occurs through percutaneous or permucosal exposure to infective body fluids. Infection can resolve spontaneously or become chronic with sequelae including cirrhosis and liver cancer. A safe and effective vaccine to prevent HBV infection has been available since 1982. In countries where the HBV prevalence is high (≥8% chronically infected), most infections are acquired perinatally or in childhood.[1] In contrast, in countries where the prevalence of HBV is low, most infections are acquired in adult life.[2-4] In The Netherlands, the incidence and prevalence of HBV infection is very low, and ›75% of infections with information are reported to have been acquired through sexual contact.[2] HBV vaccination in The Netherlands is based upon selective vaccination of individuals at high risk of infection. Examining the epidemiology of HBV contributes to the evaluation of the effectiveness of this strategy.

Routine reporting of acute HBV infections is of limited value to gain insight in the epidemiology of HBV infection. First, for about one third of reports the route of transmission is not reported. This percentage is remarkably constant across countries with different reporting systems[2,5,6] and may reflect limitations of all reporting systems or acquisition by unnoticed exposures. Second, the choice of the most likely transmission route by those reporting is based on their knowledge of risk factors and their assumed hierarchy when several are present. Last, since among adults heterosexual contact is almost ubiquitous as well as a known route of transmission of HBV, it could mask other transmission routes.

Therefore, analytical epidemiological studies are necessary to investigate transmission of HBV. Since the incidence of HBV is very low in The Netherlands, cohort studies are impracticable. We have conducted a population-based case-control study, aiming to describe routes of transmission of HBV within the Dutch population in order to inform vaccination policy.

Materials and methods

We carried out a population-based, frequency matched case-control study. A case was defined as symptoms and serology for HBsAg and HBcIgM compatible with acute HBV infection in a resident in The Netherlands. Cases notified between May 1999 and July 2000 as part of the routine Dutch notification system for communicable diseases were included.

Controls were selected from the general population through random sampling. Men and persons aged 20–39 years were oversampled (200% and 175% respectively) in order to obtain a match with expected frequencies among cases.

Written informed consent was obtained from all participants. The study was approved by the Medical Ethics Committee of The Netherlands Organisation for Applied Scientific Research.

Data collection

Information on demographics, occupation, travel, parenteral exposures and sexual partners were collected through a self-administered questionnaire. For participants aged <12 years, parents were asked to complete the questionnaire. Ethical permission was not granted to ask questions on sexual partnerships to persons aged <18 years of age. Persons who did not agree to participate in the study were asked to complete a non-response form.

More detailed questions on sexual behaviour were asked in a second questionnaire, to all cases aged >17 years and to a sample of controls aged >17 years. For cases, the second questionnaire was administered by a public health nurse, whereas controls received the second questionnaire by mail. The sample of controls who were sent the second questionnaire included all men who have (had) sex with men (MSM); all persons with a partner of non-Dutch nationality; all persons with multiple partners in the previous 6 months; and a 12% sample of all persons with at least one sexual contact during the previous 6 months. Questions in the second questionnaire included: the number of partners in the previous 6 months [and for each of the most recent three partners in this period the type of partner (casual or steady)]; sex; age; country of birth of partner; condom use (never, sometimes, mostly, always); country where sexual contact took place; injecting drug use (IDU) and commercial sex work of partner; and other sexual contacts of the partner in the last year. All exposure questions for cases and controls referred to the period 6 months prior to the date of diagnosis or of completing the questionnaire, respectively. In order to account for potential seasonal variation of exposure to risk factors for HBV infection, the mailing of questionnaires to controls was divided in four mailings of each 1800 questionnaires, in September and December 1999, and March and June 2000.

Parenteral exposures were grouped into three categories: medical (having undergone one or more of the following: injection, biopsy, operation, wound suture, renal dialysis, blood transfusion, phlebotomy); other (acupuncture, needle-stick injury, contact with another person's blood, a human bite, tattooing, piercing) and possible parenteral exposures (manicure, pedicure, beauty parlour treatment, borrowing of a toothbrush or razor, hairdresser visit abroad). Any parenteral exposure was defined as presence of a parenteral exposure as defined above.

Countries were grouped according to the prevalence of HBsAg in low (<2%), medium (2–8%) or high (>8%) endemicity.[7]

Sample size calculation

It was expected that about 200 cases of acute HBV per year would be eligible to take part in the study.[8] We calculated the number of controls needed in each subgroup of sexual behaviour in order to be able to detect an odds ratio (OR) of ≥3 for a risk factor with a prevalence of 10%, with an α of 0.05 and a power of 0.80. Subsequently, we estimated what percentage of controls, after oversampling of males and persons between 20 and 39 years, would belong to each subgroup. Finally, estimating the non-response to the first (general) and second (sexual history) questionnaire to be 1/3 and 1/6, respectively, we calculated that we needed to recruit 7200 controls.

Analysis

We used STATA statistical software, version 8.1 (StataCorp, College Station, TX, USA), except for calculating *P* values for trend, which was done in Epi-Info (CDC/WHO, version 6.04 d). Effects of exposures on risk of acute HBV were estimated by the OR and its 95% confidence interval (CI).

To adjust for oversampling of men and those aged 20–39 among controls, weights were used in all univariate analysis. Cases were assigned a weight of '1'. For controls, weights were chosen such that the age group and sex distribution among controls would fit that of the Dutch population in 2000 (http://statline.cbs.nl/). To adjust for oversampling individuals with a partner of non-Dutch nationality and/or multiple partners among controls invited to fill out the second questionnaire, a second weighing factor was introduced. Its value was calculated such that after weighing the frequency of those with a non-Dutch and/or multiple partners among those completing the second questionnaire matched this frequency among controls who completed the first questionnaire.

The effect of gender and sexual preference was examined in all adults (>17 years). The effect of other determinants was examined separately for three groups: adult women and heterosexual adult men, adult MSM, and children (participants aged <18 years).

Subsequently, we fitted multiple logistic regression models separately for these three groups to estimate effects of determinants adjusted for confounding. Prior to building the model, we classified potential determinants of infection hierarchically into groups ranging from distal to proximal to the outcome (HBV infection), as outlined in *Figures 1* and *2*. Distal determinants might have confounded more proximal ones, whilst the reverse is not the case. The building of the model consisted of as many steps as there were hierarchical levels. In the first model, only distal level determinants were added, so their effect could be estimated without inappropriately adjusting for the mediating effect of determinants situated at a more proximal level. In the next model, the determinants in the more proximal level were added, so that their effect could be estimated adjusting

for the confounding effect of the distal determinants, but not for the mediating effect of determinants situated at a more proximal level.[9] Only determinants which had a *P* value of <0.1 in the univariate analysis were included, and these variables were kept in all subsequent models, irrespective of significance levels. Age group and gender were included in all models so that weights adjusting for these variables did not have to be taken into account. For adult women and heterosexual adult men the second weighing factor was taken into account in those models where variables ascertained by the second questionnaire were included.

Figure 1. Conceptual hierarchical framework for risk factors for acquisition of HBV infection among adult females and adult heterosexual men in the Netherlands.

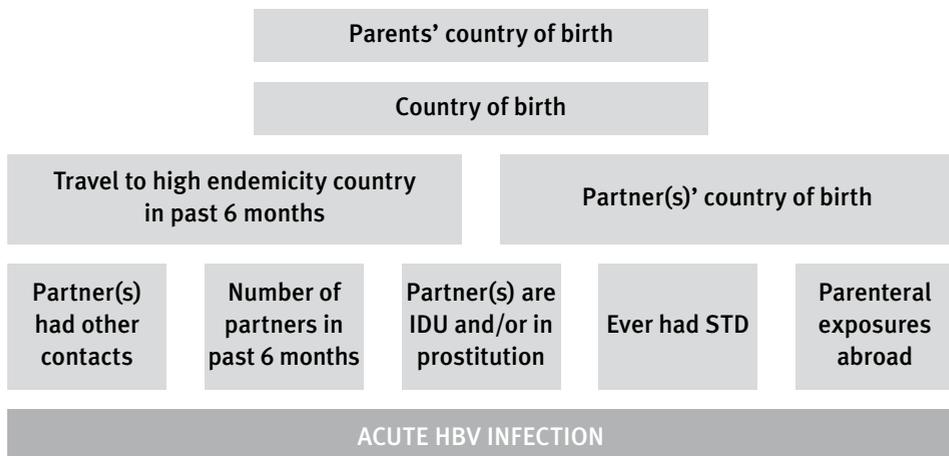|				
	Parents' country of birth			
	Country of birth			
Travel to high endemicity country in past 6 months		Partner(s)' country of birth		
Partner(s) had other contacts	Number of partners in past 6 months	Partner(s) are IDU and/or in prostitution	Ever had STD	Parenteral exposures abroad
ACUTE HBV INFECTION				

Figure 2. Conceptual hierarchical framework for risk factors for acquisition of HBV infection among individuals <18 years of age in the Netherlands.

Parents' country of birth
Country of birth
Parenteral exposures abroad
ACUTE HBV INFECTION
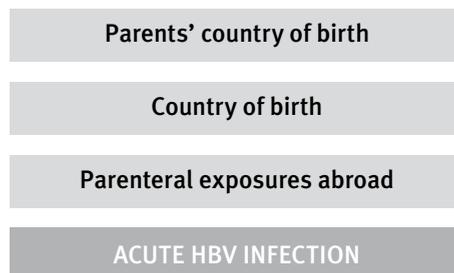
Results

Response
Cases
During the study period, 289 notifications of acute HBV infection were received. Of these, 51 did not meet the case-definition. Of the 238 remaining patients, 120 (50%) agreed to participate by completing the questionnaire and being interviewed. For 20 patients a non-response form was received.

Controls
Of 7200 initial questionnaires sent, 4353 were returned (response rate 61%). Of these, 405 (9%) were excluded, since they were not at risk for HBV infection due to HBV immunization or infection in the past, leaving 3948 controls. Of these, 2554 were adults with at least one sexual contact in the 6 months prior to completing the questionnaire. Of these, 574 controls were sent the second questionnaire, of which 491 were returned (response rate, second questionnaire 86%). Of these, 186 were female (38%), 289 were males reporting heterosexual contact only (59%), and 16 were MSM (3%).

Overall analysis
The risk of acute HBV infection was increased in individuals aged >17 years compared to younger individuals, with the highest risk in those aged between 18 and 39 years (*Table 1*). The risk of HBV infection was increased among males compared to females, with particularly high risks among MSM (OR 145.6, 95% CI 77.5–273.8).

Subgroup analyses
Group 1: Adult women and heterosexual adult men
Univariate analyses
Table 2(a) presents crude ORs for exposures grouped as 'demographic', 'parenteral', and 'sexual' among adult men reporting only heterosexual contact and adult females. Significantly associated with the risk of acute HBV infection were: being male, being aged between 18 and 24 years, having at least one parent born in a medium or high endemicity country; being born in a medium or high endemicity country; having had any parenteral exposure in a medium or high endemicity country; having had more than one partner in the past 6 months; having a non-Dutch partner; having a partner who was involved in IDU or commercial sex work; having a partner who had other partners; and having had a sexually transmitted disease (STD) in the past 5 years. IDU or commercial sex work was not reported among cases.

Table 1. Distribution of cases of acute HBV infection and controls with corresponding odds ratios (OR) and 95% confidence intervals (CI)

	Cases		Controls		OR*	95% CI	P value*
	n	%	*n*	%			
Sex							
Females	27	22.5	1.338	33.9	ref.		
Males	93	77.5	2.610	66.1	3.5	2.3 - 5.4	0.00
Age, median (95% CI)	36	(32.8–39.0)	34	(33.0–34.0)			
Age group (yr)							
0-17	9	7.5	773	19.6	ref.		
18-24	15	12.5	372	9.4	5.4	2.3 - 12.4	0.00
25-39	50	41.7	1510	38.2	5.0	2.4 - 10.2	0.00
≥40	46	38.3	1293	32.8	2.5	1.2 - 5.2	0.01
Sexual preference							
Heterosexual female	24	22.9	1143	41.8	ref.		
Heterosexual men	38	36.2	1566	57.2	2.2	1.3 - 3.6	0.00
Men having sex with men	43	41.0	28	1.0	145.6	77.5 - 273.8	0.00

The analyses included 120 cases and 3948 controls. Percentages were calculated after excluding individuals with missing information.

* *Odds ratios and P values were calculated by weighted analyses, taking into account oversampling of men and those aged 20-39 among controls.*

Multivariate analyses

Figure 1 shows the conceptual hierarchical framework for adult female and heterosexual male cases, built with determinants which were associated (*P*≤0.1) with the risk of acute HBV on univariate analysis. *Table 2(b)* lists the ORs resulting from the multivariate analyses. Model 1 indicated that descending from at least one parent born in a medium or high endemicity area for HBV is a significant risk factor for acquisition of HBV. However, the strength of this association decreases and becomes insignificant when 'country of birth' is added in a second model. In the third model, country of birth of partners and travel in the past 6 months were added. This showed that having a partner born in a medium or high endemicity country was a highly significant risk factor. Travel was not significant. In this model, the OR for country of birth decreased (from 3.6 to 0.7). On separate testing, it was found that this was largely explained by adding the country of birth of partners (results not shown). In the fourth model, the number of partners, risk behaviour of partners and parenteral exposures in medium or high endemicity countries was added. Only having two partners in the past 6 months was significant. In this model, the OR for having a partner from an endemic area fell to 8.9. On separate testing, this fall could largely be

explained by adding the variables number of partners and having had an STD in the past (results not shown).

Group 2: MSM
Univariate analyses
Table 3 presents crude ORs for exposures grouped as 'demographic', 'parenteral', and 'sexual' among adult MSM. The only significant risk factor for acquisition of HBV was reporting to have had more than two partners in the past 6 months. No multivariate analysis was done.

Group 3: Children
Univariate analyses
There were nine cases and 773 controls aged ≤18 years of age. Three of the cases reported heterosexual contact as the most likely route of transmission. Information on sexual contact was not available for controls. *Table 4(a)* presents crude ORs for demographic and parenteral exposures for those aged ≤18 years. Having at least one parent born in a medium or high endemicity country, being born in such a country, and parenteral exposure in such a country were associated with infection. Travel to a medium or high endemicity country was no more frequent among cases than among controls.

Multivariate analyses
Figure 2 shows the conceptual hierarchical framework for participants aged ≤18 years, built with determinants which were associated with the risk of acquisition of HBV on uni-variate analysis (*P*≤0.1). *Table 4(b)* lists the ORs resulting from the multivariate analyses. Model 1, including 'age', 'gender' and 'parents' country of birth', showed that having one or both parents born in a medium or high endemicity country significantly increased the risk of acquisition of HBV. The second model added 'country of birth', which was no longer significant. A subsequent model added parenteral exposures abroad. This made the OR for 'parents' country of birth' decrease, suggesting that part of the effect of the country of birth of the parents is explained by an increased frequency of parenteral exposures in medium or high endemicity countries in children with parents born in a HBV-endemic country compared to those with parents born in a non-HBV endemic country.

Table 2a. Cases of acute HBV infection in females aged >17 years, and in males aged >17 years reporting heterosexual contact only: distribution of demographic characteristics with corresponding odds ratios (OR) and 95% confidence intervals (CI)

	Cases		Controls		OR*	95% CI*	P value*
	n	%	*n*	%			
Demographic, socioeconomic determinants and travel							
Sex							
Females	21	35.6	1.143	42.2	ref.		
Males	38	64.4	1.566	57.8	2.5	1.4 - 4.2	0.00
Age group (yr)							
18-24	12	20.3	285	10.5	ref		
25-39	21	35.6	1.367	50.5	0.4	0.2 - 0.9	0.02
≥40	26	44.1	1.057	39.0	0.3	0.1 - 0.6	0.00
Age, median (95% CI)	37	(32-46)	37	(36-37)	n.a.		
Parents' country of birth							
The Netherlands or low endemicity country	48	84.2	2.497	92.2	ref.		
At least one in medium or high endemicity country	9	15.8	210	7.8	2.3	1.1 - 4.8	0.02
Country of birth							
Netherlands or low endemicity country	49	86.0	2.577	95.1	ref.		
Medium or high endemicity country	8	41.0	132	4.9	3.1	1.4 - 6.7	0.00
Employed in past 6 months							
No	17	30.4	758	28.3	ref.		
Yes	39	69.6	1.919	71.7	1.3	0.7 - 2.2	0.43
Occupation in health care							
No	40	88.9	1.689	90.1	ref.		
Yes	5	11.1	185	9.9	0.9	0.3 - 2.2	0.75
Family member in home for mentally disabled							
No	56	98.3	2.536	93.9	ref.		
Yes	1	1.8	164	6.1	0.3	0.0 - 1.9	0.18
Been abroad in past 6 months							
No, or to low endemicity country	40	70.2	2.076	77.5	ref.		
Yes, to medium or high endemicity country	17	29.8	601	22.5	1.6	0.6 - 2.9	0.09

	Cases		Controls				
	n	%	*n*	%	OR*	95% CI*	*P* value*
Parenteral exposures							
Any parenteral exposure							
None. or missing	20	33.9	965	35.6	ref.		
In the Netherlands or in other low endemicity country	32	54.2	1703	62.9	0.9	0.5 - 1.5	0.69
In medium or high endemicity country	7	11.9	41	1.5	9.6	3.8 - 24.1	0.00
Sexual exposures							
Number of partners in past 6 months							
0-1	45	76.3	2.367	96.1	ref.		
2	8	13.6	62	2.5	8.0	3.6 - 17.9	0.00
≥3	6	10.2	34	1.4	11.3	4.4 - 28.6	0.00
Country of birth last 3 partners†							
The Netherlands or low endemicity country	35	64.8	434	91.6	ref.		
At least one in medium or high endemicity country	19	35.2	40	8.4	12.1	5.8 - 24.9	0.00
Any of last three partners used drugs or was in prostitution†							
No	52	91.2	462	97.5	ref.		
Yes	4	8.8	12	2.5	10.0	2.6 - 37.7	0.01
Any of last three partners had other sexual contacts?†							
No	42	75.0	410	86.7	ref.		
Yes	14	25.0	63	13.3	6.4	3.1 - 13.6	0.00
Had STD in past 5 years?							
No	49	89.1	2665	98.6	ref		
Yes	6	10.9	38	1.4	12.0	4.8 - 30.4	0.00

The analyses included 59 cases and 2709 controls. Percentages were calculated after exclusion of individuals with missing information.

* *Odds ratios, confidence interval and P values were calculated by taking into account weights in order to adjust for oversampling of men and those aged 20–39 years among controls.*

† *Analyses included 474 controls. ORs were calculated by taking into account weights to adjust for oversampling among controls of men, those aged 20–39 years, those with a partner of non-Dutch nationality and/or with multiple partners.*

Table 2b. Cases of acute HBV infection in females aged >17 years, and in males aged >17 years reporting heterosexual contact only: results of multivariate analyses

	Model 1		Model 2		Model 3		Model 4	
	OR	P value	OR	P value	OR*	P value	OR*	P value
Gender								
Female	ref.		ref.		ref.		ref.	
Male	1.3	0.43	1.3	0.39	1.1	0.81	0.9	0.83
Agegroup								
18-24	ref.		ref.		ref.		ref.	
25-39	0.4	0.01	0.3	0.00	0.5	0.20	0.8	0.60
≥40	0.6	0.13	0.5	0.10	0.9	0.82	1.2	0.75
Parents' country of birth								
The Netherlands or low endemicity country	ref.		ref.		ref.		ref.	
Medium or high endemicity country	2.2	0.03	0.9	0.91	1.3	0.73	1.5	0.66
Country of birth								
The Netherlands or low endemicity country			ref.		ref.		ref.	
Medium or high endemicity country			3.6	0.08	0.7	0.66	0.6	0.62
Partners' country of birth								
The Netherlands or low endemicity country					ref.		ref.	
Medium or high endemicity country					14.1	0.00	8.9	0.00
Travel								
No, or to low endemicity country					ref.		ref.	
To medium or high endemicity country					0.8	0.49	0.6	0.29
Number of partners								
0-1							ref.	
2							4.1	0.04
≥3							2.7	0.30
Partner other sexual contacts								
No							ref.	
Yes							1.7	0.38

	Model 1		Model 2		Model 3		Model 4	
	OR	*P* value	OR	*P* value	OR*	*P* value	OR*	*P* value
Partner IDU or CSW								
No							ref.	
Yes							1.5	0.69
Ever had STD								
No							ref.	
Yes							3.3	0.13
Any parenteral exposures								
None, or missing							ref.	
In the Netherlands or in low endemicity country							0.7	0.41
In medium or high endemicity country							2.5	0.28

Model 1 and 2 included 57 cases and 2707 controls. Model 3 and 4 included 53 cases and 473 controls. Percentages were calculated after excluding individuals with missing information.

* *Odds ratios are calculated after taking into account weights to adjust for oversampling of those with a partner of non-Dutch nationality and one or multiple partners among controls.*

Discussion

To describe transmission of HBV, case-series are of limited value. However, in countries such as The Netherlands where the incidence of HBV infection is very low, analytical studies are difficult to perform due to the extreme range in prevalence of risk factors for transmission. Our study is the first reported population-based case-control study on risk factors for acute HBV infection in a country with a very low incidence. By including over 30 controls per case, and oversampling of known risk groups among controls, we attempted to get precise estimates of risks associated with both frequent and infrequent determinants.

The response rate among cases was 50%, and those responding may not represent all cases of acute HBV infection. However, the age and sex distribution did not differ between responders and non responders.[10]

We conclude that the most important route of transmission of HBV in The Netherlands is through male homosexual contact: this was reported for over half of male cases in our study,

Table 3. Cases of acute HBV infection in men who reported to have (had) sex with men (MSM): distribution of demographic characteristics with corresponding odds ratios (OR) and 95% confidence intervals (CI).

	Cases		Controls		OR*	95% CI*	P value*
	n	%	*n*	%			
Demographic, socioeconomic determinants and travel							
Age, median (95% CI)	36	(32-38)	37	(32-40)	n.a.		
Parents' country of birth							
The Netherlands or low endemicity country	33	86.8	26	92.9	ref.		
At least one in medium or high endemicity country	5	13.2	2	7.1	1.8	0.3 - 10.5	0.55
Country of birth							
Netherlands or low endemicity country	36	90.0	26	92.9	ref.		
Medium or high endemicity country	4	10.0	2	7.1	1.3	0.2 - 8.1	0.78
Employed in past 6 months							
No	5	12.5	4	14.3	ref.		
Yes	35	87.5	24	85.7	0.9	0.2 - 3.8	0.89
Occupation in health care							
No	32	94.1	21	87.5	ref.		
Yes	2	5.9	3	12.5	0.4	0.1 - 3.4	0.43
Family member in home for mentally disabled							
No	38	95.0	26	92.9	ref.		
Yes	2	5.0	2	7.1	0.5	0.1 - 4.0	0.52
Been abroad in past 6 months							
No, or to low endemicity country	29	70.7	17	60.7	ref.		
Yes, to medium or high endemicity country	12	29.3	11	39.3	0.5	0.2 - 1.7	0.28
Parenteral exposures							
Any parenteral exposure							
None. or missing	15	34.9	8	28.6	ref.		
In the Netherlands or in other low endemicity country	23	53.5	18	64.3	0.8	0.2 - 2.5	0.68
In medium or high endemicity country	5	11.6	2	7.1	1.3	0.2 - 10.5	0.78

	Cases		Controls				
	n	%	*n*	%	OR*	95% CI*	*P* value*
Sexual exposures							
Number of partners in past 6 months							
0-1	9	20.9	13	50.0	ref.		
2	6	14.0	2	7.7	5.7	0.9 - 35.3	0.12
≥3	28	65.1	11	42.3	4.4	1.4 - 13.4	0.02
Country of birth last 3 partners							
The Netherlands or low endemicity country	29	78.4	14	87.5	ref.		
At least one in medium or high endemicity country	8	21.6	2	12.5	2.2	0.3 - 17.4	0.47
Any of last three partners used drugs or was in prostitution							
No	40	95.2	15	93.8	ref.		
Yes	2	4.8	1	6.3	0.8	0.1 - 10.1	0.91
Any of last three partners had other sexual contacts?							
No	12	28.6	4	25.0	ref.		
Yes	30	71.4	12	75.0	1.0	0.3 - 3.9	0.98
Had STD in past 5 years?							
No	35	92.1	25	89.3	ref.		
Yes	3	7.9	3	10.7	0.7	1.2 - 3.9	0.72

The analyses included 43 cases and 28 controls. Percentages were calculated after exclusion of individuals with missing information (phase 2 questions only available for 16 controls).

* *Odds ratios, confidence interval and P values were calculated by taking into account weights in order to adjust for oversampling of men and those aged 20-39 among controls.*

and was strongly associated with infection. The number of partners in the past 6 months was the only risk factor identified among MSM. In particular, among MSM, import of HBV through partners from HBV endemic countries does not seem to play a role. This suggests that HBV transmission is sustained among MSM in The Netherlands, which is consistent with the results of a recent mathematical model of HBV transmission in The Netherlands.[11] This model also predicted that heterosexual transmission without import of new cases is not sufficient for ongoing transmission of HBV in the Dutch population.[11] Our data is also consistent with this: we found that the most important risk factor among heterosexual adults for acquisition of HBV is to have a partner born in a medium or high endemicity country.

Table 4a. Cases of acute HBV infection in children: distribution of demographic characteristics with corresponding odds ratios (OR) and 95% confidence intervals (CI).

	Cases		Controls		OR*	95% CI*	P value*
	n	%	*n*	%			
Demographic, socioeconomic determinants and travel							
Sex							
Females	6	66.7	195	25.2	ref.		
Males	3	33.3	578	74.8	0.5	0.1 - 1.9	0.30
Age, median (95% CI)	15	(13-16)	9	(8-9)			<0.001
Parents' country of birth							
The Netherlands or low endemicity country	5	55.6	706	91.3	ref.		
At least one in medium or high endemicity country	4	44.4	67	8.7	7.6	2.0 - 29.3	0.00
Country of birth							
The Netherlands or low endemicity country	8	88.9	764	98.8	ref.		
Medium or high endemicity country	1	11.1	9	1.2	9.6	1.1 - 87.7	0.05
Been abroad in past 6 months							
No, or to low endemicity country	8	88.9	671	87.4	ref.		
Yes, to medium or high endemicity country	1	11.1	97	12.6	0.8	0.1 - 6.8	0.87
Parenteral exposures							
Any parenteral exposure							
None, or missing	2	22.2	131	16.9	ref.		
In the Netherlands or in low endemicity country	6	66.7	637	82.4	0.5	0.1 - 2.6	0.42
In medium or high endemicity country	1	11.1	5	0.6	12.0	0.9 - 153.9	0.05

The analyses included nine cases and 773 controls. Percentages were calculated after exclusion of individuals with missing information.

* Odds ratios, confidence intervals and P values were calculated by taking into account weights in order to adjust for oversampling of men and those aged 20-39 among controls.

Table 4b. Cases of acute HBV infection children: results of multivariate analyses

	Model 1		Model 2		Model 3	
	OR	*P* value	OR	*P* value	OR*	*P* value
Gender						
Female	ref.		ref.		ref.	
Male	0.2	0.04	0.2	0.04	0.2	0.03
Agegroup						
0-14	ref.		ref.		ref.	
15-17	5.8	0.01	5.7	0.01	5.7	0.02
Parents' country of birth						
The Netherlands or low endemicity country	ref.		ref.		ref.	
Medium or high endemicity country	7.7	0.00	7.2	0.01	5.0	0.06
Country of birth						
The Netherlands or low endemicity country			ref.		ref.	
Medium or high endemicity country			1.5	0.77	1.6	0.74
Any parenteral exposures						
None, or missing					ref.	
In the Netherlands or in low endemicity country					0.7	0.67
In medium or high endemicity country					7.1	0.19

Model 1, 2 and 3 included nine cases and 773 controls.

Using a hierarchical method of analysis allowed us to estimate risks of HBV infection associated with having parents born abroad and being born abroad without controlling for mediating factors such as travel abroad. Due to temporal associations these mediating factors can not confound the relation between (parents') country of birth and HBV infection, and therefore should not be controlled for. Similarly, whereas the country of birth of parents can be a confounder in assessing the risk associated with country of birth, the reciprocal is not the case.

The second advantage of using a hierarchical method of analysis is that it allowed us to demonstrate that the increased risk of HBV infection among heterosexual adults associated with having parents born in a medium or highly endemic country, and being born in such a country, is explained largely through the increased likelihood of these individuals to have a partner born in an endemic country. This strongly suggests that the main route of transmission in Dutch heterosexual adults is through sexual rather than, for example, household contact.

None of our cases reported IDU or commercial sex work, suggesting that direct transmission through these routes is infrequent in The Netherlands. An alternative explanation for this observation is that IDUs and commercial sex workers may have been less likely to take part in the study, and, if taking part, may have been reluctant to admit to it. However, the high HBV seroprevalence among IDUs in The Netherlands (past infection 35–67%, carriers 4–7%) indicates low levels of susceptibility[8], which is consistent with our findings. Recently published results of molecular analyses of cases of acute HBV in Amsterdam suggests that the IDU cluster disappeared after 1998.[12] Having a partner who was involved in IDU and/or commercial sex work was a significant risk factor on univariate analysis among heterosexuals, suggesting that transmission through contact with IDUs and/or commercial sex workers is occurring.

We had few cases among children, since the majority of HBV infections in children remain asymptomatic and therefore were not included. The most important risk factor among children was to have (one or two) parents born in a medium or high endemicity country, and part of this risk was explained by a higher frequency of parenteral exposures in medium or high endemicity areas. Since there was only one case in a child with any parenteral exposures abroad, further exploration of this exposure was not possible. Some of the risk remained after including parenteral exposures abroad in the model, and excluding the childhood cases reported to have occurred through sexual contact, suggesting that other routes of transmission remain in childhood.

Implications for control of HBV infection through vaccination

In The Netherlands, at the time of our study, HBV immunization was offered to healthcare workers, individuals with certain chronic diseases, contacts of HBV carriers and babies born to infected mothers (identified by universal antenatal screening). Subsequent to our study, from autumn 2002 onwards, additional target groups have been identified, including MSM, IDUs, heterosexuals attending STI clinics and commercial sex workers. Furthermore, from March 2003 onwards, all children born to one or two parents born in intermediate or high endemicity countries, as well as all asylum seekers aged ≤18 years, are included in the target groups for immunization.[13,14] We found that MSM are at high risk of contracting HBV, and contribute to over one third of all cases. This suggests that the current Dutch programme which provides free vaccine for all MSM is appropriate.[14] Preventing infections in MSM has the potential to be highly effective since it would introduce a herd immunity effect.[11] Our study suggests that all MSM (irrespective of additional risk factors such as casual partnerships) are at increased risk of HBV infection. Indeed, having had an STD in the past 5 years was not a significant risk factor for HBV infection among MSM. This implies that delivering vaccine only through municipal health services

and STD clinics may not be sufficient, and that active outreach may be necessary. At the moment only some regions in The Netherlands provide this.[14]

Our study identified children born to parents born in medium or high endemicity countries as a high risk group for HBV infection. However, for adult heterosexuals with parents born abroad, this risk largely arises as a result of the increased likelihood of having partnerships with non-Dutch nationals: of the nine adult heterosexual cases whose parents were born abroad, seven had a partner born abroad and six were born abroad themselves. When sexual mixing with non-Dutch nationals becomes less determined by (parent's) country of birth, the effectiveness of targeting vaccination based on the latter will decrease. Part of the increased risk of having a partner born in an endemic country was explained by sexual risk behaviour. Vaccination targeted at those with sexual risk behaviour might therefore prevent some cases among those with a foreign partner.

Among heterosexual adults, only commercial sex workers and those attending an STD clinic are offered HBV vaccine free of charge. In our case-series, complete implementation of this policy would have prevented only 11% (6/55) of cases in heterosexuals. Immunization of all heterosexuals with partners of non-Dutch nationality would have prevented 36% (20/55) of cases in heterosexuals. The effectiveness of such a programme would, however, depend on whether vaccination can be given early enough to prevent transmission. Screening of individuals born in medium and high endemicity areas, and subsequent immunization of contacts of identified carriers, may be a more effective strategy to prevent heterosexual transmission of HBV. In addition, this may allow early treatment of carriers, helping to reduce the occurrence of sequelae. However, further information is necessary on feasibility, ethical issues and cost-effectiveness of such screening programmes.

Acknowledgements

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Chapter 4

Viral hepatitis in a multi-ethnic neighborhood in the Netherlands: *results of a community-based study in a low prevalence country*

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Abstract

Objectives The prevalence of viral hepatitis varies world wide. Although the prevalence of hepatitis A virus (HAV) and hepatitis B virus (HBV) infection is generally low in Western countries, pockets of higher prevalence may exist in areas with large immigrant populations. The aim of this study was to obtain more information on the prevalence of viral hepatitis in a multi-ethnic area in the Netherlands.

Methods We conducted a community-based study in a multi-ethnic neighborhood in the city of Rotterdam, the Netherlands, including both native Dutch and migrant participants, who were tested for serological markers for hepatitis A, hepatitis B and hepatitis C infection.

Results Markers for hepatitis A infection were present in 68% of participants. The prevalence of hepatitis B core antibodies (anti-HBc), a marker for previous or current infection, was 20% (58/284). Prevalence of hepatitis A and B varied by age group and ethnicity. Two respondents (0.7%) had chronic hepatitis B virus infection. The prevalence of hepatitis C was 1.1% (3/271). High levels of isolated anti-HBc were found.

Conclusions We found a high prevalence of (previous) viral hepatitis infections. This confirms previous observations in ethnic subgroups from a national general population study and illustrates the high burden of viral hepatitis in areas with large immigrant populations.

Introduction

The prevalences of hepatitis A virus (HAV), hepatitis B virus (HBV) and hepatitis C virus (HCV) infection vary worldwide. While the geographical distributions of HAV and HBV differ greatly between Western and non-Western countries, this does not hold for HCV. Hepatitis A is transmitted fecal-orally, hepatitis B through sexual contact, blood—blood contact, and perinatally from mother to child, and hepatitis C mainly parenterally. In the epidemiology of especially HAV and HBV in low endemic regions, the import of cases from intermediate or high endemic regions plays an important role.

A population based seroprevalence study in the Netherlands in 1995/1996, the so-called Pienter study, showed that 34% of the population had antibodies to HAV. The prevalence increased with age and was higher in people of non-Dutch ethnicity.[1] In the Pienter study a two-stage cluster sampling technique was used to draw a nationwide sample. In each of five geographic regions, with approximately equal numbers of inhabitants, eight municipalities were sampled proportionally to their size. Within each municipality, an age-stratified sample of 380 individuals was drawn from the population register.

Although the HBV prevalence is generally low in Western countries, pockets of higher HBV prevalence exist in areas with large immigrant populations. Therefore national prevalence figures can underestimate the burden of HBV infection. In the Netherlands, in the Pienter general population study, the prevalence of antibodies to hepatitis B core antigen (anti-HBc), a marker for past or current infection, was 2.1%, and the prevalence of hepatitis B surface antigen (HBsAg), which indicates chronic infection, was 0.2%.[2] The prevalence of chronic HBV in the Pienter study sample is believed to be an underestimate due to under-representation of the non-Dutch population. An adjusted estimate, based on the chronic HBV prevalence in the countries of origin of first generation immigrants, defined as foreign born, and their proportion in the Dutch population, resulted in a new general population HBsAg prevalence estimate of 0.36%.[3] The prevalence of hepatitis C in the general Dutch population was estimated at 0.1% in the Pienter study. Most of the HCV infected individuals had no known risk factors.[4]

In this paper we present the results of a community based study in a multi-ethnic neighborhood in the city of Rotterdam, the Netherlands. Rotterdam has a large immigrant population, with 26% of the population being first generation immigrants and 19% second generation immigrants, in contrast to 10% and 9%, respectively, in the Netherlands overall (Statistics Netherlands, data 2006). The aim of this study was to obtain further information on the prevalence of viral hepatitis in an area with a large immigrant population.

Patients and methods

Study population

A community based study in a multi-ethnic neighbourhood of nearly 13,000 inhabitants in the south of Rotterdam was carried out in 2004. We aimed at including a maximum number of 800 participants in the study, a number based on power calculations taking into consideration both feasibility and acceptable precision of prevalence rates in subgroups. People aged 18 to 65 years from the six main ethnic groups in the neighborhood, namely Dutch, Moroccan, Turkish, Surinamese, Antillean and Cape Verdean, were included in the sample. Ethnicity was based on the country of birth of the person or of one of the parents. A random sample of 1787 inhabitants of the neighborhood was drawn from the municipal administration, stratified by ethnic group. The number needed in each ethnic group was calculated based on an expected overall response of 50%, with oversampling of those ethnic groups where a lower response rate was anticipated. Foreign-born persons are referred to as first generation migrants (FGM), persons born in the Netherlands but with one of the parents born abroad, as second generation migrants (SGM). Individuals were invited by mail for a personal consultation at the community center. Reminder letters were sent to groups where response lagged. During the visit a blood sample was taken, and based on an interview in the native language, a questionnaire was filled in by the interviewer. The questionnaire included items on travel history, sexual behavior and risk factors such as intravenous drug use and other parenteral exposures (e.g., medical procedures, tattoo) in the Netherlands and abroad. Respondents provided written informed consent and the study was approved by the Medical Ethical Review Board of the Erasmus Medical Center. The respondents received their test results by mail and were offered free vaccination at the Municipal Public Health Service (MPHS) if they were susceptible to HAV/HBV.

Laboratory testing

All blood samples were tested for anti-HAV, anti-HCV, anti-HBc and HBsAg. Samples positive for anti-HBc and negative for HBsAg were further tested for antibodies to hepatitis B surface antigen (anti-HBs), and in the case of negative anti-HBs also for antibodies to hepatitis B e-antigen (anti-HBe). An anti-HBs level of less than 3 IU/l was considered negative. Anti-HAV, anti-HBc, HBsAg and anti-HBe testing was done with a microparticle enzyme immuno assay (MEIA, Axsym, Abbott Laboratories). HBsAg-positive sera were retested with a confirmation assay (Abbott Laboratories). For anti-HBs testing, a quantitative MEIA was used, and for anti-HCV testing an enzyme-linked immunosorbent assay was used (ELISA, Axsym, Abbott Laboratories). Anti-HCV positive sera were confirmed by recombinant immunoblot (Innogenetics).

Statistical analysis

Statistical analyses were performed using SPSS. To obtain prevalence figures of previous infection with HAV and HBV for the population in the neighborhood, weighted prevalences were calculated using inverse probability weighting by sex and ethnicity. In this procedure, weights are calculated for each of the 12 sex/ethnicity groups by dividing the proportion of a group in the neighborhood population by the proportion of that group in the study population. Differences in prevalence by sex and ethnicity were tested with Pearson's Chi-square test and by age group with Mantel—Haenszel Chi-square tests for linear association.

Results

Of the 1787 people invited, 288 responded and came to the community center. The overall response rate was 16%; women participated more often than men, (19% and 13%, respectively). The response rate increased with age and was 10% in 18—29 year-olds, 17% in 30—44 year-olds and 23% in 45—64 year-olds. Response also varied by ethnicity and was 9% in the Cape Verdean and Antillean groups, 17% in the Surinamese and Moroccan groups, 23% in the Dutch group, and 26% in the Turkish group. Fifty-five participants were Dutch (19%) and 233 non-Dutch (81%), of whom 90% were first generation migrants and 10% second generation migrants. Hepatitis A and hepatitis B test results were available for 284 participants, and hepatitis C results for 271 participants.

The overall prevalence of antibodies to hepatitis A was 68%, and corresponded with a prevalence of 50% (95% confidence interval (CI) 44%—55%) in the neighborhood when weighted by sex and ethnicity. The prevalence increased with age from 39% in the 18—29 years age group, to 85% in the 45—64 years age group ($p \leq 0.001$). Dutch respondents had less evidence of previous HAV infection compared to non-Dutch respondents: 36% and 76%, respectively ($p \leq 0.001$). The difference in prevalence was especially remarkable in the youngest age group, where none of the Dutch respondents had antibodies to HAV compared to 46% of the non-Dutch respondents. The anti-HAV prevalence varied in the different non-Dutch ethnic groups; while the overall prevalence was 50—55% in Surinamese and Antillean respondents, it was over 90% in the Turkish, Moroccan and Cape Verdean respondents. Among non-Dutch respondents the prevalence in FGM was higher than in SGM ($p \leq 0.001$). The overall prevalence in SGM was similar to that in Dutch respondents, but the oldest age group was not represented among the SGM. The HAV prevalence in the 18—44 years age group was higher in SGM compared to Dutch respondents but this difference was not statistically significant. (*Table 1*).

Table 1. Prevalence of anti-HAV by sex, age group, and ethnicity

	n	*N*	%		95% CI	*p*-value
Overall	193	284	68%	62%	73%	
Sex						**0.536**
Men	86	123	70%	61%	77%	
Women	107	161	66%	59%	73%	
Age group (years)						**≤0.001**
18–29	26	66	39%	29%	51%	
30–44	83	119	70%	61%	77%	
45–64	84	99	85%	76%	91%	
Ethnicity						**≤0.001[a]**
Dutch	20	55	36%	25%	50%	
Non-Dutch	173	229	76%	70%	81%	≤0.001[b]
FGM	163	205	80%	73%	84%	
SGM	10	24	42%	24%	61%	
Turkish	57	61	93%	84%	97%	
Moroccan	49	50	98%	90%	100%	
Surinamese	32	64	50%	38%	62%	
Dutch Antilles	22	40	55%	40%	69%	
Cape Verdean	13	14	93%	69%	99%	
Age by ethnicity						
Dutch						**0.003**
18–29	0	9	0%	0%	30%	
30–44	6	20	30%	15%	52%	
45–64	14	26	54%	35%	71%	
Non-Dutch						**≤0.001**
18–29	26	57	46%	33%	58%	
30–44	77	99	78%	69%	85%	
45–64	70	73	96%	89%	99%	
FGM						**≤0.001**
18–29	18	38	47%	32%	63%	
30–44	75	94	80%	72%	86%	
45–64	70	73	96%	89%	99%	
SGM						**0.934**
18–29	8	19	42%	23%	64%	
30–44	2	5	40%	12%	77%	
45–64	-	-				

Legend on next page.

The prevalence of anti-HBc, a marker for previous infection, was 20% (58/284) among respondents and corresponded with a prevalence of 16% (95% CI 12%—21%) in the neighbourhood when weighted by sex and ethnicity. The prevalence increased with age from 8% in the 18—29 years age group, to 27% in the 45—64 years age group (p=0.003). Prevalence varied by ethnicity and was the lowest in Antillean participants (3%) and highest in Cape Verdean participants (57%). The difference in prevalence between Dutch participants and non-Dutch participants, 11% and 23%, respectively, was not statistically significant. The anti-HBc prevalence in FGM was higher than in SGM (25% and 4%, respectively; p=0.02). The prevalence of anti-HBc is described in *Table 2*.

The overall prevalence of HBsAg, indicating chronic infection and infectivity, was 0.7% (2/284; 95% CI 0.1%—2.3%) — 3.4% among anti-HBc-positives (2/58; 95% CI 0.6%—10.9%). The two patients with markers for chronic hepatitis B were both FGM, a 41-year-old Turkish woman and a 53-year-old Antillean man. Except ethnicity, no other risk factors could be identified. The HBsAg prevalence in the total group of first generation immigrants was 1.0% (95% CI 0.3%—3.5%).

In 17% of anti-HBc positives no other markers of HBV infection were found. If anti-HBc is present without HBsAg, anti-HBs, or anti-HBe this is called isolated anti-HBc. The prevalence of isolated anti-HBc varied by ethnicity and was 67% (4/6) among Dutch participants and 12% (6/52) among non-Dutch participants (p=0.006).

Three respondents (1.1%, 95% CI 0.4%—3.2%) were positive for antibodies against HCV. They were a 57-year-old Surinamese man, a 20-year old Moroccan man, and a 39-year-old Antillean man. None of them reported intravenous drug use, having had a tattoo, a piercing, contact with another person's blood, or a blood transfusion. The Surinamese man had had surgery in the Netherlands; the other two men had been subject to a medical procedure with a needle, the Moroccan man abroad and the Antillean man in the Netherlands.

Of the 24 SGM, 17 (71%) had visited their parent's country of birth. Five of them (29%; two Turkish and three Surinamese) reported visiting a travel clinic and receiving vaccinations.

Table 2. Prevalence of anti-HBc by sex, age group, and ethnicity

	n	N	%		95% CI	p-value
Overall	58	284	20%	16%	25%	
Sex						**0.092**
Men	31	122	25%	19%	34%	
Women	27	162	17%	12%	23%	
Age group (years)						**0.003**
18–29	5	66	8%	3%	17%	
30–44	26	118	22%	16%	30%	
45–64	27	100	27%	19%	36%	
Ethnicity						**0.057**[a]
Dutch	6	55	11%	5%	22%	
Non-Dutch	52	229	23%	18%	29%	0.022[b]
FGM	51	205	25%	19%	31%	
SGM	1	24	4%	1%	20%	
Turkish	23	60	38%	27%	51%	
Moroccan	6	50	12%	6%	24%	
Surinamese	14	65	22%	13%	33%	
Dutch Antilles	1	40	3%	0%	13%	
Cape Verdean	8	14	57%	33%	79%	
Age by ethnicity						
Dutch						**0.618**
18–29	1	9	11%	2%	44%	
30–44	3	20	15%	5%	36%	
45–64	2	26	8%	2%	24%	
Non-Dutch						**0.001**
18–29	4	57	7%	3%	17%	
30–44	22	97	23%	15%	32%	
45–64	25	74	34%	24%	45%	

CI, confidence interval, anti-HAV, anti-hepatitis A virus antibodies; FGM, first generation migrants;

SGM, second generation migrants.

[a] *p-Value of Dutch versus non-Dutch.*

[b] *p-Value of first generation migrants versus second generation migrants.*

All respondents who were susceptible to hepatitis A and/or hepatitis B were offered vaccination against hepatitis A and/or hepatitis B free of charge. Sixty-three of 227 participants (28%) took up the offer and visited the MPHS for vaccination.

Discussion

We found high levels of previous infection with hepatitis A and B in the multi-ethnic population of a neighborhood in Rotterdam. The prevalences of chronic HBV (0.7%) and hepatitis C (1.1%) were higher than previously reported in the national general population based survey conducted in 1995/1996 (the Pienter study), which reported prevalences of 0.2% and 0.1%, respectively.

The response rate was lower than anticipated, despite the community based approach. Due to the low response rate, the total number of participants was small, which makes comparisons between subgroups difficult. However, as we do not assume that selective response by the outcome of viral hepatitis testing has occurred, we consider the results of this study as valid. Our study is of interest for the city of Rotterdam, but also for other cities with large immigrant populations.

In the Netherlands, vaccination against hepatitis A and B is not included in the National Immunization Program (NIP). However, since 2003, all children born to one or two parents born in intermediate or high endemic countries have been offered HBV immunization in the NIP. Furthermore, a vaccination programme for high risk groups (commercial sex workers, intravenous drug users, and men having sex with men) has been in place since 2002.

The prevalences of HAV and HBV markers in the study population were much higher than the general population estimates in the Netherlands, because non-Dutch respondents are over-represented in the targeted neighborhood in Rotterdam. The HAV prevalence of more than 90% among Turkish and Moroccan respondents (93% and 98%, respectively) is comparable to that among Turkish and Moroccan participants in the general population study (93% and 97%, respectively).[1] More than half of the participants of non-Dutch ethnicity in the youngest age group were susceptible to hepatitis A. The HBV prevalence found among Turkish and Moroccan respondents (38% and 12%, respectively) is comparable to that among Turkish and Moroccan participants in the general population study (39% and 19%, respectively).[2] The anti-HBc prevalence increased with age, a finding also observed in the general population study.[2] Participants aged 18—29 years have low rates of previous HBV infection, over 90% of them are probably vulnerable to HBV infection. A small part might be protected through vaccination but we have no information about this

as participants without markers for previous or current HBV infection were not tested for the presence of protective antibodies. Ninety-three percent of the participants of non-Dutch ethnicity in the youngest age group had no markers of previous HBV infection. The anti-HBc prevalence was remarkably low in SGM, with only 1/24 SGM found positive. This could suggest vaccination in this group which is however unlikely, as the pregnancy screening programme only started in 1989 and the participants in this study were born before 1986. A possible explanation might be that the risk of horizontal transmission in the Netherlands is lower compared to that in the country of origin.

Five of the 17 SGM (29%; two Turkish and three Surinamese) who had visited their parent's country of birth, went to a travel clinic and were vaccinated. Although the type of vaccination was not recorded, it most likely included hepatitis A vaccination. These results indicate a low vaccination rate in SGM, especially Moroccan SGM, and many of them are probably at risk of becoming infected with HAV while visiting their home country. Active hepatitis A vaccination of Turkish and Moroccan children has also been argued in a study among children aged 5 to 16 years in Rotterdam.[5] We would recommend a more active approach in offering children in immigrant communities who did not receive HBV immunization as part of the NIP, a combined hepatitis A and B immunization.

As could be expected, the 0.7% prevalence of chronic HBV is also higher than the national estimates. The two HBsAg positives were first generation immigrants, and the prevalence of HBsAg in the total group of first generation immigrants was 1.0%, which

Table 3. Hepatitis B prevalence (anti-HBc and HBsAg) in 2 observational studies and one theoretical estimation

Study	Year	Population	Anti-HBc (%)	HBsAg (%)
Pienter[a]	1995/1996	General	2.1	0.2
Community based study Rotterdam[b]	2004	Native Dutch	11	0
		FGM	25	1.0
		SGM	4	0
		Overall	20	0.7
Marschall, theoretical estimate[c]	2007	General	-	0.36
		FGM	-	3.77

Anti-HBc, anti-hepatitis B core antigen; HBsAg, hepatitis B surface antigen; FGM, first generation migrants; SGM, second generation migrants.

[a] *See reference 2.*

[b] *Present study.*

[c] *See reference 3.*

seems low compared to the theoretically estimated HBsAg prevalence of 3.8% in first generation immigrants in the Netherlands.[3] *Table 3* summarizes the anti-HBc and HBsAg prevalences found in two observational field studies in the Netherlands compared to the theoretical estimates.

It is not uncommon to find high rates of isolated anti-HBc. This serological profile can be the result of a previous infection with loss of measurable anti-HBs, of an ongoing low grade infection with undetectable HBsAg, or of a non-specific reaction, i.e., a false-positive test result. Especially in populations with a low prevalence of HBV markers the probability of false-positive results increases.[6] Studies in blood donors in Brazil and Denmark reported false-positivity rates of 16% and 34%, respectively.[7,8] The higher prevalence of isolated anti-HBc found in Dutch participants (4/6) compared to non-Dutch participants (6/52), and the absence of an increase in anti-HBc prevalence with age, suggests false-positive results. Therefore the prevalence of previous HBV infection of 11% in Dutch participants is most likely an overestimation. However, our study does not provide enough evidence to conclude which part of the cases of isolated anti-HBc were false-positives, and therefore we have not excluded these cases when calculating the anti-HBc prevalence. To get more information on whether a person had a previous infection with waning anti-HBs, one would have to give the person an HBV vaccination and see if the anti-HBs response resembles a primary or a secondary response. Isolated anti-HBc has been shown to be associated with HCV, but none of the participants with isolated anti-HBc found in this study had antibodies to HCV.[9]

The prevalence of HCV infection found is higher than the previous national estimate. Two of the three HCV positive participants were remarkably young at 20 and 39 years of age. It is striking that the three HCV infected respondents did not report risk factors for HCV infection. This is contrary to findings from a seroprevalence study in Amsterdam, where all HCV-positive individuals were aged 43 or older, with known HCV risk factors present in seven of nine.[10]

In conclusion, the results of this study show that within low endemic countries, pockets with higher endemicity for viral hepatitis do exist. This confirms previous observations in ethnic subgroups from a national general population study and illustrates the high burden of viral hepatitis in areas with large immigrant populations.

Acknowledgements

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Chapter 5

An improved approach to identify epidemiological and phylogenetic transmission pairs in hepatitis B source and contact tracing

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Abstract

The transmission of infectious diseases can be traced using epidemiological and molecular information. In the current study, the congruence was assessed between sequence data of the hepatitis B virus (HBV) and epidemiological information resulting from source and contact tracing of patients seen at the Municipal Public Health Service in Rotterdam between 2002 and 2005. HBV genotypes A-G were present in 62 acute and 334 chronic HBV patients. At the sequence level, the identical sequences of members of epidemiological transmission pairs and the rarity of such pairs provided strong support for correctness of the hypothesized transmission routes. The molecular support for epidemiological transmission pairs derived from source and contact tracing was further assessed by using topological constraints in parsimony analyses in agreement with epidemiological information, and by taking the presence of polymorphic sites of HBV within patients into account. This, in principle, allows mutations in epidemiological clusters. Of 22 epidemiological clusters, six could be refuted, four clusters received support from the molecular analysis, and support for the remaining twelve clusters was ambiguous. Two of the four epidemiological pairs that received molecular support had diverged (by three and fifteen mutations). These results show that levels of divergence cannot be simply used as an indicator of the likelihood that groups of sequences constitute transmission pairs. Instead, to confirm or refute transmission pairs, it is necessary to assess the likelihood of a common origin of HBV variants in epidemiologically defined transmission groups relative to the HBV diversity in the local community.

Introduction

Hepatitis B virus (HBV) infection is a major global health problem with more than 2 billion people infected worldwide and 350 million people who are chronically infected.[1] In low endemic countries such as the Netherlands sexual transmission is the major source of infection.[2] In high endemic countries the main routes of transmission are maternal transmission during the perinatal period and horizontal transmission in early life as a consequence of close family contact.[3-5] Given the large number of people of non-Dutch ethnicity in large cities, the prevalence of HBV infection is estimated to be three to five times higher in the city of Rotterdam (0.6-1.1%) than in the Dutch population as a whole (0.2%).[6] The estimated 3,500-6,500 persons in Rotterdam with a chronic HBV infection represent a major public health burden.

In the Netherlands, newly diagnosed HBV infections are notified to the Municipal Public Health Service (MPHS), which performs active source and contact tracing. The goal of source and contact tracing is to identify possible sources of infection and to prevent further transmission. Contacts at risk, for example, sexual partners, household members, other family, are screened for infection in order to identify new carriers and protect susceptible contacts through vaccination. Despite the effort placed in source and contact tracing, which may lead to the identification of epidemiological transmission pairs, the routes of transmission in both chronic and acute HBV infections in the Netherlands remain largely unknown (36% and 27% of notified cases, respectively).[7] Sequencing of the viral genome has already provided insight in the origin or transmission route of HBV infection within families,[4,8,9] in health care settings,[10-12] prisons,[13] and at the population level.[14-16] This technique might also be used to elucidate transmission of hepatitis B virus in patients with chronic and acute infections seen at the MPHS, in order to support their surveillance and intervention activities.

HBV is classified in eight genotypes, referred to as A–H, based on the level of variation in the viral genome sequence.[17-19] These genotypes have a characteristic geographic distribution[20-23] and are found predominantly in the following regions: Africa, India, Northwest Europe and North America (A); China, Japan, Southeast Asia and the Pacific (B, C); the Mediterranean and the Middle East (D); West Africa (E); Central and South America (F); the United States, Mexico and Europe (G) and Central America (H). Two subgenotypes within genotype A form two major geographical clusters, one European-North American (A2) and one mainly Afro-Asian (A1).

A priori, it is expected that infected patients from a multi-ethnic city will show a wide variation in the viral genome, which can be differentiated into genotypes easily. Sequence comparison may lead to the identification of clusters of patients infected by genetically

closely related viruses, which potentially infected each other (i.e. transmission pairs). Besides the association of genotype with geographic areas, genotypes were also found to be associated with the transmission route of HBV infections. In Denmark a large cluster of genotype D viral strains associated with injecting drug use was observed.[14] In Amsterdam, the Netherlands, in the 1990s, a cluster related to homosexual men was identified in genotype A and a cluster related to injecting drug use in genotype D.[24] In a more recent nationwide survey a large cluster of homosexual men in genotype A was again found but the cluster related to injecting drug use was not observed anymore.[25]

In spite of the successful identification of HBV transmission routes, the most common approach of linking molecular and epidemiological data, that is, through phylogenetic analysis, can be improved in several ways. First, molecular trees are constructed typically using data from larger numbers of patients than the number of contacts identified by source- and contact tracing. This imbalance makes it difficult to compare these two types of data explicitly. Second, many of the rapidly evolving and chronically infecting viruses, including HBV, show polymorphisms within patients.[26] When using direct sequencing, these sites are usually marked with ambiguity codes proposed by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). However, ambiguity codes are interpreted by phylogenetic software as reflecting a technical shortcoming of the sequencing technology, implying the presence of only one of the ordinary nucleotides. In contrast, many of the ambiguity codes in sequence data from HBV populations indicate the presence of two or more nucleotides at a single nucleotide position in the HBV sequences of a chronically infected patient.[26]

The compact HBV genome is covered almost fully with overlapping protein-coding genes resulting in evolutionary constraints at every codon position. This can be expected to exacerbate the potential for a shortage of mutations for the identification of transmission pairs.

Aim of the Study: The aim of this study was to assess the agreement between epidemiological and phylogenetic data for identifying HBV transmission pairs, while taking into account the above aspects. The detailed comparison allowed explicit epidemiological conclusions relevant to public health services.

Participants and methods

Participants
The Municipal Public Health Service in Rotterdam covers a population of 800,000 persons. All newly diagnosed HBV infections (both acute and chronic) are notified mandatorily to the MPHS and invited for serological investigation, source and contact tracing

and counseling by a public health nurse. Patients who visited the MPHS between January 2002 and December 2005 were asked to participate in the study. After permission of the patient, a blood sample obtained for routine serological analysis was also used for molecular analysis, and a survey was completed jointly by a public health nurse and the patient. Information obtained included country of birth of the patient and the parents, risk factors (e.g. exposure to medical procedures, intravenous drug use, commercial sex work, tattoo, piercing), number of years living in the Netherlands, travel behavior, sexual behavior, reason for examination, results of diagnostic tests and most likely transmission route. The country of birth of the mother determined ethnicity.

Permission for this study was received from the Medical Ethical Review Board of the University Medical Center Rotterdam (Erasmus MC). Data on transmission routes and genotype of chronic patients in this study have been described elsewhere.[27]

Statistical Analysis
Statistical analyses were performed using SPSS version 15.0. Differences in age and sex distribution by type of infection were tested with Student's *t*-test and Pearson's Chi-square test respectively.

HBV DNA Isolation and Nucleotide Sequence Accession Numbers
HBV DNA was isolated from serum using the Magnapure LC robot station (Roche Applied Science, Almere, The Netherlands) with a modified HBV-02 protocol.[28] This allowed us to sequence samples with a viral load above 1000 copies per ml. A product of 878 base pairs of the preS and part of the S gene was amplified with 20 pmol/μl of the primers HT26/5[a] and YMDD2 triple primer mix[b] to enable the sequencing of the different genotypes. If needed, a semi-nested PCR was used with HT26/2 as sense primer,[c] producing an amplicon of 805 bp. The amplicon was sequenced with 5 pmol/rx of HT26,[d] HT26/2, HT26/3,[e] S1,[f] and YMDD2 triple using the Big Dye terminator V3.1 cycle sequencing kit (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The products were separated on an ABI 3100 sequencer (Applied Biosystems) and the sequence data were analyzed using Sequence Navigator software sequencer (Applied Biosystems) and Lasergene Seqman 7.1.0 (DNASTAR, Madison, WI). The sequences were aligned using ClustalW.

[a] *HT26/5: sense, 5'-CAGGCCATGCAGTGGAA-3'*
[b] *YMDD2 triple primer mix: anti-sense, 5'-ACCCCATCTTTTTGTTTTGTTAGG-3', 5'-ACCCCATCTTTTTGTTTT-3' and 5'-ACCCCAACGTTTGGTTTTATTAGG-3'*
[c] *HT26/2: 5'-CCTGCTGGTGGCTCCAGTTC-3'*
[d] *HT26: sense, 5'-CCTGCTGGTGGCTCCAGTTC-3'*
[e] *HT26/3: anti-sense, 5'-ATAAAACGCCGCAGACACATCCAGCGA-3'*
[f] *S1: sense 5'-GTATGTTGCCCGTTTGTCCTC-3'*

The nucleotide sequence data reported in this paper have been deposited in the GenBank sequence database.[g] The reference types[h] used have previously been included as references in several studies.[19]

Definitions of Epidemiological and Molecular Clusters and Phylogenetic Analysis

For each patient it was determined whether he/she was part of an epidemiological and/or a molecular transmission pair, based on the following criteria. HBV patients were considered an epidemiological transmission pair when information from source and contact tracing suggested that the patients could have infected each other (e.g. sexual partners or children) or if a possible common source of transmission could be suggested (e.g. siblings of the index patient).

Because, among others, the mutation rate of HBV varies widely from person to person,[26] the use of a divergence cut-off to identify potential transmission pairs is compromised potentially. The only exceptions are fully identical sequences, because these mark the limits of resolution provided by molecular markers. Whether patients were part of a molecular cluster was assessed in two different ways. First, all identical sequences pairs were identified in the six globally most common HBV genotypes (barring ambiguity codes in pair wise comparisons). Identical sequence pairs were identified based on the distance matrix using uncorrected distances barring ambiguity codes. Identical clusters comprise sequences whose minimal absolute distance to one of the members is zero. This definition allows sequences to be classified in the same identical cluster, even if they differ, if both of them lack differences when compared to other sequences of the cluster. Among patients in molecular clusters transmission pairs might be present, but without epidemiological information, molecular support for transmission pairs cannot be assessed accurately. Second, the level of molecular support for patients included in epidemiologically defined transmission pairs was assessed. Patients were considered to differ in the transmission route if they had different HBV genotypes. Within genotypes, molecular transmission pairs were assessed based on the agreement between epidemiological and molecular classifications using parsimony.[29] Parsimony is a widely used method for the construction of trees, especially when dealing with (a combination of) different types of data. Generally, under parsimony, the total number of mutations (or changes) across a tree is minimized and many trees with the same total number of mutations can be collected. The difference between numbers of mutations in constrained (forced clustering of

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[g] *Accession numbers: DQ403173-DQ403176 and DQ412133-DQ412386*

[h] *Reference types: genotype A X02763, genotype A-Africa AF297621, genotype Ba D00330, genotype Bj AB073858, genotype C AB033556, genotype Caus AB0478704, genotype D X02496, genotype E X75657, genotype F X69798, genotype G AF160501, genotype H AY090454*

epidemiological transmission pairs) and unconstrained (forced spread of epidemiological transmission pairs so that these are not each others' closest relative) analyses was used to assess the level of support for epidemiological transmission pairs.[30] Constrained analyses were conducted using PAUP*.[29] If all three coding methods (described below) required fewer mutations in constrained compared to unconstrained analyses, the transmission pair was considered to be supported by the sequence data. Alternatively, if all three coding methods required most mutations in constrained analyses, the transmission pair was considered to be refuted by the sequence data. Lastly, if there was no difference in the number of mutations in constrained and unconstrained analyses in at least one of the coding methods, molecular support for the transmission pair was considered ambiguous. During tree searches, 1000 replicates of random additions of taxa using TBR swapping were used to avoid getting trapped in a limited number of islands of trees. Therefore, the inferences regarding transmission pairs do not depend on a single tree estimate.

Ambiguities—nucleotide positions at which more than one nucleotide occurs—typically reflect a technical shortcoming of the sequencing reaction, but in many chronically infecting viral populations, such "multiple-nucleotide positions" reflect the presence of closely related but genetically different variants. Although these positions are not resolved at the nucleotide level, they may still be informative with regard to the routes of HBV infection, if these mixes of nucleotides are stable during prolonged time intervals. To incorporate as much information as possible in the detection of transmission pairs, three coding schemes were used to accommodate nucleotide ambiguities. Firstly, sites with ambiguity codes were coded as additional "bases" for the reconstruction of gene tree topologies, resulting in ten "nucleotides" (four standard and six ambiguity nucleotides). This coding scheme is referred to as ten states. Alternatively, sites with ambiguity codes were treated as sites with both nucleotides present (in the data only twofold redundant ambiguity codes were present). Under this coding scheme, which is referred to as "poly", phylogenetic trees are reconstructed using the nucleotide that can be added most parsimoniously to the phylogeny, thereby resulting in the smallest increase of the total number of mutations of the tree relative to the shortest tree. The alternative nucleotide is then treated as a mutant that originated in terminal branches (i.e. within patients). Finally, it was assessed whether the transition/transversion (Ts/Tv) ratio could increase the level of resolution of the HBV genotype phylogenies and, thereby, improve the detection of transmission pairs. Typically, transitions occur much more frequently than transversions, with the former being least reliable for phylogeny reconstruction.[31] Weighting transitions and transversions differently may therefore improve tree reconstructions. Here, the Ts/Tv ratio was estimated under the HKY substitution model. Constrained analyses described above were performed under all three coding schemes.

Table 1. Patient Characteristics by Genotype(n, %)

	A1		A2		B		C		D	
Type of infection										
Acute	2	(5)	33	(52)	0	(0)	5	(13)	20	(13)
Chronic	39	(95)	31	(48)	53	(100)	35	(88)	133	(87)
Sex										
Male	26	(63)	55	(86)	22	(42)	22	(55)	89	(58)
Female	15	(37)	9	(14)	31	(58)	18	(45)	64	(42)
Ethnicity										
Western Europe	1	(2)	42	(66)	3	(6)	3	(8)	12	(8)
Mediterranean	1	(2)	5	(8)	0	(0)	1	(3)	97	(65)
Central/South America	20	(49)	8	(13)	10	(19)	0	(0)	12	(8)
Africa	19	(46)	5	(8)	0	(0)	1	(3)	3	(2)
Asia	0	(0)	1	(2)	40	(75)	35	(88)	10	(7)
Other[a]	0	(0)	2	(3)	0	(0)	0	(0)	15	(10)
Missing[b]	0		1		0		0		4	
Total	**41**	**(10)**	**64**	**(16)**	**53**	**(13)**	**40**	**(10)**	**153**	**(39)**

To compare the usual approach to the analysis of viral transmission-phylogenetic analysis- and to highlight the shortcomings of this approach for the delimitation of identical and near-identical sequence variants, two distance-based phylogenetic trees were constructed using MEGA4[32] using the neighbor-joining option based on Tamura-Nei distances with rate heterogeneity following a gamma distribution with shape parameter 0.25.

Results

Between January 2002 and December 2005, 837 HBV infections were reported to the MPHS (625 chronic, 138 acute and 74 with unknown type of infection). A total of 545 HBV patients (68 acute and 477 chronic) were enrolled in the study (response rate 65%). However, the study population consisted only of patients whom HBV sequences could be obtained (396 patients, 62 acute and 334 chronic, 73% in total, see *Table 1*). Sixty-one percent of the study population was male. The sex distribution differed by type of infection, 79% (49/62) of acute infections were in males, compared to 57% (191/334) of the chronic infections (*P* = 0.001). The mean age of the patients was 34 years (range 8–80 years, SD 11).

E		F+G		Total	
0	(0)	2	(67)	62	(16)
42	(100)	1	(33)	334	(84)
25	(60)	1	(33)	240	(61)
17	(40)	2	(67)	156	(39)
0	(0)	3	(100)	64	(16)
0	(0)	0	(0)	104	(27)
0	(0)	0	(0)	50	(13)
42	(100)	0	(0)	70	(18)
0	(0)	0	(0)	86	(22)
0	(0)	0	(0)	17	(4)
0		0		5	
42	**(11)**	**3**	**(1)**	**396**	**(100)**

[a] *Other includes North America, the Middle East, Eastern Europe and (former) Russia*

[b] *Patients of whom the ethnicity was missing were excluded in the calculation of the distribution of ethnicity by genotype*

The majority of patients with detectable HBV-DNA had a non-Dutch ethnicity (327 out of 396; 83%) and included mainly people from the Mediterranean, Asia and Africa (*Table 1*). The variety of identified HBV genotypes reflected these different ethnic backgrounds; genotype A (105; 27%, 41 A1 and 64 A2), B (53; 13%), C (40; 10%), D (153; 39%), E (42; 11%), F (2; 0.6%) and G (1; 0.3%). In the chronic patients genotypes A1, A2, B, C, D and E were well represented (39; 12%, 31; 9%, 53; 13%, 35; 11%, 133; 40% and 42; 13% respectively), while genotype A2 (33; 52%) and D (20; 32%) were represented mainly in the acute HBV patients. There was a significant difference between the proportion of acute infections in genotype A2 (52%) compared to the other genotypes (9%, *P* < 0.001).

Acute HBV Infections
The majority of the 62 patients with acute HBV included in this study was male (49; 79%). The patients originated from Western Europe (36; 58%), the Mediterranean region (10; 16%), Central and South America (9; 15%), Asia (3; 5%) and other countries (2; 3%). The patients with the two main genotypes, A2 and D, differed in sexual behavior. Patients infected with genotype A2 (33) were mainly homosexual men (25) and the remaining 8 were heterosexual, of whom 74% had had multiple sexual contacts in the 6 months before infection. Patients infected with genotype D (20) were primarily heterosexual (18). The sexual preference was unknown in the remaining two cases. Seven (39%) of the

18 heterosexuals with genotype D had 2 or more sexual partners in the last 6 months. For most of the acute patients (49; 72%), the assumed route of transmission, based on source and contact tracing, was sexual contact. All but one women with acute HBV (12/13; 92%), were infected with a genotype from abroad (mainly D), mostly by sexual contact.

Sequence Analysis and Molecular Clusters

The genotypes A through F comprised a substantial proportion of sequences with ambiguity codes (37%, 21%, 37%, 48%, 47%, and 40%, respectively) and proportion of sites with ambiguity codes (9%, 7%, 5%, 9%, 18%, and 8%, respectively). Only two of the sequences used had the lamivudine YSDD (tyrosine-serine-aspartate-aspartate) resistance signature in the S gene (not shown), which did not affect the identification of transmission pairs, as these sequences were not found in patients included in the epidemiological transmission pairs.

A total of 24 clusters including 86 patients with identical sequences were identified comprising genotypes A, B, C, D, E and F. The number of patients with identical sequences within a cluster ranged from 2 to 20 (*Table 2*). The cluster of 20 persons with genotype A2 was the largest cluster found in this study, including 7 chronic and 13 acute HBV patients. The seven clusters of in total 34 patients with genotype A2 mainly consisted of males (32; 94%), the majority (28; 88%) being homosexual men and four men claiming heterosexual contacts only. Of the patients with an acute HBV infection, 66% (41/62) was part of a molecular cluster, compared to 13% (45/334) of the chronic patients ($P < 0.001$). This difference remained when patients with genotype A2 were excluded in which case 59% (17/29) of acute patients and 12% (35/303) of chronic patients clustered ($P < 0.001$). Twenty-six percent of the patients with identical sequences were included in an epidemiological cluster (22/86). This was more often the case for chronic patients (33%, 15/45), compared to acute patients (17%, 7/41; $P = 0.084$).

Transmission Pairs

Twenty-two epidemiologically defined transmission pairs were found collectively including 48 patients. Fourteen of the 22 clusters (64%) consisted of a patient and his/her sexual partner, of which in 8 clusters (57%) at least one partner had an acute infection. The other eight clusters consisted of seven patients with a chronic HBV infection and one with an acute infection and their chronically infected family members (12). For the Rotterdam sample of sequences, the support for some transmission pairs was very similar among the coding schemes, whereas others gave ambiguous results. In *Table 3*, the epidemiological clusters are described separately for the different levels of molecular support. Six of the epidemiologically defined transmission pairs (27%) were refuted by the molecular data. Three were refuted because the patients had different HBV genotypes, and an additional three pairs—whose members had the same genotype—were refuted because

the constrained HBV tree required additional steps relative to the shortest tree under all three coding schemes. The proportion of epidemiological transmission pairs that could not be considered a molecular transmission pair was similar for pairs consisting of sexual partners and family members (29% (4/14) and 25% (2/8) respectively, P = 0.86). This proportion differed, although not statistically significant, when only transmission pairs consisting of at least 2 chronically infected patients with non-Dutch ethnicity were taken into account (13 pairs). Then three of the five sexual partner transmission pairs (60%) were refuted, compared to 1 of 8 (13%) family pairs (P = 0.11). Four epidemiological transmission pairs (18%) received support from the molecular analysis and the remaining 12 (55%) were ambiguous in that there was no consistent difference between the shortest tree and the tree with the transmission pair constrained. Seven of the 12 clusters (58%) with ambiguous support consisted of patients with identical sequences. In four of these seven clusters at least one of the patients had an acute infection. Two of the four epidemiological transmission pairs that received molecular support had diverged considerably. One pair was a family cluster of a Chinese mother and her two sons. The two brothers had identical HBV sequences, while the virus of the mother differed by three mutations. The other transmission pair involved a Turkish brother and sister with 15 mutations difference in their HBV sequences.

Phylogenetic Trees
The various HBV strains identified in the chronic and acute patients were used for the construction of phylogenetic trees of genotype A and D, as these genotypes were most common and demonstrate little variation (genotype A2, *Fig. 1*), and more variation (genotype D, *Fig. 2*). Because of the preponderance of ambiguity codes, pair wise comparisons of sequences need not be compatible with the phylogenetic tree, because they may give rise to incompatible distances (e.g., violation of triangle inequality), even among sequences that are otherwise identical. These sequences may then end up in branches in the tree with a non-zero branch length. This counterintuitive pattern can be identified most easily for cluster M-19, a cluster of sequences that lacked mutations in pair wise comparisons, but which had nevertheless slightly diverged and were spread across the tree (genotype D).

Table 2.   Molecular Clusters with Identical Sequences

Geno-type	# patients	HBV infection		Details of relationship	Cluster nr.	
		Acute	Chronic		Mol	Epi
A1	3 pt	1	2	Surinamese woman and 2 men (heterosexual), unknown contact	1	
A2	20 pt	13	7	Primarily homosexual men, all but 2 unknown contact	2	3
A2	4 pt	4	0	Dutch homosexual men, all but 2 unknown contact	3	4
A2	2 pt	2	0	Dutch homosexual men, unknown contact	4	
A2	2 pt	2	0	Dutch and Venezuelan homosexual men, unknown contact	5	
A2	2 pt	2	0	Dutch and Indonesian homosexual men, partners	6	1
A2	2 pt	0	2	Algerian and Ukrainian heterosexuals, unknown contact	7	
A2	2 pt	1	1	Algerian homosexual man and Antillean heterosexual man, unknown contact	8	
B	2 pt	0	2	Chinese females, unknown contact	9	
C	2 pt	0	2	Chinese brothers, epidemiological cluster	10	6
C	3 pt	3	0	Dutch homosexual man, Dutch and French heterosexual men, unknown contact	11	
C	2 pt	0	2	Chinese father and son, epidemiological cluster	12	7
D	9 pt	1	8	Dutch, Antillean, Dominican heterosexual partners and mother and sons, epidemio-logical clusters. Antillean and Turkish heterosexual females, unknown contact	13	10, 11, 12
D	4 pt	2	2	Dutch heterosexuals, unknown contact	14	
D	3 pt	1	2	Surinamese and Pakistani heterosexual partners, and Chinese woman with unknown contact	15	13
D	2 pt	1	1	Egyptian (M) and Dutch (F) heterosexual partners	16	14

Geno-		HBV infection			Cluster nr.	
type	# patients	Acute	Chronic	Details of relationship	Mol	Epi
D	2 pt	0	2	Turkish heterosexuals, unknown contact	17	
D	2 pt	1	1	Turkish heterosexual partners	18	9
D	4 pt	4	0	Dutch, Turkish, Moroccan, Bulgarian heterosexual males, unknown contact	19[a]	
D	2 pt	1	1	Turkish (M) and Dutch (F) heterosexuals, unknown contact	19[b]	
D	3 pt	0	3	Turkish heterosexuals (2 M, 1 F), unknown contact	19[c]	
D	3 pt	0	3	Turkish heterosexual women, unknown contact	20	
E	3 pt	0	3	Angolan heterosexuals, unknown contact	21	
E	2 pt	0	2	Ghanaian male and Congolese female, unknown contact	22	
E	2 pt	0	2	Ghanaian women, unknown contact	23	
F	2 pt	2	0	Dutch heterosexual women, unknown contact	24	

Discussion

Epidemiology of HBV in Rotterdam

The large cohort of patients (n = 545) studied here resulted in a unique series of HBV-DNA samples from 396 patients in a large multi-ethnic city in the Netherlands. The relatively large proportion (27%) of unsuccessful attempts to obtain sequence information can be attributed to the fact that a large proportion of the patients seen at MPHS are inactive carriers with low HBV titres.[33] This is common in patients seen at public health services that are not representative of the clinical population.

Collectively, the acutely and chronically infected patients comprised seven of the eight HBV genotypes known currently, thereby providing an overview of the genetic reservoir of HBV infections in this city and of the most important transmission routes. The acute infections are mostly in Dutch homosexual men infected with the endemic genotype A2, and to a lesser extent in Mediterranean and Dutch heterosexuals infected with genotype

Table 3. Epidemiological Transmission Pairs by Level of Molecular Support.

Epi cluster number	Geno- type	Ts/Tv		Poly		Ten states		molecular support
		C	U	C	U	C	U	
16	A/D	A/D	A/D					No
18	B/C	B/C	B/C					No
21	B/Cᵇ	495	495	310	310	272	271	No / Ambiguous
8	D	1123	1120	797	797	634	633	No
19	D	1125	1121	798	797	634	633	No
22	D	1127	1120	799	797	635	633	No
1	A2	207	210	117	119	117	119	Yes
6	C	313	314	221	222	181	182	Yes
7	C	313	314	221	222	181	182	Yes
15	D	1120	1126	797	801	633	636	Yes
2	A2	207	207	117	117	117	117	Ambiguous
3	A2	207	207	117	117	117	117	Ambiguous
4	A2	207	207	117	117	117	117	Ambiguous
5	B	496	495	310	310	271	271	Ambiguous
9	D	1120	1121	797	797	633	634	Ambiguous
10	D	1121	1120	797	797	633	633	Ambiguous
11	D	1120	1120	797	797	633	633	Ambiguous
12	D	1121	1121	797	797	633	634	Ambiguous
13	D	1121	1121	797	797	633	633	Ambiguous
14	D	1121	1121	797	797	633	634	Ambiguous
17	A1	467	467	257	257	257	257	Ambiguous
20	D	1120	1121	798	798	634	633	Ambiguous

"Pairwise differences between constrained (C) and unconstrained (U) analyses for 3 coding schemes[a]"

[a] *Because the estimated transitition-transversion rates were 2.99 (Aafr), 3.08 (A2), 2.38 (B), 3.20 (C), 2.59 (D), and 2.99 (E), for parsimony analyses, a ratio of three was used throughout.*

molecular remarks	Mol cluster number	HBV infection[c]		Epidemiological description
		A	C	
		0	2	Turkish heterosexual partners
		0	2	Chinese heterosexual partners
		1	2	Chinese father (acute, genotype C), son and daughter (both genotype B)
1 mutation, 1 ambiguity		1	1	Dutch heterosexual partners
8 mutations		0	2	Turkish heterosexual partners
10 mutations, 8 ambiguities		0	2	Turkish mother and son
Y/T (1 ambiguity, 1 substitution)	6	2	0	Dutch and Indonesian homosexual partners
3 substitutions	10	0	3	Chinese mother and 2 sons
Identical	12	0	2	Chinese father and son
15 mutations		0	2	Turkish brother and sister
		1	1	Dutch male homosexual partners
	2	0	2	Dutch and Somali male homosexual partners
	3	2	0	Dutch homosexual partners
		0	2	Surinamese heterosexual partners
	18	1	1	Turkish heterosexual partners
	13	1	1	Dutch and Antillean heterosexual partners
	13	0	2	Antillean and Dominican heterosexual partners
	13	0	3	Antillean mother and 2 sons. Another son is part of epidemiological cluster 10.
	15	1	1	Surinamese and Pakistani heterosexual partners
	16	1	1	Dutch and Egyptian heterosexual partners
		0	2	Cape Verdian sisters
		0	3	Turkish brothers and sister

[b] *The pairwise differences and molecular support are for the two patients with genotype B.*

[c] *Type of HBV infection abbreviated as 'A' for acute and 'C' for chronic infection*

Figure 1
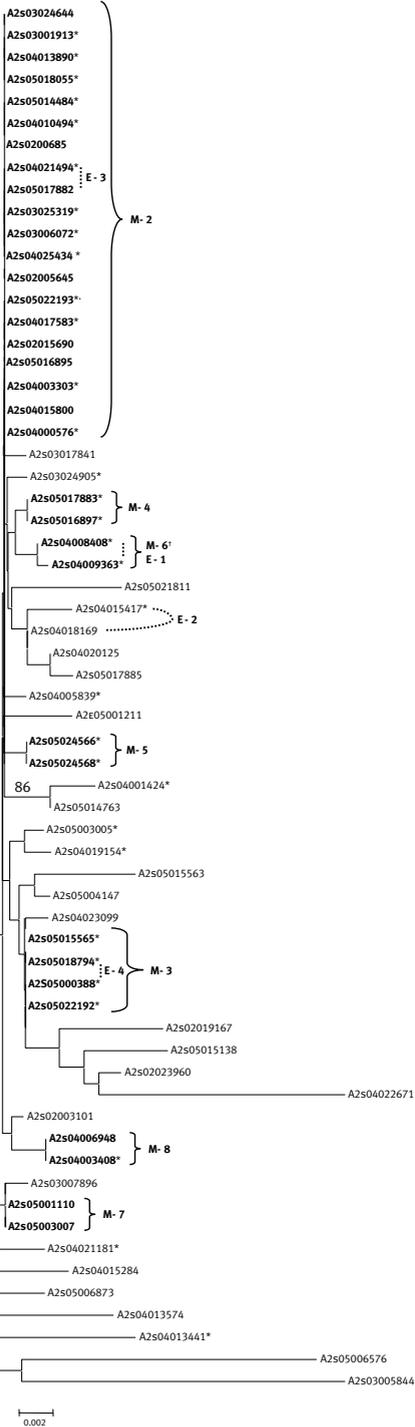
Figure 2
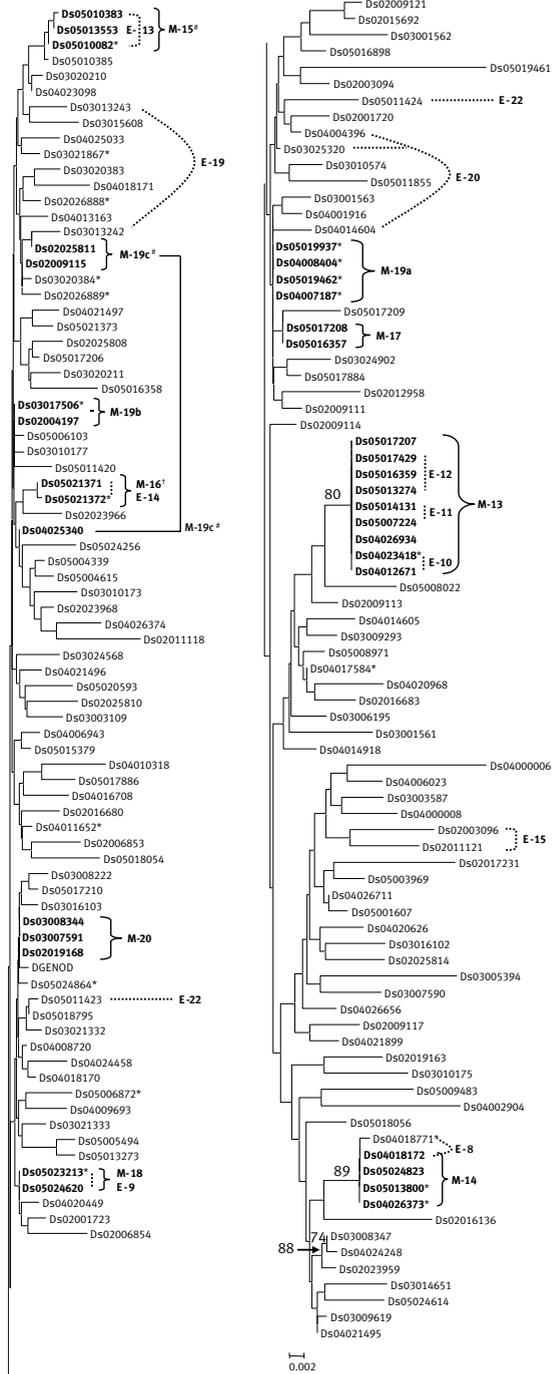
D. The chronic HBV population was represented mainly by heterosexual immigrants infected by genotype B, C, D and E. The finding that most women with an acute HBV infection were infected with genotype D through sexual contact suggests that a partner from abroad is an important source of infection for heterosexually infected women. This is supported by an epidemiological study of acute HBV infections in the Netherlands, in which 57% of heterosexually infected women had been infected by a partner originating from a hepatitis B endemic region.[34] This is consistent with the finding that heterosexual HBV transmission alone unlikely leads to a sustained transmission without new infections from abroad.[35] Particularly striking is the relatively large number of acute infections in the A2 (homosexual men) cluster, which indicates that the virus is actively spreading in this community. Many newly diagnosed chronic infections are also found in this cluster, which markedly illustrates the large extent of the epidemic. Overall, the findings in this study resemble those of a recent study in Amsterdam in which a large cluster of acute infections in homosexual men was described.[25]

Prospects for Identifying HBV Transmission Pairs

Patients were identified as possible transmission pairs based on epidemiological information from source and contact tracing. However, in case of chronically infected patients who were born in HBV endemic areas the most likely transmission route is difficult to establish as these patients might be infected years ago. More than half (13/22) of all epidemiological clusters consisted of such patients. Especially when there is doubt about the transmission route, molecular analysis can be used to refute or confirm the epidemiological hypothesis. Although the small number of clusters makes it difficult to compare subgroups, the data indicate that transmission pairs of chronically infected non-Dutch patients who were linked through sexual contact, relatively often do not receive support from the molecular analysis. Further research is needed to determine whether chronically infected transmission pairs are more difficult to detect than those associated with acute infections.

Figure 1 and 2

Phylogenetic tree of reported cases of acute and chronic HBV-infections with genotype A2 (Figure 1) and genotype D (Figure 2) using the Kimura two-parameter neighbor-joining method (Mega 4.0 software) with 1000 bootstrap replications. Only internodes with bootstrap support exceeding 70% are shown. Sequences included in epidemiological (E) and molecular (M) clusters are marked with the cluster numbers as used in Tables 2 and 3.

* Asterisks mark acute cases

† The sequences in this cluster are identical in pairwise comparison barring ambiguity codes.

 # The sequences in this cluster are identical in pairwise comparison to at least one of the members.

The diversity-ridden cluster of identical sequences of genotype A2 in homosexual men marks an extended HBV epidemic in the gay community. Because the contacts of these men are often anonymous, and because many of the HBV viruses transmitted in this group are genetically identical, it is usually not possible to identify the source(s) of infection. At the population level, instead, the genetic diversity of A2 clearly identifies the transmission route and risk group.[25] Finding clusters of identical sequences that are not epidemiologically linked might indicate the shortcomings of source and contact tracing, but it is also likely that the virus had a common origin and that multiple intermediate transmissions may be involved.[25] This can especially be the case if sequences belong to the so-called conserved sequences as described for genotypes D and E.[17] It would be interesting to verify these clusters, for example by using sequence data from another genome region such as the C region. Using this region, Boot et al. found that each genotype D isolate they studied had a unique sequence.[36]

Contrary to studies on HBV transmission in which identical sequences are considered the most likely candidate transmission pairs,[14] in the present study divergence among members of transmission pairs was allowed, because the degree of divergence of HBV can be expected to depend on unknown variables such as the time since transmission, the (variation of the) HBV mutation rate, and the level of heterogeneity of intra-patient HBV populations. By allowing mutations, molecular support was found for several epidemiologically defined transmission pairs of which some had diverged considerably. As a consequence, levels of divergence cannot be used as an indicator of the likelihood that pairs or groups of sequences constitute transmission pairs. The different numbers of mutations in transmission pairs tentatively suggest that mutation rates may differ considerably among patients. Highly variable[26] and extremely low mutation rates of HBV have been found (Dept. of Virology, Erasmus MC, Rotterdam; unpublished data). For example, in a serial study of chronically infected heart transplant recipients, mutations did not accumulate over the course of 10 years. The method of analysis used here can confirm the correctness of the hypothesized transmission route convincingly when molecular support is present or absent. However, the majority of the epidemiologically defined transmission pairs in this study received ambiguous support from molecular analysis, which is not unexpected when dealing with closely related sequences. Fortunately, in many cases transmission pairs with ambiguous molecular support may still be informative with respect to HBV transmission route if their sequences are identical. For example, in genotype D most of the epidemiologically defined transmission pairs that received ambiguous molecular support are genetically identical (*Table 3*). The combination of acute infections in one or more members of an epidemiological cluster provides further non-statistical support for HBV transmission.

This is the first epidemiological study that explicitly compares epidemiological and molecular data on HBV transmission while taking into account aspects of the molecular evolution of HBV patients, such as the presence of multiple nucleotides at a single position and the different phylogenetic reliability of different types of mutations. Nevertheless, several improvements and caveats of the identification of transmission pairs can be mentioned. First, the HBV DNA region studied here comprises overlapping reading frames and it would be helpful to assess diversity in different genome regions.[36] This would also aid the general interpretation of identity as a criterion for transmission (e.g., Fisker, 2004) and to elucidate the value of conserved HBV sequences of genotype D and E.[17] Overall, the number of mutations supporting or rejecting epidemiological pairs is disappointingly low (*Table 3*). This, it turns out, is not a consequence of an overall lack of variation in the S region, but results from the presence of a few hyper variable sites (not shown). The resulting large number of shortest trees might not occur when using another region of the HBV genome. It is not clear, however, whether such a strategy would allow molecular identification of transmission pairs in genotype A2, in which the lack of diversity largely precludes the use of diverged transmission pairs. Second, although each branch of a resolved phylogeny could be treated as representing transmission pairs, it is important to realize that phylogenetic groupings are formed in the process of building a tree. As a consequence, without epidemiological information one should refrain from treating anonymous clusters in trees as if they were transmission pairs. In this respect, analyzing only transmission groups supported by epidemiological data and relating the sequence variation among patients to a large reference data set with closely related sequences is crucial to prevent spurious results. In addition, a large comparative data set is required because the detection of genuine transmission pairs depends on the factual inclusion of transmission pairs. The realistic assumptions underlying the combined analysis of epidemiological and molecular data, and the availability of a large reference database can be expected to allow an efficient tracing of sources of infections.

Acknowledgements

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Chapter 6

Accuracy of a referral guideline for chronic hepatitis B patients in primary care to select patients eligible for evaluation by a specialist

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Abstract

Objective
To assess the accuracy of a simple guideline based on hepatitis B e-antigen (HBeAg) status and single alanine aminotransferase (ALT) determination to predict hepatitis B virus (HBV) viral load in chronic HBV patients.

Design
Observational analytic study of chronic HBV patients in primary care.

Setting
Municipal Public Health Service (MPHS) in Rotterdam, a city with a large multi ethnic population in the Netherlands.

Patients
420 patients with newly diagnosed chronic HBV infection seen at the MPHS from 2002 to 2005.

Main outcome measures
Accuracy of a guideline based on HBeAg positivity and/or elevated ALT level to predict high HBV DNA levels (defined as more than 105 copies/ml). Positive and negative predictive value, sensitivity, and specificity of the guideline were calculated.

Results
43% (181/420) of patients were eligible for referral to specialist care based on a positive HBeAg test or elevated ALT. The positive predictive value of the referral guideline was 45% (82/181, 95% confidence interval 38%-53%). The negative predictive value, i.e. the proportion of patients with low viral loads who were not selected for referral, was 95% (227/239, 95% CI 71%-97%). Sensitivity was 87% (95% CI 80%-93%); the patients selected included 82 of 94 patients with high HBV DNA. Of the 12 patients with high viral loads not selected by the guideline, 11 had a viral load of between 10^5-10^6 copies/ml.

Conclusions
A guideline based on HBeAg and a single ALT determination can successfully predict viral load in chronic HBV patients and can be used in primary care to select patients for referral to specialist care.

Introduction

Persons with chronic hepatitis B virus infection (HBV) are at risk of developing serious sequelae, such as cirrhosis and hepatocellular carcinoma.[1] The phases in chronic HBV infection are characterised by the presence or absence of hepatitis B e-antigen (HBeAg), the level of serum alanine transaminase (ALT), and serum HBV DNA.[2,3]

A large cohort study in Taiwan showed that, in addition to seropositivity for HBeAg and elevated serum ALT level, the level of HBV DNA in serum was a strong independent predictor of cirrhosis and hepatocellular carcinoma.[4,5] The level of HBV DNA in serum is a marker for viral replication and is used to evaluate the ability of antiviral treatment to suppress viral replication, slow disease progression, and prevent complications in decompensated liver disease.

Antiviral treatment is only indicated for a small proportion of patients, i.e. those with active HBV replication and ongoing inflammation of the liver, and practice guidelines have been developed to select patients who might benefit from treatment based on the probability of response to therapy, the severity of liver disease, and the likelihood of adverse events and complications.[6-8] These sophisticated guidelines are directed at specialist care, and include the assessment of serum HBV DNA. However, most chronic HBV patients are diagnosed in primary care where, unlike HBeAg and ALT, HBV DNA is usually not assessed as it is an expensive test which is not routinely performed in most laboratories.

A simpler, less expensive guideline that can be used at the primary care level to select patients for referral to specialists care can reduce the number of patients referred who, after evaluation by a specialist, are ineligible for treatment. By preventing the referral of ineligible patients, the disappointment patients with high expectations experience after referral to a specialist is avoided. Furthermore, follow-up efforts for patients going from primary to specialist care is limited to selected patients, and lastly, it reduces the burden on specialists. Patients who are not selected for referral by the guideline should be monitored regularly at the primary care level.[6]

A guideline for the referral of chronic hepatitis B patients in primary care based on HBeAg status and ALT level has been in use in Rotterdam since 2002.[9] In this paper, we assess the accuracy of the referral guideline to identify patients with high HBV DNA levels above the cut-off point who are indicated for treatment by a specialist.

Participants and methods

Participants

The study population was an unselected population based cohort of newly diagnosed chronic HBV patients in Rotterdam, The Netherlands. New chronic HBV patients (HBsAg positive for ›6 months) are notified obligatory to the Municipal Public Health Service Rotterdam Area (MPHS) and are invited to an appointment with a public health nurse. Of patients who visited the MPHS between January 1, 2002 and October, 2005 for source and contact tracing, counseling, and serological investigation a peripheral blood sample obtained for routine serological analysis was also used for molecular analysis and to quantify serum HBV DNA. Information on HBeAg status, ALT level, ethnicity, demo-graphics, sexual and travel behavior, and possible mode of infection was collected through a structured questionnaire, or extracted from the patient's files. Permission for the study was received from the Medical Ethical Commission of the University Medical Center Rotterdam (Erasmus MC).

Methods

Patients were considered eligible for referral if they were positive for HBeAg or had elevated ALT levels. Patients not selected for referral were referred to their general practitioner (GP) for annual monitoring of their ALT level. Elevated ALT level was defined as ALT ≥ 42 IU/ml in men, and ≥ 32 IU/ml in women. The HBV DNA viral load was deter-mined as described previously.[10] Patients with HBV DNA levels ≥ 10^5 copies/ml, also referred to in this paper as having a high viral load, were considered to be potentially eligible for treatment, as this cut-off point is recommended in clinical practice.[6,7] The patients' mothers country of birth, or if this information was missing, the country of birth of the patient, determined ethnicity. Descriptive statistical analyses were performed using SPSS 12.0. Positive predictive value (PPV), negative predictive value (NPV), sensi-tivity, and specificity of the referral guideline and its components, HBeAg and ALT, were calculated with 95% confidence intervals (95% CI).

Results

During the study period, serological and questionnaire data were collected from 464 chronic HBV patients. For 420 patients, information on HBeAg, ALT, and HBV DNA was available. Half of the study population was female. The age range of patients was from 8 to 80 years and included patients of various ethnic backgrounds. The general character-istics of the patients are described in *Table 1*.

Table 1.   Characteristics of 420 chronic HBV patients

	n	%
Sex		
Men	215	51.2
Women	205	48.8
Age (years)		
mean (SD)	34.2	12.5
median	32.0	
range	8 – 80	
Ethnicity[*]		
Western Europe	25	6.0
Eastern Europe/Russia	17	4.1
Mediterranean	127	30.5
North America	1	0.2
Central/South America and Caribbean	56	13.5
Africa	91	21.9
Middle East	4	1.0
Asia	95	22.8
HBeAg		
negative	355	84.5
positive	65	15.5
ALT[†]		
normal	264	62.9
elevated	156	37.1
Selected for referral[‡]		
no	239	56.9
yes	181	43.1
ALT (IU/l)		
mean (SD)	52.7	107.2
median	31.5	
range	9 – 1493	
HBV DNA (copies/ml)		
undetectable (<10^3)	163	38.8
$1.0 \times 10^3 - 9.9 \times 10^4$	163	38.8
$\geq 1.0 \times 10^5$	94	22.4
HBV DNA (log copies/ml)[§]		
mean (SD)	4.3 (2.2)	
median	3.4	
range	2.7 – 10.5	

[*] *mediterranean includes Turkey and Morocco*

[†] *elevated when ≥ 32 IU/l in women and ≥ 42 IU/l in men*

[‡] *selected when HBeAg is positive and/or ALT is elevated*

[§] *undetectable HBV DNA level was given a value of 500 copies/ml*

According to the referral guideline based on a positive HBeAg or elevated ALT, 43% (181/420) of the patients were eligible for referral to specialist care. The positive predictive value of the referral guideline was 45% (95% CI 38%–53%), as 82 of 181 patients selected for referral had high viral loads. The negative predictive value was 95% (95% CI 91%–97%), as 227 of 239 patients not selected for referral had low viral loads. The patients selected for referral included 82 of 94 patients with high serum HBV DNA, which corresponds with a sensitivity of 87% (95% CI 80%–93%). The procedure is illustrated in a flow chart (*Figure 1*), and the results are summarised in *Table 2*.

The first component of the referral guideline, positive HBeAg, was present in 15% of the patients and had a PPV of 88% (95% CI 78-94). The sensitivity of a positive HBeAg was 61% (95% CI 51-70). The second component of the guideline, elevated ALT, was present in 37% of the patients and had a PPV of 39% and a sensitivity of 65%. When the criterium 'elevated ALT' was only applied in HBeAg negative patients, 33% of HBeAg negative patients would be selected, with a PPV of 22% (95% CI 15-30) and a sensitivity of 68% (95% CI 51-80).

Of 12 patients with high viral loads who were not selected for referral by the guideline, 11 had a viral load of 10^5-10^6 copies/ml. We checked whether these patients had in one way or another been seen by a specialist at the department of Gastroenterology and

Figure 1. Flowchart of patients by HBeAg status, ALT level, and serum HBV DNA level
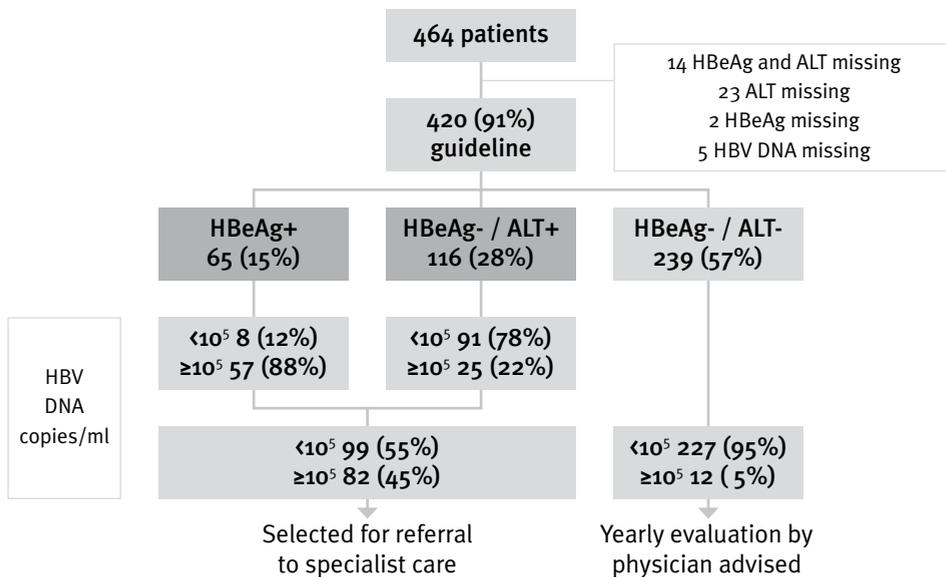
Hepatology of ErasmusMC, which was the case for three of them. All three were referred back to their GP after exclusion of liver disease. Another patient had an extremely high viral load of 2×10^{10} copies/ml. He was a 65 year old Dutch male patient who was tested due to dialysis, and was already under the care of a specialist.

Discussion

By using a guideline based on HBeAg and a single ALT determination, a meaningful pre-selection of chronic HBV patients can be made at the primary care level, referring less than half of the patients to specialist care. Of the referred patients, 87% of those with high viral loads were included.

Combining the results of the HBeAg and a single ALT determination improved the ability of the guideline to predict which patients had high viral loads better than either test alone. When only patients with a positive HBeAg test are referred, the number eligible for referral could be reduced to the 15% of patients who are HBeAg positive. However, although the predictive value of a positive HBeAg test is high at 88%, the sensitivity is unacceptably low, and almost 40% of patients with a high viral load would be missed. The sensitivity of an elevated ALT level is also low at 65%, but the combination of both test results in the guideline improved the sensitivity to an acceptable 87%.

We used the HBV DNA level as a proxy for eligibility for antiviral therapy, but while patients with HBV DNA levels $\geq 10^5$ copies/ml and elevated ALT levels should generally be treated, additional diagnostic procedures might be needed for HBeAg positive patients with normal ALT levels to define candidates for therapy, such as a liver biopsy to assess

Table 2. Characteristics of different determinants for selection for referral (% and 95% CI)

	HBeAg+ or ALT+	HBeAg+	ALT+	ALT+ in HBeAg-
Prevalence of determinant	43% (38–48)	15% (12-19)	37% (33-42)	33% (28-38)
Positive Predictive Value*	45% (38–53)	88% (78-94)	39% (32-37)	22% (15-30)
Negative Predictive Value†	95% (91–97)	90% (86-92)	88% (84-90)	95% (91-97)
Sensitivity‡	87% (80–93)	61% (51-70)	65% (55-74)	68% (51-80)
Specificity§	70% (64–74)	98% (95-99)	71% (66-76)	71% (66-76)

* *proportion of patients selected for referral that have high viral loads*

† *proportion of patients not selected for referral that have low viral loads*

‡ *proportion of patients with high viral loads that are selected for referral*

§ *proportion of patients with low viral loads that are not selected for referral*

liver damage. Only 5% of patients not selected for referral appeared to have high serum HBV DNA levels. Closer inspection reveals that the majority of these patients have intermediate elevated viral loads, and as they are HBeAg negative and have normal ALT levels, they would most probably not be found eligible for treatment after an evaluation by a specialist. However, these patients could have a precore mutation that prevents HBeAg formation, with replicating HBV and intermittent ALT flares.[11] We used only a single ALT determination, which could cause the misclassification of such patients. All patients who were negative for HBeAg and who had normal ALT levels were, however, advised to have their ALT level evaluated yearly by their physicians for at least 3 years after diagnosis, so these patients might very well be referred to a specialists care at a later time.

This is the first study to assess the ability of combined HBeAg and ALT to predict high viral load in chronic hepatitis B patients seen in primary care. The referral guideline began as an expert opinion, and is now validated with population based data. We found one Scandinavian study which looked at ALT levels as a predictor of high viral load in HBeAg negative pregnant women, and found a low correlation.[12]

By using the guideline based on both HBeAg and a single ALT determination, the majority of patients with high viral loads are referred to a specialist's care. We can conclude that the additional value of HBV DNA viral load is low for the selection of patients in primary care for referral. Although our study population had a varied ethnic background, the referral guideline should be validated in other populations. In the Netherlands, where current guidelines for GPs advise referring all chronic HBV patients to specialist care, introduction of the referral guideline is likely to considerably improve the efficiency of care for chronic HBV patients.

Acknowledgement

Ethical approval and funding

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Potential impact of long-term nucleoside therapy on the mortality and morbidity of active chronic hepatitis B

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Abstract

The potential impact of long-term antiviral therapy on the burden of chronic hepatitis B has hardly been documented. The aim of this study was to estimate the effects of prolonged antiviral therapy and antiviral resistance on the mortality and morbidity of active chronic hepatitis B patients. A population cohort of chronic hepatitis B patients in the Netherlands was constructed and stratified according to 10-year age groups, prevalence of hepatitis B surface antigen, hepatitis B virus DNA level, alanine aminotransferase level, hepatitis B e antigen status, and presence of cirrhosis. A Markov model was created to mathematically simulate the cohort's progression through a finite series of health states. The analysis was performed on the basis of four scenarios: natural history, long-term therapy with a high-resistance profile drug without or with salvage, and therapy with a low-resistance profile drug. It has been estimated that there were 64,000 people (0.4%) suffering from chronic hepatitis B infection in the Netherlands in 2005, with 6521 (10%) of them having high viremia and elevated alanine aminotransferase levels. Within a 20-year period, 1725 (26%) of the 6521 patients in the active chronic hepatitis B cohort will die because of liver-related causes. Of the 5685 without cirrhosis at entry, 1671 (29%) will develop cirrhosis. Of those 836 with cirrhosis at entry, 619 (74%) will die within a 20-year period. If this active chronic hepatitis B cohort is fully detected and treated, mortality related to liver disease can be reduced by 80% if a low-resistance profile drug is chosen from the start. The effect is due to both the reduction in complications of cirrhosis and the prevention of the development of cirrhosis.
Conclusion: Long-term antiviral therapy with a strategy that minimizes or controls resistance will have a major preventive effect on liver-related mortality and morbidity.

Introduction

Worldwide, about 360 million people have a chronic hepatitis B (CHB) infection and each year, 500,000 to 700,000 deaths are estimated to arise from hepatitis B virus (HBV)–related cirrhosis and hepatocellular carcinoma (HCC); therefore, CHB ranks as the 10th leading cause of death worldwide.[1] Most HBV-related deaths occur in developing countries. However, in many developed countries, mortality from hepatitis B-related cirrhosis and HCC is also substantial and exceeds that of other infectious causes, including human immunodeficiency virus.[2,3]

Vaccination is often seen as the key intervention to address the problem of HBV-related mortality over time. Although HBV vaccination programs clearly contribute to the reduction of new cases of HBV infection,[4] vaccination does not have any impact on preexisting CHB. Antiviral therapy is the only option to control and prevent progression of disease in patients with active CHB. Evidence has accrued for the efficacy of continuous nucleot(s)ide analogue therapy, which provides highly effective HBV suppression.[5-7] However, antiviral therapy has its limitations; with long-term use, it can be associated with the development of viral resistance that eventually can create serious clinical problems.[5,8]

Public health planners would benefit from knowing the possible future outcome of antiviral therapy in active CHB infection in terms of reductions in morbidity and mortality and the impact of antiviral resistance in a low endemic country with migration from highly endemic countries. The impact of antiviral therapy can be assessed in a mathematical model, with cohort studies providing progression rate estimates for the natural history, and information on the outcome of treatment with different antiviral drugs from clinical trials is now available. The aim of this study was to quantify with a mathematical model the morbidity and mortality of active CHB infection and to evaluate the potential impact of long-term nucleot(s)ide analogue therapy and antiviral resistance in a population of active CHB patients for a median follow-up period of 20 years.

Patients and methods

Cohort definition
A population cohort of CHB patients was constructed with the recently updated age-stratified prevalence of hepatitis B surface antigen (HBsAg) in the Dutch population.[9] We projected the age-stratified HBsAg prevalence to the total Dutch population, which was 16 million in 2005 (Statistics Netherlands).[10]

Table 1.   Annual transition estimates of the natural history of chronic hepatitis B by initial state and age group

Initial state	Outcome	Age group: 0-24 years		Age group: 25-65+ years	
		Estimate (%)*	Reference	Estimate (%)*	Reference
Chronic hepatitis B e+	Spontaneous virologic response	9.4 (8.3-23)	22,23	6.9 (2.0-23)	16,17
	Cirrhosis	0.1 (0.0-0.1)	22,23	2.7 (1.6-3.8)	21
	Hepatocellular carcinoma	0.1 (0.0-0.1)	22,23	0.4 (0.3-0.6)	21
	Chronic hepatitis B e-	0.4 (0.2-0.6)	22,23	1.9 (1.0-3.8)	21
Chronic hepatitis B e-	Spontaneous virologic response	9.4 (8.3-11)	22,23	1.6 (0.0-11)	16,17
	Cirrhosis	0.1 (0.0-0.1)	22,23	6.2 (2.8-9.7)	21
	Hepatocellular carcinoma	0.1 (0.0-0.1)	22,23	0.4 (0.3-0.6)	21
Cirrhosis e+	Decompensated cirrhosis	3.9 (2.0-7.9)	24-26	3.9 (2.0-7.9)	24-26
	Hepatocellular cancer	1.8 (0.9-3.8)	24-26	1.8 (0.9-3.8)	24-26
	HBV-related death	3.1 (3.1-3.8)	21,24-26	3.1 (3.1-3.8)	21,24-26
Cirrhosis e-	Decompensated cirrhosis	2.7 (1.4-5.4)	24-26	2.7 (1.4-5.4)	24-26
	Hepatocellular cancer	2.9 (1.0-5.6)	24-26	2.9 (1.0-5.6)	24-26
	HBV-related death	3.1 (3.1-3.8)	21,24-26	3.1 (3.1-3.8)	21,24-26
Decompensated cirrhosis	Liver transplantation	3.3 (1.0-8.4)	28,29	3.3 (1.0-8.4)	28,29
	HBV related death	26 (15-62)	21	26 (15-62)	21
Hepatocellular carcinoma	Liver transplantation	1.2 (0.2-5.0)	28,29	1.2 (0.2-5.0)	28,29
	HBV-related Death	35 (20-60)	16,17	35 (20-60)	16,17
Liver transplant	HBV-related death	6.6 (2.0-12)	16,17	6.6 (2.0-12)	16,17

Abbreviation: HBV, hepatitis B virus

* Ranges are shown in parentheses

The HBsAg-positive cohort was first divided into two groups, active CHB and inactive CHB, based on hepatitis B e antigen (HBeAg) status, HBV DNA level, and serum alanine aminotransferase (ALT) level. The age-specific distribution of these factors was derived from a large database with 479 newly diagnosed CHB patients who were seen at the Municipal Public Health Service (Rotterdam-Rijnmond), the Netherlands.[11] The differentiation between active CHB and inactive CHB is essential because progression of the disease is different in these two groups. Patients with high HBV DNA levels (HBV DNA ≥10^4 copies/mL for HBeAg-negatives and ≥10^5 for HBeAg-positives) and elevated ALT (>2 times the upper limit of normal) are classified as having active CHB, have potentially progressive liver disease, and are candidates for HBV antiviral therapy,[12,13] whereas those with low or undetectable HBV DNA and normal ALT levels usually are inactive HBsAg carriers with a low risk of disease progression. Lastly, we classified the active CHB patients into two categories, with cirrhosis and without cirrhosis, using age group-specific proportions from large HBeAg-positive and HBeAg-negative clinical trials, respectively.[14,15]

Markov model

A Markov mathematical simulation was used to model the outcome of the Dutch cohort of treatment-naïve active CHB patients with high viremia for each age group over a time period of 20 years, which is sufficient time to allow for all possible HBV-related outcomes (development of cirrhosis, liver failure, HCC and death) to occur. The model describes disease progression and determines the long-term morbidity and mortality of the cohort during follow-up. The model uses annual probabilities of transition from CHB to virologic response and of progression to cirrhosis, decompensated liver disease or HCC, and finally death; these were obtained mostly from systematic reviews published in the literature (*Table 1* and *2*).[16-26] These include both cohort studies describing the natural history of CHB and clinical trials reporting the effect of treatment. As the probability estimates for progression from chronic CHB are clearly different in younger patients and adults, the cohort was split by age, with age groups of 0 to 24 years and 25 to 65+ years. When progression rates were reported, these were transformed into annual probabilities using a standard formula: $P=1-e^{-r \times t}$, where P is the probability, e is the base of the natural logarithm, r is the event rate, and t is the time interval.[27] The term *morbidity* was defined as events related to decompensated cirrhosis, HCC, and liver transplantation, whereas *hepatic death* was death related to liver failure or other liver-related complications. Other causes of death not related to liver disease are included in the model as age-specific mortality rates derived from Statistics Netherlands.[10] Mortality is the model's major outcome, but the expected number of cirrhosis, decompensated cirrhosis, HCC and liver transplant cases is also quantified. The model was built with TreeAge Pro 2006 (TreeAge Software, Inc., Williamstown, MA).

Table 2. Treatment-related annual transition estimates[†]

Initial state	To	High-resistance profile drug		Low-resistance profile drug		Salvage therapy	
		HBeAg+	HBeAg-	HBeAg+	HBeAg-	HBeAg+	HBeAg-
CHB Initial therapy[¶]	Sustained virological response	20	10	22[‡]	11[‡]	12	10
	Cirrhosis[*]	0.5	1.2	0.2	0.6	0.5	1.2
	Hepatocellular carcinoma[**]	0.2	0.2	0.2	0.2	0.2	0.2
CHB long-term therapy	Sustained virological response	24	10	27[‡]	11[‡]	12	10
	Cirrhosis[*]	0.5	1.2	0.2	0.6	0.5	1.2
	Resistance	23	23	1	1	1.3	1.3
	Hepatocellular carcinoma[**]	0.2	0.2	0.2	0.2	0.2	0.2
Resistant CHB long-term therapy	Sustained virological response	4.5	0	5[‡]	0.5[‡]	4.5	0
	Cirrhosis[*]	2.7	6.2	2.7	6.2	2.7	6.2
	Hepatocellular carcinoma[**]	0.4	0.4	0.4	0.4	0.4	0.4
Cirrhosis Initial therapy	Sustained virological response	20	10	22[‡]	11[‡]	12	10
	Hepatocellular carcinoma[**]	0.9	1.5	0.9	1.5	0.9	1.5
Cirrhosis long-term therapy	Sustained virological response	24	1	27[‡]	11[‡]	12	1
	Resistance	2	2	1	1	5	5
	Decompensated Cirrhosis	1.9	1.9	1.9	1.9	1.9	1.9
	Hepatocellular carcinoma	1.6	1.6	1.6	1.6	1.6	1.6
	HBV-related death	2.4	2.4	2.4	2.4	2.4	2.4
Resistant Cirrhosis long-term therapy	Sustained virological response	4.5	0	5[‡]	0.5[‡]	4.5	0
	Decompensated Cirrhosis	7.9	7.9	7.9	7.9	7.9	7.9
	Hepatocellular carcinoma	1.8	2.9	1.8	2.9	1.8	2.9
	HBV-related death	3.1	3.1	3.1	3.1	3.1	3.1
Decompensated Cirrhosis	Liver Transplantation[§]	3.3	3.3	3.3	3.3	3.3	3.3
	HBV-related death	26	26	26	26	26	26
Hepatocellular carcinoma	Liver Transplantation[§]	1.2	1.2	1.2	1.2	1.2	1.2
	HBV-related death	35	35	35	35	35	35
Liver Transplantation	HBV-related death	6.6	6.6	6.6	6.6	6.6	6.6

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Scenario analyses

Four different scenarios were analyzed in the study. In the first scenario, the natural history of active CHB was simulated: patients received all medical care except antiviral medication to suppress the viral infection. In the second scenario, patients received antiviral treatment with a high-restance profile drug.[5] In the third scenario, patients received the high-restance profile drug and salvage therapy[17] upon the development of resistance. In a fourth scenario, patients received antiviral medication with a low-resistance profile.[20] In all four scenarios we followed the cohort over a period of 20 years through a series of Markov cycles governing patients' transitions between relevant health states.

Scenario 1: Natural History

In this scenario, active CHB patients progressed according to the natural history; annual rates of progression derived from systematic reviews were followed (*Table 1*).[16,17] *Spontaneous virologic response* was defined as seroconversion to antibody against hepatitis B e antigen (anti-HBe) for HBeAg-positive patients and as persistent HBV DNA suppression and ALT normalization for HBeAg-negative patients. Deviations in the transmission estimates from Kanwal et al.[16,17] were introduced when new information on the progression rates of specific diseases states or the impact of antiviral drugs became available. Such deviations are mentioned in the text and in *Table 2*.

The probabilities of receiving a liver transplant for decompensated cirrhosis and liver cancer were calculated on the basis of data from the European Liver Transplant Registry and the Dutch Transplantation Organization.[28,29] Yearly, four liver transplants are estimated to take place because of liver cancer, 80% of which is HCC. This corresponds to an annual probability of receiving a liver transplant for liver cancer of 1.2% as 264 cases of HCC were reported in 2005. The annual probability of receiving a liver transplant for decompensated

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Abbreviations: CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

† *Estimates were taken from Kanwal et al.[16,17]*

‡ *Estimates were taken from recent clinical trials by Chang et al.,[18] Lai et al.,[19] and Colonno et al.[20]*

* *Estimates were calculated under the assumption that the natural progression rates of chronic hepatitis B taken from Kanwal et al.[16,17] are reduced by antiviral therapy. Similar to Kanwal's assumption of no progression of disease in HBeAg seroconversion, we assumed no progression of disease when HBV DNA was undetectable by polymerase chain reaction. In the studies from Chang et al.[18] and Lai et al.,[19] full suppression of HBV DNA was observed in 80% with a high-resistance profile drug and in 90% with a low-resistance profile drug. We took these percentages for our calculations.*

** *Estimates were based on the reduction of progression rates by nucleoside analogue therapy of 50%.[5]*

§ *Liver Transplantation estimates for the Netherlands.*

¶ *Initial therapy was the first 12 months (48 weeks) of therapy.*

cirrhosis was calculated to be 3.3%; this was based on an estimated number of 300 cases of decompensated cirrhosis and 10 liver transplantations for this condition.

Scenario 2: High-resistance profile drug

In this scenario, patients received long-term therapy with the first licensed antiviral HBV drug, which is associated with a high incidence of resistance[5]; such monotherapy is still being practiced in many countries with limited resources.[30] In HBeAg-positive patients, *virological response* was defined as HBe antigen loss and development of anti-HBe. In HBeAg-negative patients, *virological response* was defined as HBV DNA levels undetectable by polymerase chain reaction. We assigned different rates of virologic response under long-term therapy between resistant and nonresistant patients (*Table 2*). Following current practice guidelines, we assigned patients who did not respond to initial therapy or who experienced relapse after initial response to long-term therapy.

According to the assumptions in the systematic reviews,[16,17] the disease stops progressing in patients who develop virologic response, whereas in cases of nonresponse and viral resistance, the disease progresses as in the natural history. However, the rate of progression from compensated cirrhosis to decompensated cirrhosis is higher in patients presenting with resistance versus those following the natural history.[17]

Scenario 3: High-resistance profile drug followed by salvage therapy

In this scenario, the same high-resistance profile drug used in scenario 2 was given. Once resistance occurred, patients were salvaged by the addition of a second antiviral drug with potency against the resistant strain (salvage therapy).[17] Patients without resistance continued to receive the initial drug.

Scenario 4: Low-resistance profile drug

The same patient management strategy used in scenario 2 was applied. The annual probability of resistance developing in those receiving a low-resistance profile drug was much lower than in scenario 2 and was set at 1% per year on the basis of a recent study that reported data after 4 years of follow-up.[20] The treatment-related probability estimates are shown in *Table 2*.

Sensitivity analysis

To study the robustness of our results, we performed a sensitivity analysis on the low and high ranges of the transition estimates in the natural history scenario (*Table 1*). First, a so-called best case scenario was assessed by the application of the high ranges of progression to spontaneous virological response and by the application of the low ranges of the estimates of disease progression. Second, a worst case scenario was assessed by the application of the low progression rates to spontaneous virological response and by the application of the high ranges of the disease progression estimates.

Our assumption that disease progression to decompensated cirrhosis is higher in patients with cirrhosis who develop resistance versus patients who progress according to the natural history might underestimate the efficacy of the high-resistance profile drug. To judge the impact of this possible underestimation, we calculated liver-related mortality under the assumption that progression from cirrhosis to decompensated cirrhosis in case of resistance equals the progression in natural history.

Results

Cohorts and natural history

Table 3 shows the total population of the Netherlands in 2005 with the age-specific prevalence of HBsAg. Around 64,000 individuals (0.4% of the total population) are estimated to be HBsAg carriers, with 10,802 (17%) of them having HBeAg-positive CHB and 53,046 (83%) having HBeAg-negative CHB. The total number of patients with active CHB was 6521 or 10% of the total HBsAg-positive cohort, 26% of HBeAg positives, and 7% the HBeAg-negatives. The proportion of cirrhosis increased by age: from 2% to 34% among HBeAg-positive CHB patients and from 5% to 56% among HBeAg-negative CHB patients.

Natural History of the active CHB cohort

The estimated burden of active CHB infection in 20 years of follow-up is shown in Fig. 1 for the natural history scenario. If the active cohort of 6521 individuals remains untreated, 1725 (26%) will die because of liver-related complications. Within 20 years, there will be 1283 (20%) morbidity events, with 575 decompensation events (9%) and 670 HCC events (10%), and 38 cases will undergo liver transplantation (0.6%). At entry into the cohort in the year 2005, 836 cases (13%) are already in the cirrhotic stage (*Table 3*). By the year 2025, another 1671 (29%) of 5685 cases will have developed cirrhosis, and this will have led to a cumulative number of 2507 cirrhotics (38%) in the eligible cohort.

Subgroups: noncirrhosis vs cirrhosis

At entry 5685 (87%) of 6521 cases had no signs of cirrhosis, with 47% being HBeAg-positive and 53% being HBeAg-negative. If these noncirrhotic cases are left untreated, 1106 (19%) of the 5685 cases will die because of liver-related complications within 20 years. This proportion differs by HBeAg status and is 9% for HBeAg-positives and 28% for HBeAg-negatives. About 1671 (29%) of the noncirrhotic cases will develop cirrhosis (12% and 44% for HBeAg-positives and HBeAg-negatives, respectively), 360 (6%) will develop decompensated cirrhosis (4% and 9% for HBeAg-positives and HBeAg-negatives, respectively), 481 (8%) will develop HCC (4% and 13% for HBeAg-positives and HBeAg-negatives, respectively), and 30 (0.5%) will undergo liver transplantation (0.3% and 0.7% for HBeAg-positives and HBeAg-negatives, respectively).

Table 3. Age group-specific distribution of chronic hepatitis B in the Netherlands by HBeAg and stage of liver disease

Age group (years)	Population	HBsAg+	(%)	HBeAg+	HBeAg-	Active CHB HBeAg+	HBeAg-
‹15	3,009,000	2,708	(0.09%)	812	1,896	211	133
15-24	1,948,000	14,415	(0.74%)	4,325	10,091	1,124	706
25-34	2,185,000	10,051	(0.46%)	2,010	8,041	523	563
35-44	2,622,000	16,519	(0.63%)	2,643	13,876	687	971
45-54	2,315,000	16,437	(0.71%)	822	15,615	214	1,093
55-64	1,938,000	2,713	(0.14%)	190	2,523	49	177
65+	2,288,000	1,005	(0.08%)	0	1,005	0	70
Total	**16,305,000**	**63,848**	**(0.39%)**	**10,802**	**53,046**	**2,808**	**3,713**

Table 4. Morbidity and mortality of active chronic hepatitis B by HBeAg status in the natural history scenario

CHB stage at entry	N	Cirrhosis (%)		Decompensated cirrhosis (%)		HCC (%)	
No cirrhosis							
HBeAg+	2,634	317	(12%)	94	(4%)	93	(4%)
HBeAg-	3,051	1,354	(44%)	266	(9%)	388	(13%)
all no cirrhosis	5,685	1,671	(29%)	360	(6%)	481	(8%)
Cirrhosis							
HBeAg+	174	174	(100%)	55	(32%)	17	(10%)
HBeAg-	662	662	(100%)	160	(24%)	172	(26%)
all cirrhosis	836	836	(100%)	215	(26%)	189	(23%)
Total	**6,521**	**2,507**	**(38%)**	**575**	**(9%)**	**670**	**(10%)**

Thirteen percent of the cohort had cirrhosis at entry (836/6521), with 21% being HBeAg-positive and 79% being HBeAg-negative. If left untreated, 619 (74%) of the 836 members of the cirrhotic cohort will die because of liver-related complications. This proportion does not differ by HBeAg status. The morbidity in this cohort will be 412 (50%) of 836 (again the same for HBeAg-positives and HBeAg-negatives); 215 (26%) cases will develop decompensated cirrhosis (32% and 24% for HBeAg-positives and HBeAg-negatives, respectively), 189 (23%) will develop HCC (10% and 26% for HBeAg-positives and HBeAg-

	Cirrhosis			Chronic hepatitis (no cirrhosis)	
	HBeAg+	(%)	HBeAg-	(%)	HBeAg+	HBeAg-
	4	(2%)	7	(5%)	207	126
	22	(2%)	35	(5%)	1,102	671
	31	(6%)	39	(7%)	491	524
	48	(7%)	146	(15%)	639	825
	53	(25%)	306	(28%)	160	787
	16	(33%)	90	(51%)	33	87
	0	(0%)	39	(56%)	0	31
	176	**(6%)**	**663**	**(18%)**	**2,633**	**3,051**

Abbreviations: CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

	Liver transplant (%)		Death (%)	
	8	(0.3%)	248	(9%)
	22	(0.7%)	858	(28%)
	30	(0.5%)	1,106	(19%)
	2	(1.1%)	127	(73%)
	6	(0.9%)	492	(74%)
	8	(1.0%)	619	(74%)
	38	**(0.6%)**	**1,725**	**(26%)**

Abbreviations: CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma.

negatives, respectively), and 8 (1%) will undergo liver transplantation (1% and 1% for HBeAg-positives and HBeAg-negatives, respectively). *Table 4* and *Fig. 1* show the different outcomes in all scenarios for the noncirrhotic and cirrhotic subgroups.

If the active CHB cohort is not treated, 138 individuals (3%) of the 0 to 24 age group (*n* = 2174) and 1661 (38%) of the 25 to 65+ age group (*n* = 4345) will die because of liver related complications within a 20-year period. The morbidity will be 34 cases (2%) in the 0 to 24 age group and 1249 (29%) in the 25 to 65+ age group.

Impact of treatment

A reduction of hepatitis B-related mortality and morbidity was observed in model projections when treatment was applied. Treating the cohort with a high-resistance profile antiviral drug will decrease the mortality to 971 cases (15%; *Fig. 1*) and the number of morbidity events to fewer than 822 (13%). Treating the same patients with a low-resistance profile drug will further decrease the mortality to 339 liver related deaths (5%) and the morbidity events to around 229 (3%). Eight hundered sixty-one (15%) new cases of cirrhosis are to be expected if the active CHB cohort is treated with a high-resistance profile drug, whereas treatment with a low-resistance profile drug will yield only 112 (2%) new cases of cirrhosis after 20 years of follow-up. When salvage therapy is applied without delay to the cases that become resistant, mortality and morbidity will be 333 (5%) and 386 (6%), respectively.

Figure 1. Liver related mortality and morbidity of the hypothetical Dutch cohort with active CHB (period 2005-2025) by four different scenarios.
(NH=Natural History, HRD=High-resistance profile drug, SRx=Salvage therapy, LRD=low-resistance profile drug)
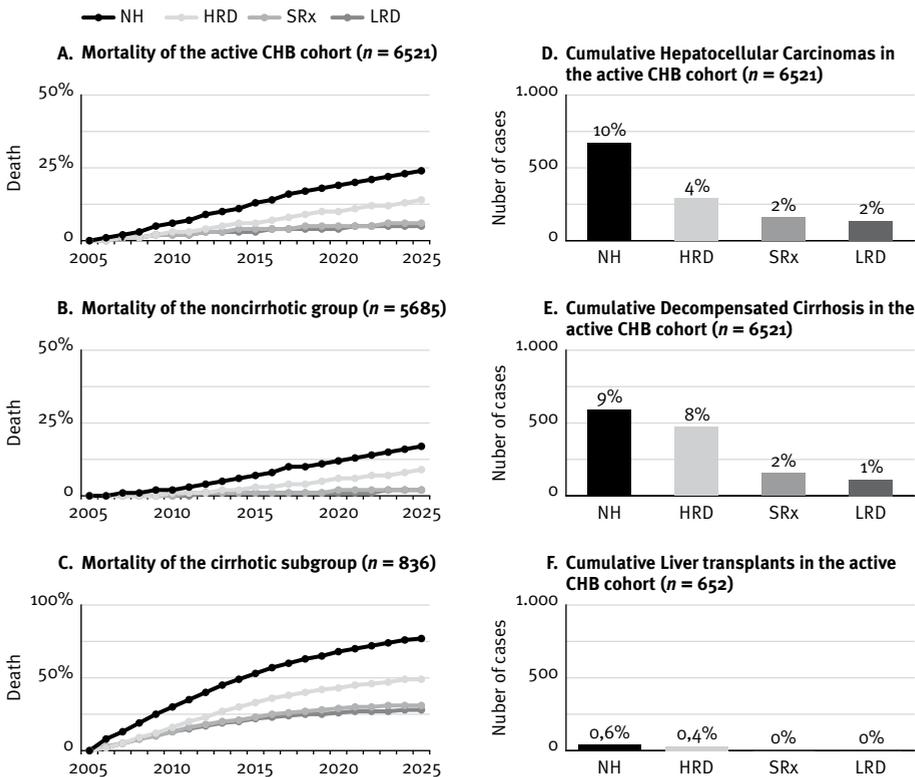
Comparing the four scenarios, we find that a low-resistance profile drug will prevent 1386 cases (80%) of liver-related death, whereas an antiviral with a high incidence of resistance will prevent only 754 cases (44%) of CHB-related deaths. Applying salvage therapy without delay to the cases who become resistant will prevent 1339 cases (77%) of CHB-related death. The burden of antiviral resistance in this model is 632 deaths (36% of the total number of liver-related deaths).

Comparing the scenarios in terms of morbidity, we find that a low-resistance profile drug would prevent 1054 cases (82%) from proceeding to complications, whereas an antiviral drug with high-resistance will prevent 461 cases (36%) from proceeding to CHB-related complications. The burden of antiviral resistance in terms of morbidity is 593 cases (46%). If salvage therapy is applied, this scenario will prevent 950 cases (74%) from proceeding to liver-related complications.

Sensitivity analysis
The sensitivity analysis for the natural history scenario show that, in comparison with the base case, in which the mortality of the active CHB cohort is 26%, the mortality ranges from 13% in the best case scenario to 39% in the worst case scenario. When assessed by subgroups, in the best and worst case scenarios, mortality ranges from 3% to 26% for HBeAg-positive chronic hepatitis, from 8% to 36% for HBeAg-negative chronic hepatitis, and from 60% to 91% for cirrhosis independent of HBeAg status.

Combining these ranges with the treatment-related outcomes indicates that a low-resistance profile drug can prevent 59% of liver-related deaths in the best case scenario for natural history and 87% in the worst case scenario with its high disease progression rate.

The sensitivity analysis regarding progression from cirrhosis to decompensated cirrhosis in the case of resistance shows that mortality in the high-resistance profile drug scenario decreased from 15% to 13% when the rate of progression in resistance is changed from higher than that in natural history to equal to that in natural history.

Discussion

If all patients with high viremia and elevated ALT from the total cohort of CHB patients are fully treated with a low-resistance profile drug, liver-related mortality can be reduced by 80% (sensitivity analysis range, 59%-87%). Because liver-related mortality is approximately 26% (sensitivity analysis range, 13%-39%), a high relative reduction in mortality will also translate into a high absolute number of cases in which mortality can be prevented.

Treating the cohort with a high-resistance profile drug will reduce the liver-related mortality and morbidity by only 47%. The burden of antiviral resistance if no salvage therapy is applied is considerable: about 42% of the potential benefit of antiviral therapy is lost by resistance. In the Netherlands, in which reimbursement of salvage therapy is without restraint, adding a second antiviral agent in case of resistance appears as good as starting with a low-resistance profile drug. In the model, the efficacy of salvage therapy might be overestimated because the start of salvage therapy is programmed at the time of occurrence of resistance. In practice, the start of salvage therapy will often be delayed. In addition, current evidence shows that salvage therapy can become ineffective in the long run.[31]

The beneficial effect of antiviral therapy is due to both the reduction in complications of cirrhosis and the prevention of the development of cirrhosis. Liaw et al.[5] documented the beneficial effect of long-term nucleoside analogue therapy on clinical outcome in patients with cirrhosis. Our study underlines the potential efficacy of long-term antiviral therapy in patients with potentially progressive disease who are still in the noncirrhosis stage.

The aforementioned findings are related only to the subgroup of CHB patients with potentially progressive disease, that is, those with high viremia and elevated ALT. In a low endemic country such as the Netherlands, 10% of newly diagnosed CHB cases fall into this category, with about equal numbers of HBeAg-positives and HBeAg-negatives.

The active CHB cohort was constructed in a way that captured relevant aspects related to disease progression and response to treatment, that is, age, HBe antigen status, HBV DNA and ALT levels, and presence of cirrhosis. In assessing the cohort's progression through the various health states, we used transition estimates that were based on extensive systematic reviews[16,17] and updated with recently available robust findings.[5,18-26] We performed the analyses by simulating the cohort separately for each age-specific group, HBe antigen status, and stage of liver disease, as these factors affect prognosis, thereby approaching the real-life situation as much as possible. For the treatment scenarios, the model simulated long-term treatment as it is now emerging in guidelines.[13] We developed the model

in such a way that with small adaptations it can be used to estimate the hepatitis B burden and impact of antiviral therapy in various countries or regions according to their profiles, such as prevalence by age-specific group, and treatment characteristics, such as the drug type chosen for initial therapy and percentage application of salvage therapy.

We chose to apply the simulation to the specific cohort of high-viremia patients with elevated ALT because our main goal was to define the impact of antiviral drugs on clinical outcome and these patients would qualify for treatment according to recent guidelines.[12,13] Patients with high viremia and ALT levels within the normal range were not included in the cohort as these patients are often in the immune-tolerant phase of their infection and treatment is currently not recommended for this group. However, the context of how antiviral therapy should be used remains a difficult question, particularly with respect to which patients with CHB should be treated and what ALT level (abnormal, twice normal, or greater than 5 times normal) should be used for the criteria.[32] The transition estimates for disease progression in our natural history model were taken from various international studies. Although the patients included in these studies were mainly patients with active CHB (i.e., with elevated ALT levels), some studies were based on a mixed population of CHB patients and also included patients with normal ALT levels. For this reason, the progression rates used in the natural history model might underestimate the morbidity and mortality of a strict cohort of active CHB patients with a high viral load and elevated ALT levels in natural history, and this implies an even higher impact of treatment.

In a further study, the outcome of patients with low viremia and those with high viremia but normal ALT will be assessed as well as the effect of antiviral therapy in specific sub-groups such as noncirrhotic and cirrhotic patients, with transition estimates specifically applicable to these patients that are in different disease phases.

A limitation of our study is that we used simplified assumptions (e.g., we did not consider coinfection with other viruses or toxins such as alcohol that will accelerate progression), and we assumed the cohort to be static, so there were no new cases added to the cohort. Assuming that the development of resistance with a low-resistance profile drug for the coming 20 years will stay at 1% per year likely underestimates what will happen as longer term data are collected.

The proportion of patients without cirrhosis in our cohort at baseline is comparable to that found in a recent Italian longitudinal cohort study of untreated adult Caucasian patients with CHB, 87% of whom presented without cirrhosis at diagnosis.[33] In the Italian study, 27% of the CHB patients developed cirrhosis during the follow-up period, and this is similar to our study, in which 29% of CHB cases develop cirrhosis over a period of 20 years. At the end of 20 years of follow-up, 26% of patients in the active CHB cohort will die

of CHB-related causes, whereas this was 16% in the Italian cohort. However, the Italian cohort consisted of only 70 patients and the 16% mortality rate fell within the sensitivity range of our mortality estimate. A possible explanation for the difference in mortality between our study and the Italian cohort study is that our cohort consists of patients with high viremia and ALT › 2 the upper limit of normal, whereas the Italian study used HBeAg status as an indicator of high viremia. HBeAg-negative patients were not included in the Italian study, and these patients have a higher progression rate to cirrhosis.

Even the most precise mathematical modeling is only an estimate of the real-life situation.[34] However, in the case of CHB, modeling might reflect real life better than official mortality data because epidemiological data based on death certificates have been shown to be extremely unreliable in estimating mortality from CHB.[35,36]

This study, like a preliminary one in Spain,[37] focused on quantifying the impact of therapy and antiviral resistance at the population level. Other studies of hepatitis B have used mathematical modeling to compare cost effectiveness among various antiviral drugs,[16,17] and to predict the impact of vaccination programs in preventing HBV-related death.[38] These studies mainly give an economic message, whereas we focused our study on the health aspect of the burden of disease. Apparently, CHB is not only a problem of the less developed countries with high HBV endemicity but also a problem of countries with a low endemicity because there is a high absolute number of preventable liver-related deaths in a 20-year period.

The important clinical benefits described in this study can be obtained only if the full subgroup of active CHB patients is detected and treated for many years with high compliance. Currently, most of the eligible individuals are not treated because of limited screening activities to identify eligible cases. There are also major shortcomings in referral to specialist care,[39] where treatment can be started and monitored.

On the basis of this study, public health organizations should turn their attention, means, and actions increasingly to CHB and take the responsibility for identifying CHB and selecting those patients with potentially progressive disease for referral to treatment centres.

Acknowledgements

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Chapter 8

Screening of migrants for chronic hepatitis B virus infection:
a cost effectiveness analysis

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Abstract

Background

Persons with chronic hepatitis B virus infection (HBV) are at risk of developing cirrhosis and hepatocellular carcinoma. Early detection of chronic HBV infection through screening and treatment of eligible patients has the potential to contribute to secondary prevention of HBV.

Objective

To assess the costs-effectiveness of systematic screening for chronic hepatitis B (CHB) of first generation migrants in the Netherlands from intermediate and high endemic countries. migrants.

Methods

Epidemiological data of expected numbers of patients with active CHB in the target population and data on the costs of a screening program were combined with the outcomes of a Markov model in terms of costs and QALYs for patients with and without treatment, using a life time horizon. The base-case assumptions were an HBV prevalence of 3.35%, 35% participation in screening, specialist consultation for 58% of patients selected for referral, and 75% of eligible patients starting treatment.

Results

Compared to the status quo, a one-time screening can reduce mortality of liver related diseases by 10%, from 1,073 to 965 deaths. Using base case estimates, the incremental cost effectiveness ratio (ICER) of screening compared to the status quo is € 8,966.- per QALY gained. The ICER varied between € 7,936.- and € 11,705.- per QALY gained in univariate sensitivity analysis, varying parameter values of HBV prevalence, participation rate, successful referral and treatment compliance. In multivariate sensitivity analysis for treatment effectiveness the ICER varied between € 7,222.- and € 15,694.-, and for disease progression in natural history from € 5,568.- to € 60,418.-.

Conclusion

Early detection and treatment of eligible patients has a large impact on liver related health outcomes. A systematic screening program for chronic HBV infection targeted at first generation migrants is likely to be cost effective, even at low estimates for the HBsAg prevalence, participation, referral and treatment compliance.

Introduction

Hepatitis B is an important public health problem, with an estimated 350 million persons chronically infected worldwide.[1] Persons with chronic hepatitis B virus infection (HBV) are at risk of developing serious sequelae, such as cirrhosis and hepatocellular carcinoma. A mathematical modeling study estimated that 620,000 people died worldwide from HBV-related causes in the year 2000.[2] Primary prevention of HBV infection is available in the form of a highly effective vaccine, but since universal HBV vaccination has only been introduced about a decade ago in many countries,[3] the problem of people already infected with HBV remains. Screening for hepatitis B is a form of secondary prevention, aimed at early disease detection to allow antiviral treatment to prevent HBV related liver disease. The possibilities for antiviral treatment have greatly improved over the past decade: several registered drug therapies for chronic HBV, which have been shown to be cost-effective, are now available.[4-7] We recently estimated that treatment with a low resistance profile drug of chronic HBV patients with active disease could reduce mortality related to liver disease in this group by 80%.[8]

However, while the potential impact of treatment is large, the current benefit is not optimal for several reasons. Firstly, the proportion of patients actually receiving treatment of those who might benefit is low due to the largely asymptomatic nature of chronic HBV infection, which makes case detection difficult. Patients often have progressed liver disease when they are detected based on symptoms. Secondly, as the management of patients after detection in primary care is not optimal, patients are often not seen by a specialist.[9] Lastly, not all patients that are eligible for treatment will start treatment. Early detection of chronic HBV infection through screening, with follow-up and treatment of eligible patients therefore has the potential to contribute to secondary prevention of HBV.

In countries with low HBV endemicity the prevalence of chronic HBV infection varies widely between population subgroups. Migrants from countries with a relatively high HBV endemicity are the largest at risk group, with a prevalence of chronic infection that is up to 25 times higher than the prevalence in the indigenous population.[10-13] Surveillance data show that 77% of chronic HBV patients notified in the Netherlands were born abroad, almost all in intermediate or high endemic countries.[14] First generation migrants (FGM) are therefore an important target group for screening for chronic HBV. Hutton et al. recommended screening of Asian and Pacific Islander adults in the United States, which they showed is likely to be cost-effective.[15] In the Netherlands, recommendations for HBV screening of migrants do not exist. To support policy making, we carried out a cost-effectiveness analysis (CEA) of systematic screening and treatment for chronic hepatitis B of FGM in the Netherlands from intermediate and high endemic countries.

Methods

We used a Markov chain model to assess the costs and health outcomes of a cohort of patients that either followed the natural history of HBV infection or received antiviral treatment. Comparative outcomes of these models, in terms of mortality, quality of life and health care costs, were entered in a separate cost-effectiveness model containing all relevant variables of the screening program. The status quo includes a baseline level of detection of chronic HBV infections through the existing pregnancy screening program, through testing due to medical complaints, contact tracing, or a check-up for sexually transmitted infections. Our analysis was performed using a health care perspective. We consider as the target population for screening all FGM in the Netherlands born in inter-mediate and high prevalence countries, based on data of the World Health Organization.[16] This target population consists of about 1.3 million persons, which is 8% of the Dutch population (Statistics Netherlands, 1 January 2006).

Assumptions regarding detection and patient management in status quo
To estimate the detection rate in the status quo, we divided the number of chronic HBV patients that are notified over a 5-year period (2002-2006) by the expected number of HBsAg positive persons in the target population. We assumed that there are currently 44,117 HBsAg positive persons in the target population, based on the recently estimated

Table 1.   **Estimated HBsAg prevalence in FGM from intermediate and high endemic countries (derived from Marschall et al, 2008, ref. 12) by disease status and estimated number of patients starting treatment in the status quo and following screening.**

Age group (years)	Population	HBsAg+	(%)	Active CHB[*]	(%)	Patients starting treatment Status quo	(%)	Base-case	(%)
‹15	66,267	1,829	2.76%	232	13%	6	3%	49	21%
15-24	164,167	6,551	3.99%	832	13%	51	6%	94	11%
25-34	291,540	9,665	3.31%	1,044	11%	59	6%	123	12%
35-44	318,519	13,486	4.23%	1,354	10%	36	3%	215	16%
45-54	220,816	9,467	4.29%	753	8%	13	2%	123	16%
55-64	139,834	2,475	1.77%	206	8%	6	3%	42	20%
65+	114,807	645	0.56%	45	7%	2	4%	7	15%
Total	**1,315,950**	**44,117**	**3.35%**	**4,466**	**10%**	**173**	**4%**	**652**	**15%**

[*] *Active CHB is HBV DNA ›10e5 AND ALT › 2 ULN for HBeAg+ and HBV DNA ›10e4 AND ALT › 2 ULN for HBeAg-*

HBsAg prevalence of 3.35%.[12] Over 5,500 patients from the target group were notified over a 5-year period, corresponding to a detection rate of 12.6% in the status quo.

Subsequent to notification of a new HBV infection, either the MPHS or the GP invites the patients for additional serological investigation, source and contact tracing and counseling. The patient is referred for further care according to a national referral guideline.[9,17] This guideline, based on a positive HBeAg test and/or elevated ALT level, can successfully identify patients with a high viral load, who might qualify for antiviral treatment and should be seen by a medical specialist.[18] Based on data from a recent study in Rotterdam, updated with 59 patients and now including 479 newly detected chronic HBV patients, we assumed that 48% of the patients who are detected in primary care meet the referral criteria and are referred for specialist care, including all HBeAg positive patients, and 36% of HBeAg- negative patients.[18] Patients who do not meet the referral criteria, i.e. those who are HBeAg negative and have normal ALT levels, are advised to see their GP for yearly ALT checks. A study following patients after referral for specialist care conducted in 1998-1999, showed that only 39% of the patients who met the referral criteria were actually seen by a specialist.[9] Based on this study, we assume that in the status quo referral is successful for 39% of patients meeting the referral criteria.

Current Dutch guidelines for treatment of chronic HBV infection recommend that patients with HBV DNA >10^5 copies/mL (for HBeAg positives) or HBV DNA >10^4 copies/mL (for HBeAg negatives) and ALT levels of at least 2 times the upper limit of normal (ULN) are eligible for antiviral treatment.[19] Based on data from 479 patients seen at the MPHS Rotterdam we calculated that 26% of HBeAg positive patients and 19% of HBeAg negative patients that meet the referral criteria are eligible for treatment according to these treatment guidelines and can be considered to have active CHB. Lastly, we assume that 75% of the patients that are seen by the specialist and found eligible for treatment actually start treatment (R. de Man, personal communication). This assumption will be referred to as 'treatment compliance'

Intervention and assumptions regarding participation and referral
The intervention we evaluate consists of a one-off systematic screening effort and subsequent treatment of eligible patients. The target population for screening will be identified in the municipal population registry, which contains information on the country of birth and the current postal address. People in the target population will receive an invitation by mail with information and a laboratory form which they can take to a laboratory nearby to get tested. A reminder is sent after 6 weeks. Participants are tested according to the following test algorithm: anti-HBc, if positive: HBsAg and HBsAg confirmation, with HBeAg testing when HBsAg positive and ALT when HBeAg negative. Participants are asked to fill in the name of their GP on the laboratory form. The test result is sent to

Table 2. Annual progression estimates in natural history of active CHB

Initial state	To	Estimate (range) in %		Reference
Chronic hepatitis B e+ (adults >24 years)	Spontaneous virologic response	6.9	(2.0-23)	4, 21
	Cirrhosis	2.7	(1.6-3.8)	22
	Hepatocellular carcinoma	0.4	(0.3-0.6)	22
	Chronic hepatitis B e-	1.9	(1.0-3.8)	22
Chronic hepatitis B e+ (children 0-24 years)	Spontaneous virologic response	9.4	(8.3-23)	23, 24
	Cirrhosis	0.1	(0.0-0.1)	23, 24
	Hepatocellular carcinoma	0.1	(0.0-0.1)	23, 24
	Chronic hepatitis B e-	0.4	(0.2-0.6)	23, 24
Chronic hepatitis B e- (adults >24 years)	Spontaneous virologic response	1.6	(0.0-11)	4, 21
	Cirrhosis	6.2	(2.8-9.7)	22
	Hepatocellular carcinoma	0.4	(0.3-0.6)	22
Chronic hepatitis B e- (children 0-24 years)	Spontaneous virologic response	9.4	(8.3-11)	23, 24
	Cirrhosis	0.1	(0.0-0.1)	23, 24
	Hepatocellular carcinoma	0.1	(0.0-0.1)	23, 24
Cirrhosis e+	Decompensated cirrhosis	3.9	(2.0-7.9)	25-27
	Hepatocellular cancer	1.8	(0.9-3.8)	25-27
	HBV related Death	3.1	(3.1-3.8)	22, 25-27
Cirrhosis e-	Decompensated cirrhosis	2.7	(1.4-5.4)	25-27
	Hepatocellular cancer	2.9	(1.0-5.6)	25-27
	HBV related Death	3.1	(3.1-3.8)	22, 25-27
Decompensated Cirrhosis	Liver transplantation	3.3	(1.0-8.4)	28, 29
	HBV related Death	26	(15-62)	22
Hepatocellular carcinoma	Liver transplantation	1.2	(0.2-5.0)	28, 29
	HBV related Death	35	(20-60)	4, 21
Liver transplant	HBV related death	6.6	(2-12)	4, 21

the participants and their GP. HBsAg positive participants are advised to visit their GP (or the MPHS in case the MPHS in their region has a program for direct referral of HBsAg positive individuals) for further management and referral to secondary care if necessary. A study in Rotterdam found that an enhanced referral project resulted in an increase of the proportion of referred patients that was seen by a specialist from 39% to 58%.[9] Since the referral guideline was recently included in the patient management guidelines for GPs,[17] and attention for HBV will be raised by the screening program, we assume that in the context of a systematic HBV screening program 58% of patients meeting the referral criteria is successfully referred. We took the 39% from the study by Mostert et al. as the lower bound for this estimate, and 75% (similar difference) for the upper bound.

To estimate the participation in screening we took the age specific response rates in FGM from a population based sero-prevalence study conducted in 2006, the Pienter study (L. Mollema, RIVM, personal communication). Adjusted for the age distribution of the target population the response was 21%. As the Pienter study also required taking a blood sample but was proposed as a research study and participants did not receive their test results, we assume that the response in the Pienter study can be taken as the lower bound estimate for the expected response to screening. For the upper bound, we took the participation in cervical cancer screening in migrant women, which was 48%.[20] We took 35% as the base-case estimate for participation, which is the mid-point between the lower and upper bound.

Cohort

We calculated the number of chronic HBV patients in the target population based on recently published estimates for the Netherlands.[12] The HBsAg prevalence was estimated at 3.35%, corresponding to a total number of 44,117 HBsAg positive persons in the target group (*Table 1*). Ten percent of these is expected to have active CHB (based on the HBV DNA and ALT level), corresponding to almost 4,500 persons eligible for treatment. The number of patients actually receiving treatment is based on base-case assumptions regarding participation in the screening program and management of patients with chronic HBV, i.e. referral, eligibility for treatment and compliance, as specified above. The costs and health outcomes for the patients that are actually treated, and for those who remain untreated are calculated in the Markov models for treatment and natural history, respectively.

Markov model and clinical probability estimates

To compare the incremental clinical and economic outcomes of screening and subsequent treatment of eligible HBV carriers in the status quo (no screening), we used a Markov model that describes disease progression and assesses the long-term morbidity and mortality of a cohort of patients during follow up. The estimates for the progression

Table 3. Treatment-related annual transition estimates[*]

Initial state	To	Estimate in % (range)			
		HBeAg+		HBeAg-	
CHB Initial therapy[†]	Sustained virological response[‡]	22	(17-27)	11	(5.5-22)
	Cirrhosis[§]	0.2	(0.1-0.5)	0.6	(0.3-1.2)
	Hepatocellular carcinoma[ǁ]	0.2	(0.1-0.5)	0.2	(0.1-0.5)
CHB long-term therapy	Sustained virological response[‡]	27	(17-27)	11	(5.5-22)
	Cirrhosis[§]	0.2	(0.1-0.5)	0.6	(0.3-1.2)
	Resistance[‡]	1	(0.0-2.0)	1	(0.0-2.0)
	Hepatocellular carcinoma[ǁ]	0.2	(0.1-0.5)	0.2	(0.1-0.5)
Resistant CHB long-term therapy	Sustained virological response[‡]	5	(2-7)	0.5	(0.2-1.0)
	Cirrhosis	2.7	(1.6-3.8)	6.2	(2.8-9.7)
	Hepatocellular carcinoma	0.4	(0.3-0.6)	0.4	(0.3-0.6)
Cirrhosis Initial therapy	Sustained virological response[‡]	22	(17-27)	11	(5.5-22)
	Hepatocellular carcinoma[ǁ]	0.9	(0.3-1.4)	1.5	(0.7-3.0)
Cirrhosis long-term therapy	Sustained virological response[‡]	27	(17-27)	11	(5.5-22)
	Resistance	1	(0.0-10)	1	(0.0-10)
	Decompensated Cirrhosis	1.9	(0.9-3.8)	1.9	(0.9-3.8)
	Hepatocellular carcinoma	1.6	(0.8-3.2)	1.6	(0.8-3.2)
	Death HBV	2.4	(1.2-4.8)	2.4	(1.2-4.8)
Resistant Cirrhosis long-term therapy	Sustained virological response[‡]	5	(2-7)	0.5	(2-7)
	Decompensated Cirrhosis	7.9	(4-15)	7.9	(4-15)
	Hepatocellular carcinoma	1.8	(0.9-3.8)	2.9	(1.0-5.6)
	Death HBV	3.1	(3.1-3.8)	3.1	(3.1-3.8)
Decompensated Cirrhosis	Liver Transplantation[¶]	3.3	(1.0-8.4)	3.3	(1.0-8.4)
	Death HBV	26	(15-62)	26	(15-62)
Hepatocellular carcinoma	Liver Transplantation[¶]	1.2	(0.2-5.0)	1.2	(0.2-5.0)
	Death HBV	35	(20-60)	35	(20-60)
Liver Transplantation	Death HBV	6.6	(2-12)	6.6	(2-12)

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probabilities were obtained from the literature. The structure of the model and the estimates used have been described in detail elsewhere,[8] and are included in *Table 2* and 3.[4,21-33] We assume that eligible patients are treated with entecavir, an antiviral drug with a low resistance profile.[34] For HBeAg positive patients, we consider sustained HBeAg seroconversion the end point after which treatment can be stopped.[35,36] For HBeAg negative patients we assume that long term or indefinite treatment is necessary to sustain virologic response.[37]

Costs

We included direct health care costs of the screening program, consultations, diagnostic tests, medical management and entecavir therapy. Patients who do not participate in the screening and are not detected by other means follow the natural history. As we assume these patients do not have symptoms in the first stages of disease, we did not include costs for management in the CHB and compensated cirrhosis health states. For participants with chronic HBV, the costs for a consultation for referral and source and contact tracing were included, as well as the costs of follow up by the GP for patients not referred to specialist care. For patients that were found ineligible for treatment after specialist evaluation the costs for specialist evaluation and subsequent follow up by the GP were taken into account. As the initial screening consists of an anti-HBc test and further testing is only carried out for anti-HBc positives, the prevalence of anti-HBc was calculated assuming 6.5% of anti-HBc positives is HBsAg positive.[38,39]

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[*] *Estimates from Kanwal et al. 2005 and 2006, and Fattovich et al. 2008 Ref. 4, 21, 22.*

[†] *Initial therapy is 12 months (48 weeks) of therapy.*

[‡] *Estimates from recent clinical trials: Chang et al. 2006, Lai et al. 2006 and Colonno et al. 2007, Ref. 30-32.*

[§] *Estimates calculated by the author, based on the assumption that the natural progression rates of chronic hepatitis B are reduced by antiviral therapy. Estimates derived from natural history estimate. Similar to Kanwal's assumption of no progression of disease in HBeAg seroconversion, we assume no progression of disease in case HBV DNA is undetectable by PCR. In the papers from Chang and Lai full suppression of HBV DNA was observed in 90% with a low resistance profile drug. We took these percentages for our calculations. Ref. 31, 32.*

[||] *Estimates based on reduction of progression rates by nucleoside analogue therapy of 50 % (Liaw et al. 2004, Ref. 33).*

[¶] *Liver Transplantation estimates for the Netherlands, Ref. 28.*

Table 4.   Costs estimates[*]

Overall program costs†	€
Campaign, personnel	500,000.-
range for sensitivity analyses	± 250,000.-
Invitation and reminder	1.80
Test and follow up costs[‡]	
Blood test administration costs	12.60
Anti-HBc test	13.80
HBsAg test (including confirmation test)	40.35
HBeAg test	13.45
ALT test	2.55
Source and contact tracing[†]	63.13
Follow up (patients not meeting referral criteria)[§]	72.45
Consultation referral (patients meeting referral criteria)	9.00
Consultation specialist but not eligible for treatment[§]	765.45
Annual CHB medical management costs[‡]	
Monitoring of CHB	693.-
Compensated cirrhosis	2,035.-
Decompensated cirrhosis	7,068.-
Hepatocellular carcinoma	15,600.-
Liver transplantation	125,000.-
Treatment with low resistance profile drug[ǁ]	5,875.-

[*] *For test and medical costs ranges were not applicable*

[†] *estimated by the authors*

[‡] *data from the Dutch Healthcare Authority (NZa)*

[§] *costs of 3 annual ALT checks through the GP are included*

[ǁ] *data for entecavir from the Dutch Health Care Insurance Board (CVZ)*

The cost estimates are described in *Table 4*. The costs of laboratory tests and of medical care were based on data from the Dutch Healthcare Authority (NZa).[40] Medical costs are expressed as Diagnosis Treatment Combinations (DBCs) which are used in the Netherlands for the registration and reimbursement of hospital and medical specialist care since 2005.[41] DBCs are defined as the whole set of activities and interventions of the hospital and medical specialist resulting from the first consultation and diagnosis of the medical specialist in the hospital. The entecavir costs are obtained from the Dutch Health Care Insurance Board (CVZ).[42] We discounted all costs at 3% per year.

Health outcomes and utilities
Health outcomes were estimated in the Markov model as discounted quality-adjusted life-years (QALYs). Age-specific utility estimates (*Table 5*) were obtained from a recent multi-national study on chronic hepatitis B.[43] We discounted all utilities at 3% per year.

Sensitivity analysis
We explored the impact on the cost-effectiveness estimates of the following assumptions: the prevalence of chronic HBV in the target population, the overall program costs, participation in the screening program, percentage of successful referrals and treatment compliance, by performing univariate sensitivity analyses taking the low and high ranges of each estimate (*Table 5*).

The impact of assumptions on the effectiveness of treatment and on disease progression in natural history was assessed separately. As disease progression is not a single variable, we performed multivariate sensitivity analyses for all variables included in the Markov model describing disease progression and utilities both in natural history and under treatment. The ranges for each variable used in the sensitivity analysis are listed in *tables 2, 3* and *5*. The effect of applying discounting according to the Dutch guidelines, costs at 4% and effects at 1.5%, was also assessed.

Table 5. Assumption and range for sensitivity analysis regarding prevalence, the screening program, subsequent patient management and utilities

Variable	Base-case estimate	(range)	Reference
Epidemiological, screening, and patient management			
HBsAg prevalence	3.35	(2.23-4.47)	12
Participation	35	(21-48)	20, personal communication L. Mollema
Successful referral	58	(39-75)	9
Treatment compliance	75	(50-100)	personal communication R. de Man
Utilities*			
Durable response to treatment	1.00	(0.95-1.00)	43
Chronic HBV	0.68	(0.66-0.70)	43
Compensated cirrhosis	0.69	(0.66-0.71)	43
Decompensated cirrhosis	0.35	(0.32-0.37)	43
Hepatocellular carcinoma	0.38	(0.36-0.41)	43
Liver transplantation	0.67	(0.64-0.69)	43

* *See Levy et al. (ref 43) for the age-specific utilities*

Table 6. Costs and Health Outcomes of Screening and Treatment

Costs, € 1,000.-	Status Quo	Screening
Program costs		3,580
Test costs	458	15,954
Referral and follow up costs	838	3,074
Source and contact tracing	296	806
Healthcare costs of patients receiving treatment	17,177	64,753
Healthcare costs of patients in natural history	90,409	80,312
Total costs	109,178	168,480
Health Outcomes		
HBsAg positive patients identified in target population	5,565	15,159
Active CHB patients in target population	4,466	4,466
Active CHB patients receiving treatment	173	652
Active CHB patients following natural history	4,293	3,813
HBV related deaths	1,073	965
QALYs experienced	113,411	120,025

Results

The target population of 1.3 million first generation migrants includes an estimated 44 thousand HBsAg carriers of whom 4,466 are estimated to have active CHB. In the status quo, only 4% of patients with active CHB (173/4,466) are expected to start treatment; the remainder follow the natural history of disease progression (*Table 1*). Lifetime follow up of the cohort of active CHB patients suggests that 1,073 (24%) of the 4,466 patients will die of HBV related diseases.

With a one-off screening program, under base-case assumptions, the proportion of patients with active CHB that starts treatment increases to 15% (652 patients) (*Table 1*). During lifetime follow up of the total cohort of active CHB patients after a one time screening, 965 patients will have died. Screening can thus prevent 108 HBV related deaths, corresponding to a reduction in mortality of 10% (*Table 6*).

Assuming base-case assumptions, the costs of the screening program (including costs of testing, referral, follow-up and source- and contact tracing) amount to 23.4 million euro and the health costs related to disease progression and treatment to 145 million euro. The average life time costs of treatment amount to almost € 100,000.- per patient. The life-time incremental cost difference between the status quo and a scenario following screening and subsequent treatment of eligible patients amounts to € 59.3 million (*Table 6*). In the base-case estimate, the incremental difference in health gains between the two scenario's is 6,614 QALYs, resulting in an Incremental Cost-Effectiveness Ratio (ICER) of € 8,966.- per QALY gained. Applying discounting according to the Dutch guidelines, costs at 4% and effects at 1.5%, resulted in a slightly lower ICER of € 8,823.- per QALY gained.

Sensitivity analyses
Univariate analysis showed that the ICER for screening varied between € 7,936.- and € 11,705.- per QALY gained (*Figure 1*). The assumptions regarding the proportion of successful referral of patients to specialist care and the proportion of eligible patients that actually start treatment had the largest effect on the ICER. When the lower bound estimate was assumed for the prevalence of HBsAg, the ICER would increase to € 10,998.- per QALY gained.

The multivariate sensitivity analyses for treatment effectiveness and disease progression in natural history showed ICER estimates ranging between € 5,568.- and € 60,418.- per QALY gained (*Figure 2*). The higher range estimate was obtained when assuming relatively slow disease progression in natural history.

Figure 1. Incremental Cost Effectiveness Ratio (ICER) for screening in univariate
sensitivity analyses for the lower and upper bound of the estimates related
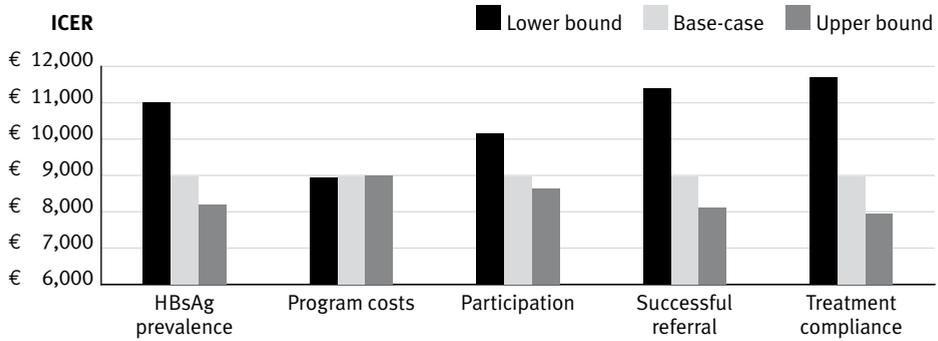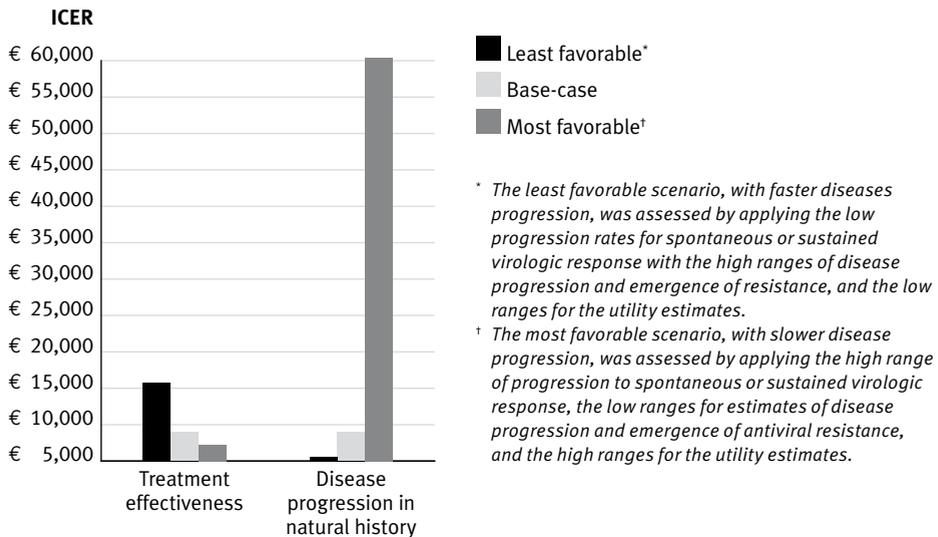to prevalence, the screening program and patient management.

Figure 2. Incremental Cost Effectiveness Ratio (ICER) for screening in multivariate
sensitivity analyses for most favorable (slower progression) and least
favorable (faster progression) scenarios of the estimates related to
treatment effectiveness and disease progression in natural history.

* The least favorable scenario, with faster diseases
  progression, was assessed by applying the low
  progression rates for spontaneous or sustained
  virologic response with the high ranges of disease
  progression and emergence of resistance, and the low
  ranges for the utility estimates.
† The most favorable scenario, with slower disease
  progression, was assessed by applying the high range
  of progression to spontaneous or sustained virologic
  response, the low ranges for estimates of disease
  progression and emergence of antiviral resistance,
  and the high ranges for the utility estimates.

Discussion

We found that screening first generation migrants for chronic HBV infection, aimed at improving outcome of CHB by early detection and treatment, is cost effective compared to the status quo. We estimate that the ICER of screening is around € 9,000.- per QALY gained, which is well below the threshold of € 20,000.- per QALY gained that is commonly accepted in the Netherlands.[44] In the status quo, we estimated that only 4% of the population eligible for treatment is actually treated. By improving case detection through the screening program and improving referral to specialist care of eligible patients, approximately 15% of the cohort with active CHB would receive treatment, resulting in fewer deaths and complications due to CHB. The univariate and multivariate sensitivity analyses suggested that screening and treatment are likely to be cost-effective even when varying main assumptions.

This is the first study to explore the possible public health impact and cost effectiveness of interventions aimed at identifying and treating eligible patients with CHB. Previous cost-effectiveness studies on interventions have either assessed the outcome of treating all patients in the cohort, or assumed relatively high compliance with the screening intervention and 100% acceptance of medical management of persons found to be chronically infected.[4,15] These assumptions might well be too optimistic considering the complexity of the medical management of chronically infected patients.[35,36] From previous research into the referral from primary to specialist care, we know that a relatively small proportion of identified patients actually consults a specialist after referral.[9] We used this information to make more realistic assumptions. One could argue that the proportion of patients that is referred to a specialist and actually gets a consultation might be higher for patients identified through a screening program in which they voluntary decided to participate, compared to patients who are identified in other ways. In that case, our base-case assumption of the proportion that is seen by a specialist might be an underestimation. On the other hand, we assumed that all patients that are seen by the specialist and qualify for treatment based on HBV DNA and ALT level are offered treatment, even though practice guidelines include additional diagnostic tests, e.g. a liver biopsy, to assess eligibility of a patient for treatment.[19,36]

Previous studies of the cost-effectiveness of treatment or screening and treatment took the costs of medical care into account for patients following natural history, except for the costs for antiviral treatment.[4,15,45] However, as the patients in the natural history model are assumed not to be identified as CHB patients, this implies an overestimation of the costs of active CHB patients in natural history. To avoid this overestimation we did not include costs for medical management of CHB and compensated cirrhosis in natural history.

As many countries have started universal HBV vaccination in the past decade, we might have overestimated the prevalence of HBsAg in young FGM. However, this will probably not have a large impact on the cost-effectiveness of screening in this group, as sensitivity analysis showed that influence of the prevalence of HBsAg on the ICER was relatively small.

A limitation of our study is the lack of data to support the assumptions regarding participation in the screening program. One can only speculate about the compliance to systematic screening for HBV as long as screening programs have not been implemented. Furthermore, no data is available on the proportion of patients that actually starts treatment of those who qualify for treatment. Sensitivity analysis for treatment compliance indicated that this factor, together with the proportion of patients successfully referred, had a relatively large impact on the ICER. Although treatment compliance can probably not be influenced by a screening program, this finding emphasizes the importance of effective referral for the success of a screening program. The ICER for screening went up to € 60,000.- per QALY gained when hepatitis B sequelae in natural history were assumed to occur at a lower rate, resulting in a lower disease burden. However, this is the result of an extreme scenario, where we assumed all factors describing disease progression to take the most favorable range.

Cost-effectiveness is an import aspect in policy making. However, in the decision on the appropriateness of a screening program, other criteria, as described by Wilson and Junger should also be taken into account.[46] For hepatitis B screening, most of their criteria are met as CHB is an important public health problem for which an acceptable test for detection in an early stage is available. The natural history of the disease is well known, but although the effectiveness of treatment on intermediate outcomes has been shown, evidence of effect on clinical outcomes is still insufficient.[47] Also, the ethics around identifying patients with a chronic HBV infection of whom only a subset is eligible for treatment need to be explored.

We show the impact and cost effectiveness of a screening program at population level. Screening can achieve considerable health gains, but compared to the size of the problem, even with the upper bound response rate, approximately 80% of the total population with active CHB would still not be identified and hence would not benefit from treatment. Therefore, other methods of improving access to treatment, e.g. adding opportunistic screening to systematic screening, need to be explored.

Acknowledgements

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Chapter 9

General discussion

General discussion

The research described in this thesis covers different aspects of hepatitis B in the Netherlands, varying from epidemiological studies into risk factors and prevalence of hepatitis B virus infection, to mathematical modelling studies into cost effectiveness of secondary prevention through screening and treatment. In this chapter we answer the research questions posed in the introduction with the results of the different studies and findings from the literature. We conclude with recommendations for future implementation of interventions.

Research question 1:   **What are the transmission routes, sources of infection and risk factors for acute hepatitis B virus infection in the Netherlands?**

Most acute hepatitis B virus infections are transmitted through sexual contact. The main risk factor among heterosexual adults is having a partner from a country where hepatitis B is endemic, and among men who have sex with men having more than two sexual partners in the past 6 months.

The results of a case-control study into risk factors for acute hepatitis B are described in *chapter 2 and 3* and are consistent with the expected transmission patterns in a low endemic country. Fifty-nine percent of the patients were infected through sexual contact, slightly more than half (52%) of those reporting homosexual contact, and 48% heterosexual contact. The contribution of sexual transmission to acute infections was similar in notification data from the period 2002-2005, with 58% through homosexual-, and 42% through heterosexual contact.[1] Comparing the patients with acute hepatitis B with healthy controls showed that the risk of infection was particularly high in men having sex with men (MSM), with an odds ratio of 146 compared to heterosexual women. For heterosexual men and women, having a partner born in an intermediate or high endemic country and having more than 1 partner in the past 6 months were independent risk factors for acute hepatitis B virus infection. For MSM, having more than two sexual partners in the past 6 months was the only significant risk factor. In contrast to heterosexuals, import of hepatitis B virus infection through partners from endemic countries does not seem to play a role in MSM. This suggests that there is sustained transmission of hepatitis B virus in the MSM population, whereas import of infections plays an important role in transmission in the heterosexual population. These findings are supported by mathematical modelling and molecular epidemiology studies.[2-4]

Injecting drug use does not seem to contribute to new hepatitis B virus infections. It was not reported in the cases in our study and accounts for only 1% of notified acute infections.[1] This in contrast to for example the United Kingdom where injecting drug use was the most frequently reported route of transmission.[5,6] Possibly injecting drug users are underrepresented in our study and in the notification data, but the needle exchange programme in combination with a decline in injecting drug use practices could also explain the low number of acute hepatitis B infections attributable to injecting drug use.[7,8]

For almost 20% of patients the most likely transmission route was through medical or dental treatment, blood contact or a percutaneous procedure. However, the case-control study showed that although (possible) parenteral exposures abroad were associated with acute hepatitis B virus infection in heterosexual adults in univariate analysis, the association became insignificant in combination with other explanatory factors. In children, parenteral exposures in medium or high endemic countries partly explained the association of having parents born in these countries with the risk of acquisition of hepatitis B.

For a relatively large number of patients the transmission route remains unknown. In our study risk factors could not be identified in 15% of patients. In the notification data this proportion is around 25%, indicating that enhanced surveillance can further reduce the proportion of unknown transmission routes.[1] Sexual transmission might explain part of the infections with unknown transmission route, as shown in a study from the United States.[9] Molecular analysis of acute hepatitis B patients in the Netherlands identified a cluster of identical sequences of genotype A, of which 61% was found in MSM. The transmission route in other patients with identical sequences in this cluster was unknown or 'other' and heterosexual in some. Strikingly, these patients were mostly men, possibly illustrating the difficulties in voluntary reporting homosexual contacts. That the contribution of homosexual transmission to acute hepatitis B infections might be higher than observed is also suggested by another finding from this study, i.e. that the median age of homosexual patients was significantly higher than that of heterosexual patients, but did not differ from the group with an unknown mode of transmission.[10,11]

A problem associated with studies based on notification data is that acute patients are mainly diagnosed because of symptomatic disease, and asymptomatic patients are missed. Since the proportion of patients with asymptomatic infection decreases with age, especially infections in children are more likely to be under-reported.

Research question 2: What is the prevalence of hepatitis B virus infection in different ethnic groups in Rotterdam?

Of people of non-Dutch ethnicity in Rotterdam, 23% had markers of a previous infection with the hepatitis B virus, ranging from 12% in Moroccans, to 57% in Cape Verdeans. Chronic hepatitis B virus infection was found in 1% of first generation migrants.

The incidence of new hepatitis B infections in the Netherlands is low, and therefore the prevalence of hepatitis B in the general Dutch population is low. However, due to migration, the prevalence in the general population does not reflect the prevalence in area's with a large migrant population from countries with a higher level of hepatitis B endemicity. It is difficult to estimate precisely the prevalence of chronic hepatitis B in the Netherlands. In a general population study into the prevalence of vaccine preventable diseases, the prevalence of chronic hepatitis B was underestimated because migrants were under-represented.[12] The data of a second round of this large general population study have been collected but have not yet been analysed. The results are expected in 2009 and should give better estimates of the prevalence in migrant groups, as these groups have now been over-sampled.

Information on the prevalence in migrant groups is available from a study from Amsterdam with almost 900 persons of non-Dutch ethnicity.[13] The prevalence of chronic hepatitis B in the three largest groups of first generation migrants was 4.8% for Turks, 1.9% for Surinamese and Antilleans, and 0.4% for Moroccans. This illustrates the large difference in prevalence in migrants from countries classified as intermediate endemic for hepatitis B. In the study described in *chapter 4*, 205 first generation migrants participated. Of these, only 2 (1%; 95% CI: 0.3-3.5%), had evidence of a chronic infection.[14] Due to the relatively low number of participants, the confidence interval around this estimate is wide.

Recently, a theoretical estimate of the hepatitis B prevalence in the Netherlands was published.[15] This study was based on an extrapolation of the prevalence of hepatitis B in the countries of origin of migrants in the Netherlands to their relative number in the Netherlands. The prevalence in first generation migrants was estimated at 3.77%. In combination with the results of the general population study, the prevalence of HBsAg in the general Dutch population was estimated between 0.32 and 0.51%.

The prevalence of hepatitis B virus infection in second generation migrants appears to be comparable to that of the native Dutch population. The number of second generations migrants in the study in Rotterdam was only 24, of which 1 (4%) was anti-HBc positive.[14] In the study from Amsterdam, 64 second generation migrants participated and 1 was found anti-HBc positive, corresponding to a weighted prevalence of 0.6%.[13]

This is surprising because the second generation migrants in both studies were born before the introduction of the pregnancy screening programme. Possibly, the risk of horizontal transmission in the Netherlands is lower compared to that in the country of origin, due to improved hygienic standards, less crowding and other as yet unidentified environmental factors.

Research question 3: What is the added value of molecular analysis in hepatitis B source and contact tracing?

Molecular analysis can be used to find support for epidemiological transmission pairs found in source and contact tracing, but its value depends on the availability of a large reference data set.

Molecular analysis has been used to study the origin and transmission routes of hepatitis B virus infection. A nationwide study into the molecular epidemiology of acute hepatitis B virus infection showed that genotypes A–F were present and that genotype A was found most often, especially in homosexual men.[10] In Rotterdam, the genotypes of both acute and chronic patients found in the period 2002–2005 were studied and the distribution of genotypes of acute patients resembled the findings of the national study. The genotypes found in chronically infected patients corresponded well with the genotypes generally found in the countries of origin of the patients or their mothers.[16]

Although molecular epidemiological studies give valuable information for the surveillance of hepatitis B virus infections, the added value for source and contact tracing is less clear. In the study described in *chapter 5*, we assessed the level of molecular support for patients found as a result of contact tracing, so-called epidemiological transmission pairs. To do so, we analysed each epidemiological transmission pair separately, together with the other sequences from patients with the same genotype. We used parsimony to construct phylogenetic trees and compared the number of mutations between trees with and without forced clustering of epidemiological transmission pairs. Because the degree of divergence of hepatitis B virus sequences can be expected to to depend on unknown variables such as the time since transmission and the mutation rate, we allowed mutations among members of transmission pairs.

Our study showed that about one quarter of the transmission pairs found in contact tracing were refuted by the molecular analysis, about one fifth received support and support for slightly more than half was ambiguous. When molecular support is present or absent, which was the case in almost half of the investigated transmissions pairs, molecular analysis can convincingly confirm or refute the hypothesized transmission route. However, the other half of transmission pairs received ambiguous support from

molecular analysis. Molecular support is ambiguous when there is no difference between the shortest tree and the tree with the transmission pair constrained, in which case the transmission pair is not statistically refuted, but cannot be confirmed either. Fortunately, if the sequences of epidemiological transmission pairs with ambiguous molecular support are identical, this does provide non-statistical support for the transmission route. Further non-statistical support for the transmission route is provided when acute infections are part of the transmission pair because this implies recent transmission, which facilitates establishing the most likely transmission route.

In our analyses, we allowed divergence among members of epidemiological transmission pairs and we did find molecular support for transmission pairs that had diverged, sometimes considerably with up to 15 mutations difference between sequences. As a consequence, we conclude that levels of divergence cannot be used to confirm or reject epidemiological transmission pairs. Likewise, because molecular support for epidemiological transmission pairs with identical sequences was often ambiguous, we conclude that lack of divergence cannot be used to confirm a transmission pair. Especially without epidemiological information, identical sequences should not be interpreted as genuine transmission pairs. Finding clusters of identical sequences that are not epidemiologically linked might indicate the shortcomings of source and contact tracing, but it is also likely that the virus had a common origin and that multiple intermediate transmissions may be involved. Furthermore, the sequences of epidemiological transmission pairs should be related to a large reference data set with closely related sequences to prevent spurious results.

Research question 4: What is the impact of secondary prevention of hepatitis B?

This research question has been subdivided in three sub questions.
Is selection of patients in primary care for referral to specialist care possible?
What is the potential public health impact of different treatment strategies?
What is the cost effectiveness of systematic screening of migrants for chronic hepatitis B virus infection?

The potential impact of secondary prevention of hepatitis B is large. Selection in primary care of chronic hepatitis B patients for specialist evaluation is possible with a referral guideline that selects 87% of patients with high HBV DNA. Long-term antiviral therapy of patients with active chronic hepatitis can potentially have a major preventive effect on liver-related mortality and morbidity. Systematic screening of migrants for chronic hepatitis B infection is cost-effective.

Secondary prevention is the early detection of disease, followed by an intervention to prevent disease progression and the occurrence of symptoms. In patients with chronic

hepatitis B virus infection it generally takes many years before symptoms develop, which creates the opportunity for secondary prevention by screening for chronic infection. After chronically infected patients have been identified, it is necessary to distinguish those patients for whom intervention—in the form of antiviral treatment—is helpful. Patients in the immune active phase of their hepatitis B virus infection and patients with HBeAg negative chronic hepatitis are the ones who might benefit from antiviral treatment.[17,18] As this applies to only a small proportion of the patients detected in primary care, a guideline that can select these patients for referral to specialist care optimizes patient management and avoids unnecessary referral. In *chapter 6*, the accuracy of a referral guideline to select patients with high HBV DNA levels is described. High HBV DNA levels—defined here as more than 10^5 copies/mL—are a first prerequisite for patients to qualify for antiviral treatment.[18,19] We showed that with a guideline that refers HBeAg positive patients and HBeAg negatives with elevated ALT levels, 87% of patients with high HBV DNA level are selected for referral. The referral guideline has been included in the recently revised practice guideline for Dutch general practitioners.[20]

Patients that do not match the referral criteria, i.e. those that are HBeAg negative and have normal ALT, are advised to visit their general practitioner for yearly ALT follow op. This is necessary because ALT values may fluctuate in patients with HBeAg negative chronic hepatitis B and therefore the value of a single ALT determination is limited.[21]

Although current guidelines do not recommend treatment for patients in the immune tolerant phase, it is argued by some that treating these patients is very cost-effective and should be considered.[22] If treatment of immune tolerant patients would become more common in clinical practice, the referral guideline would still be sufficient. Because virtually all patients in the immune tolerant phase are HBeAg positive, they would still be referred to specialist care according to the present referral guideline.

In *chapter 7*, we assessed the potential impact on liver related morbidity and mortality of long term treatment of patients with active chronic hepatitis B. A mathematical model was used to describe the disease progression of a hypothetical cohort of patients with active chronic hepatitis B over a period of 20 years. We estimated that without antiviral treatment 26% of the cohort will die due to liver-related disease in this period. Mortality would decrease to 5% (a reduction of 80%), by treating all patients with an antiviral drug with a low resistance profile.

The progression estimates for natural history are based on reviews of long term follow op studies of hepatitis B patients, updated with recent studies.[23-28] However, estimates for the progression under treatment are difficult to obtain as treatment duration is limited, especially for new generation antiviral drugs such as entecavir, which has a very low

risk of antiviral resistance.[29] The first clinical trials of entecavir were only published in 2006 and 2007.[30-32] The problem of limited follow up of patients on treatment was illustrated in a recently published extensive review of the effectiveness of antiviral treatment for chronic hepatitis B patients, conducted for the National Institutes of Health.[33] The authors identified 60 unique randomised controlled trials (RCTs) but concluded that none demonstrated an effect on important clinical outcomes such as cirrhosis, hepatocellular carcinoma or death. This is due to the fact that the studies were not designed to assess clinical outcomes—as these may take many years to occur—but to assess the effect of treatment on intermediate outcomes such as HBeAg seroconversion, reduction of HBV DNA, normalisation of ALT, and histological improvement. For our Markov model, we could use data from clinical trials to estimate the probabilities of virological response under treatment. For progression to health states for which direct estimates could not be derived from clinical trials, we calculated the estimates based on reduced rates observed for intermediate outcomes.

There is no consensus on the duration of treatment, but it is becoming more clear that treatment in HBeAg positive patients can be stopped after a virological response is achieved, in this case HBeAg seroconversion, which is sustained off treatment.[18,19] For HBeAg negative patients however, a recently published study showed very high relapse rates after treatment was discontinued in patients who achieved virological response on treatment.[34] This implies that for HBeAg negative patients usually prolonged or indefinite treatment is necessary.[35]

Several studies have calculated the cost-effectiveness of various treatment strategies for both HBeAg positive and HBeAg negative chronic hepatitis B patients.[23,24,36-39] These studies generally demonstrated cost-effectiveness ratios below the commonly accepted threshold of $US 50,000.- per quality-adjusted life-year (QALY) gained. For the Netherlands, this is € 20,000.- per QALY gained.[40] From a public health perspective, the available knowledge that antiviral treatment in selected patients with chronic hepatitis B is cost-effective and can have a major impact on the burden of chronic hepatitis B, emphasizes the need for interventions increasing the detection of patients with chronic hepatitis B. A strategy to improve detection of cases is active screening of risk groups. In the Netherlands, almost three quarter of chronic hepatitis infections is found in migrants from countries with a relatively high HBV endemicity.[1] To inform public health policy, we investigated the cost-effectiveness of a systematic screening programme targeted at first generation migrants in the Netherlands. This study, combining epidemiological information and a model estimating costs and QALYs, is described in *chapter 8*. So far, only one study looked into the cost-effectiveness of screening and subsequent vaccination and treatment. Hutton et al. showed that a screening programme targeted at the Asian and Pacific Islander population would likely be cost-effective.[41] This study was

based on unrealistic optimistic assumptions regarding uptake of screening and compliance with the intervention.

We attempted more realistic assumptions regarding participation, referral and treatment compliance based—among others—on previous research into the referral from primary to specialist care.[42] Using mathematical modelling we compared the costs and heath effects of a systematic screening programme for migrants to the status quo, which included a certain detection rate of patients through the pregnancy screening programme and because of screening for other reasons. The screening programme is cost-effective with an incremental cost-effectiveness ratio (ICER) compared to the status quo of around € 9,000.- per QALY gained. In sensitivity analysis varying parameter values of chronic hepatitis B prevalence in the target group, participation in screening, successful referral and treatment compliance, the ICER varied between approximately € 8,000.- and € 12,000.- per QALY gained.

A limitation of our study is the lack of data to support the assumption regarding participation in the screening programme. One can only speculate what the compliance to systematic screening for HBV would be, as screening programmes have not been implemented. Furthermore, no data are available on the proportion of patients that actually starts treatment once referred from primary to specialist care. However, by doing sensitivity analysis for a range of possible values for these factors we showed that screening remained cost-effective even at the lower ranges for prevalence, participation, successful referral and treatment compliance.

Conclusions and recommendations

From the studies presented in this thesis, it can be concluded that acute hepatitis B virus infections are mainly transmitted through sexual contact. Having multiple sexual partners is the main risk factor among men who have sex with men, while among heterosexual men and women this is having a partner from a hepatitis B endemic country. The Dutch Health Council recently concluded that despite great efforts, the high-risk group approach for vaccination against hepatitis B is insufficiently effective in reducing disease burden, and advised general vaccination of infants with a catch-up campaign for twelve-year-olds.[43]

Although general vaccination can be expected to reduce the incidence of acute hepatitis B virus infections, it does not have an impact on chronic infections that were contracted abroad. The majority of the disease burden of hepatitis B in the Netherlands is due to chronic hepatitis B virus infections, which are mainly found in migrants from countries where hepatitis B is more prevalent. The only option to control and prevent progression of disease in patients with active chronic hepatitis B is antiviral treatment. To increase access to antiviral treatment for patients with active chronic hepatitis B, the detection of chronic hepatitis B patients should be improved. Besides the benefit for the individual patient, increasing the number of patients that are aware of their hepatitis B virus infection can also be expected to lower the chance of secondary transmission. This is because newly detected patients will be counselled on how to prevent infecting others, and contacts at risk for infection will be screened and vaccinated when necessary. Furthermore, the viral load and thereby the level of infectivity will be reduced in successfully treated patients.

To increase the detection of chronic hepatitis B patients in migrant groups in the Netherlands, a proactive approach is necessary as most patients do not have symptoms. An example of a proactive approach is systematic screening, which means that people in the target population are actively identified in a systematic way and invited for screening, for instance by an invitation letter. Another option is opportunistic screening, for example by general practioners (GPs), who offer screening to patients from the target population, even if the reason for consultation is unrelated to the disease being screened for. A method related to opportunistic screening is the so-called supported diagnosis for GPs. Computer-based tools are being developed that are integrated into the GP's information system and which alert the GP to test for hepatitis B virus infection, if for example a patient's liver enzymes are elevated. We showed that a systematic screening programme targeted at first generation migrants is cost-effective. However, in practice a large part of the patients with active chronic hepatitis B will still not be identified when screening is implemented because the estimated participation is not likely to exceed 50%. Therefore, other options such as adding opportunistic or outreach screening to systematic screening need to be explored. Furthermore, adding screening for hepatitis C

might improve the cost-effectiveness of the screening programme as hepatitis C is also more prevalent in some migrant populations and effective treatment for hepatitis C is also available.

Recommendations
- We endorse the advice of the Dutch Health Council to start general vaccination against hepatitis B.

- The detection of chronic hepatitis B patients should be improved so that they can be offered antiviral treatment and the burden of disease can be reduced.

- A proactive approach to screening should be adopted as most chronic hepatitis B patients do not have symptoms.

- Effective referral to specialist care should be ensured for patients detected by screening.

- A pilot study implementing systematic hepatitis B screening targeted at first generation migrants from endemic countries should be conducted.

- Other methods to increase detection of chronic hepatitis B patients such as opportunistic or outreach screening strategies should be explored.

- The added value of adding screening for hepatitis C in a systematic screening programme should be investigated.

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Summary
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Summary

The aim of this thesis is to study the epidemiology of acute and chronic hepatitis B in the Netherlands, with a focus on the impact of secondary prevention of hepatitis B.

A general introduction of the natural history of hepatitis B virus infection, the (molecular) epidemiology of hepatitis B in the Netherlands, and the possibilities for primary and secondary prevention is given in *chapter 1*. An infection with hepatitis B virus affects the liver and can result in a broad spectrum of disease outcomes. Acute hepatitis B virus infection can resolve and lead to protective immunity, but it can also result in a chronic infection and, in rare cases, cause acute liver failure with a high risk of dying. People with chronic hepatitis B virus infection remain infectious to others and are at risk of serious liver disease such as liver cirrhosis or liver cancer later in life. In the Netherlands, hepatitis B is low endemic and acute infections are mainly transmitted through sexual contact. Chronic infections are found mostly in migrants born in countries where hepatitis B is relatively prevalent.

Primary prevention of hepatitis B is possible through vaccination, but vaccination does not have an impact on pre-existing chronic infections. Screening for hepatitis B is a form of secondary prevention, whereby antiviral treatment can be used to prevent the occurrence of hepatitis B related liver disease. As the possibilities of antiviral therapy to prevent liver disease caused by chronic hepatitis B have greatly improved, it becomes more important to identify chronic hepatitis B patients.

In this thesis, we address the following research questions:
1. What are the transmission routes, sources of infection and risk factors for acute hepatitis B virus infection in the Netherlands?
2. What is the prevalence of hepatitis B virus infection in different ethnic groups in Rotterdam?
3. What is the added value of molecular analysis in hepatitis B source and contact tracing?
4. What is the impact of secondary prevention of hepatitis B?

In *chapter 2* we describe the transmission routes and source of infection of 144 patients with acute hepatitis B virus infection. By enhanced surveillance of hepatitis B through public health services, detailed information of patients with an acute infection was collected. It appeared that the majority (59%) acquired the infection through sexual contact; 52% of these by homosexual and 48% by heterosexual contact. In 60% of the heterosexual cases, the source of infection was a partner originating from a hepatitis B-endemic region. We conclude that sexual transmission is the most common route of transmission of acute hepatitis B in The Netherlands and introduction of infections from abroad plays a key role in the current epidemiology of HBV.

In *chapter 3* the relative importance of risk factors for acute hepatitis B infection is stud-ied in a population-based case-control study. Participants were 120 patients with acute hepatitis B, and 3948 randomly selected population controls. Risk factors were studied using logistic regression, distinguishing confounders and mediators through hierarchical analysis. The risk of acute hepatitis B was increased in men who have sex with men, with reporting to have had more than two partners in the past 6 months the only significant risk. In children, adult females and heterosexual males, having parents born in a hepatitis B endemic country was a significant risk. For adult females and heterosexual males, this was largely explained by having a foreign partner. For children this was partly explained by parenteral exposures abroad.

Although the prevalence of viral hepatitis is generally low in Western countries, pockets of higher prevalence may exist in areas with large immigrant populations. In *chapter 4*, we describe the prevalence of previous and current infections with hepatitis A, B, and C found in a community-based study in a multi-ethnic neighbourhood in Rotterdam. Markers for hepatitis A infection were present in 68% of participants. The prevalence of hepatitis B core antibodies (anti-HBc), a marker for previous or current infection, was 20% (58/284). Prevalence of hepatitis A and B varied by age group and ethnicity. Two respondents (0.7%) had chronic hepatitis B virus infection. The prevalence of hepatitis C was 1.1% (3/271). High levels of isolated anti-HBc were found. The high prevalence of (previous) viral hepatitis infections found confirms previous observations in ethnic subgroups from a national general population study and illustrates the high burden of viral hepatitis in areas with large immigrant populations.

When a patient is diagnosed with hepatitis B, the Municipal Public Health Service performs source and contact tracing. In the study described in *chapter 5*, we studied sequence data of the hepatitis B virus and epidemiological information from patients with acute and chronic infections found in Rotterdam. We assessed the level of molecu-lar support for patients found as a result of contact tracing, so-called epidemiological transmission pairs. To do so, we used parsimony to construct phylogenetic trees and compared the number of mutations between trees with and without forced clustering of epidemiological transmission pairs. HBV genotypes A-G were present in 62 acute and 334 chronic HBV patients. Of 22 epidemiological transmission pairs, six could be refuted, four clusters received support from the molecular analysis, and support for the remain-ing twelve clusters was ambiguous. When molecular support is present or absent, which was the case in almost half of the investigated transmissions pairs, molecular analysis can convincingly confirm or refute the hypothesized transmission route. In our analyses, we allowed divergence among members of epidemiological transmission pairs and we did find molecular support for transmission pairs that had diverged, sometimes considerably with up to 15 mutations difference between sequences. As a consequence, we conclude

that levels of divergence cannot be used to confirm or reject epidemiological transmission pairs. Likewise, because molecular support for epidemiological transmission pairs with identical sequences was often ambiguous, we conclude that lack of divergence cannot be used to confirm a transmission pair. Especially without epidemiological information, identical sequences should not be interpreted as genuine transmission pairs. Finding clusters of identical sequences that are not epidemiologically linked might indicate the shortcomings of source and contact tracing, but it is also likely that the virus had a common origin and that multiple intermediate transmissions may be involved. Furthermore, the sequences of epidemiological transmission pairs should be related to a large reference data set with closely related sequences to prevent spurious results.

In *chapter 6*, we studied whether a referral guideline for chronic hepatitis B patients can be used in primary care to select patients eligible for evaluation by a specialist. The accuracy of a simple guideline based on HBeAg positivity and/or elevated ALT level to predict high HBV DNA levels (defined as more than 105 copies/ml) was assessed by calculating the positive and negative predictive value, sensitivity, and specificity of the guideline. We found that 43% (181/420) of patients were eligible for referral to specialist care based on a positive HBeAg test or elevated ALT. The positive predictive value of the referral guideline was 45% (82/181, 95% confidence interval 38%-53%). The negative predictive value, i.e. the proportion of patients who were not selected for referral that had low viral loads, was 95% (227/239, 95% CI 71%-97%). Sensitivity was 87% (95% CI 80%-93%); the patients selected included 82 of 94 patients with high HBV DNA. Of the 12 patients with high viral loads not selected by the guideline, 11 had a viral load of between 105-106 copies/ml. We conclude that a guideline based on HBeAg and a single ALT determination can successfully predict viral load in chronic HBV patients and can be used in primary care to select patients for referral to specialist care.

In *chapter 7*, we estimated the effect of long-term antiviral treatment and antiviral resistance on the mortality and morbidity of active chronic hepatitis B patients. To do so, we used a mathematical model including progression estimates of chronic hepatitis B patients with and without treatment. A population cohort of chronic hepatitis B in the Netherlands was constructed and stratified according to 10-year age groups, the prevalence of HBsAg, HBV DNA level, ALT level, HBeAg status and the presence of cirrhosis. A Markov model was created to mathematically simulate the cohort's progression through a finite series of health states. The analysis was performed based on four scenarios; natural history, long-term therapy with a high-resistance profile drug without and with salvage, and a low-resistance profile drug. It is estimated that there are 64,000 people (0.4%) suffering from chronic hepatitis B infection in the Netherlands in 2005, with 6,521 (10%) of them having high viremia and elevated ALT levels. Within a 20-year period, 1,725/6,521 (26%) of the active chronic hepatitis B cohort will have died due to liver-

related causes. Of the 5,685 without cirrhosis at entry, 1,671 (29%) will develop cirrhosis. Of those 836 with cirrhosis at entry, 619 (74%) will die within a 20-year period. If this active chronic hepatitis B cohort is fully detected and treated, mortality related to liver disease can be reduced by 80% if a low-resistance profile drug is chosen from the start. The effect is due to both the reduction in complications of cirrhosis and to the prevention of the development of cirrhosis. We conclude that long-term antiviral therapy with a strategy that minimizes or controls resistance will have a major preventive effect on liver-related mortality and morbidity.

Antiviral treatment can prevent HBV related liver disease but although the potential impact of treatment is large, the benefit is not optimal as the proportion of patients that need to be treated and who actually receive treatment is low. Screening can improve the detection of HBV patients. In *chapter 8*, we assessed the costs-effectiveness of systematic screening for chronic hepatitis B of first generation migrants from mid- and highly endemic countries in the Netherlands. To do so, we combined epidemiological data of expected numbers of patients with active CHB in the target population and data on the costs of a screening program with the outcomes of a Markov model in terms of costs and quality-adjusted life-years (QALYs) for patients with and without treatment for a life time horizon. The base-case assumptions were an HBsAg prevalence of 3.35%, 35% participation in screening, 70% successful referral, and 75% treatment compliance. At the base case estimates, screening can reduce mortality of HBV related diseases with 10% compared to the status quo. The incremental cost effectiveness ratio (ICER) of screening compared to the status quo is € 8,966.- per QALY gained. The ICER varied between € 7,936.- and € 11,705.- per QALY gained in univariate sensitivity analysis, varying parameter values of HBV prevalence, participation rate, successful referral and treatment compliance. In multivariate sensitivity analysis for treatment effectiveness the ICER varied between € 7,222.- and € 15,694.-, and for disease progression in natural history from € 5,568.- to € 60,418.-. A systematic screening program for chronic HBV infection targeted at first generation migrants is likely to be cost effective, even at low estimates for the HBsAg prevalence, participation, referral and starting treatment.

Chapter 9 (General discussion) summarizes the main findings and answers the research questions. We conclude that to increase access to antiviral treatment for patients with active chronic hepatitis B, the detection of chronic hepatitis B patients should be improved. To do so, a proactive approach is necessary as most patients do not have symptoms. We showed that a systematic screening programme targeted at first generation migrants is cost-effective. However, in practice a large part of the patients with active chronic hepatitis B will still not be identified when screening is implemented because the estimated participation is not likely to exceed 50%. Therefore, other options such as adding opportunistic or outreach screening to systematic screening need to be explored.

Furthermore, adding screening for hepatitis C might improve the cost-effectiveness of the screening programme as hepatitis C is also more prevalent in some migrant populations and effective treatment for hepatitis C is also available.

We present the following *recommendations* for future implementation of interventions:

- We endorse the advice of the Dutch Health Council to start general vaccination against hepatitis B.

- The detection of chronic hepatitis B patients should be improved so that they can be offered antiviral treatment and the burden of disease can be reduced.

- A proactive approach to screening should be adopted as most chronic hepatitis B patients do not have symptoms.

- Effective referral to specialist care should be ensured for patients detected by screening.

- A pilot study implementing systematic hepatitis B screening targeted at first generation migrants from endemic countries should be conducted.

- Other methods to increase detection of chronic hepatitis B patients such as opportunistic or outreach screening strategies should be explored.

- The added value of adding screening for hepatitis C in a systematic screening programme should be investigated.

Samenvatting

In dit proefschrift wordt de epidemiologie van acute en chronische hepatitis B in Nederland beschreven, met de nadruk op de impact van secundaire preventie van hepatitis B.

In *hoofdstuk 1* wordt een algemene beschrijving gegeven van het natuurlijk beloop van een infectie met het hepatitis B virus, de (moleculaire) epidemiologie van hepatitis B in Nederland, en de mogelijkheden tot primaire en secundaire preventie. Een infectie met het hepatitis B virus tast de lever aan, wat uiteindelijk tot verschillende ziekteverschijnselen kan leiden. Een acute infectie kan verdwijnen waarna blijvende immuniteit tegen het hepatitis B virus ontstaat, maar kan ook overgaan in een chronische infectie. In zeldzame gevallen ontstaat acuut leverfalen na een hepatitis B virus infectie, met een hoog overlijdensrisico. Mensen met een chronische hepatitis B virus infectie blijven besmettelijk voor andere personen en lopen de kans later in hun leven ernstige leverziekten te ontwikkelen zoals levercirrose en leverkanker. In Nederland komt hepatitis B relatief weinig voor en wordt het virus voornamelijk overgedragen door seksueel contact. Chronische infecties worden het meest gevonden bij migranten geboren in landen waar hepatitis B relatief veel voorkomt.

Primaire preventie van hepatitis B is mogelijk door vaccinatie, maar vaccinatie heeft geen invloed op reeds bestaande chronische infecties. Screening op hepatitis B is een vorm van secundaire preventie, waarbij antivirale middelen gebruikt kunnen worden om het ontstaan van hepatitis B gerelateerde leverziekten te voorkomen. Omdat de behandelmogelijkheden met antivirale middelen sterk zijn toegenomen, wordt het belangrijker om patiënten met chronische hepatitis B tijdig op te sporen.

In dit proefschrift richten we ons op de volgende onderzoeksvragen:
1 Wat zijn de transmissieroutes, bronnen van infectie en risicofactoren voor acute hepatitis B virus infectie in Nederland?
2 Wat is de prevalentie van hepatitis B in mensen met verschillende etnische achtergrond in Rotterdam?
3 Wat is de toegevoegde waarde van moleculaire analyse voor bron- en contactopsporing bij hepatitis B?
4 Wat is de impact van secundaire preventie van hepatitis B?

In *hoofdstuk 2* wordt de transmissieroute en bron van infectie beschreven van 144 patiënten met een acute hepatitis B virus infectie. Gedetailleerde informatie over de patiënten is verzameld door het uitbreiden van de surveillance van hepatitis B door de gemeentelijke gezondheidsdiensten (GGD). De meerderheid (59%) van de infecties bleek door seksueel contact te zijn verkregen; 52% daarvan door homoseksueel en 48%

door heteroseksueel contact. In 60% van de door heteroseksueel contact overgedragen infecties was de bron een partner afkomstig uit een hepatitis B endemisch gebied. We concluderen dat seksuele transmissie de meest voorkomende overdrachtsroute van acute hepatitis B in Nederland is en dat de introductie van nieuwe infecties vanuit het buitenland een sleutelrol speelt in de huidige epidemiologie van hepatitis B.

In *hoofdstuk 3* wordt het relatieve belang van verschillende risicofactoren voor acute hepatitis B virus infectie onderzocht in een patiënt-controle-studie. Deelnemers in de studie waren 120 patiënten met acute hepatitis B en 3948 willekeurig geselecteerde controle personen uit de algemene bevolking. Risicofactoren werden bestudeerd door middel van logistische regressie. Met behulp van hiërarchische analyse kon onderscheid gemaakt worden tussen verstorende variabelen (confounders) en mediërende variabelen (mediatoren). Het risico op acute hepatitis B bleek verhoogd onder homo- en biseksuele mannen, waarbij het hebben van meer dan twee partners in de afgelopen 6 maanden de enige significante risicofactor was. Voor kinderen, volwassen vrouwen en heteroseksuele mannen was het hebben van ouders geboren in een gebied waar hepatitis B endemisch is een significante risicofactor. Voor volwassen vrouwen en heteroseksuele mannen werd deze associatie voornamelijk verklaard door het hebben van een buitenlandse partner. Voor kinderen werd het gedeeltelijk verklaard door parenterale blootstelling in het buitenland.

Hoewel de prevalentie van virale hepatitis in het algemeen laag is in westerse landen, kunnen er ook gebieden zijn met een hogere prevalentie. In *hoofdstuk 4* beschrijven we de prevalentie van hepatitis A, B en C, gevonden in een bevolkingsonderzoek in een multiculturele wijk in Rotterdam. Bij 68% van de deelnemers konden in het bloed antistoffen tegen het hepatitis A virus aangetoond worden. De prevalentie van antistoffen tegen het hepatitis B core-antigeen (anti-HBc), een kenmerk van een eerder doorgemaakte of huidige infectie, was 20% (58/284). De prevalentie van hepatitis A en B varieerde per leeftijdsgroep en etniciteit. Twee respondenten (0,7%) hadden een chronische hepatitis B virus infectie. De prevalentie van hepatitis C was 1,1% (3/271). Er werd een hoog niveau van geïsoleerde anti-HBc gevonden. De hoge prevalentie van (vroegere) virale hepatitis infecties bevestigt eerdere bevindingen in etnische subgroepen uit een landelijke algemene populatiestudie, en illustreert de hoge ziektelast door virale hepatitis in gebieden met grote migrantenpopulaties.

Als bij een patiënt de diagnose hepatitis B gesteld wordt, voert de GGD bron- en contactopsporing uit. In de studie beschreven in *hoofdstuk 5*, zijn van patiënten met acute en chronische hepatitis B virus infecties in Rotterdam de sequentie gegevens van het virus genoom en de epidemiologische informatie onderzocht. We hebben de mate van moleculaire ondersteuning vastgesteld voor patiënten die gevonden zijn door contactonderzoek, de zogeheten epidemiologische transmissieparen. Dit is gedaan door met behulp

van de parsimony methode fylogenetische bomen te maken mét, en zonder geforceerde clustering van epidemiologische transmissieparen. Vervolgens is het verschil in het aantal mutaties tussen de bomen vergeleken. In de 62 acute en 334 chronische hepatitis B geïnfecteerden werden de hepatitis B virus genotypen A t/m G gevonden. Van de 22 epidemiologische transmissieparen werden er 6 weerlegd door de moleculaire analyse, 4 bekrachtigd en was de ondersteuning voor de overige 12 paren ambigu. Als moleculaire ondersteuning aan- of afwezig is, wat het geval was in bijna de helft van de onderzochte transmissieparen, dan kan de veronderstelde transmissieroute overtuigend bevestigd of weerlegd worden door de moleculaire analyse. In onze analyse werd divergentie tussen leden van epidemiologische transmissieparen toegestaan, en hebben we ook moleculaire ondersteuning gevonden voor paren die aanzienlijk, soms tot wel 15 mutaties, van elkaar verschilden. Dit leidt tot de conclusie dat de mate van divergentie niet gebruikt kan worden om individuele transmissieparen te bevestigen of te weerleggen. Omdat moleculaire ondersteuning voor transmissieparen met identieke sequenties vaak ambigu was, concluderen we net zo, dat afwezigheid van divergentie ook niet gebruikt kan worden om een transmissiepaar te bevestigen. Vooral zonder epidemiologische informatie zouden identieke sequenties niet opgevat mogen worden als echte transmissieparen. Het vinden van clusters met identieke sequenties die niet epidemiologisch aan elkaar gelinkt zijn, zou kunnen duiden op tekortkomingen van de bron- en contactopsporing. Maar het zou ook kunnen dat het virus een gemeenschappelijk bron had en dat er meerdere transmissies bij betrokken zijn. Bovendien zouden de sequenties van epidemiologische transmissieparen gerelateerd moeten worden aan een grote referentie dataset om schijnresultaten te voorkomen.

In *hoofdstuk 6* is onderzocht of een verwijsrichtlijn voor chronische hepatitis B patiënten in de eerste lijn gebruikt kan worden om patiënten te selecteren die in aanmerking komen voor beoordeling door een specialist. Hiervoor is de nauwkeurigheid bepaald waarmee een eenvoudige richtlijn, gebaseerd op HBeAg positiviteit en/of verhoogde leverenzymen (ALAT), kan voorspellen of er sprake is van een verhoogd HBV DNA (gedefinieerd als meer dan 105 kopieën/ml serum). De nauwkeurigheid is bepaald door de positieve- en negatieve voorspellende waarde, de sensitiviteit, en de specificiteit van de richtlijn te berekenen. We vonden dat 43% (181/420) van de patiënten in aanmerking kwam voor doorverwijzing naar de specialist op basis van een positieve HBeAg test of een verhoogde ALAT. De positief voorspellende waarde van de verwijsrichtlijn, dat wil zeggen het deel van de doorverwezen patiënten dat een hoge viral load heeft, was 45% (82/181, 95% betrouwbaarheidsinterval 38%-53%). De negatief voorspellende waarde, dat wil zeggen het deel van de patiënten dat niet geselecteerd is voor doorverwijzing en dat een lage virale load heeft, was 95% (227/239, 95% BI 71%-97%). De sensitiviteit was 87% (95% BI 80%-93%); onder de geselecteerde patiënten bevonden zich 82 van de 94 patiënten met een verhoogde virale load. Van de 12 patiënten met een verhoogde

virale load die niet geselecteerd werden door de richtlijn hadden er 11 een virale load van 105-106 copies/ml. We concluderen dat een verwijsrichtlijn gebaseerd op HBeAg en eenmalige ALAT bepaling gebruikt kan worden in de eerste lijn om patiënten te selecteren voor doorverwijzing naar de specialist.

In *hoofdstuk 7* hebben we het effect geschat van langdurige behandeling met antivirale middelen en het onstaan antivirale resistentie op de mortaliteit en morbiditeit van patiënten met actieve chronische hepatitis B. Hiervoor hebben we een mathematisch model gebruikt dat schattingen van de ziekteprogressie van patiënten mét en zonder behandeling bevat. Er is een cohort geconstrueerd van patiënten met chronische hepatitis B in Nederland, dat is gestratificeerd naar 10-jaars leeftijdsgroepen, HBsAg prevalentie, HBV DNA niveau, HBeAg status, en de aanwezigheid van cirrose. Vervolgens is een Markov model gebouwd dat de progressie van het cohort door een beperkt aantal ziektestadia wiskundig simuleert. De analyse is gebaseerd op 4 scenario's; natuurlijk beloop, langdurige behandeling met een antiviraal middel met een hoog resistentieprofiel, scenario 2 waarbij een tweede middel wordt toegevoegd indien resistentie optreedt (salvage), en behandeling met een middel met een laag resistentieprofiel. In 2005 zijn er in Nederland naar schatting 64.000 mensen (0,4%) met een chronische hepatitis virus infectie, waarvan 6.521 (10%) een hoge viral load en verhoogde ALAT heeft. In een periode van 20 jaar zal 26% (1.725/6.521) van het cohort patiënten met actieve chronische hepatitis B overleden zijn aan de gevolgen hiervan. Van de 5.685 patiënten zonder cirrose in het begin zullen 1.671 (29%) cirrose ontwikkelen. Van de 836 patiënten die al cirrose hadden, zullen er 619 (74%) overlijden binnen 20 jaar. Als het cohort patiënten met actieve chronische hepatitis B in zijn geheel ontdekt en behandeld zou zijn, dan kan de lever gerelateerde sterfte met 80% gereduceerd worden als vanaf het begin een antiviraal middel met een laag resistentieprofiel gekozen wordt. Dit effect is toe te schrijven aan zowel de vermindering van de complicaties van cirrose, als het voorkomen van het ontstaan van cirrose. We concluderen dat langdurige antivirale behandeling met een strategie die de kans op resistentie minimaliseert, een belangrijk preventief effect zal hebben op de aan leverziekten gerelateerde sterfte en ziektelast.

Antivirale behandeling kan door hepatitis B veroorzaakte leverziekten voorkomen. Maar ook al is de impact potentieel groot, er wordt niet optimaal van geprofiteerd aangezien slechts een klein deel van de patiënten dat behandeling nodig heeft ook daadwerkelijk behandeld wordt. Screening kan de opsporing van hepatitis B patiënten verbeteren. In *hoofdstuk 8* hebben we de kosteneffectiviteit vastgesteld van systematische screening op chronische hepatitis B van eerste generatie migranten in Nederland, afkomstig uit landen waar hepatitis B middel en hoog endemisch is. Hiervoor hebben we epidemiologische gegevens over het verwachtte aantal patiënten met actieve chronische hepatitis B in de doelgroep en gegevens over de kosten van een screeningsprogramma, gecombineerd

met de uitkomsten van een Markov model voor patiënten met en zonder behandeling in termen van kosten en voor kwaliteit van leven gecorrigeerde levensjaren (QALY's). De basis aannames in het screeningsscenario waren een HBsAg prevalentie van 3,35%, 35% deelname aan screening, 70% succesvolle doorverwijzing, en 75% start met behandeling. Onder de basis aannames kan screening de aan hepatitis B gerelateerde sterfte met 10% doen afnemen in vergelijking met de status quo. De incrementele kosteneffectiviteitsratio (ICER) van screening ten opzichte van de status quo is € 8.966,- per gewonnen QALY. De ICER varieerde tussen € 7.936,- en € 11.705,- per gewonnen QALY tijdens univariate sensitiviteitsanalyses waarbij de parameter waarden van de hepatitis B prevalentie, deelname aan screening, doorverwijzing en start met behandeling gevarieerd werden. In multivariate sensitiviteitsanalyse voor de effectiviteit van behandeling varieerde de ICER tussen € 7.222,- en € 15.694,-, en voor variatie in de ziekteprogressie van € 5.568,- tot € 60.418,-. Een systematisch screeningsprogramma voor chronische hepatitis B virus infectie gericht op eerste generatie migranten is waarschijnlijk kosteneffectief, zelfs bij lage schattingen voor de HBsAg prevalentie, deelname aan screening, verwijzing en het starten van behandeling.

In *hoofdstuk 9* (Algemene discussie) worden de belangrijkste bevindingen samengevat en de onderzoeksvragen beantwoord. We concluderen dat om de toegang tot antivirale behandeling voor patiënten met actieve chronische hepatitis B te vergroten, de opsporing van deze patiënten verbeterd moet worden. Om dit te bereiken is een actieve benadering nodig omdat de meeste patiënten geen symptomen hebben. We hebben laten zien dat een screeningsprogramma gericht op eerste generatie migranten kosteneffectief is. Hoe dan ook, in de praktijk zal een groot deel van de patiënten met actieve chronische hepatitis B nog steeds niet geïdentificeerd worden, aangezien de deelname aan de screening naar schatting niet boven de 50% uit zal komen. Daarom is het nodig naast systematische screening ook andere mogelijkheden te verkennen, zoals het toevoegen van opportunistische screening of een outreach . Verder kan het toevoegen van screening op hepatitis C de kosteneffectiviteit van het screeningsprogramma mogelijk nog verbeteren aangezien hepatitis C ook meer voorkomt in sommige migrantenpopulaties en er voor hepatitis C effectieve behandeling beschikbaar is.

We komen tot de volgende aanbevelingen ten aanzien van het implementeren van toekomstige interventies:

- We onderschrijven het advies van de Gezondheidsraad om algemene vaccinatie tegen hepatitis B in te voeren.

- De opsporing van chronische hepatitis B patiënten moet verbeterd worden zodat hen behandeling aangeboden kan worden en de ziektelast omlaag gebracht kan worden.

- Screening dient te gebeuren met een pro-actieve benadering omdat de meeste chronische hepatitis B patiënten geen symptomen hebben.

- Effectieve doorverwijzing naar de specialist moet gegarandeerd zijn voor patiënten die met screening opgespoord worden.

- Er moet een pilot studie uitgevoerd worden naar systematische hepatitis B screening gericht op eerste generatie migranten.

- Andere methoden die de opsporing van hepatitis B patiënten kunnen verbeteren, zoals opportunische of outreach screeningsstrategieën moeten onderzocht worden.

- De toegevoegde waarde van het toevoegen van hepatitis C aan een systematisch screeningsprogramma dient onderzocht te worden.

Dankwoord

De laatste loodjes wegen het zwaarst, dat geldt zeker voor het afmaken van een proef-schrift! Maar loodzwaar heb ik het werken aan mijn proefschrift de laatste jaren gelukkig niet ervaren, en ik wil graag iedereen bedanken die daarbij geholpen heeft.

Allereerst wil ik mijn copromotoren Rob de Man en Jan-Hendrik Richardus bedanken bij wie ik altijd terecht kon voor goede raad. Beste Rob, vaak ging ik op weg naar het zieken-huis met het idee dat er niet veel te bespreken was. Maar natuurlijk kwamen er al pratende toch vragen naar boven. Jouw doeltreffende opmerkingen hielpen me altijd weer op de goede weg zodat ik fluitend terugging naar de GGD. Beste Jan Hendrik, bedankt voor de sturing en adviezen vanaf het begin dat ik op de GGD kwam werken, en in het bijzonder tijdens de laatste jaren. Je bent altijd bereid mee te denken, zelfs als je aan de andere kant van de wereld zit, veel dank daarvoor. Mijn promotor Harry Janssen wil ik bedanken voor alle vrijheid en het vertrouwen dat ik kreeg bij mijn promotieonderzoek. De leden van de promotiecommissie dank ik voor hun bereidheid zitting te nemen in de commissie.

Onderzoek doe je nooit alleen, en ik wil dan ook alle coauteurs bedanken voor hun constructieve bijdragen. Ik heb door de samenwerking met collega's uit verschillende vakgebieden veel geleerd. Beste Mehlika, dankzij jou kon ik mijn proefschrift afsluiten met twee mooie artikelen gebaseerd op mathematische modellen. We hebben er vele uren over gediscussieerd, waarbij we soms helemaal de weg kwijt raakten maar uiteinde-lijk toch steeds weer verder kwamen. Ik hoop dat we nog veel zullen samenwerken en ik kijk al uit naar jouw proefschrift! Beste Ted, jouw vernieuwende aanpak heeft de analyse van de transmissieparenstudie vlot getrokken. Bedankt ook voor je geduldige uitleg van de fylogenetische analyse, voor mij een helemaal nieuw onderwerp. Verder wil ik Susan en Jolanda bedanken voor het analyseren van alle hepatitis B samples.

Ik wil ook graag iedereen van zowel het secretariaat van het cluster Infectieziekte-bestrijding op de GGD, als van de afdeling Maag-, Darm- en Leverziekten op het EMC bedanken voor de ondersteuning bij het praktische regelwerk. Astrid, bedankt ook voor je hulp bij het organiseren van het symposium.

Alle verpleegkundigen van de sectie Infectieziekten wil ik bedanken voor hun hulp bij het onderzoek door het verzamelen van de bloedmonsters van hepatitis B patiënten.

Mijn lieve collega's op de GGD, Marijke, Ytje, Mireille, Jeanelle, Hélène en Marloes, bedankt voor alle gezelligheid en de fijne lunchwandelingen. Marijke, naast mijn kamer-genoot ben jij de pionier van het hepatitis B onderzoek op de GGD. Zonder de studie die jij hebt opgestart was dit proefschrift niet mogelijk geweest. Mireille en Ytje, jullie GVO kijk

op onderzoek heeft mijn onderzoeksblik verbreed. Het was fijn alle promotiebeslommeringen met jullie te kunnen bespreken, nog even, en dan zijn jullie ook klaar! Hannelore, bedankt voor de fijne samenwerking door de jaren heen. Onno, onder jouw inspirerende leiding is IZB een geweldige plek geworden om onderzoek te doen. Bedankt dat je me de kans hebt gegeven om ook te promoveren.

Mijn Utrechtse vrienden wil ik bedanken voor de gezellige etentjes, films, avonden in het café, het hardlopen op zondagochtend en niet te vergeten het geduldig aanhoren van mijn promotieperikelen. Fiona, bedankt voor alle gezellige muzikale en culinaire wuit-stapjes.

Lieve Susan, zet 'm op, jouw proefschrift komt er ook! Dat er nog maar vele gezellige avonden mogen volgen waarbij we de tijdslimiet die we over hepatitis B mogen praten niet teveel overschrijden. Gaan we volgend jaar voor een nieuw PR bij de Marikenloop?

Ook alle HYS-fijne vrienden uit Nijmegen en verre omstreken bedankt voor de fantastische vakanties. Het is elke keer weer geweldig om mee te maken hoe harmonieus we met meer dan 20 mensen een week samen kunnen genieten van het goede leven. Dat we dat nog maar lang mogen volhouden, ook nu het aantal kinderen exponentieel aan het toenemen is.

Mijn allerliefste vriendinnen Bregje en Inez, ik ben heel blij dat jullie mijn paranimfen willen zijn! De week in New York was wel het hoogtepunt van onze weekendjes weg! Gelukkig lukt het ondanks dat we niet bij elkaar om de hoek wonen en de volle agenda's toch om elkaar regelmatig te zien. Dat er nog maar vele drie-dubbel-dates mogen komen!

Mirjam, je bent de beste en liefste zus! Gelukkig zien we elkaar nog steeds vaak nu je in Frankrijk woont, lang leve Skype en de Thalys. Samen skaten langs de Amstel inruilen voor fietsen langs de Loire is helemaal zo slecht nog niet! De bezoekjes aan jou, Fred en Anand voelen altijd als vakantie. Ook mijn ooms en tantes en schoonfamilie wil ik bedanken voor de getoonde interesse.

Lieve papa en mama, dankzij jullie steun ligt dit boekje hier nu! Bedankt voor al het vertouwen dat jullie me altijd gegeven hebben. Jullie zijn er altijd voor me en dat is een heel fijn gevoel.

Lieve Walco, de laatste regels zijn voor jou, om te zeggen dat ik van je hou! Jij hebt van het dichtstbij de ups en downs van het promoveren meegemaakt. Bedankt dat je me af en toe rustig liet uitrazen en zorgde dat ik het snel weer zag zitten. Zullen we nu het klaar is dan echt eens samen een liedje opnemen?

Curriculum vitae

Irene Veldhuijzen werd in 1975 geboren in De Bilt. Na het behalen van het VWO diploma op het Christelijk Lyceum Zeist in 1993, ging ze gezondheidswetenschappen studeren aan de Katholieke Universiteit Nijmegen. Haar interesse in infectieziekten en reizen voerden haar naar Tanzania waar ze haar kleine stage deed voor TANESA, een project gericht op ondersteuning van de HIV/AIDS bestrijding in de Mwanza regio. Voor haar wetenschappelijke stage verbleef Irene op het Centrum voor Infectieziekten Epidemiologie (CIE) van het RIVM. Vervolgens deed ze nog een extra stage in Zambia waar ze het bereik van thuiszorgprogramma's voor aidspatiënten onderzocht.

Na het afronden van haar studie met afstudeerrichting epidemiologie, ging ze als junior-epidemioloog voor het RIVM werken. Daar heeft ze meegewerkt aan onderzoek naar de evaluatie van het Rijksvaccinatieprogramma, antimicrobiële resistentie in Europa, en hepatitis B. Na drie jaar maakte ze de overstap naar de GGD Rotterdam. Ze deed daar onderzoek naar verschillende onderwerpen zoals chlamydia screening, de hiv-sneltest, risicoperceptie van SARS en vogelgriep, om uiteindelijk weer bij hepatitis B uit te komen. Hepatitis B kreeg de focus vanaf 2006, om uit te groeien tot het onderwerp van haar promotie. Tijdens haar promotieonderzoek zag ze ook nog kans om een Master in Public Health te volgen.

Curriculum vitae

Irene Veldhuijzen was born in 1975 in De Bilt. After completing her secondary education at the Christelijk Lyceum Zeist in 1993, she started to study Biomedical Sciences at the Radboud University in Nijmegen. Her interest in infectious diseases and traveling brought her to Tanzania where she did her minor internship for the Tanzania-Netherlands Project to Support HIV/AIDS control in Mwanza Region (TANESA). For her research internship Irene stayed at the Epidemiology and Surveillance Unit of the National Institute of Public Health and the Environment (RIVM). She arranged an additional internship in Zambia where she studied the coverage of a project offering home care for AIDS patients.

After graduating with a major in Epidemiology in 1998, she started to work at the RIVM. First on vaccine preventable diseases, then on antimicrobial resistance and lastly on hepatitis B. In 2002, Irene started working for the Division of Infectious Diseases Control of the Municipal Public Health Service Rotterdam-Rijnmond. She worked on several subjects including Chlamydia screening, HIV rapid testing, risk perceptions of SARS and avian influenza, and hepatitis B. From 2006 onwards, she focussed mainly on hepatitis B and started to work on her PhD thesis. As part of her PhD studies, Irene completed a Master of Science in Public Health at the Netherlands Institute of Health Science in 2009, the year in which she also finished her thesis.

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PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student:	Irene Veldhuijzen	PhD period:	2006 - 2009
Erasmus MC Department:	MDL	Promotor:	prof.dr. H.L.A. Janssen
Research School:	Nihes	Supervisors:	dr. J.H. Richardus
			& dr. R.A. de Man

1. PhD training	Year	Workload (Hours/ECTS)
Research skills and in-depth courses		
Master in Public Health (Nihes)	2007-2009	27 ECTS
Presentations		
- Lunchreferaat GGD Cluster Infectieziektenbestrijding	2007	4 hours
- Najaarsvergadering Nederlandse Vereniging voor Gastro-enterologie (NVGE)	2008	1 ECTS
- Viral Hepatitis Prevention Board Meeting (VHPB)	2008	8 hours
International conferences		
- European Public Health Association (EUPHA), oral presentation	2006	1 ECTS
- 59th annual meeting of the American Association for the Study of Liver Diseases (AASLD), poster presentation	2008	1 ECTS
Seminars and workshops		
- SARSControl Integration Workshop, oral presentation	2006	1 ECTS
- 4e Post AASLD symposium	2006	4 hours
- 5e Landelijke Hepatitisweek, workshop gegeven	2007	1 ECTS
- Cephir seminar Infectieziektebestrijding, oral presentation	2007	8 hours
- 22e Erasmus Liver Day en 5e Post AASLD symposium,	2007	6 hours
- Symposium moleculaire epidemiologie van hepatitis B in Nederland, RIVM, oral presentation	2008	20 hours
Didactic skills		
Other		

2. Teaching activities	Year	Workload (Hours/ECTS)
Lecturing		
Supervising practicals and excursions		
Supervising Master's theses		
- MSc-internship student VU University Amsterdam	2008	20 hours
Other		