Epidemiology of Hepatitis B Virus Infection in The Netherlands and Beyond

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Epidemiology of Hepatitis B Virus Infection in The Netherlands and Beyond

Epidemiologie van Hepatitis B Virus Infectie in Nederland en Elders

(met een samenvatting in het Nederlands)

Proefschrift

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Chapter 1

Introduction

Chapter 1.1

Hepatitis B virus: a general introduction

Based on: Modern Infectious Disease Epidemiology, part III, chapter 19.2

Based on Modern Infectious Disease Epidemiology, part III, chapter 19.2 (1) © Springer 2010

Hepatitis B virus: a general introduction

Hepatitis is a clinical entity due to infectious and non-infectious causes. Most of the global hepatitis burden can be attributed to hepatitis B virus (HBV), one of the five viruses that cause hepatitis (hepatitis A-E virus). The causal agent for hepatitis B was identified in 1965 by Blumberg and colleagues (2). The burden of liver disease and death associated with HBV infection mainly results from sequelae of chronic infection including hepatocellular carcinoma (HCC) and cirrhosis. An estimated 400 million people worldwide are infected with HBV, accounting for an estimated one million deaths annually (3-5). A safe and effective vaccine to prevent HBV infection has been available since 1982. It is included in universal vaccination programmes of more than 170 countries (6). Treatment options to prevent sequelae of chronic infections are improving rapidly. Despite the fact that HBV infection and a large part of its sequelae are preventable, it is still associated with a high burden of morbidity and mortality. This makes prevention and control of HBV infection one of the major public health priorities.

EPIDEMIOLOGY

Transmission routes and global patterns of infection

HBV is acquired by percutaneous or permucosal exposure to blood or body fluids from an infected person. Transmission usually takes place through sexual contact, blood contact or perinatally. Perinatal transmission usually occurs at the time of delivery, although infection in utero is also possible (7). The risk of perinatal transmission is related to the HBeAg status of the mother: without treatment, children born to HBeAg positive mothers virtually all acquire perinatal infection compared to about 25% of children born to HBeAg negative mothers (8,9). HBV is also transmitted within households through close non-sexual contact (10). An infection with HBV can resolve but it can also lead to chronic infection. The main determinant of this is the age at infection: infants and young children who are HBV infected are at much higher risk of chronic infection than older people (11). The most striking feature of the global distribution of HBV is the large difference in prevalence of infection. The lifetime risk of acquiring HBV infection varies between around 0.4% in western Europe to 90% in East Asia (12,13). In high endemic areas, 8% or more of the population is chronically infected. In medium and low endemic areas this is 2-7% and <2%, respectively (Figure 1) (12). In the Netherlands, the prevalence of chronic HBV infection in its population of about 17 million inhabitants is 0.2%, which is among the lowest prevalences reported worldwide (14,15).

The main explanation for the extreme variation in HBV prevalence between geographical regions is that the HBV incidence, average age of infection and age-related probability of developing chronic infection are linked in a positive feed-back loop. This leads to 'catastrophic' behaviour of HBV transmission dynamics, whereby non-linear behaviour of prevalence patterns over time are instable at intermediate levels and stabilise at discrete, highly different, levels (13). This phenomenon can also be observed in other systems where positive feed-back loops cause catastrophic dynamics, where it e.g. explains the earth differentiation in ecological zones, the spread of wildfires, or, in geopolitics, the different states of war and peace (16,17). Since several aspects of the natural history of HBV infection, including the age at HBeAg seroconversion, are genotype dependent, the differences in genotype distribution between geographical regions likely contributes to heterogeneity in prevalence (8).

Risk groups for HBV infection

The prevalence of HBV in a country is associated with its predominant transmission modes and the duration and quality of its vaccination strategy. In countries with high prevalence (HBsAg seroprevalence ≥ 8%), most infections are acquired perinatally or in early childhood (12). In these countries, health-care associated HBV transmission is also frequent (19). In low prevalence countries, most infections are acquired through adult risk behaviour including male homosexual contact and injecting drug use (IDU) (20). Other groups with increased risk for acquiring HBV include close contacts of people with chronic infection, infants born to infectious mothers, health care workers, children with developmental disabilities, patients undergoing haemodialysis, close contacts of infected people, migrants and long-term travellers to endemic areas (21-24).

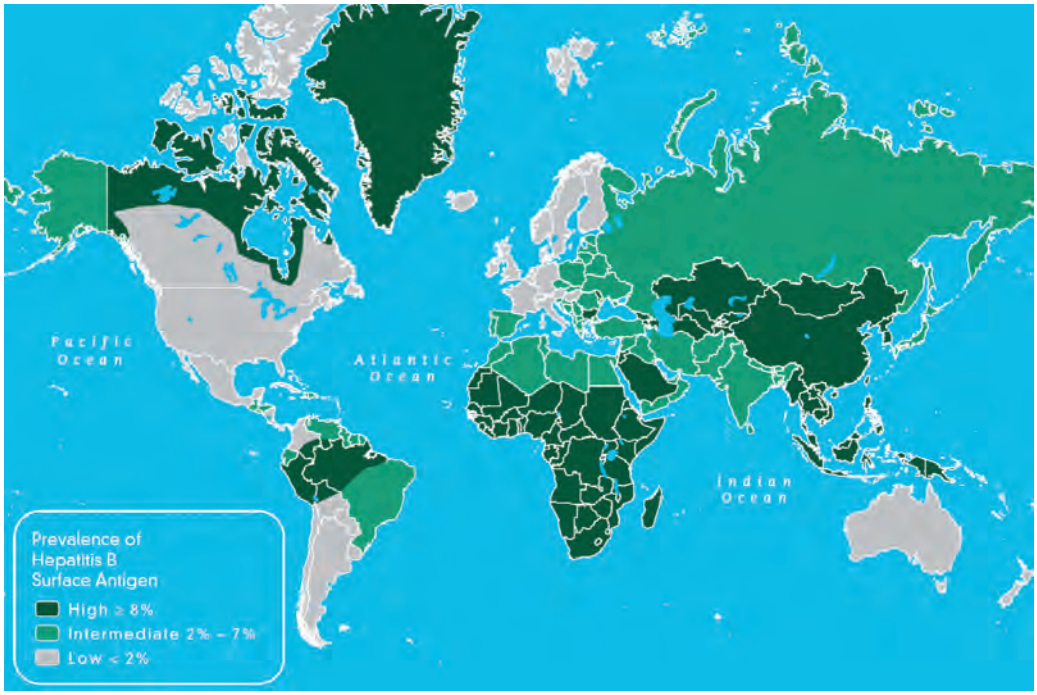

Figure 1. Prevalence of chronic HBV infection, 2006 (18)

Diagnosis and clinical course

Diagnosis

HBV infection can be diagnosed by testing serum for antibodies against the virus, detection of viral antigens, or by detecting HBV DNA using nucleic acid amplification tests. Serologically, several hepatitis B antigens (HBsAg, HBeAg) and antibodies (anti-HBc, anti-HBs, anti-HBe) can be distinguished. Based on the pattern of these the phase of infection can be determined (Table 1). Once chronic infection is diagnosed, further clinical follow-up is required including testing of liver biochemistry, virus serology, viral load and abdominal imaging. The viral load can be quantified by HBV-DNA detection by PCR, and is used to assess infectiousness and therapeutic effect. Patients who do not have an indication for antiviral treatment should be monitored because disease activity can increase (38).

Clinical disease

HBV is non-cytopathic to the hepatocyte. The pathogenesis of HBV therefore mostly results from the host's immune response, although the virus is likely to be also directly oncogenic (7,8). Infection with HBV can result in a broad spectrum of disease outcomes. After an incubation period of on average 2-3 months, symptoms of acute HBV infection can occur, including jaundice, nausea, upper abdominal pain and malaise. About 1% of acute infections results in fulminant liver failure, for which liver transplant is usually indicated. The probability of developing symptoms of acute HBV infection is age-dependent: among adults about a third of those infected develop symptoms whilst among children this is less than 10% (25-27). The majority of neonates who become infected perinatally remain asymptomatic (7).

Chronic HBV infection is defined as persistence of HBsAg (and/or HBV-DNA) for over 6 months after infection. The risk of developing chronic infection decreases with age of acquisition of HBV infection. Infants who acquire the infection perinatally develop chronic infection in 90% of cases. This risk is 20-50% in children aged 1-5 years, and less than 5% in adults (11,28). The risk is increased in those with impaired immunity or when there is co-infection with hepatitis C virus (29). A recent study among MSM and IDUs in Amsterdam, found an unexpected high proportion (about 25%) of HBV infected individuals developing chronic infection (30). For IDUs this

Table 1. Serological status in HBV infection (1)

	Phase 1	Phase 2	Phase 3	Phase 4		Prolonged Phase 1	Prolonged Phase 2	Prolonged Phase 3	Not infected
	Late in incubation period	Acute hepatitis B	Late acute hepatitis B	Recent hepatitis B	Ever hepatitis B	Chronic active hepatitis B		Chronic inactive hepatitis B	After successful vaccination
Time since infection	*2-12 weeks*	*2-4 months*	*3-6 months*	*<1 year*	*> 1year*	*HBeAg pos* > 6 months	*HBeAg neg* > 6 months	*> 6 months*	*n.a.*
ALAT	normal	increased	normal/increased	normal	normal	increased	increased	normal	normal
HBsAg	+	+	+	-	-	+	+	+	-
HBeAg	+ / -	+	-	-	-	+	-	-	-
IgM-anti-HBc	-	++	++	+	-	- (+)	- (+)	-	-
Total anti-HBc	-	+	++	++	++	++	++	++	-
Anti-HBe	-	-	+	+	+	-	+	+	-
Anti-HBs	-	-	+ / -	++	+	-	-	-	++
HBV-DNA	+	+	+ / -	-	-	+	+	+ / -	-
Infectiousness	+	++	+	-	-	++	++	+	-
Symptoms	-	+	+ / -	-	-	+ / -	+ / -	-	-

N.a. = not applicable.

may be partly explained by HIV/HCV co-infection. The explanation for the high rate among MSM remains unclear.

Each year, about 1% of people with chronic HBV infection clear HBsAg and HBV-DNA, resulting in a much improved prognosis (31). In those not clearing the infection, the natural history of disease is characterised by four phases, which can be distinguished by serological, virological and clinical markers (32). The transition through these phases depends mainly on the age at acquisition of infection, and highly influences the outcome of infection.
The main sequelae of chronic HBV infection are cirrhosis and HCC. The risk of developing these increases with the duration and type of chronic infection and the viral load (33). The 5-year risk of cirrhosis in patients with chronic HBV infection ranges from 8-20% (34). The annual risk of HCC in patients with chronic HBV infection ranges from 0.1% to 10% depending mainly on the underlying stage of liver disease (35). The outcome and treatment effectiveness of chronic HBV infection is negatively affected by co-infection with HIV, hepatitis A virus and HCV (34). HCC is the fifth most common cancer among men and the seventh among women. It is a leading cause of cancer-related mortality in many developing countries. In the US, the 5-year survival rate of HCC is less than 12% (36).

Therapy
Acute hepatitis B is a self-limiting disease. In the rare case of fulminant liver failure antiviral therapy may be beneficial but liver transplantation is usually the only life saving treatment (37). The indication for pharmaceutical treatment of chronic hepatitis virus infection depends on the presence of cirrhosis and/or active hepatitis, disturbances of the liver function (which can be assessed by serum serum alanine aminotransferase (ALAT) levels) and HBV DNA levels (38). Treatment options include immune modulation (with interferon alfa or peginterferon alfa) and antiviral treatment with nucleoside/nucleotide analoges (such as lamivudine, entecavir, telbivudine, adefovir and tenofovir) (39). Pegulated interferon alfa during 12 months leads to sustained viral response in 35% of patients and complete cure in 7% (40). However, inconvenient administration (subcutaneous) and frequent side effects hamper wide use. Antiviral nucleos(t)ide analoges can be given for prolonged periods: one year of treatment leads to reduction in viral load, lowering of transaminases, and improvement of the liver histology in almost all patients. However, after discontinuation of antiviral therapy the viral load may increase to pre-treatment values. A serious risk associated with long term use of antivirals (especially lamivudine, but also adefovir and telbivudine) is the occurrence of viral resistance. Resistance against the newer entecavir and tenofovir is at present limited, but the follow-up period is still short.

Prevention and control of HBV infection at a population level
Immunisation
HBV vaccines, available since 1982, are safe and effective in preventing HBV infection, and were the first vaccines against a virus that can cause human cancer. The first HBV vaccines were made from plasma from chronically infected individuals. Currently, mostly recombinant vaccines are used, derived from yeast or mammalian cells (40a). Several injections over months are needed for an effective antibody response to vaccination. Hepatitis B immunoglobulin

(HBIg) is derived from humans and has high levels of antibody to hepatitis B surface antigen. HBIg is immediately protective after injection.
Since vaccination only prevents new infections, impact on the burden of disease can only be expected several decades after implementation of programmes, depending on the pre-vaccination HBV epidemiology, age-groups targeted and coverage achieved (41). In low prevalence countries such as the Netherlands, most of the disease burden now and in the immediate future is in migrants from endemic areas who acquired the infection before migrating. In 1992, the World Health Organization (WHO) called for all countries to implement universal vaccination, and over 170 of 192 member states comply (6). However, in areas with very low prevalence the cost-effectiveness of universal vaccination is under debate. Universal vaccination in high and medium prevalence countries has led to great reductions in prevalence, morbidity and mortality (42,43). Costs are the major barrier for vaccine introduction in developing countries. Fortunately, the Global Alliance for Vaccines and Immunization, a coalition between public and private institutions, and the Global Fund for Children's Vaccines is making HBV vaccine available for 74 low-income countries (44).
Some low prevalence countries such as the United Kingdom do not have universal vaccination but instead target groups at increased risk of infection (21). Irrespective of universal vaccination, programmes targeting behavioural high-risk groups will be needed for the coming decades, depending on the coverage achieved and the age groups targeted for universal vaccination. Selective HBV vaccination of population subgroups at increased risk of HBV infection are therefore recommended in all European countries and the USA (45,46). Selective programmes have, however, frequently been criticised as ineffective since the reported vaccine coverage achieved in the target populations was considered too low (47-50). The impact on HBV transmission of selective vaccination programmes targeting behavioural high-risk groups was, prior to work presented in this Thesis in Chapter 2.4, never demonstrated on a national level.

Prevention of perinatal transmission
Prevention of perinatal transmission is a cornerstone in the control of HBV infection, as the risk of perinatal infection from HBsAg positive mothers is high, infected neonates usually develop chronic infection and of those about 25% eventually develop severe, often fatal, liver disease (12). Hepatitis B vaccine, HBIg, and a combination of both are effective to prevent hepatitis B occurrence in newborns of HBsAg positive mothers (51,52). Failure of these interventions is associated with a high viral load during pregnancy (53). Lamivudine treatment of chronic HBV infection during pregnancy for women with a high viral load can reduce the risk of perinatal transmission (53), and is recommended in Dutch and European guidelines for pregnant women with a high viral load (34,38,54). HBIg treatment of HBV infected pregnant women was also shown to reduce the risk of perinatal transmission (55). However, the relative effectiveness compared to antiviral treatment is unclear, and HBIg treatment during pregnancy is not included in clinical practice guidelines (34,38,54).

HBV notification and post-exposure prophylaxis
In areas of low endemicity notification remains important for HBV control. For reported patients, serological markers are evaluated and source and contacts are traced and vaccinated if susceptible. Needle stick injuries and other percutaneous and permucosal exposures, including sexual risk exposures warrant risk-assessment for immediate post exposure prophylaxis (with HBV vaccine and possibly HBIG).

Hygienic precautions
For control of blood born pathogens such as HBV, hepatitis C virus (HCV) and human immunodeficiency virus (HIV), hygienic precautions in the healthcare setting are essential. This includes screening of blood products, tissues and organs, avoiding the use of contaminated instruments and other universal precautions for invasive procedures. Outside health care, the use of contaminated instruments by e.g. tattoo, piercing, nail studios and IDU can be avoided, e.g. by offering needle exchange programmes for IDUs. Condom use is effective to prevent sexual transmission of HBV.

Secondary prevention
Secondary prevention is the early detection of disease to allow interventions to prevent disease progression and death. Possibilities for secondary prevention of HBV are improving, as treatment options are advancing rapidly. Several new antiviral drugs have become available in the past decade. Evidence is accumulating that these therapies are cost-effective to reduce the morbidity and mortality associated with chronic infection with HBV and HCV (56-

58). In addition to improving the outcome of chronic hepatitis, antiviral treatment is likely to reduce transmission by reducing the viral load and therefore infectivity of chronic carriers, similar to what has been documented for HIV (59-61). For HBV, vaccination of susceptible contacts of identified carriers can prevent new infections. Non-pharmaceutical interventions, such as the advice to limit alcohol intake and cease smoking, can improve outcomes for people living with chronic viral hepatitis (62,63).
Since the acquisition of HBV is often asymptomatic or subclinical, and sequelae take several decades to develop, between 40% and 80% of people with chronic hepatitis are unaware of their infection (14,64-70). Therefore, screening programmes for chronic HBV infection have the potential to contribute considerably to primary and secondary prevention. However, existing HBV screening programmes in Europe stem from an era when treatment options for were limited. Hence they are mainly aimed at primary prevention, targeting blood donors, pregnant women, and behavioral high-risk groups (71). Now that secondary prevention of HBV is possible, there is an urgent need to identify chronic carriers who may benefit from treatment (72).

National HBV vaccination programmes in the Netherlands
Several groups are targeted for HBV vaccination in the Netherlands, including health care workers and certain patient groups. The four HBV vaccination programmes funded by the Dutch Ministry of Health are the main topic of this Thesis:
(1) *Selective vaccination for infants born to HBsAg positive mothers (started in 1989)*
This programme is arguably the most important as infants born to HBsAg positive mothers are at high risk of infection, chronic infection and death due to HBV related liver disease. The Netherlands was among the first countries in the world to offer universal screening for HBV to all pregnant women, a key component of the programme to prevent perinatal transmission. The vaccination schedule for this group has varied. Currently, infants born to HBsAg positive women receive HBIg and HBV vaccination at birth, with 4 subsequent doses of HBV vaccine.
(2) *Selective vaccination of population subgroups at increased risk of HBV due to risk behaviour (started in 2002)*
This programme provides free HBV vaccination for men having sex with men (MSM) and commercial sex workers (CSWs). Heterosexuals who change partners frequently were excluded from the target population from 2007 onwards as there was insufficient evidence of an increased risk in this group. From 2012 onwards, drug users were excluded since cases of HBV infection among drug users were no longer reported. As a precautionary measure, vaccination of drug users is now provided through drug treatment services.
(3) *Selective vaccination of infants of whom one or both of the parents was born in a midor highly endemic country (started in 2003, ceased in 2011)*
This programme followed an advice by the Health Council in 2001 which assumed these children were at increased risk of HBV infection (73). The programme was ceased upon implementation of universal infant HBV vaccination in August 2011.
(4) *Universal infant HBV vaccination (started in August 2011)*
This was introduced following an assessment by the Dutch Health Council that included a cost-effectiveness analysis with favourable results (74).

Surveillance of hepatitis B virus in low endemic countries
Public health surveillance is "the ongoing, systematic collection, analysis and interpretation of health data, essential to the planning, implementation and evaluation of public health practice, closely integrated with the dissemination of these data to those who need to know and linked to prevention and control" (75). The main intervention for primary prevention of HBV is vaccination. Since this can only prevent transmission, it is important that new infections are distinguished within HBV surveillance. Secondary prevention is possible through early diagnosis and treatment. An important focus of surveillance of chronic HBV infection should therefore be the monitoring of timely and equal access to care.
Tools used in the surveillance of HBV are case-based reporting, sero-epidemiology, immunogenicity studies, vaccine coverage monitoring, monitoring adverse event following immunisation, molecular epidemiology, phylodynamics and behavioural surveillance.

Outline of this Thesis
This Thesis resulted from a decade of studying the epidemiology of HBV in the Netherlands, England & Wales and at a European level, starting in 2003. The studies were mainly aimed at improving the knowledge of the local epidemiology of HBV and to assess the effectiveness of HBV vaccination

programmes, in order to inform public health policy for prevention and control of HBV and its associated burden of disease and death in the Netherlands and elsewhere. Study methods included sero-epidemiology, case-control analyses, cross-sectional studies, systematic literature review, immunogenicity studies, molecular epidemiology and phylodynamics.

Following a General Introduction of the HBV in Chapter 1, I focus on the Netherlands in Chapter 2. Here included papers are dedicated to studying the prevalence and routes of transmission of HBV (2.1, 2.2) and the effectiveness of three national HBV vaccination programmes: the programme targeting behavioural high-risk groups (2.3), the programme to prevent perinatal transmission (2.4, 2.5) and the programme for children of migrants (2.6-2.8). Chapter 3 focuses on England and Wales, including a study on the incidence and routes of transmission, both overall (3.1) and in a population subgroup at increased risk of infection (3.2). Chapter 4 presents the results of a study commissioned by the European Centre for Disease Control and Prevention (ECDC) regarding the prevalence of HBV/HCV and the cost effectiveness of screening in 34 countries in the European neighbourhood. In the final chapter (Chapter 5) I synthesize the previous by outlining what the Thesis adds to what was already known regarding HBV and discussing its implications for HBV surveillance, prevention, control and research.

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Chapter 2.1

Prevalence of hepatitis B virus infection in The Netherlands in 1996 and 2007

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Prevalence of hepatitis B virus infection in The Netherlands in 1996 and 2007

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SUMMARY

We aimed to assess differences in the prevalence of hepatitis B virus (HBV) infection in The Netherlands between 1996 and 2007, and to identify risk factors for HBV infection in 2007. Representative samples of the Dutch population in 1996 and 2007 were tested for antibodies to hepatitis B core antigen (anti-HBc), hepatitis B surface antigen (HBsAg) and HBV-DNA. In 2007, the weighted anti-HBc prevalence was 3·5% (95% CI 2·2–5·5) and the HBsAg prevalence was 0·2% (95% CI 0·1–0·4). In indigenous Dutch participants, the anti-HBc prevalence was lower in 2007 than in 1996 (P=0·06). First-generation migrants (FGMs) had a 13-fold greater risk of being HBsAg and/or HBV-DNA-positive than indigenous Dutch participants. In indigenous Dutch participants, risk factors for anti-HBc positivity were older age and having received a blood product before 1990. In FGMs, being of Asian origin was a risk factor. In second-generation migrants, having a foreign-born partner and injecting drug use were risk factors. FGMs are the main target group for secondary HBV prevention in The Netherlands.

Key words: Anti-HBc, HBsAg, HBV-DNA, hepatitis B, prevalence, The Netherlands.

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INTRODUCTION

Hepatitis B virus (HBV) infects the liver and can cause chronic infection, resulting in a broad spectrum of disease outcomes including liver cirrhosis, liver carcinoma and death. It is estimated that about 25% of persons who become chronically infected in childhood and 15% of those who become infected later in life die from cirrhosis or liver cancer [1]. Globally, an estimated 360 million people are chronically infected with HBV [2]. The prevalence of HBV in adults varies markedly by country: over 90% of the population in some countries in the Far East have serological markers indicating past or active infection compared to less than 5% in some Western European countries [2, 3].

Even in low-endemic countries such as The Netherlands, HBV prevention and control is a public health priority, particularly since safe and effective vaccines are available. In The Netherlands, HBV vaccination has been recommended since 1983 for high-risk occupations and certain patient groups. In 1989, universal antenatal HBV screening was implemented with passive and active

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immunization of infants born to mothers with chronic HBV infection [4]. In 2002, a national programme was introduced for vaccination of behavioural high-risk groups [including men who have sex with men (MSM), drug users (DUs), prostitutes and heterosexuals with a high rate of partner change]. Since 2003, children of migrant(s) from countries with a moderate or high HBV prevalence have been offered HBV vaccination within the national immunization programme. In autumn 2011, universal infant HBV vaccination was implemented [5, 6].

In addition to primary prevention, recent advancements in the treatment of chronic HBV infection now allow secondary prevention. Currently, hepatitis B screening programmes in The Netherlands target individuals who are most at risk of transmitting HBV, such as blood donors and pregnant women, rather than groups with the highest prevalence of infection [7].

Since new and chronic HBV infections are often asymptomatic, seroepidemiology, which studies serological markers of HBV infection in a population sample, is needed to identify population subgroups with an increased prevalence of infection. In The Netherlands, a nationally representative serological survey was conducted in 1995/1996 and 2006/2007, primarily for the evaluation of the national immunization programme (the ' Pienter ' studies) [8, 9]. With the aim of evaluating and informing national policy on primary and secondary HBV prevention, we used these surveys to assess whether the prevalence of HBV infection in The Netherlands changed between 1996 and 2007, and to identify risk factors for HBV infection in 2007.

MATERIALS AND METHODS

Study population and sample design

In the Pienter studies, cross-sectional samples of the Dutch population aged 0–79 years were taken from municipal registers in 1995/1996 and 2006/2007. For ease of reference, in this article the two surveys will be referred to as having taken place in 1996 and 2007. In each of five geographical regions, eight municipalities were randomly drawn with probability proportional to size. In each of these municipalities, individuals were randomly selected within 17 age groups. Further details of the sampling design can be found elsewhere [9]. In 1996 and 2007, 15 189 and 19 781 individuals were invited, respectively [9], including in 2007 oversampling of the largest migrant groups in The Netherlands. Participants completed a questionnaire and an informed consent form, and visited a clinic for venepuncture. A separate questionnaire was used for participants aged 0–14 years, to be completed by their parents. The questionnaire included questions on demographic characteristics, vaccination history, activities possibly related to infectious diseases and information related to sexually transmissible diseases for 15 to 79-year-olds. Residents born in a foreign non-Western country received a letter of invitation in their own language (Turkish) or a partly translated letter in English, French and Arabic along with a Dutch version. Participants received a gift voucher of €10. People who declined participation were asked to complete a short questionnaire including questions on demographic characteristics, reasons for non-participation, educational degree and general health status. The study proposal was approved by the Medical Ethics Testing Committee of the Foundation of Therapeutic Evaluation of Medicines (METC-STEG) in Almere, The Netherlands (ISRCTN 20164309).

Laboratory methods

Serum was tested for antibodies to HBV core antigen (anti-HBc) using the AxSYM Core assay (Abbott Laboratories, USA). In both surveys the same test was used with identical specifications. Samples positive for anti-HBc were tested for HBV surface antigen (HBsAg) using the AxSYM HBsAg (V2) assay (Abbott Laboratories). Anti-HBc-positive samples taken in the 2007 survey were also tested for presence of HBV-DNA using a S-region-based PCR method with a lower limit of detection of 50 genomic equivalents/ml serum [10]. PCR-positive samples were genotyped on the basis of the S-region sequence. AntiHBs tests were not performed.

Definitions

The Dutch population was defined as individuals registered in municipal registers. Countries of origin were divided in two groups: those with a low prevalence of HBV (HBsAg prevalence <2%) and HBVendemic countries [those with a moderate to high HBV prevalence (HBsAg >2%)] [1]. Indigenous Dutch participants were born in The Netherlands to parents born in The Netherlands. A first-generation

migrant (FGM) was a person born in a HBV-endemic country, of whom at least one parent was born outside The Netherlands. A second-generation migrant (SGM) was a person born in The Netherlands, of whom at least one parent was born in an HBVendemic country. Past or present HBV infection was defined by the presence of anti-HBc. Chronic HBV infection was defined by the presence of anti-HBc and HBsAg and/or HBV-DNA.

Statistical analyses

All analyses took account of the survey design. We estimated the hepatitis B prevalence weighted to the Dutch population, taking into account sex, age group and migrant status. We used 1997 and 2007 population estimates from the National Statistics Office (CBS). For all analyses of anti-HBc results, including prevalence estimates, we excluded children aged <18 months as it can not be excluded that their anti-HBc reflects maternal infection [11].

We assessed differences in the hepatitis B prevalence between 1996 and 2007 by calculating prevalence ratios (PRs). We tested whether these PRs differed significantly from 1 using the Delta method [12]. We assessed determinants for HBV infection only for the 2007 survey data, as in 1996 potentially important determinants such as injecting drug use, having received blood products and partner's country of birth were not ascertained. We used univariable and multivariable Poisson regression to estimate PRs for anti-HBc positivity and HBsAg and/or HBVDNA positivity. Risk factor analyses were performed separately for children (aged <15 years) and older participants, for the overall dataset, as well as stratified by migrant status. Variables with a P value <0·1 in univariable analyses were included in a multivariable model. The effect of migrant status and country of birth was estimated adjusting for age and sex. The effect of other determinants was assessed by a multivariable model, which included age, sex, migrant status and all determinants with a univariable P value >0.1, The final model was selected by removing de-terminants with a P value >0·05, unless they changed the effect parameter of one or more of the remaining variables by >10%. Only determinants included in the final model are included in the tables. We estimated population attributable fractions (PAFs), which represent the estimated proportion of HBV infections that is attributable to a determinant, for determinants that were significantly associated with HBV infection on multivariable analysis. These were derived from the multivariable model by changing the actual individual value for the determinant to that of the reference category [13]. Bootstrapping was used to obtain 95% confidence intervals (CIs) using SAS version 9.2 (SAS Institute Inc., USA) [14]. All other analyses were performed in Stata 10.0 (StataCorp LP, USA).

RESULTS

Information from the questionnaire and an anti-HBc test result were available for 7249 individuals in 1996 and for 6246 individuals in 2007, representing a response of 47·7% and 31·6%, respectively. In both surveys, men, certain age groups and FGMs were less likely to participate. Educational degree was not an independent determinant for non-participation. Those who perceived themselves as less healthy were less likely to participate.

The proportion of Dutch citizens born in an HBV-endemic country increased from 7·2% in 1996 to 8·7% in 2007 (P<0·0001) (source data: CBS).

The overall population

In 1996, of 7249 sera tested, 150 anti-HBc positives were found. For 142 of these an HBsAg result was available, of which six were HBsAg positive. The estimated population prevalence of anti-HBc and HBsAg was 2·9% and 0·1%, respectively.

In 2007, of 6246 sera tested, 211 anti-HBc positives were found. For 203 of these, an HBsAg result was available, of which 14 were HBsAg positive and 11 were HBV-DNA positive. Nine of the 14 HBsAg positives and two of the 189 HBsAg negatives were HBV-DNA positive. For 10 of the 11 HBV-DNApositive samples, the genotype could be determined [A (n=4), B (n=1), D (n=4) and E (n=1)]. The estimated population prevalence of anti-HBc and HBsAg was 3·5% and 0·2%, respectively. The prevalence of HBsAg and/or HBV-DNA was also 0·2%. This corresponds to 39 469 persons with chronic HBV infection (HBsAg and/or HBV-DNA positive) in the Dutch population in 2007 (95% CI 18 572–83 721).

The prevalence of HBsAg and anti-HBc did not statistically differ between 1996 and 2007 (Table 1). In both 1996 and 2007, the prevalence of anti-HBc increased with age until age 45 years (Fig. 1*a*).

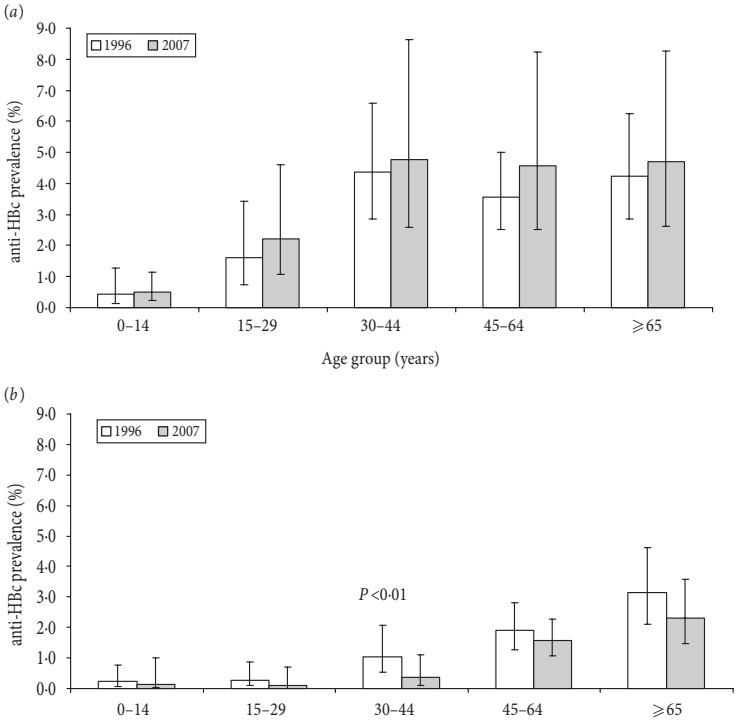

Fig. 1. Prevalence of anti-HBc in the Dutch population by age group, The Netherlands, 1996 and 2007. (a) All participants aged >18 months (1996: N=7015; 2007: N=5930). (b) Indigenous Dutch participants aged >18 months (1996: N=6209; 2007: N=4414).

The 2007 anti-HBc prevalence in FGMs and SGMs was, respectively, 32·7 and 2·9 times higher than in indigenous Dutch participants (P<0·01 and P=0·06, respectively). The HBsAg prevalence in FGMs was 10·4 times higher than in indigenous Dutch participants (P=0·2). The HBsAg and/or HBV DNA prevalence was 1·4% in FGMs. This corresponds to 20 284 FGMs with chronic HBV infection in the Dutch population in 2007 (95% CI 10 399–39 301). This constitutes 51% of the estimated total number of people with chronic HBV infection while only 9% of the population were FGMs. The anti-HBc prevalence in SGMs was much lower than in FGMs (PR 0·09, P<0·01). There were no HBsAg and/or DNA positive SGMs in 1996 or 2007.

Combining data from both surveys, 4% of the anti-HBc positives (10/240 with information on this question) and 20% (3/15) of the HBsAg and/or HBVDNA positives reported they had previously been diagnosed with hepatitis B.

In 2007, independent risk factors for anti-HBc positivity in adults were being a FGM (PAF 70%) or a SGM (4%), having a foreign-born partner (33%) and having received a blood product before 1977 (3%). The PR for being a FGM was more than halved when the variable 'partner born abroad' was added to the model. This suggests that a considerable proportion of HBV infections in FGMs are sexually acquired. For SGMs the PR changed less. Independent risk factors for HBsAg and/or HBV-DNA positivity were being a FGM (PAF 58%) and having received a blood product (31%) (Table 2b).

In 2007, there were 11 anti-HBc-positive and two HBsAg-positive children among the participants. Independent risk factors for anti-HBc positivity in children were being a FGM or a SGM (PAF 59% and 56%, respectively). By adding travel to the model, the PR for migrant status decreased, suggesting part of the increased risk in migrant children is explained by travel to endemic countries (Table 3).

Indigenous Dutch participants

There were 6410 and 4649 indigenous Dutch participants in 1996 and 2007, respectively. There was a borderline significantly lower prevalence of anti-HBc in 2007 compared to 1996 (0·9% and 1·2%, respectively, P=0·06). The difference was largest in the 30–44 years age group where the prevalence decreased from 1·0% to 0·4% (P<0·01) (Table 1, Fig. 1b). When stratified by educational level, the anti-HBc PR between 2007 and 1996 was 0·6, 0·8 and 0·5 in participants with a low, medium and high educational level, respectively (P=0·08, 0·79 and 0·04, respectively). The HBsAg prevalence in indigenous Dutch participants did not differ in 2007 from 1996 (0·1% in both years). The proportion of indigenous Dutch participants with a record of at least three doses of HBV vaccination was higher in 2007 than in 1996 (3·50% and 0·07%, P=0·03).

In 2007, older age and having received a blood product before 1977 or between 1977 and 1990 were the only significant independent risk factors for antiHBc positivity in indigenous Dutch participants (PAF 14% and 6%, respectively) (Table 4). Having received a blood product was the only significant risk factor for being HBsAg and/or HBV DNA positive [adjusted PR 11·7, P=0·01, PAF 46% (95% CI –12 to 100)].

FGMs from endemic countries

There were 240 and 673 FGM participants in 1996 and 2007, respectively. There was no difference between the anti-HBc and HBsAg prevalence in FGMs in 2007 compared to 1996 (Table 1). In 2007, being of Asian origin was a risk factor for anti-HBc positivity in FGMs (PAF 15%). Having a partner born in an endemic country was a borderline significant risk factor (P=0·06) (Table 5). None of the determinants studied was an independent risk factor for HBsAg and/or DNA positivity in FGMs.

SGMs from endemic countries

There were 243 and 495 SGM participants in 1996 and 2007, respectively. There was no difference between the anti-HBc prevalence in SGMs in 2007 compared to 1996 (Table 1). In 2007, independent risk factors for anti-HBc positivity in SGMs were having a foreign-born partner (PR 9·1, P=0·04, PAF 31%, 95% CI-17 to 100) and a history of injecting drug use (PR 32·4, P=0·01). There was only one SGM who reported injecting drug use, and this participant was anti-HBc positive. The PAF could not be estimated due to low numbers.

DISCUSSION

Our analyses of two large, population-based HBV seroprevalence studies showed that the prevalence of HBV infection in the Dutch population did not differ between 1996 and 2007, and remained in 2007 among the lowest worldwide (anti-HBc 3·5%, HBsAg 0·2%) [15–17]. FGMs had a much higher HBV prevalence than the indigenous Dutch participants. Moreover, in SGMs the anti-HBc prevalence in 2007 was higher than in the indigenous population, although their prevalence was much lower than in FGMs. This is the first time the increased risk of hepatitis B in SGMs compared to indigenous Dutch people has been documented [18]. Since 2003, SGMs have been targeted for HBV vaccination within the national immunization programme [19]. As our study was conducted only 4 years later, the impact of this targeted vaccination programme can not yet be assessed.

In indigenous Dutch participants, the prevalence of anti-HBc was lower in 2007 than in 1996 (P=0·06), with the largest and significant difference in 30 to 44 year-olds. It may be argued that this is a biased observation due to a lower representation of high-risk groups in indigenous Dutch participants in 2007 compared to 1996. However, the proportion of participants reporting risk behaviours that may be related to acquisition of HBV (male homosexual contact and a high rate of partner change) was not lower in 2007 compared to 1996. Given also that the laboratory tests used did not differ, the lower anti-HBc prevalence in indigenous Dutch participants in 2007 compared to 1996 probably reflects a genuine difference. The higher HBV vaccination coverage in indigenous Dutch participants in 2007 compared to 1996 is a probable explanation for the reduced anti-HBc prevalence, and may reflect the impact of targeted vaccination programmes such as for travellers and behavioural and occupational high-risk groups. However, the lower prevalence in the more recent survey could also reflect other prevention strategies such as improved screening of blood products. Surveillance of acute hepatitis B infections coupled with phylogenetic analyses and behavioural surveillance will be crucial to monitor and disentangle the impact of different prevention strategies [20].

Table 1. *Prevalence of hepatitis B virus (HBV) infection by age group and migrant status, The Netherlands, 1996 and 2007*
(*a*) Prevalence of past of present HBV infection (*anti-HBc*) in individuals aged ⩾18 months

	1996 ($n=7015$)					2007 ($n=5930$)						
				Population prevalence					Population prevalence		Prevalence ratio Pienter-II/	
Variable	Sample size*	Anti-HBc positive	Crude prevalence %	%	95% CI	Sample size*	Anti-HBc positive	Crude prevalence (%)	%	95% CI	Pienter-I	*P* value†
Overall population	7015	145	2·1	2·9	2·2–3·7	5930	206	3·5	3·5	2·2–5·5	1·2	n.s.
Sex‡												
Male	3293	67	2·0	3·1	2·2–4·2	2674	109	4·1	3·9	2·4–6·0	1·3	n.s.
Female	3710	77	2·1	2·7	1·9–3·6	3256	97	3·0	3·1	1·8–5·3	1·1	n.s.
Age, years												
0–14	1589	5	0·3	0·4	0·1–1·3	1476	11	0·7	0·5	0·2–1·1	1·2	n.s.
15–29	1104	9	0·8	1·6	0·7–3·4	1002	12	1·2	2·2	1·1–4·6	1·4	n.s.
30–44	1357	37	2·7	4·4	2·9–6·6	1021	31	3·0	4·8	2·6–8·6	1·1	n.s.
45–64	1825	51	2·8	3·5	2·5–5·0	1457	92	6·3	4·6	2·5–8·2	1·3	n.s.
⩾65	1140	43	3·8	4·2	2·8–6·2	974	60	6·2	4·7	2·6–8·3	1·1	n.s.
Indigenous Dutch participants	6209	82	1·3	1·2	0·9–1·6	4414	39	0·9	0·9	0·7–1·2	0·7	n.s.
Age, years												
0–14	1348	3	0·2	0·2	0·1–0·8	877	1	0·1	0·1	0·0–1·0	0·6	n.s.
15–29	969	3	0·3	0·3	0·1–0·9	817	1	0·1	0·1	0·0–0·7	0·3	n.s.
30–44	1199	13	1·1	1·0	0·5–2·1	823	3	0·4	0·4	0·1–1·1	0·3	<0·01
45–64	1656	31	1·9	1·9	1·3–2·8	1131	17	1·5	1·6	1·1–2·3	0·8	n.s.
⩾65	1037	32	3·1	3·1	2·1–4·6	766	17	2·2	2·3	1·5–3·6	0·7	n.s.
FGM	238	49	20·6	23·1	18·3–28·9	669	147	22·0	28·7	21·9–36·7	1·2	n.s.
Age, years												
0–14	41	0	0·0	0·0	0–8·6§	258	6	2·3	2·3	1·1–5·1	∞	n.s.‖
15–29	43	5	11·6	14·3	6·4–28·9	49	8	16·3	22·3	11·1–40·0	1·6	n.s.
30–44	68	20	29·4	31·1	21·0–43·4	85	27	31·8	32·6	20·8–47·2	1·1	n.s.
45–64	55	16	29·1	29·2	19·1–42·0	177	66	37·3	32·7	21·0–47·1	1·1	n.s.
⩾65	31	8	25·8	26·1	11·6–48·7	100	40	40·0	40·8	29·0–53·7	1·6	n.s.
Country of birth												
Suriname	24	6	25·0	21·1	10·8–37·3	153	38	24·8	28·7	19·9–39·4	1·4	n.s.
Turkey	36	12	33·3	34·1	22·4–48·1	110	27	24·5	34·8	18·8–55·3	1·0	n.s.
Morocco	32	6	18·8	14·8	7·4–27·3	92	14	15·2	22·1	11·4–38·5	1·5	n.s.
Dutch Antilles and Aruba	14	1	7·1	12·2	1·4–56·9	64	3	4·7	4·6	1·2–15·5	0·4	n.s.
Indonesia	78	13	16·7	17·1	10·7–26·1	55	11	20·0	14·7	7·6–26·4	0·9	n.s.

Table 1 (*cont.*)

(*a*) Prevalence of past of present HBV infection (*anti-HBc*) in individuals aged ⩾18 months

	1996 ($n=7015$)					2007 ($n=5930$)						
Variable	Sample size*	Anti-HBc positive	Crude prevalence %	Population prevalence %	95% CI	Sample size*	Anti-HBc positive	Crude prevalence (%)	Population prevalence %	95% CI	Prevalence ratio Pienter-II/ Pienter-I	*P* value†
SGM	223	2	0·9	0·8	0·2–3·2	429	11	2·6	2·5	1·3–4·7	3·0	n.s.
Age, years												
0–14	135	2	1·5	1·6	0·4–0·6	243	3	1·2	1·7	0·5–5·1	1·0	n.s.
15–29	42	0	0·0	0·0	0·0–8·4§	73	2	2·7	2·4	0·6–9·5	∞	n.s.‖
30–44	28	0	0·0	0·0	0·0–12·3§	58	1	1·7	1·9	0·2–13·3	∞	n.s.‖
45–64	11	0	0·0	0·0	0·0–28·5§	38	4	10·5	7·2	2·1–21·8	∞	n.s.‖
⩾65	7	0	0·0	0·0	0·0–41·0§	17	1	5·9	5·6	1·3–20·6	∞	n.s.‖

(*b*) Prevalence of chronic HBV infection (HBsAg)

	1996 ($n=7241$)					2007 ($n=6238$)						
	Sample size	HBsAg positive¶	Crude prevalence %	Population prevalence %	95% CI	Sample size	HBsAg positive#	Crude prevalence %	Population prevalence %	95% CI	Prevalence ratio Pienter-II/ Pienter-I	*P* value
Overall population	7241	6	0·1	0·1	0·0–0·3	6238	14	0·2	0·2	0·1–0·4	1·5	n.s.
Indigenous Dutch participants	6404	3	0·0	0·1	0·0–0·2	4648	4	0·1	0·1	0·0–0·4	1·8	n.s.
FGM	239	1	0·4	0·7	0·1–5·4	668	9	1·3	1·1	0·4–2·7	1·5	n.s.
SGM	242	0	0·0	0·0	0·0–1·5§	493	0	0·0	0·0	0·0–0·7	—	—

FGM, First-generation migrant; SGM, second-generation migrant; CI, Confidence interval; n.s., not significant.

* This excludes infants aged <18 months (234 in 1996 and 316 in 2007).

† Determined by the Delta method.

‡ For 12 participants in 1996 the sex was unknown.

§ Estimated with the exact method.

‖ Estimated with Fisher's exact test.

¶ For two HBsAg-positive individuals migrant status could not be classified [country of birth missing ($n=1$), born in India to Dutch parents ($n=1$)].

\# For one HBsAg-positive individual migrant status could not be classified (no information on country of birth of the mother).

Table 2. *Prevalence rate ratios (PRs) and 95% confidence intervals (CIs) for determinants of hepatitis B virus infection, 2007*

(*a*) All participants aged ⩾15 years (*N*=4454), determinants for anti-HBc positivity

		Anti-HBc positive								PAF	
Determinants	*N*	*n*	%	PR	*P* value	aPR	*P* value	aPR	*P* value	%	95% CI
Gender											
Male	1931	101	5·2	Ref.		Ref.		Ref.			
Female	2523	94	3·7	0·7	0·02	0·8	n.s.	0·7	n.s.		
Age group (yr)											
15–29	1002	12	1·2	Ref.		Ref.		Ref.			
30–44	1021	31	3·0	2·4	0·02	2·0	0·05	1·3	n.s.		
45–59	1044	68	6·5	4·4	<0·01	3·2	<0·001	2·3	0·05		
60–79	1387	84	6·1	4·0	<0·01	3·4	<0·001	1·8	n.s.		
Migrant status											
Indigenous Dutch participants	3537	38	1·1	Ref.		Ref.		Ref.			
FGM	411	141	34·3	31·9	<0·001	29·3	<0·001	13·2	<0·001	70	43–82
SGM	186	8	4·3	4·0	<0·001	5·0	<0·001	3·9	<0·01	4	0–11
County of birth of partner											
The Netherlands or low endemic country	3088	48	1·6	Ref.				Ref.			
Medium or high endemic country	267	77	28·8	17·2	<0·001			2·5	<0·01	33	8–55
Received transfusion of blood products											
No	3430	132	3·8	Ref.				Ref.			
In The Netherlands before 1977	93	11	11·8	3·4	<0·001			3·9	<0·01	3	0–10
In The Netherlands after/in 1977, before 1990	110	5	4·5	1·4	n.s.			1·4	n.s.		
In The Netherlands after/in 1990	272	10	3·7	0·9				1·5	n.s.		
Abroad	23	6	26·1	5·1	<0·01			2·4	n.s.		

(*b*) All participants aged ⩾15 years (*N*=4454), determinants for HBsAg and/or HBV-DNA positivity

		HBsAg and/or HBV-DNA positive								PAF	
Determinant	*N*	*n*	%	PR	*P* value	aPR	*P* value	aPR	*P* value	%	95% CI
Gender											
Male	1928	7	0·4	Ref.		Ref.		Ref.			
Female	2518	7	0·3	0·7	n.s.	0·8	0·64	0·5	n.s.		
Age group (yr)*				1·0	n.s.	1·0	0·34	1·0	n.s.		
15–29	1002	0	0·0								
30–44	1020	3	0·3								
45–59	1041	5	0·5								
60–79	1383	6	0·4								
Migrant status											
Indigenous Dutch participants	3536	5	0·1	Ref.		Ref.					
FGM	406	9	2·2	14·3	<0·001	13·4	<0·001	1·9	n.s.	58	−5 to 100
SGM	184	0	0·0	<0·01	n.s.	<0·01	n.s.	<0·01	n.s.		
County of birth of partner											
The Netherlands or low endemic country	3085	5	0·2	Ref.				Ref.			
Medium or high endemic country	265	4	1·5	8·9	<0·01			2·7	n.s.		
Received transfusion of blood products											
No	3424	7	0·2	Ref.				Ref.			
Yes	526	4	0·8	4·1	0·05			5·8	0·03	31%	−29 to 100

Ref., Reference category; n.s., not significant; aPR, adjusted prevalence ratio; PAF, population attributable fraction; FGM, first-generation migrant; SGM, second-generation migrant.

Determinants that were significant only in univariable analyses were for anti-HBc: sexual preference, travel to Asia, travel to Central or South America, net monthly income and educational level; for HBsAg and/or DNA none of the determinants was only significant in univariable analyses.

* Age included as a continuous variable since effects for grouped estimates could not be calculated.

Table 3. *Prevalence rate ratios (PRs) and 95% confidence intervals (CIs) for determinants of hepatitis B virus infection (anti-HBc positivity) in children aged <15 years, 2007 (N=1476)*

		Anti-HBc positive								PAF	
Determinant	*N*	*n*	%	PR	*P* value	aPR	*P* value	aPR	*P* value	%	95% CI
Gender											
Male	743	8	1·1	Ref.		Ref.					
Female	733	3	0·4	0·3	n.s.	0·4	n.s.				
Age group (yr)											
1–4	434	2	0·5	Ref.		Ref.					
5–9	612	6	1·0	0·5	n.s.	0·6	n.s.				
10–14	430	3	0·7	1·0	n.s.	0·7	n.s.				
Migrant status											
Indigenous Dutch participants	877	1	0·1	Ref.		Ref.		Ref.			
FGM	258	6	2·3	21·3	<0·01	23·2	<0·01	16·9	0·01	59	−2 to 100
SGM	243	3	1·2	10·8	0·04	11·6	0·03	8·1	n.s.	56	−16 to 100
Travel outside Europe											
Not to Africa	1300	8	0·6	Ref.				Ref.			
To Africa	152	3	2·0	2·3	n.s.			2·2	n.s.		
Not to Asia	1226	7	0·6	Ref.				Ref.			
To Asia	226	4	1·8	3·2	n.s.			2·7	n.s.		

Ref., Reference category; n.s., not significant; aPR, adjusted prevalence ratio; PAF, population attributable fraction; FGM, first-generation migrant; SGM, second-generation migrant.

In indigenous Dutch participants, no current independent risk factor was identified. However, blood transfusion before 1990 was a significant risk for being HBsAgand/or anti-HBc-positive, with the highest risk when transfused before 1977. The actual question in the 2007 questionnaire asked about the most recent year a blood transfusion was received. The number of transfusions and first year of transfusion were not ascertained. It is therefore not possible to establish the exact period associated with an increased risk of hepatitis B.

In FGMs and SGMs, having a partner from an endemic country was an important risk factor, consistent with our earlier work [21]. It suggests that a considerable proportion of FGMs and SGMs resident in The Netherlands acquire HBV through sexual contact. This conclusion is further supported by the observation that in FGMs and SGMs only 5% of the anti-HBc positives were HBsAg positive (10/209, Table 1), suggesting acquisition of infection took place during or after adolescence [22]. Current vaccination for FGMs and SGMs is only targeted at children. Our results suggest an assessment of the need for catch-up vaccination of older FGMs and SGMs may be required.

The Pienter studies are the only source of information on the prevalence of HBV infection in the general Dutch population. Marschall et al. estimated the general population HBsAg prevalence in The Netherlands as between 0·4% and 0·6%, based on the 1996 Pienter survey data with an adjustment for underrepresentation of high-risk groups including migrants [23]. This estimate is considerably higher than our current weighted estimate for 1996 of 0·1%. A likely explanation is that Marschall et al. assumed an HBsAg prevalence of 3·8% in FGMs, whereas in our 1996 and 2007 data we estimated this as around 1%. In 2004, the HBsAg prevalence in FGMs in Amsterdam ranged from 0·6% to 4·8% in a survey where only adults were included [18].

The main limitation of our study is the relatively low response, particularly in the 2007 survey. Underrepresentation of males and certain age groups and migrant groups was adjusted for by weighting prevalence estimates. However, our HBV prevalence estimates probably underestimate the true population prevalence, as high-risk groups for HBV such as undocumented migrants and injecting drug users are likely to be under-represented. Non-response analyses indicated that those who perceived their health status as relatively unfavourable were less likely to participate. However, among participants, this was not an independent risk factor for being anti-HBc positive. Conversely, our estimate of the population antiHBc prevalence could be somewhat

Table 4. *Prevalence rate ratios (PRs) and 95% confidence intervals (CIs) for determinants of hepatitis B virus infection (anti-HBc) in indigenous Dutch participants ⩾15 years of age, 2007 (N=3537)*

		Anti-HBc positive						PAF	
Determinant	*N*	*n*	%	PR	*P* value	aPR	*P* value	%	95% CI
Gender									
Male	1526	16	1·0	Ref.		Ref.			
Female	2011	22	1·1	1·0	n.s.	1·1	n.s.		
Age group (yr)									
15–29	817	1	0·1	Ref.		Ref.			
30–44	823	3	0·4	2·9	n.s.	2·5	n.s.		
45–59	811	13	1·6	12·6	0·01	9·6	0·02		
60–79	1086	21	1·9	15·1	0·01	10·2	0·02		
Received transfusion of blood products									
No	2749	21	0·8	Ref.		Ref.			
In The Netherlands before 1977	79	7	8·9	11·4	<0·001	7·0	<0·001	14%	0 to 33
In The Netherlands after/in 1977, before 1990	94	4	4·3	5·5	<0·01	3·5	0·02	6%	−4 to 25
In The Netherlands after/in 1990	228	4	1·8	2·3	n.s.	1·7	n.s.		
Abroad	7	0	0·0	<0·1	n.s.	<0·01	n.s.		

Ref., Reference category; n.s., not significant; aPR, adjusted prevalence ratio; PAF, population attributable fraction.
Travel to Africa was only significant in univariable analysis.

Table 5. *Prevalence rate ratios (PRs) and 95% confidence intervals (CIs) for determinants of hepatitis B virus infection (anti-HBc) in first generation migrants (FGMs) aged ⩾15 years, 2007 (N=411)*

		Anti-HBc positive								PAF	
	N	*n*	%	PR	*P* value	aPR	*P* value	aPR	*P* value	%	95% CI
Gender											
Male	174	76	43·7	Ref.		Ref.		Ref.			
Female	237	65	27·4	0·6	<0·01	0·7	0·03	0·7	n.s.		
Age group (yr)											
15–29	49	8	16·3	Ref.		Ref.		Ref.			
30–44	85	27	31·8	2·0	n.s.	2·0	n.s.	1·3	n.s.		
45–59	130	48	36·9	2·1	n.s.	1·8	n.s.	1·4	n.s.		
60–79	147	58	39·5	2·1	n.s.	1·9	n.s.	1·1	n.s.		
County of birth											
Other	169	44	26·0	Ref.		Ref.		Ref.			
Asia	167	68	40·7	1·9	0·01	1·6	0·03	1·9	n.s.*	15	−4 to 52
Africa	75	29	38·7	1·6	n.s.	1·5	n.s.	1·8	n.s.		
County of birth of partner											
The Netherlands or low endemic country	83	12	14·5	Ref.				Ref.			
Medium or high endemic country	182	74	40·7	2·4	<0·01			1·9	n.s.*		

Ref., Reference category; n.s., not significant; aPR, adjusted prevalence ratio; PAF, population attributable fraction.
Determinants that were significant only in univariable analyses were: having a net income <€1750 per month, having male homosexual contact and having a low educational level.
* $p=0{\cdot}06$.

overestimated due to the relatively low positive predictive value of a positive anti-HBc test due to non-specific reactivity [24]. This affects low-risk populations more than those with a high prevalence. Indeed, in our 2007 sample, indigenous Dutch participants had more quantitative anti-HBc values close to the cut-off than FGMs (data not shown). Since this bias may have led to an underestimation of the difference in anti-HBc prevalence between FGMs and indigenous Dutch participants, it is unlikely to have affected our conclusions.

Last, a limitation of our study is that despite

oversampling of migrants, the power of our study to identify risk factors for chronic HBV infection was limited as only 16 chronically infected individuals were found in 2007.

In summary, our study has confirmed that The Netherlands remains a very low-prevalence country for HBV, despite increases in the proportion of the population born in endemic countries. We identified FGMs as the most important high-risk group, accounting for 70% of prevalent infections. Hepatitis B screening and treatment of Dutch FGMs was recently deemed a cost-effective intervention to prevent morbidity and mortality from sequelae of chronic hepatitis B [25]. Further work is urgently needed to collate the evidence for screening programmes so that policy-making on secondary hepatitis B prevention can proceed.

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DECLARATION OF INTEREST

None.

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Chapter 2.2

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

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

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SUMMARY

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 (o8% 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

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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 o3 for a risk factor with a prevalence of 10%, with an a 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.

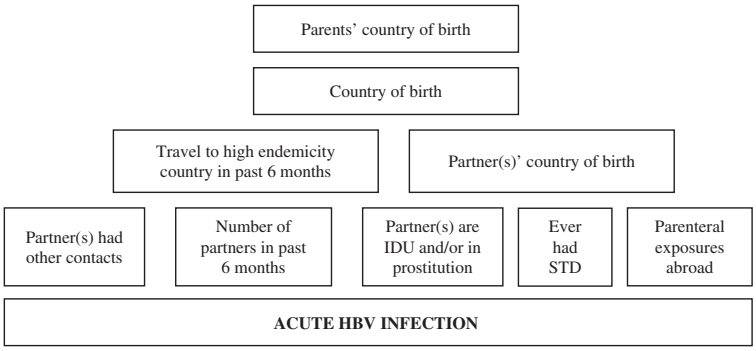

Fig. 1. Conceptual hierarchical framework for risk factors for acquisition of HBV infection among adult females and adult heterosexual men in The Netherlands.

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 EpiInfo (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

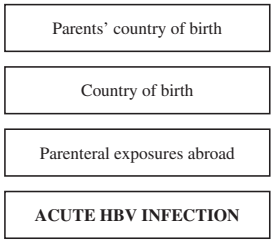

Fig. 2. Conceptual hierarchical framework for risk factors for acquisition of HBV infection among individuals aged <18 years in The Netherlands.

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

	Cases		Controls				
	n	%	*n*	%	OR*	95% CI	*P* value*
Sex							
Females	27	*22·5*	1338	*33·9*	ref.		
Males	93	*77·5*	2610	*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 male	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 years among controls.

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.

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

Table 2(*a*). *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				
	n	%	*n*	%	OR*	95% CI*	*P* value*
Demographic, socioeconomic determinants and travel							
Sex							
Females	21	*35·6*	1143	*42·2*	ref.		
Males	38	*64·4*	1566	*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*	1367	*50·5*	0·4	0·2–0·9	0·02
⩾40	26	*44·1*	1057	*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*	2497	*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							
The Netherlands or low endemicity country	49	*86·0*	2577	*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*	1919	*71·7*	1·3	0·7–2·2	0·43
Occupation in health care							
No	40	*88·9*	1689	*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*	2536	*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*	2076	*77·5*	ref.		
Yes, to medium or high endemicity country	17	*29·8*	601	*22·5*	1·6	0·6–2·9	0·09
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*	2367	*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 endemic country	35	*64·8*	434	*91·6*	ref.		
At least one in mid or high endemic country	19	*35·2*	40	*8·4*	12·1	5·8–24·9	0·00

Table 2(*a*) (*cont.*)

	Cases		Controls				
	n	%	*n*	%	OR*	95% CI*	*P* value*
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.

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.

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

Table 2(*b*). *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*
Age group (yr)								
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 in past 6 months								
0–1							ref.	
2							4·1	*0·04*
⩾3							2·7	*0·30*
Partner other sexual contacts								
No							ref.	
Yes							1·7	*0·38*
Partner IDU or commercial sex worker								
No							ref.	
Yes							1·5	*0·69*
Ever had sexually transmitted disease								
No							ref.	
Yes							3·3	*0·13*
Any parenteral exposures								
None, or missing							ref.	
In The Netherlands or in low endemic country							0·7	*0·41*
In medium or high endemic country							2·5	*0·28*

Models 1 and 2 included 57 cases and 2707 controls. Models 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.

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 univariate 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 acqui-

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				
	n	%	*n*	%	OR*	95% CI*	*P* value*
Demographic, socioeconomic determinants and travel							
Median age (yr) (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.		
Medium or high endemicity country	5	*13·2*	2	*7·1*	1·8	0·3–10·5	0·55
Country of birth							
The 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 exposures							
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
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 years among controls.

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

	Cases		Controls				
	n	%	*n*	%	OR*	95% CI*	*P* value*
Demographic characteristics and travel							
Gender							
Female	6	*66·7*	195	*25·2*	ref.		
Male	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.		
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 endemic country	8	*88·9*	671	*87·4*	ref.		
Yes, to medium or high endemic 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 other 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 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.

sition 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.

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 nonresponders [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, 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

Table 4(*b*). *Cases of acute HBV infection in 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*
Age group (yr)						
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	ref.		ref.		ref.	
Medium or high endemicity	7·7	*0·00*	7·2	*0·01*	5·0	*0·06*
Country of birth						
The Netherlands or low endemicity			ref.		ref.	
Medium or high endemicity			1·5	*0·77*	1·6	*0·74*
Any parenteral exposures						
None, or missing					ref.	
In The Netherlands or low endemicity country					0·7	*0·67*
In medium or high endemicity country					7·1	*0·19*

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

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.

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 ex-

cluding 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 health-care 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 highrisk 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 costeffectiveness of such screening programmes.

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DECLARATION OF INTEREST

None.

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Chapter 2.3

Selective vaccination has reduced hepatitis-B-virus transmission in the Netherlands: results of molecular epidemiological surveillance from 2004 to 2010

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Selective vaccination has reduced hepatitis-B-virus transmission in the Netherlands: results of molecular epidemiological surveillance from 2004 to 2010

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ABSTRACT

BACKGROUND In the Netherlands, a selective hepatitis-B-virus (HBV) vaccination programme commenced in 2002, initially targeting men having sex with men (MSM), drug users, commercial sex workers and heterosexuals who change partners frequently. We used surveillance data and molecular typing to assess the programme's effectiveness.

METHODS We analysed acute HBV cases reported in the Netherlands between 2004 and 2010 requesting serum samples from all patients for HBV-genome S-region sequencing, and, for some, C region sequencing. We used coalescence analyses to assess temporal changes in genetic diversity of nonimported genotype-A cases.

RESULTS 1687 patients with acute HBV infection were reported between 2004 and 2010. The incidence of reported acute HBV infection decreased from 1.8 to 1.2 per 100,000 inhabitants in this period, mostly due to a reduction in the number of cases in MSM. Men were overrepresented among cases with an unknown route of transmission, especially among genotype A2 cases mainly associated with transmission through male homosexual contact. The genetic diversity of nonimported genotype-A strains obtained from MSM decreased from 2006 onwards, suggesting the HBV incidence among MSM decreased.

CONCLUSIONS The selective HBV-vaccination programme for behavioural high-risk groups reduced the incidence of acute HBV infection in the Netherlands mainly by preventing HBV infections in MSM. A considerable proportion of cases in men who did not report risk behaviour was probably acquired through homosexual contact. Our findings support continuation of the Dutch programme, and adopting similar approaches in other countries where HBV transmission is focused in high-risk adults.

INTRODUCTION

Hepatitis B virus (HBV) is a major cause of liver disease and death primarily resulting from sequelae of chronic HBV infection including liver cancer and cirrhosis. The HBV is transmitted perinatally and through sexual and parenteral contact. An estimated 400 million people worldwide are infected with HBV, accounting for an estimated one million deaths annually [1, 2]. A safe and effective vaccine against HBV has been available since 1982 and is

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included in universal vaccination programmes in more than 170 countries [3].
The estimated prevalence of chronic HBV infection in the Dutch population of about 17 million inhabitants is 0.2%, which is among the lowest reported prevalences worldwide [4, 5]. Universal infant HBV vaccination was introduced in the Dutch national immunisation programme in August 2011 after an assessment by the Dutch Health Council that included a favourable cost-effectiveness analysis [6]. In addition to the recent introduction of universal vaccination, the Netherlands has a selective vaccination programme that provides HBV vaccination free of charge for population subgroups at increased risk of HBV due to risk behaviour, including men having sex with men (MSM), drug users, commercial sex workers (CSWs) and heterosexuals who change partners frequently. After a pilot programme in seven regions between 1998 and 2000, the permanent programme was implemented nationally from 2002 onwards. From 2007 onwards, heterosexuals with multiple partners were excluded from the target population as there was insufficient evidence of an increased risk in this group. From 2012 onwards, drug users were excluded for the same reason. Vaccination of drug users is now provided through drug treatment services.
Selective HBV vaccination of population subgroups at increased risk of HBV infection is recommended in all European countries and the USA [7, 8]. Irrespective of universal vaccination, programmes targeting behavioural high-risk groups will be needed for the coming decades, depending on the coverage achieved and the age groups targeted for universal vaccination. Selective programmes have, however, frequently been criticised as ineffective since the reported vaccine coverage achieved in the target populations was considered too low [9-12]. The impact on HBV transmission of selective vaccination programmes targeting behavioural high-risk groups has never been demonstrated on a national level.
Traditional epidemiological methods have several limitations in assessing the impact of selective vaccination. Vaccine coverage in the target populations is an obvious but problematic indicator, as estimates of the size of these hidden populations are very uncertain [13, 14]. Routine surveillance based on the reporting of acute HBV infection is incomplete, as less than half the cases of acute HBV infection among adults are symptomatic [15], not all patients with symptoms seek healthcare, and health care professionals do not manage to report all cases, reporting variably over time. Furthermore, for about a quarter of the cases, the route of acquisition of the virus remains unknown despite patient interviews [16]. Lastly, it is difficult to draw conclusions about the impact of vaccination since its effects can be counterbalanced by increased risk behaviour. Molecular epidemiological and phylodynamic methods take the genetic variability of a pathogen and its evolutionary processes into account, and as a result, low reporting quality and sampling intensity have less effect. These methods are therefore complementary to report-based surveillance in providing insight into the transmission dynamics of HBV [17].
A preliminary assessment of the selective vaccination programme in the Netherlands in 2007 showed that its effectiveness to reduce HBV transmission was limited [13]. Using a more complete dataset and applying additional analytical methods, we re-assessed the effectiveness of this programme to guide its implementation and to inform prevention and control of HBV in other countries where transmission is concentrated in risk groups.

METHODS

Study population

We studied all reported cases of acute HBV infection in the Dutch population between 2004 and 2010. Cases of acute HBV infection are statutorily notifiable in the Netherlands. The case definition for reporting was a positive laboratory result for immunoglobulin M antibody to the hepatitis B core antigen (IgM anti-HBc) and/or hepatitis B surface antigen (HBsAg; the latter only after exclusion of hepatitis A and C viruses) in a person with an acute onset of symptoms compatible with acute hepatitis and jaundice and/or increased serum aminotransferase. Following reporting of cases by clinicians and laboratories, public health nurses interviewed patients with acute HBV infection to ascertain risk exposures and possible source(s) of infection. On the basis of the interview, the most probable mode of transmission, together with other patient data, was registered in an anonymous, on-line, national database. In the analyses, we distinguished the following transmission modes: heterosexual contact, male homosexual contact, injecting drug use (IDU), and 'other' modes of transmission (including parenteral procedures abroad, needle stick accidents, and multiple probable routes of transmission). If it was unknown

whether the sexual contact was heterosexual or homosexual, the case was included in the unknown transmission route group. From 2004 onwards, we asked medical microbiology laboratories to submit serum samples from all patients with a reported case of acute HBV to one of the three participating laboratories [the National Institute for Public Health and the Environment (RIVM), the Public Health Laboratory Amsterdam, and the Erasmus Medical Centre in Rotterdam]. All laboratories isolated HBV DNA and a 648-nucleotide (nt) fragment of the S region. The RIVM only amplified and sequenced a 655 nt fragment of the C region as previously described [18, 19]. We described cases with the age and sex of the patient and the probable mode of transmission, and we studied trends over time. Differences and trends in proportions were assessed for statistical significance using the chi-square test and Pearson's correlation, respectively. We assessed differences in continuous variables using the Wilcoxon rank sum test. Stata version 11.0 was used for these analyses (StataCorp, Texas).

Genotypes and genosubtypes were assigned to cases by entering their S-region sequences in a typing tool that uses reference strains as described by Norder et al. [20, 21]. We assessed clustering of strains by aligning sequences with BioEdit 7.0.9.0 [22], removing relatively short sequences. We used BioNumerics version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium) to construct a maximum parsimony tree based on the S-region sequence. We selected a subgroup of cases for which both the Sand C-region sequences were available; these cases were of genotype A and were reportedly acquired in the Netherlands (i.e. nonimported). We distinguished two groups in this subset: MSM cases and cases acquired by heterosexual contact. We studied temporal changes of the effective population size of HBV causing acute infections in each of these groups by constructing Bayesian skyride plots in BEAST version 1.7.1 [23]. Hereby we assumed a general-time-reversible substitution model, a γ-shaped site heterogeneity model, and a relaxed molecular clock with a lognormal distribution. We studied the statistical support of any observed changes in the skyride plots over time by comparing its median posterior likelihood (quantified by its Bayes factor) with likelihoods from analyses assuming a constant population size. We considered differences greater than three in Bayes factors to be significant. All sequences used in our analysis were deposited in GenBank (accession numbers: to be completed once received from GenBank). Ethical approval was sought but deemed not necessary by the ethics committee of the University of Amsterdam.

Role of the funding source

The Netherlands Organisation for Health Research and Development (ZonMW) sponsored the study (research grant 125010004). This sponsor had no role in the study design, collection, analyses or interpretation of data, writing of the report or the decision to submit the paper for publication.

RESULTS

Vaccination programme

Regional Public Health Services implement the selective vaccination programme for behavioural high-risk groups [24], which the Centre for Infectious Disease Control of the RIVM centrally coordinates. Vaccinations are given at diverse locations, including public health premises, in prisons, drug service locations, and outreach locations, including gay men's saunas and CSW locations (Table 1). Serum is drawn from each participant for HBV serology (anti-HBc-IgG, and if positive, HBsAg) at the first vaccination visit. Anti-HBc-positive individuals do not receive subsequent vaccinations, while HBsAg-positive individuals are referred to routine clinical care. All vaccinations and serological test results are registered in an on-line, national database. From 1998 to 2011, about 105,000 individuals received at least one HBV vaccination within the programme. Of these, about one-third were MSM. On average, 19% of the MSM received their vaccinations in outreach locations. Nearly three-quarters of the HBV-susceptible MSM completed the series of three doses; 0·6% of the MSM were found to be chronically infected (Table 1).

Reports of acute HBV infection

Between January 2004 and the end of December 2010, 1696 cases of acute HBV infection were reported in the Netherlands. We excluded nine cases because the gender of the patients was not reported, leaving 1687 patients, of whom 1320 (78·3%) were men. Male cases were older than female cases (median age 40 and 29 years, respectively; $p<0{\cdot}001$). Six patients were reported to have died due to the HBV infection (0·4% of 1676 cases with information about survival), and 344 patients (21%) were admit-

Table 1. Characteristics of the selective HBV vaccination programme for behavioural high-risk groups, The Netherlands, 1998–2010

	Men who have sex with men	Heterosexuals with frequent partner change†	Drug users	Commercial sex workers
Number receiving first dose	32,746	40,717	17,127	14,518
Vaccination locations‡				
Public Health Service (%)	51·4	39·1	5·3	23·5
STD clinic (%)	27·4	29·8	2·5	10·7
Outreach§ (%)	19·0	9·0	10·2	59·9
Drug services (%)	0·3	0·7	57·5	0·5
Prison (%)	0·7	21·0	24·4	5·3
General Practice (%)	1·3	0·4	0·1	0·2
Prevalence¶				
anti-HBc prevalence (%) [95% CI]	11·3 [11.0–11·7]	5.4 [5·1–5·6]	14·5 [13·9–15·0]	16·0 [15·4–16·7]
HBsAg prevalence (%) [95% CI]	0·6 [0·6–0·7]	0·6 [0.5–0·7]	0·8 [0·7–1·0]	1·2 [1·0–1·4]
Compliance (%)‖	73·7	60·2	58·0	50·7

Anti-HBc=antibody to the hepatitis B core antigen; CI=Confidence interval; HBsAg= hepatitis B surface antigen; STD=sexually transmitted disease

† Heterosexuals with frequent partner changes were no longer included in the programme from 2007 onwards

‡ Proportion of the first vaccinations given at different locations, in percentages. Note: information about the vaccine location was missing for 3856 vaccinations

§ Outreach locations included bars and saunas frequented by MSM, shelters for homeless people, and commercial sex worker locations

¶ Prevalence was calculated by dividing the number of patients with a positive test by the total number with a test result

‖ Compliance is defined as the proportion of those susceptible at the first vaccination completing three doses. Data up to 30 June 2011 were included

ted to hospital. The incidence of reports declined from 1.8 to 1.2 per 100,000 population between 2004 and 2010, mainly because of the declining incidence among men from 3.1 to 1.9 per 100,000. For women, the incidence remained constant at around 0.7 per 100,000. Of all patients, 78% were reported to have acquired the infection in the Netherlands. Male homosexual contact was the most frequently reported route of transmission (32% of the cases; Figure 1). Male patients with acute HBV infection more frequently reported an unknown route of transmission than female patients (20% and 5%, respectively, $p<0.001$).

Most of the decrease in the number of acute HBV reports could be attributed to a declining number of reports for MSM (Figure 1). The number of infected men with an unknown mode of transmission also diminished from about 70 annually in 2004 and 2005 to about 45 annually from 2007 onwards. The number of cases attributed to male heterosexual contact increased slightly from 2007 onwards, while there was a small decline in the number of cases in women. There were only seven cases in injecting drug users (IDUs), three of which were reported in 2004. Relative to all cases reported by year, the proportion for MSM showed a decreasing trend between 2004 and 2010, while the proportion of male cases attributed to heterosexual contact increased (both $p<0.01$).

Genotype analyses

S-region sequences were available for 902 of the 1687 reports (53%). For four additional cases, information about the genotype, but not the genosubtype, was available. The proportion of reports for which information about genotype was available varied by Public Health Service (ranging from 22% to 100%; $p<0.001$) and was lower for patients born abroad than for those born in the Netherlands (46% and 56%, respectively; $p<0.01$). The presence of genotype information did not vary by most probable mode of transmission, gender, or year of report ($p>0.1$). Genotype A was the most frequent genotype among the cases of MSM, for men and women with heterosexually acquired HBV, and cases with an unknown route of transmission (Figure 2a-c). Genotype D was the second most frequent genotype for all three of these subgroups, although it only accounted for a small proportion of cases among MSM. Among heterosexually acquired cases, the proportion of cases with genotype A increased over time until 2009,

while the proportion that was genotype D decreased (both: p for trend ≤0.05). Among cases with an unknown route of transmission, the proportion with genotype A also increased (p=0.03). The genotype distribution did not change among MSM. Men were over-represented among genotype A cases acquired by heterosexual or unknown routes of transmission (55% and 88%, respectively).
Genosubtype A2 was by far the most prevalent (94%) among genotype A cases. Its proportion varied by risk group, ranging from all cases in IDUs (2 cases) and 98% in MSM (261 cases) to 79% in women heterosexuals (54 cases; p<0.001). Genotype D1 was the most prevalent (66%) among genotype-D cases. This proportion did not differ by risk group (p=0.4). The genotype-D3 strain that used to be associated with IDUs in the Netherlands was no longer detected [25]. Phylogenetic analysis demonstrated a large cluster (353 cases) of indistinguishable strains within genotype A2 (Figure 3). Nearly half (48%) of the cases with this clonal strain concerned MSM; 13%, heterosexual men; and 9%, heterosexual women. Cases with this strain who did not report a route of transmission were 11 times more likely to be male than female (p<0.0001). The clonal strain was indistinguishable from a strain reported in the USA, the UK, and Japan [26-28]. For heterosexuals and those with an unknown route of transmission, a significantly increasing trend appeared in the proportion of cases carrying this clonal strain (p<0.01). There was no trend in the occurrence of this clonal strain among MSM.

Coalescent analyses of genotype-A cases acquired in the Netherlands

Both S-region and C-region sequences were available for 283 genotype-A cases acquired in the Netherlands. Of these, 121 were in MSM and 86 were heterosexually acquired HBV infections. All except three of the smaller Public Health Services reported the cases in this selection. The skyride plot of strains in cases of MSM showed a small increase in effective population size (i.e. genetic diversity) starting in 2000, followed by a large decrease from 2007 onwards. The plot for heterosexuals showed a small decrease starting from 2006 onwards (Figure 4a,b). Comparing the significance of the observed changes over time by comparing Bayes factors of the skyride analyses with those assuming a constant population size showed that only the changes in the plot for MSM were significant.

DISCUSSION

Our analyses of the largest molecular epidemiological study of acute HBV infection ever suggests that the Dutch selective vaccination programme for behavioural high-risk groups reduced transmission of HBV in the Netherlands, primarily by reducing the HBV incidence among MSM. This is the first time effectiveness of such a programme has been demonstrated. The incidence of acute HBV reports diminished by about one-third in this period. Most of this decline was attributed to a reduction in the number of cases in MSM. This coincided with a marked decrease in the genetic diversity of HBVs sampled from MSM, suggesting that the incidence in this group had genuinely diminished. Taken together with the large number of MSM vaccinated against HBV since the start of the programme, we conclude that this programme has effectively reduced transmission of HBV among MSM. It does remain uncertain, however, which part of the observed decline in HBV transmission among MSM is due to direct or indirect (herd immunity) effects of the vaccination programme. It is possible that treatment of chronically infected MSM identified through the programme also contributed to preventing new infections.
Our results and conclusions contrast with an earlier evaluation of the Dutch selective HBV vaccination programme [13], probably because new methods were used to analyse more years of data. An evaluation of the programme in Amsterdam with data up to 2006 indicated that the programme was effective in preventing transmission among MSM, an effect that may not have been visible in case-based surveillance because it coincided with an increase in risk behaviour [17]. Our findings are important since selective vaccination programmes will need to continue for several decades, irrespective of universal HBV vaccination. Extending universal vaccination to adult populations as suggested in Japan is unlikely to be cost-effective [29].
The conclusion that the selective vaccination programme rather than possible reductions in risk behaviour reduced HBV transmission among MSM is corroborated by observations from surveillance data regarding other sexually transmitted infections and monitoring of risk behaviour among MSM. The annual number of reports of MSM with gonorrhoea doubled between 2004 and 2010 [30, 31]. Risk behaviour among MSM in the Amsterdam Cohort Studies steeply increased during the second half of the 1990s and early 2000s, then levelled off during our study period [32].

Figure 1. Number of cases of acute HBV infection by most probable mode of transmission and year of report, the Netherlands, 2004–2010 (n=1687).

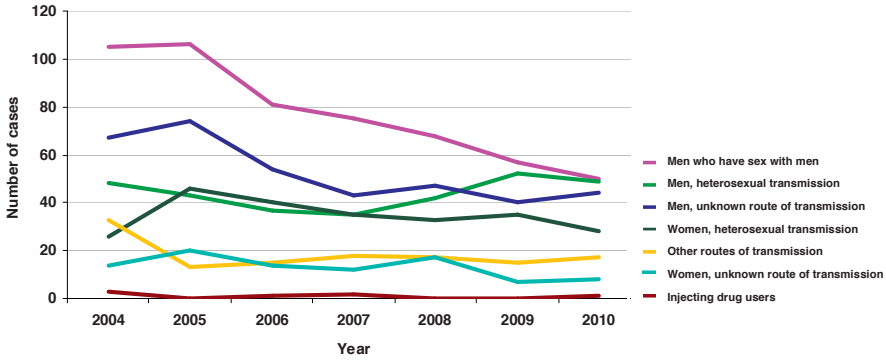
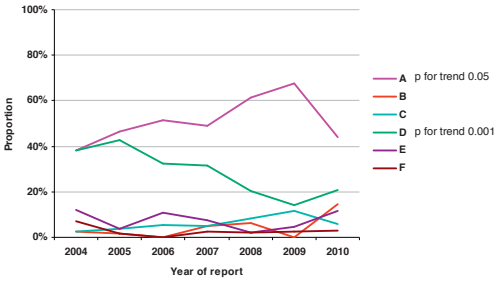
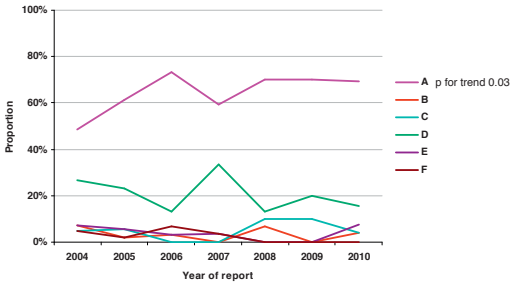


Figure 2a-c. Genotype distribution by year of reporting of acute HBV infections, the Netherlands, 2004-2010

a. Cases acquired heterosexually by men and women (n=300)



b. Cases with an unknown route of transmission acquired by men and women (n=226)



c. Cases acquired by men who have sex with men (n=305)

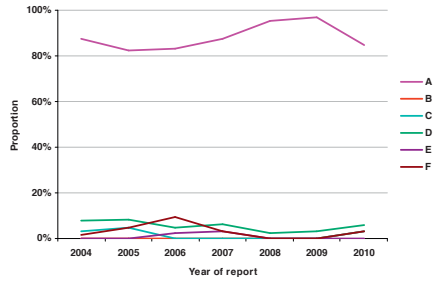


Figure 3. Maximum parsimony tree based on the hepatitis-B-virus S region sequence of acute cases of hepatitis-B-virus infection (n=894), by most probable mode of transmission and gender, the Netherlands, 2004–2010, and selected reference strains (n=19)

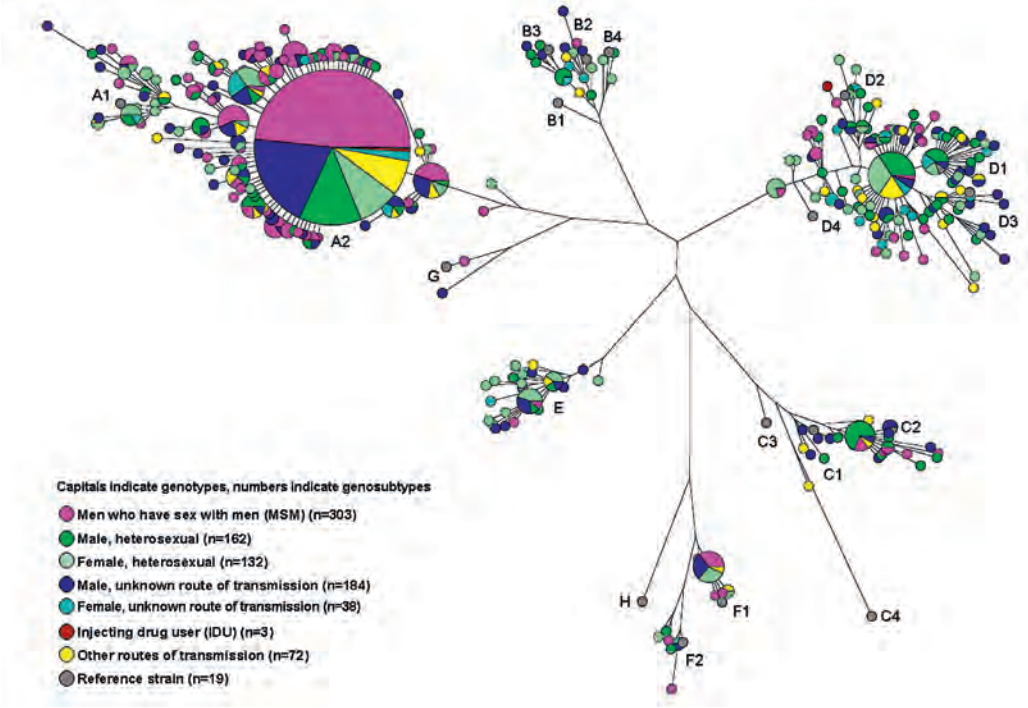
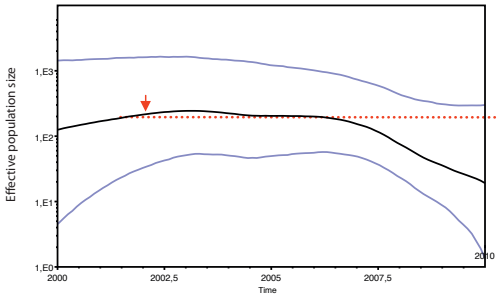


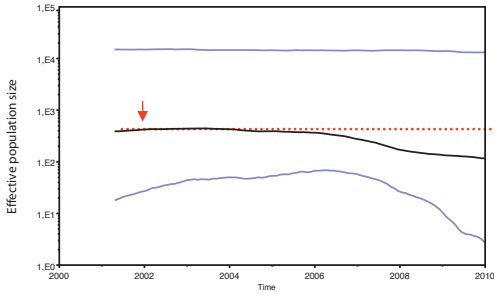
Figure 4a,b. Bayesian skyride plot: estimated genetic diversity over time of the S and C-region sequences of genotype-A acute hepatitis-B-virus infections acquired in the Netherlands, 2004–2010

a. Hepatitis-B-virus infections in men who have sex with men (n=121)



Bayes factor: -3160. Bayes factor for constant population size: -3164

b. Hepatitis-B-virus infection in heterosexual men and women (n=86)



Bayes factor: -3022. Bayes factor for constant population size: -3023

Legend

- Effective population size
- 95% Bayesian credible interval
- Start of HBV vaccination programme (2002)
- Effective population size at start of vaccination programme

If we assume that all MSM in the Netherlands were in the target population for the selective vaccination programme [13] the estimated vaccination coverage would accrue to around 1% per year. Earlier modelling of HBV transmission among MSM in the Netherlands predicted that, with a coverage of 2% per year, the HBV incidence among MSM could be halved in 10 years if specifically MSM at high-risk of HBV acquisition were vaccinated [33]. Our observation that the number of reports of cases in MSM was halved after 9 years of programmatic HBV vaccination suggests that the programme was successful in reaching high-risk MSM. The large proportion of vaccines that were given at outreach locations may have been essential to this success. The relative absence of homophobia, marginalisation and stigmatisation of MSM in the Netherlands compared to most other countries in the world is likely to have had a beneficial effect on improving access to prevention services [34].

The distribution of genotypes sampled from MSM did not change over time, which confirms that HBV-vaccine-induced immunity protects against all genotypes [21]. In contrast, there was a relative increase of genotype A among heterosexuals up to 2009 and a decrease in genotype D. The clonal A2 strain that caused this increase was indistinguishable from the strain found in the cluster of MSM, and there is an identical S-region sequence present in the clonal strain documented to have spread among adults in the UK, USA and Japan (the 'UK prison variant') [26-28]. This spread may be explained by factors including a relative advantage of this strain in causing chronic infection or a higher viral load [28, 35-37]. The relative and absolute decreases of genotype D among heterosexuals in our study population may have been caused by demographic changes in the Netherlands: migration from Mediterranean countries such as Morocco and Turkey, where genotype D is endemic [20, 21], has considerably decreased in the last decade [38]. The observation that the overall number of cases of HBV among heterosexuals did not increase after this group was excluded from the selective vaccination programme in 2007 provides a justification for this change in policy. There was a non-significant reduction of genetic diversity among heterosexually acquired cases starting after 2006. This indicates possible herd-immunity effects of the selective vaccination programme. It may also reflect the spreading of the clonal A2 strain in these populations.

Regarding the cases with an unknown route of transmission, the observation that men are over-represented, particularly among genotype A and subtype A2 strains, but even more so among the clonal A2 strain, suggests that a considerable proportion of these cases is acquired through male homosexual contact that is undisclosed in interviews with public health nurses. The decreasing trend over time in the number of men's cases with an unknown route of transmission that parallels the decrease in cases in MSM further corroborates this hypothesis.

In contrast with many European countries including the UK, Denmark, and the Baltic states [39-41], drug use no longer plays a role in the transmission of HBV in the Netherlands. Furthermore, the specific strain formerly associated with injecting drug use has disappeared. Explanations for this include the reduction of injection among drug users, ageing of the population, and high mortality among those at highest risk [25]. The effectiveness of the selective vaccination programme for preventing HBV among IDUs could not be assessed as the incidence of HBV among IDUs in the Netherlands had already decreased before the implementation of the programme in 2002.

The strength of our study is that it is based on nationwide, population-based, surveillance data of acute hepatitis B cases and hepatitis B viruses for a 7-year period. We maximised information obtained from the data by combining epidemiological analyses of cases and genotypes with phylodynamic analyses of viral sequences. The synergy of combining surveillance methods was used to evaluate the effectiveness of the selective vaccination programme and to generate hypotheses about viral characteristics that may favour transmission. Coalescence-based studies may be particularly important in situations where traditional epidemiological surveillance is less developed, e.g. in other countries or for other infections. Nevertheless, more methodological work is needed to further validate this method for different epidemiological situations.

Our study has some limitations. The proportion of cases for which a sequence was available varied considerably between Public Health Services. This was caused mainly by differences in local practices, e.g. how long serum samples taken for primary diagnostics were stored. The relatively few samples is not problematic for the coalescent analyses, as long as the sampling intensity is fairly constant over time [42]. Comparing the results of coalescent analyses with epidemiological analyses and observations from surveillance of behaviour and of another sex-

ually transmitted disease proved the validity of these coalescent analyses. However, inferences from coalescence analyses have to be drawn with caution since they can lead to a biased estimate of the effective population size in certain conditions [43].
To conclude, we have demonstrated the synergy of combining report-based surveillance combined with molecular epidemiology and phylodynamics in understanding HBV transmission, evaluation of a public health programme, and generating hypotheses about viral characteristics. These methods are likely to be useful for many other infectious diseases and contexts. By doing so, we found evidence that the selective HBV vaccination programme for behavioural high-risk groups reduced HBV transmission in the Netherlands primarily by preventing infections among MSM. This supports continuation of the programme. It also provides important information to guide control of HBV infection in other countries where HBV transmission occurs predominantly in adult high-risk groups.

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Chapter 2.4

Prevention of perinatal hepatitis B virus transmission in the Netherlands, 2003–2007: Children of Chinese mothers are at increased risk of breakthrough infection

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Prevention of perinatal hepatitis B virus transmission in the Netherlands, 2003–2007: Children of Chinese mothers are at increased risk of breakthrough infection

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ABSTRACT

Background: In the Netherlands, different hepatitis B vaccination schedules have been used for children born to HBV-infected mothers. All schedules included a birth dose of hepatitis B immunoglobuline (HBIg). We assessed determinants of perinatal HBV transmission and determinants of anti-HBs titers in infants born to HBsAg positive mothers.

Methods: We included infants born to HBV infected mothers between 1.1.2003 and 30.6.2007, using national databases and a separate database for Amsterdam. Risk factors for perinatal transmission and determinants of the anti-HBs titer were studied using logistic and linear regression, respectively. Results: Of 2657 infants registered in the national database, 91% were registered to have received HBIg and at least three hepatitis B vaccinations. In Amsterdam, this coverage among 413 children at risk was higher (96%, $p < 0.01$). Serological test results for 2121 infants (80%) indicated that 13 (0.6%) were HBsAg positive. A mother of Chinese descent was the only risk factor for perinatal HBV infection identified (RR 9.1, 95% CI 3.1–26.8). Receiving a birth dose of hepatitis B vaccine later than in the first week of life was not associated with an increased risk of perinatal HBV infection. A shorter period between last vaccination and testing, and having received more doses of hepatitis B vaccine were independently associated with a higher anti-HBs titer.

Conclusions: Infants born to Chinese mothers were at increased risk of perinatal HBV infection. All HBsAg positive pregnant women of Chinese origin should be assessed to determine whether there is an indication for anti-viral treatment during pregnancy. Among infants who received HBIg at birth, we did not detect an increased risk of perinatal HBV infection when the first dose of hepatitis B vaccine was administered after the first week of life.

Keywords: Hepatitis B virus, Vaccination, Perinatal transmission, Hepatitis B vaccine, Hepatitis B immunoglobulin

1. INTRODUCTION

The prevalence of chronic HBV infection in the Netherlands is very low compared to most other countries. Estimates of the general population prevalence of hepatitis B surface antigen (HBsAg) positivity range from 0.2 to 0.5% [1,2]. Nevertheless, HBV prevention and control is of public health importance, since there are subgroups in the population with an increased risk of infection and

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Table 1
Recommendation for HBV immunisation of infants born to HBV infected mothers, the Netherlands, birth cohort 1.1.2003–30.6.2007.

Region	Birth date	HBIg[a]	Hepatitis B vaccine at birth			Subsequent HBV vaccinations		
			Age (d)	Vaccine	Dose HBsAg	Age (months)	Vaccine	Dose HBsAg
Netherlands except Amsterdam	01.01.2003	300 IU	–	–		2, 4, 11	HBvaxPRO	5 µg
	01.01.2006	300 IU	1	HBvaxPRO	5 µg	2, 4, 11	HBvaxPRO	5 µg
	01.06.2006	300 IU	1	HBvaxPRO	5 µg	2, 3, 4, 11	Infanrix hexa	10 µg
	01.01.2007	150 IU	1	HBvaxPRO	5 µg	2, 3, 4, 11	Infanrix hexa	10 µg
Amsterdam	01.01.2003	300 IU	eAg–: <7 d eAg+: <2 h	Engerix-B	20 µg	1, 6	Engerix-B	20 µg
	01.01.2006	300 IU	eAg–: <7 d eAg+: <2 h	HBvaxPRO	5 µg	1, 6	HBvaxPRO	5 µg
	01.01.2007	150 IU	eAg–: <7 d eAg+: <2 h	HBvaxPRO	5 µg	1, 6	HBvaxPRO	5 µg

h: hours; d: days.
[a] Within 2 h after birth.

effective and safe interventions for prevention are available. In July 2010 the Dutch Ministry of Public Health, Welfare and Sportsdecided to introduce universal HBV vaccination in the national immunisation programme for all infants [3]. This was implemented for infants born from 1st August 2011 onwards. Regardless of this decision, prevention of perinatal transmission of HBV remains of great importance, as infants born to HBV infected mothers are at very high risk of developing chronic HBV infection [4]. Perinatal HBV transmission can be prevented by immunisation of newborns of HBV infected mothers by either active immunisation (with hepatitis B vaccine), passive immunisation (with hepatitis B immunoglobulin (HBIg)), or a combination of both. A recent metaanalysis of randomized controlled trials found that HBV vaccination at birth reduced the risk of perinatal HBV transmission by 72% [5]. The combination of HBIg and hepatitis B vaccine was more effective than vaccine alone. However, in none of the 29 trials included in this meta-analysis was the first dose of vaccine given later thanat 2 weeks of age. Based on observational data, the WHO concluded that delaying the birth dose increases the risk of perinatal HBV transmission [6,7].

The Netherlands was in November 1989 among the first countries to recommend antenatal HBsAg screening for all pregnant women. This is recommended at 16 weeks of gestation. The participation rate to this screening is very high, with estimates increasing from 97% in 2003 to 100% in 2006/7 [8]. The estimated prevalence of HBsAg among pregnant women is 0.3–0.4% [9]. Up to 2010, the recommended immunisation schedule for infants born to HBV infected mothers differed between the Amsterdam region and the rest of the country (Table 1). In both Amsterdam and the rest of the Netherlands, all recommended vaccination schedules for infants born to HBsAg positive mothers included a birth dose of 300 IU HBIg up to 2007, and 150 IU from 2007 onwards. In Amsterdam, he vaccination schedule for infants born to positive mothers has always included a birth dose of vaccine [10]. In the rest of the Netherlands, however, the birth dose was only introduced in January 2006. The introduction of a birth dose followed a Dutch Health Council advice [11]. Between 1989 and 2003 the first dose of vaccine was given at 3 months of age. Between 2003 and 2006 this was at 2 months of age. The schedule without a birth dose was justified by results of three trials carried out in the 1980s [12], and allowed giving HBV vaccine at the same time as the other infant vaccines (against diphtheria, pertussis, poliomyelitis and tetanus). However, with only eight HBsAg positive children, these trials were underpowered to reveal any differences in effectiveness of schedules.

The aim of our current study was to assess risk factors for perinatal HBV infection and determinants of the anti-HBs titers, in infants born to HBsAg positive mothers.

2. STUDY POPULATION AND METHODS

We defined infants at risk as those born to a HBV infected woman. Maternal HBV infection was defined as a confirmed positive HBsAg test during pregnancy. We included infants at risk born between 1.1.2003 and 30.6.2007 in the analyses.

Nationally, data on antenatal screening results and vaccinations given in the National Immunisation Programme are stored in a central database ('Praeventis'). During the study period, this database did not allow linking HBV infected mothers to their infants. Unfortunately, antenatal HBeAg status was not systematically recorded in the national database. In Amsterdam, the Public Health Service (PHS) records antenatal screening results linked to data on

infants at risk in a dedicated database. We estimated the HBIg and HBV vaccination coverage for children at risk in the Netherlands and Amsterdam using the national database Praeventis and the Amsterdam PHS database, respectively.

All infants at risk are recommended to be tested for HBV infection 4–6 weeks after the last dose of hepatitis B vaccine [13]. In Amsterdam, children were first tested for anti-HBs. If the anti-HBs titer was below 10 IU/l, their sample was tested for anti-HBc. If this was positive, the sample was tested for HBsAg and HBeAg. Test results were registered in a database at the PHS. In the rest of the Netherlands, children born to HBV infected mothers were invited by the National Institute for Public Health and the Environment (RIVM) to participate in serological screening for HBV infection, 4–8 weeks after the last hepatitis B vaccination. Parents were requested to fill in a questionnaire, including questions on demography and information on their infant. All blood samples received were tested for anti-HBs, anti-HBc and HBsAg. For all serologic analyses, commercial Abbott AxSYM assays were used. Anti-HBs titers >1000 IU/l were not determined further. For HBsAg positive children, molecular typing of the S and C region was performed using methods described elsewhere [14]. Test results were stored in a database at RIVM. Perinatal HBV infection in children born to HBV infected mothers was defined by a positive HBsAg test and a positive confirmatory HBsAg test, or a positive HBV DNA test, in an infant's serum sample.

We studied determinants of perinatal HBV infection and of the anti-HBs titer in children for whom a HBV serological test result was available, had a record of receiving HBIg at birth and of receiving their first HBV vaccination within 90 days of birth and who received in total 3, 4 or 5 HBV vaccinations. We assessed whether any of the four different vaccination schedules recommended for children in our study population (see Table 2) was associated with an increased risk of perinatal HBV. If the age at the first dose of vaccine was ≤7 days, we classified the child as having received a birth dose. We disregarded the timeliness of subsequent doses. We also assessed whether the infant's sex and the country of birth of the mother were associated with an increased risk of perinatal HBV infection. The effect of the maternal HBeAg status and viral genotype on the risk of breakthrough infection could not be assessed as this information was only available for HBsAg positive children.

Table 2
HBV vaccination schedules and outcomes for infants born to HBV infected mothers between 1.1.2003–30.6.2007 in The Netherlands. Source data: Praeventis (national vaccination data), PHS Amsterdam (Amsterdam vaccination data and test results), RIVM (test results children rest of the Netherlands).

Area	Birth cohort	Recommended Schedule		*N*	Infants who received HBIg and at least the number of vaccines according to schedule		Infants who received HBIg and ≥3 doses of hepatitis B vaccine		Infants with a HBsAg and anti-HBs test result[a]		HBsAg positive infants			Infants with anti-HBs <10 IU/l	
		HBIg	Hepatitis B vaccine (months)		*n*	%[b]	*n*	%[b]	*n*	%[b]	*n*	%[c] 95% CI		*n*	%[c] 95% CI
Netherlands	01.01.2003–31.12.2005	Birth	2, 4, 11	1759	1553	88.3%	1553	88.3%	1196	68.0%	9	0.8% 0.3%–1.4%		133	11.1% 9.4%–13.0%
	01.01.2006–31.05.2006	Birth	0, 2, 4, 11	212	194	91.5%	203	95.8%	145	68.4%	0	0.0% 0.0%–2.5%		3	2.1% 0.4%–5.9%
	01.06.2006–30.06.2007	Birth	0, 2, 3, 4, 11	686	621	90.5%	660	96.2%	445	64.7%	3	0.7% 0.1%–2.0%		4	0.9% 0.3%–2.3%
Amsterdam	01.01.2003–30.6.2007	Birth	0, 1, 6	413	396	95.9%	396	95.9%	335	81.1%	1	0.3% 0.0%–1.7%		8	2.4% 1.0%–4.7%

[a] In Amsterdam only children with an anti-HBs <10IU/l were tested for anti-HBc, and only those positive for anti-HBc are tested for HBsAg.
[b] Percentage of *N*.
[c] Percentage of those with a test result.

We studied the following determinants of the anti-HBs titer using linear regression: The time between sampling and last vaccination, the vaccination schedule (whether or not a birth dose was received and the number of vaccine doses received), birth weight, gestational age at birth and sex. Birth weight (grams) and duration of gestation (weeks) were grouped (<2000 g, 2000–2500 g, 2500–3000 g, 3000–4000 g and >4000 g; and <32, 32–36, 36–40, 40–42 weeks). Amsterdam born children were not included in these analyses as the PHS database did not contain information on duration of gestation and birth weight. Children who acquired perinatal HBV infection were also not included in these analyses. First we performed single variable analyses. Variables with a p-value <0.1 were included in a multivariable model.

We used STATA 10 for all analyses [15] For the linear regression the censored function was used, to account for the lack of quantitative information on titers >1000 IU/l.

3. RESULTS

The national vaccination register Praeventis included 2657 infants at risk registered as born to a HBV infected mother between 1.1.2003 and 30.6.2007. Of these, 2416 (91%) were registered to have received HBIg and at least three HBV vaccinations. The latter proportion increased from 83.7% in 2003 to 98.1% in 2007 (p for trend <0.001). The Amsterdam PHS database included 452 infants at risk born in the same study period. Of these, 30 were recorded to have left the region, one died and for eight infants the mother was shown to be HBsAg negative on retesting, leaving 413 infants for the analyses. Of these, 96% (396) were registered to have received HBIg and at least three HBV vaccinations, a higher coverage than estimated in the national programme ($p < 0.01$) (Table 2).

Information was available on HBV serological test results for 2123 children (335 in Amsterdam and 1788 in the rest of the Netherlands). Two HBsAg positive children were excluded from the analyses (one since it was born and vaccinated outside of the Netherlands and one since it was diagnosed outside of our screening programme), leaving 2121 children for analyses. Among the 2121 children with a test result, information on their vaccination status was available for 1978 children. Of these, 1945 (98%) received HBIg at birth and at least three hepatitis B vaccinations. Of the 2121 children with a test result, 13 were HBsAg positive (0.6%) (Table 2). In addition, three children (0.2%) of those with an anti-HBc result had a high titer suggestive of resolved breakthrough infection [16].

Of the 13 HBsAg positive children, 11 had an anti-HBs titer <10 IU/l and two had a titer above this threshold (68.3 IU/l and 605.2 IU/l). All HBsAg positive children had received HBIg and at least the recommended number of vaccines. Two of the 13 HBsAg positive children were born to the same mother. Of the 12 mothers of the 13 HBsAg positive children, 10 were HBeAg positive and two were HBeAg negative. Viral sequence information was available for all HBsAg positive children, of whom three, including one child of an HBeAg negative mother, were shown to have mutations in the HBV S or C-region.

A relatively high proportion of high-risk children born in 2003–2005 had a low anti-HBs titer (<10 IU/l) (Table 2). This can be explained by waning of antibody titers as these children were retrospectively

Table 3
Potential risk factors for perinatal HBV infection in at children born to HBV infected mothers, The Netherlands, birth cohort 1.1.2003–30.6.2007 ($n = 1891$).[a]

Determinant	Region of birth	Number of children	HBsAg positive children Number (%)	RR	95%CI	*p*-value
Hepatitis B vaccination schedule[b]						
2, 4, 11 months	Rest of Netherlands	1044	8 (0.8%)	Ref.	–	–
0, 2, 3, 4, 11 months	Rest of Netherlands	364	3 (0.8%)	1.1	0.3–4.0	0.91
0, 2, 4, 11 months	Rest of Netherlands	109	1 (0.9%)[c]	1.2	0.2–9.5	0.87
0, 1, 6 months	Amsterdam	265	1 (0.3%)	0.5	0.1–3.9	0.50
Gender[d]						
Female	Netherlands	920	3 (0.3%)	Ref.		
Male	Netherlands	967	10 (1.0%)	3.2	0.9–11.5	0.09
Mother born in China[e]						
No	Netherlands	1662	6 (0.4%)	Ref.		
Yes	Netherlands	213	7 (3.2%)	9.1	3.1–26.8	<0.001

[a] Includes only children who received HBIg on the day of birth, who received the first dose of hepatitis B vaccine within 90 days of age and who received >2 and <6 hepatitis B vaccines.
[b] For 109 children a vaccination schedule could not be defined (e.g. children born in the Amsterdam region with a first dose given later than in the first week of life).
[c] This child received four HBV vaccinations with the first one at birth, even though it was born in the period that the recommended HBV vaccination schedule was 2, 4, 11 months. This explains the discrepancy between Tables 2 and 3 regarding the number of perinatal infections for schedule 0, 2, 4, 11 months.
[d] For 4 individuals the sex was unknown.
[e] For 16 individuals the country of birth of the mother was unknown.

included in the testing programme, with a much longer time than recommended between the last vaccination and taking of the blood sample.

Of the 2121 children with a test result, 1891 (89.2%) with complete information were included in the analyses to identify determinants of perinatal HBV infection. Having a mother who was born in China was the only significant risk factor for perinatal HBV infection. The vaccination schedule in which the first dose of hepatitis B vaccine was given after the first week of life was not associated with an increased risk of perinatal HBV infection (Table 3). Assuming that 9% of pregnant HBsAg positive women are HBeAg positive [10], the breakthrough infection rate among HBeAg positives would be 7% (11/160) and among HBeAg negatives 0.1% (2/1622) (Table 3).

We included 1496 children with complete information in the analyses to study determinants of the anti-HBs titer. A shorter period between last vaccination and testing and having received more doses of vaccine were independently associated with a higher anti-HBs titer (Table 4).

4. DISCUSSION

The Dutch programme of tracing children born to HBV infected mothers and offering them HBIg and hepatitis B vaccination has been highly successful. The vaccination coverage among registered at risk children was high (91%) compared to that reported from other countries, and it increased in recent years to 97% [17–19]. The rate of perinatal infection among infants who received HBIg at birth was very low, regardless of whether vaccination was started in the first week of life or after. This suggests that infants who received the first dose of vaccine after the first week of life were sufficiently protected for the first months by the passive immunisation with HBIg. Since our study only assessed the risk of breakthrough infection among infants who received HBIg, our results are not applicable to countries without an antenatal HBV screening programme, or where HBIg is not used.

Our findings are consistent with the results of the meta-analyse by Lee et al, who found that HBIg, without vaccination, significantly reduced the risk of perinatal HBV compared to a placebo [5]. Our results do not agree with findings by Marion et al, who found that late administration of the first dose of vaccine was associated with an increased risk of perinatal infection, even after adjustment for HBIg administration [6]. However, as some of the children with perinatal infection in this study received the first dose of vaccine much later than children included in our study, results are not directly comparable. The dose of HBIg (300 IU) recommended for most infants included in our analyses is relatively high compared to recommendations elsewhere [20]. This may explain the relatively low transmission rate we observed.

Our results need to be interpreted with caution, as the number of perinatal infections was very low leading to low power in the analyses. Second, we unfortunately had information on the maternal

Table 4
Determinants of the anti-HBs titer in children born to HBV infected mothers, The Netherlands, birth cohort 1.1.2003–30.6.2007 (n = 1496).[a]

Determinant	Number	Univariate analyses		Multivariate analyses	
				Pseudo R^2 = 0.20	
		Coefficient	p-value	Adjusted Coefficient	p-value
Period between last vaccination and date of sampling (days)	–	−0.0030	<0.001	−0.0023	<0.001
HBV vaccination schedule[b]					
0, 2, 3, 4, 11 months	347	Ref.		Ref.	
0, 2, 4, 11 months	106	−0.800	<0.001	−0.6141	<0.001
2, 4, 11 months	991	−1.8144	<0.001	−0.9482	<0.001
Birth weight					
<2000 gram	30	Ref.	–		
2000–2500 gram	56	0.1981	0.53		
2500–3000 gram	275	0.4427	0.10		
3000–4000 gram	994	0.4847	0.06		
>4000 gram	142	0.5216	0.06		
Gestational age at birth					
<32 weeks	9	Ref.	–		
32–36 weeks	43	−0.0815	0.88		
36–40 weeks	762	0.1005	0.84		
40–42 weeks	683	0.1184	0.81		
Gender					
Female	730	Ref.	–		
Male	767	−0.1103	0.14		

[a] Includes only children who received HBIg on the day of birth and the first dose of hepatitis B vaccine within 90 days of age.
[b] For 53 children a vaccination schedule could not be defined (e.g. children who received the first dose of vaccine within 7 days of birth but received only three vaccinations in total).

HBeAg status only for infants with perinatal infection, not for the entire Dutch population of HBsAg pregnant women. An earlier evaluation of the Amsterdam antenatal screening programme showed that only 8.9% of pregnant HBsAg positive women were 1719 HBeAg positive [10]. Our results may therefore not apply in countries where HBV infected pregnant women generally have a higher viral load.

The only significant risk factor for perinatal transmission we identified was Chinese descent of the mother. This may be associated with the high prevalence of genotype C viruses in this group [21]. Chronic infection with this genotype takes a longer time to seroconversion of HBeAg to anti-HBeAg, and is associated with a higher viral load [22]. Our results are therefore consistent with other studies suggesting that perinatal HBV transmission is associated with high maternal viral load [23]. Three out of the 13 infants with perinatal infection had a mutated HBV viral strain. It is unknown whether this is associated with a higher risk of perinatal infection [23,24].

A limitation of our study is that the different vaccine schedules we compared contained a variation of hepatitis B vaccines, differing in the amount of HBsAg included (Table 1). We were not able to disentangle effects of this from effects of the schedule used.

Consistent with other publications, time between last vaccination and testing and having received more doses of hepatitis B vaccine were independent determinants of a higher anti-HBs titer [25]. There was an indication that a birth weight over 3000 g was associated with a higher anti-HBs titer, but this was not significant.

Both in the group of infants who received a birth dose of vaccine, and those who started vaccination later, the rate of perinatal infection was less than 1%. In the UK, 4.9% of children born to HBV infected mothers were found to have evidence of current infection [26]. This study included mainly HBeAg positive women. Our estimate of 6.9% breakthrough infections among HBeAg positive women is consistent with their findings. In the US, a perinatal transmission rate of 2.2% was found [27]. As the maternal prevalence of HBeAg was not recorded in this study, these results cannot directly be compared with ours.

Achieving an even lower rate of perinatal HBV infections may be possible by antiviral treatment for HBsAg positive pregnant women with a high viral load [28]. This is currently recommended in the Netherlands [29,30]. Data regarding the uptake of this recommendation is not available. Based on these results, HBsAg positive pregnant women of Chinese origin should be assessed to determine whether there is an indication for anti-viral treatment during pregnancy [29].

Among registered children at risk the coverage for HBIg and at least three hepatitis B vaccinations compares favourably with coverages reported elsewhere [17,18]. However, multiplying the estimated antenatal prevalence (0.34–0.40%) [8,31] with the number of live births in the Netherlands in the study period (n = 855,598), between 2909 and 3,422 infants born to HBV infected mothers were expected in the study period. The national vaccination register therefore included only an estimated 78–91% of all infants born to HBV infected mothers. We do not know whether the non-registered children received HBIg and vaccination or were simply not registered. The completeness of the registration of children born to HBsAg positive women should therefore be improved.

An additional limitation of the programme is that participation to the serological screening to detect perinatal infection was incomplete (around 80% of registered children in Amsterdam and 66% in the rest of the Netherlands, Table 2). It is likely that the rates of perinatal infection found in our study population cannot be extrapolated to the entire population of children at risk, as the vaccination coverage in the group who participated to the screening was higher than in the entire registered population of at risk infants (98% and 91%, respectively). We are currently investigating whether less invasive methods (e.g. dried blood spots) can be used to detect HBV-infected children and anti-HBs levels, as availability of these are likely to increase the participation to the screening.

The coverage we report for the birth period 2003–2005 is lower than that reported for the birth cohort 2000 (88.3% and 95%, respectively) [32]. This may be related to the 2005 transfer of retrospective vaccination data to the national database Praeventis. Our results show that the registered vaccine coverage in Amsterdam is higher than the national coverage, consistent with a previous evaluation of the Amsterdam programme for prevention of perinatal HBV transmission [10]. It is difficult to compare results of the programme in Amsterdam with the rest of the Netherlands, however, as characteristics of the population and working procedures differ. The local Amsterdam data is, however, likely to be more robust than the national data, as a direct link between the

laboratory carrying out antenatal tests and the PHS exists. The higher coverage found in Amsterdam may partly be due to the fact that a dedicated public health nurse ensured that children who had left the region were excluded from the database and that all vaccines delivered were registered.

Remarkably, two of 13 children who were HBsAg positive had an anti-HBs titer ≥10 IU/l. Unfortunately, no follow-up HBsAg test results are available. It suggests that testing only children with a titer <10 IU/l for HBsAg, as was done in Amsterdam up to 2010, may have been insufficient to detect all HBsAg positive children. This may have led to an underestimation of the proportion of perinatal infections in Amsterdam, which may partly explain the relatively low proportion found there relative to the rest of the Netherlands.

From January 2010 onwards, the same vaccination schedule for at risk children is used in Amsterdam and the rest of the Netherlands.

The programme to prevent perinatal HBV transmission remains a key priority to control HBV infection in the Netherlands, regardless of introduction of universal hepatitis B vaccination for infants in 2011 [3,13]. Our study confirms that among children registered as at risk the current programme of providing HBIg and HBV vaccine at birth, with subsequent completion of the HBV vaccination schedule is effective, achieving high coverage with very few perinatal infections. Testing of adequately immunised infants born to HBsAg positive mothers was therefore considered to be no longer among the national public health priorities in the Netherlands and the national programme for this was ceased from 2010 onwards. Regardless of this, it is still recommended that every child born to an HBsAg positive mother is tested for HBsAg, to assess whether it has escaped chronic HBV infection.

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Chapter 2.5

Persistent and transient hepatitis B virus (HBV) infections in children born to HBV-infected mothers despite active and passive vaccination

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Persistent and transient hepatitis B virus (HBV) infections in children born to HBV-infected mothers despite active and passive vaccination

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SUMMARY.
Combined passive and active immunization for newborns very effectively prevents perinatal hepatitis B virus (HBV) infections. In the Netherlands, babies born to hepatitis B surface antigen (HBsAg)-positive women receive passive immunization with hepatitis B and at least three active HBsAg vaccinations. Serological testing for the presence of HBV markers was offered for all infants born to HBsAg-positive mothers between January 2003 and July 2007, after completion of their vaccination schedule. About 75% of the infants (n = 1743) completed their HB-vaccination schedule and participated in the serologic evaluation. Twelve of them (0.7%) were found to be HBV infected. Furthermore, we identified three older children with high levels of anti-HBc, anti-HBs and anti-HBe, while they were HBsAg and HBV DNA negative. This serologic profile is evidence for a resolved HBV infection. In the group of older children (1.5–5 years of age, n = 728), about half of the HBV-infected children (3 of 7) had already cleared their infection at the time of sampling. For a proper evaluation of the efficacy of a new intervention programme to prevent vertical HBV transmission, it is also important to analyse the HBV markers in serum collected when the children are older than 1.5 years. In a programmatic setting, all children born to HBV-infected mothers should be tested not only for the level of anti-HBs but also for the absence of HBsAg, because 2 of the 12 HBV-infected children (17%) had a high level of anti-HBs.

Keywords: anti-HBc, anti-HBs, hepatitis B virus, maternal antibodies, perinatal transmission.

INTRODUCTION

Vaccination with hepatitis B surface antigen (HBsAg), the major protein of the hepatitis B virus (HBV), induces the production of specific hepatitis B antibodies (hepatitis B surface protein antibodies or anti-HBs). Such antibodies are an indicator of long-term protection against clinically relevant HBV infections. Infants vaccinated at birth who develop anti-HBs above the cut-off value of 10 IU/L at 1 to 3 months after their last vaccination are expected to be protected for minimally 15 years, and probably for life [1–3]. Unlike HB vaccination, natural HBV infection induces antibodies against the core protein of the HBV (anti-HBc) and, in most cases, against the HBV e-antigen (anti-HBe). Large-scale population serosurveys of past or current HBV infections are generally based on assessment of the presence

Abbreviations: HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

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of anti-HBc rather than anti-HBs, as anti-HBs declines more rapidly than antiHBc. Furthermore, vaccination-induced anti-HBs cannot be distinguished from anti-HBs induced by natural infection.
The risk of chronic infection depends largely on the age of infection: ~90% for newborns, ~50% for 3 years old and ~5% for adults [4]. Because chronic infection can cause severe liver problems, the average life-time expectancy of chronically infected individuals is severely reduced [5]. Perinatally acquired infections cause an estimated 21% of HBV-related deaths worldwide [6]. Prevention of perinatal HBV infections is a key priority in controlling HBV transmission. Active vaccination of newborns within 48 h of delivery is very effective in preventing vertical transmission [7], and the World Health Organization (WHO) recommends it for all neonates worldwide. Many developed countries supplement active vaccination with anti-HBs (passive vaccination) given directly after birth (<2 h).
Although HB vaccination effectively prevents HBV transmission at the population level [8], up to 10% of the adolescent and young adult vaccinees do not respond well (<10 IU/L) or at all (<1 IU/L) to HB vaccination, and the proportion increases to 35% for people over 60 years old [9]. Despite timely passive and active vaccination, newborns may still become HBV infected because of either intrauterine infection [10,11] or nonresponse to vaccination [12]. The risk of infection despite vaccination is directly proportional to the size of the mother's viral load [13–15]. To determine the optimal strategy to prevent perinatal transmission (vaccine, vaccination schedules; anti-HBs for the child; antivirals and/ or anti-HBs for the mother), the efficacy of the different intervention programmes must be determined. The most important parameter is the presence of HBsAg at 1 year of age, which is proof of an HBV infection. In most cases, it will become chronic in these young children [16]. However, the newborn may have a transient HBV infection and clear it by the age of 1 year. Both chronic and transient infections should be taken into account in the evaluation of new prevention strategies, as a short-term subclinical infection indicates that the child was not optimally protected and could have transmitted the virus to others (e.g. in a day-care setting). Furthermore, a transient hepatitis B infection might not be cleared completely and could reactivate later in life, causing complications [17,18]. Therefore, statistically significantly more transient infections in a new prevention strategy mean that the strategy is inferior.
Some HB-vaccination studies of children with HBVinfected mothers use the anti-HBc level as a marker to determine whether a child had been HBV infected [7,19,20]. However, because the anti-HBc level can be very high in chronically infected mothers, the anti-HBc in the blood of young children could be of maternal origin. Currently, it is impossible to distinguish between maternally derived and infection-induced anti-HBc. We undertook our study to evaluate the decline of anti-HBc over time in vaccinated children born to HBV-infected mothers and to determine whether anti-HBc levels can identify transient HBV infections in these children.

MATERIAL AND METHODS

Identification of HBV-infected mothers

Since 1989, all pregnant women in the Netherlands have been offered serological screening for HBsAg at around 12 weeks of pregnancy. Women who test positive for HBsAg for the first time ever are asked to provide a second serum sample to confirm the positive result and to exclude contamination or sampling errors. Vaccination for the newborn, both passive (HBV hyperimmunoglobulin, hereafter referred to as HBIg, within 2 h after birth) and active (HB vaccine, >=3 shots), is then offered to all newborns. All Dutch children born to HBV-infected mothers between 1 January 2003 and 1 July 2007 were invited to participate in the serological screening after completion of their HB-vaccination schedule in conformance to the national immunization schedule. Children born in the Amsterdam area, who have been vaccinated according to a different schedule (HBIg directly after birth and active vaccination at 0, 1 and 6 months), were excluded from our study.

Serum analyses of children born to HBV-infected mothers

All children born to HBV-infected mothers were eligible for both passive and active HB vaccination and subsequent serum analysis for the HBV markers and response to HB vaccination. All undiluted sera were analysed for the presence of anti-HBs (AUSAB Abbott AxSYM; given in international units per litre [IU/L]; with a maximum of 1000 IU/L) and HBsAg (HBsAg V2 Abbott AxSYM; sera with a sampleover-index-calibrator mean rate [S/N] equal to or greater than 2.00 are considered reactive). If serum was available, the presence of anti-HBc (Core, Abbott

AxSYM; sera with a sample over cut-off ratio [S/CO] equal to or less than 1.00 are considered reactive) was also determined. Sera that were positive for HBsAg were only recorded as such if a subsequent HBsAg confirmation test of these sera was also positive. Either the Abbott AxSYM HBsAg confirmatory test or a nested polymerase chain reaction analysis specific for the S region of HBV [21] was used for this purpose. Sera of older children (1.5–5 years) with an intermediate or high level of anti-HBc were also analysed for the presence of HBeAg (HBe 2.0, Abbott AxSYM; sera with an S/CO equal to or >1.00 are considered reactive) and anti-HBe (Anti-HBe 2.0, Abbott AxSYM; sera with an S/CO equal to or <1.000 are considered reactive), if sufficient material was available. Furthermore, HBeAg and anti-HBe were also determined from anti-HBc-level-matched sera of children younger than 1.5 years (hereafter referred to as 'infants'), and from agematched older children who were anti-HBc negative. All assays were carried out according to the manufacturer's instructions. The phrase 'more than 1.5 years old' is used when serum was collected 550 days or more after birth. An 'intermediate level' of anti-HBc was defined as 2–5 times the cut-off level for 1/anti-HBc (i.e. 2.0–5.0); while a 'high level' of anti-HBc was defined as equal to or more than five times the cut-off value for 1/anti-HBc (i.e. >=5.0).

Vaccination schedules

During the study period, several HB vaccines and vaccination schedules were used in the Dutch national immunization programme (Table 1). The vaccination coverage for all the vaccination schedules was high. In the period 2003–2005, over 90% of the children born to registered HBsAg-positive mothers received all scheduled HB vaccinations. Children who were HBsAg negative and who had an anti-HBs level of less than 10 IU/L were offered revaccination in an accelerated schedule: HBVaxPro (5 lg) at 0, 1 and 2 months. Response to revaccination was analysed as described earlier.

Statistical analysis

We determined the 99th percentile for the anti-HBc level using the Generalized Additive Models for Location, Scale and Shape. These models are implemented in the GAMLSS package for the statistical software package R [22]. We compared multiple distributions for the response variable by running the GAMLSS model with each distribution and evaluating Schwartz's Bayesian Criterion. This value was lowest with the four-parameter Box–Cox t-distribution, so we used this distribution for estimating the percentile. We assumed that the location and scale parameters depended on age, and we estimated them by using cubic smoothing splines on age. To simplify matters, we assumed that the skewness and kurtosis parameters were constant.

To estimate the relationship between age and the anti-HBc levels on the HB-measured anti-HBs, we categorized the continuous age variable by dividing age into groups of 50 days. We also divided the anti-HBc level into groups of 0.5 IU. We chose to model these categorized variables rather than to use smoothing terms from the Generalized Additive Models, as these categorizations allow for exploring the relationship in the data while maintaining a clearer interpretation. We used a survival regression model (available in the R library survival), under assumption of Weibull distributed response variable (anti-HBs level), with both age and anti-HBc level as explanatory variables, which allowed us to look at the marginal influence of each explanatory variable of the response.

RESULTS

HBV-infections in young children

All children of HBV-infected mothers born between January 2003 and July 2007 in the Netherlands were invited to participate in the serologic screening 6 weeks–4 years after completion of their vaccination schedule (Table 1). In total, we received 1743 serum samples from the estimated 2280 children (75.4%). The sera were collected when the children were aged between 205 and 1727 days (average = 622; median 482). Twelve of these sera contained HBsAg (0.69%). All twelve HBsAg-positive children had completed their HB-vaccination schedule, which included administration of HBIg within 2 h after birth. Five of these 12 children had an anti-HBs antibody titre greater than 1.0 IU/L (Fig. 1). Remarkably, two of these had a titre greater than 10 IU/L (i.e. 68.3 and 605.2 IU/L), a level that is generally regarded as being protective against a HBV infection [3].

Anti-HBc levels

To further assess the effectiveness of HBV-vaccination programmes for newborns at risk of perinatal HBV infection, we determined the anti-HBc level

Table 1 Hepatitis B-vaccination schedule for newborns of hepatitis B virus (HBV)-infected mothers in the Netherlands from March 2003 onward

			Vaccination 1		Vaccination 2		Vaccination 3		Vaccination 4		Vaccination 5	
Starting date	Number of children*	HBIg† (IU)	Age (days)‡	Vaccine	Age (months)	Vaccine	Age (months)	Vaccine	Age (months)	Vaccine	Age (months)	Vaccine
01-03-2003	~1500	300	–	–	2	HBvaxPRO	–	–	4	HBvaxPRO	11	HBvaxPRO
01-01-2006	~180	300	1	HBvaxPRO‡	2	HBvaxPRO	–	–	4	HBvaxPRO	11	HBvaxPRO
01-06-2006	~330	300	1	HBvaxPRO	2	Infanrix hexa‡	3	Infanrix hexa	4	Infanrix hexa	11	Infanrix hexa
01-01-2007	~270	150	1	HBvaxPRO	2	Infanrix hexa	3	Infanrix hexa	4	Infanrix hexa	11	Infanrix hexa

*Number of children who were born to registered HBV-infected mothers who were eligible for the indicated vaccination schedule. †HBV-specific immunoglobulins (HBIgs) were administered within 2 h after birth. ‡The HBvaxPRO vaccine contains 5 μg HBsAg, while Infanrix hexa contains 10 μg HBsAg.

in the same sera that was used for HBsAg and anti-HBs analyses (Fig. 2). Almost one-third (29%) of the infants were positive for anti-HBc, while this was true for only 3% of the older children (aged 1.5–5). However, the average anti-HBc level of infants is significantly lower ($P < 0.05$) in successfully vaccinated children in this age group (1/anti-HBc average = 1.97; n = 928) than in HBV-infected children (1/anti-HBc average = 9.6; n = 7). For older children, the difference in anti-HBc levels is even more pronounced ($P < 0.05$): uninfected children had an average 1/anti-HBc level of 0.62 (n = 728) compared to 11.9 (n = 5) for HBV-infected children. Four of the 5 older children (80%) with an HBV infection had a high level of anti-HBc (1/anti-HBc > 5, Table 2), while of the 728 uninfected older children only 3 (0.4%) had a high level of anti-HBc ($P < 0.001$). To determine whether the anti-HBc level of the newborn influences the HB-vaccination response, we predicted the anti-HBs level as a function of anti-HBc level while adjusting for age. As a control, we also predicted the anti-HBs level as a function of age while adjusting for the anti-HBc level (Fig. 3). All vaccination schedules are completed at 11 months of age (Table 1) so that the child's age at the time of sampling is directly related to the time after vaccination. There is a clear correlation between the child's age and the anti-HBs level, while the anti-HBc level and the HB vaccine response did not correlate.

HBeAg and anti-HBe levels

Sera from the 12 older children with intermediate and high anti-HBc levels (1/anti-HBc > 5) were also analysed for the presence of HBV e-antigen (HBeAg) or antibodies against HBeAg (anti-HBe). As expected, high levels of HBeAg were found in the sera of all four HBV-infected older children (Table 2). Anti-HBe was absent in the sera of the HBVinfected older children, but was detectable in all three uninfected older children with a high anti-HBc level. The sera of children comparable to those listed in Table 2 were also analysed for the presence of HBeAg and anti-HBe. No anti-HBe was present in any of the sera from age-matched children with low levels of anti-HBc (1/anti-HBc < 2; data not shown). Some, but not all, of the 1/anti-HBc-matched HBsAg-negative infants also possessed anti-HBe (data not shown). Because we do not know the anti-HBc and anti-HBe levels of the corresponding mothers, we were unable to determine whether these antibodies were maternally

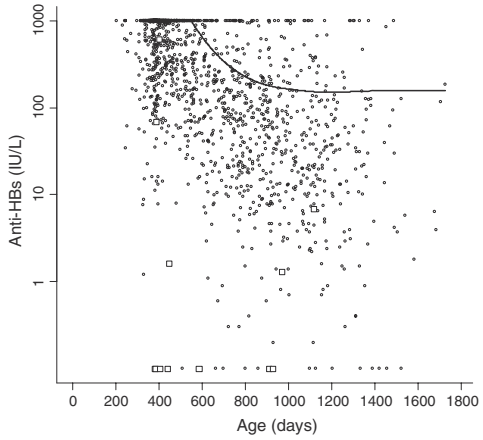

Fig. 1 Relationship between age and antibody response to HB vaccination of children born to hepatitis B virus (HBV)-infected mothers. The *solid line* represents the average anti-HBs level; each *dot* represents a single uninfected child; and each *open square* represents one of the 12 HBV-infected children.

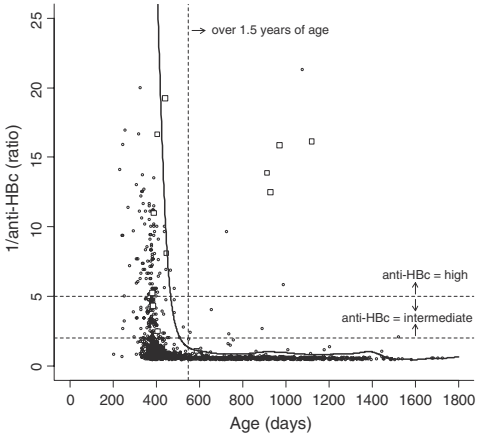

Fig. 2 Relationship between age and antibodies against the core protein of hepatitis B virus (HBV) in children born to HBV-infected mothers. The *solid line* represents the 99th percentile for the anti-HBc levels; each *dot* represents a single uninfected child; and each *open square* represents one of the 12 HBV-infected children.

derived or whether they were caused by a transient HBV-infection. All three HBsAg-negative, anti-HBc/anti-HBe-positive older children also had high levels of anti-HBs (Table 2).

DISCUSSION

Vertical transmission, a major transmission route of HBV, can largely be prevented by vaccinating newborns directly after birth. Although newborn vaccination might not prevent infections because of prenatal (i.e. intrauterine) transmission, infections because of perinatal or postnatal transmission should be preventable, if the vaccine and vaccination schedule induce rapid and good response in every newborn. In developed countries, many newborns of HBVinfected mothers receive HBV-specific immunoglobulin (HBIg) in addition to active vaccination. In the view of WHO, administration of HBIg directly after birth does not significantly improve protection against perinatally acquired infections of full-term newborns vaccinated immediately (<24 h) after birth [3]. However, Lee and colleagues' metaanalysis finds that the addition of HBIg to schedules of HB vaccination with recombinant HBV vaccines reduces the relative risk to 0.52 (95% confidence interval 0.41–0.67) [7].

To determine which vaccination programme is most efficacious in preventing vertical HBV infections, it is essential to identify children not responding to HB vaccination and HBV-infected children in a uniform and sensitive way. For logistic reasons, most of the children in our study who were born in 2003 and 2004 were sampled long after completion of their vaccination schedule (up to 50 months after their last vaccination). Furthermore, all sera in which the level of anti-HBs exceeded the maximum level of the assay were recorded as 1000 IU/L. Therefore, comparison of the numbers of HB-infected children and the levels of induced antiHBs in the different vaccination schedules is complicated. It is estimated that 80–90% of the newborns who become infected with HBV will develop a chronic infection [16]. However, no data about the relative frequency of chronic HB infections in newborns are available when different interventions such as HBIg administration and/or HB vaccination are applied. Transient HBV infections in HB-vaccinated children can be determined retrospectively by the detection of anti-HBc at an age when maternally derived anti-HBc has waned. On the basis of the data presented in Fig. 2, we conclude that a child should be at least 1.5 years old before a high level of anti-HBc can be used as a reliable indicator for a transient HBV infection. In the sub-cohort of 728 children who were above 1.5 years of age when their sera were collected, four were HBV infected, while three more had had

Table 2 HBV markers in serum of children (1.5–5.0 years of age) who are HBV infected (shaded rows) or have intermediate or high antibody levels against the core protein of the HBV

Child	Age (days)	HBsAg* (S/N)	HBV DNA†	Anti-HBs* (IU/L)	1/anti-HBc* (S/CO)	HBeAg* (S/CO)	1/anti-HBe* (S/CO)
1	442	**499.2**	Positive	0.0	**19.2**	0.4	**16.1**
2	404	**178.6**	Positive	**605.2**	**16.7**	n.a.‡	n.a.
3	1120	**3.3**	Positive	**6.8**	**16.1**	**178.4**	0.2
4	971	**88.1**	Positive	1.3	**15.9**	**178.6**	0.2
5	913	**217.3**	Positive	0.0	**13.9**	**322.1**	0.0
6	929	**218.8**	Positive	0.0	**12.5**	**362.6**	0.1
7	389	**191.7**	Positive	**68.3**	**11.0**	n.a.	n.a.
8	447	**306.5**	Positive	1.6	**8.1**	**233.1**	0.1
9	382	**200.9**	Positive	0.0	**5.3**	**182.0**	0.2
10	385	**312.2**	Positive	0.0	**4.3**	n.a.	n.a.
11	396	**283.1**	Positive	0.0	**2.5**	**266.5**	0.1
12	587	**311.2**	Positive	0.0	**1.1**	**233.6**	0.1
13	1077	0.7	Negative	**>1000.0**	**21.3**	0.2	**31.3**
14	728	1.0	Negative	**158.1**	**9.6**	0.2	**2.9**
15	992	0.9	Negative	**>1000.0**	**5.8**	0.2	**7.3**
16	656	0.9	Negative	**9.8**	**4.0**	0.3	0.3
17	893	0.8	Negative	**73.8**	**2.7**	n.a.	n.a.
18	558	0.8	Negative	**>1000.0**	**2.4**	0.3	0.5
19	739	0.7	Negative	**81.9**	**2.3**	0.2	0.4
20	1524	0.8	Negative	**126.5**	**2.1**	0.2	0.5

HBV, hepatitis B virus; anti-HBc, antibodies against the hepatitis B core antigen; anti-HBs, antibodies against the hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBe, antibodies against hepatitis B e antigen; IU/L, international units per litre; S/CO, sample over cut-off control of the specific AxSYM assay (see Materials and methods section); S/N, sample over Index Calibrator mean rate of the specific AxSYM assay (see Materials and methods section); n.a., not applicable. *Samples that are reactive in the AxSYM analysis are given in bold face. †Only children who were positive for HBsAg were found positive in a HBV-specific S-region polymerase chain reaction. ‡Serum was not available for these analysis.

a transient HBV infection as shown by their high anti-HBs, anti-HBc and anti-HBe levels, in the absence of HBV-specific proteins and DNA. The risk of the HBV infection becoming chronic in our sub-cohort of older children was thus only 57% (4 of 7). This reduced risk of the infection becoming chronic, considered in the light of previous studies, could be because of the intervention; i.e. HBV-specific antibodies directly after birth and at least three active vaccinations in the 1st year of life. Another possible explanation is that transient infections are more prevalent than previously assumed, and the estimated chance that 80–90% of the infections become chronic is actually an overestimation because subclinical transient infections were missed. A recent publication by Walz et al. [23] indicated that HBV markers can also be detected early after birth (≤4 months) in a small fraction (6.6%) of infants born to mothers who were HBsAg negative but antiHBc positive. However, all HBV-DNA positive children from this study who could be test-

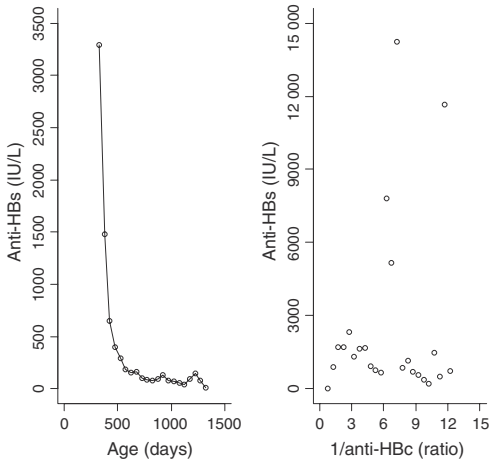

Fig. 3 Predicted values for anti-HBs levels plotted against age (left panel) and anti-HBc level (right panel) with adjustment for their respective influences.

ed were HBsAg and HBV-DNA negative within 15 months after birth. Whether these children were truly infected or whether the HBV markers were maternally derived remains uncertain.

Once an HB-vaccination programme is implemented in a programmatic setting, it is important to evaluate the individual response to active vaccination. Because anti-HBs levels in young children diminish quite rapidly in time (Fig. 1) [2,24], it is preferable to determine the anti-HBs level within 3 months after the last vaccination. A short time span between the last vaccination and the analysis of the vaccination response will also reduce the loss to follow-up in a programmatic setting. Aiming at serum analysis within 3 month after HBV vaccination is therefore optimal in a programmatic vaccination setting. In our cohort, two of the 12 HBV-infected children (17%) had a high level of anti-HBs (i.e. 68 IU/L and 605 IU/L), while their samples were taken within the 3-month postvaccination period. Thus, good response to the HB vaccination clearly does not exclude HBV infection. Therefore,children born to a HBV-infected mother should not only have their anti-HBs levels assessed, but the absence of HBsAg should also be determined, after completion of the HB-vaccination schedule.

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Chapter 2.6

Effectiveness and impact of hepatitis B virus vaccination of children with at least one parent born in a hepatitis B virus endemic country: an early assessment

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Effectiveness and impact of hepatitis B virus vaccination of children with at least one parent born in a hepatitis B virus endemic country: an early assessment

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Objective To determine the effectiveness and impact of the Dutch childhood hepatitis B virus (HBV) vaccination policy targeted at children with at least one parent born in a HBV endemic country.

Methods The Dutch vaccination registration database was used to determine vaccine coverage for HBV and DTP-IPV-Hib in the target population. HBV notifications were used to estimate the impact. The HBV incidence was determined in children aged 0-4 years and born after (2003-7) and before (1990-2002) the introduction of the HBV vaccination programme.

Results HBV vaccine coverage in the target population was 89.6% (96 186/107 338) in the period 2003-5. There were 37 notified acute infections in the prevaccination birth cohort 1990-2002 (incidence 2.9/10^6 person-years), compared with one in the postvaccination birth cohort 2003-7 (incidence 0.3/10^6 person-years). The incidence rate ratio for the 2003-7 birth cohort compared with the 1990-2002 birth cohort was 0.12 (95% CI 0.02 to 0.87; p=0.04).

Conclusions This paper shows that the incidence of HBV notifications in children born after the introduction of targeted childhood HBV vaccinations is lower compared with the incidence in children born before the start of this vaccination programme. Although this is consistent with a good HBV vaccine coverage, the interpretation is hampered by a change in case definition for notification in 1999. The results are of importance to policy makers in both The Netherlands and other countries that have a targeted HBV vaccination programme.

Hepatitis B is an important disease worldwide, responsible for approximately 500 000-700 000 deaths each year.[1] It is estimated that approximately two billion people worldwide have evidence of exposure to the hepatitis B virus (HBV), with approximately 400 million actively infected.[2] Most of the morbidity and mortality caused by HBV infections can be attributed to chronic infections, which can lead to liver cirrhosis and hepatocellular carcinoma.[3] Patients with chronic HBV infections are often infected as infants or young children.[2 4 5]

HBV prevalence in countries is categorised as high endemic (>8%), intermediate endemic (2-8%) or low endemic (<2%), based on the seroprevalence of the hepatitis B surface antigen (HBsAg).[1] The Netherlands is a low-endemic country for HBV, with an estimated HBsAg seroprevalence of 0.3-0.5%.[6] So far, The Netherlands has not implemented universal HBV vaccination.[7]

Universal antenatal screening and subsequent vaccination of the infant when the mother is HBsAg positive was introduced in 1989. A nationwide programme for vaccination of behavioural risk groups was introduced in 2002.[8]

This was further expanded in 2003 by the introduction of HBV vaccination in the national immunisation programme for children with at least one

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parent born in an intermediate or high-endemic country. Within Europe, such a programme also exists in Norway and Sweden.[9 10] The majority of first-generation immigrants in The Netherlands originate from Morocco, Turkey and Suriname, all three countries of intermediate HBV endemicity.[6 11] The rationale for the vaccination of children from first-generation migrants is that these children are at increased risk of contact with chronic HBV carriers, either within the family or in their social environment. The epidemiology in these groups reflects the patterns observed in their country of origin.[8 12] The Dutch Health Council recently recommended that universal HBV vaccination be implemented in The Netherlands.[13] So far, the Minster of Health has not decided whether this will be implemented.

Compulsory registration of all newborns in the Dutch municipal population register (GBA) is used to invite children to participate in the national immunisation programme. The child's birth notification in the GBA is entered into the database 'Praeventis'. Two weeks after birth, invitations for the child to participate in the national immunisation programme are generated from this database. The Praeventis database contains information on vaccines received as well as demographic information on all newborns registered in The Netherlands. HBV vaccination is offered when the GBA shows that at least one parent is born in a country that is intermediate or high endemic for HBV.

For children eligible for HBV vaccination born in 2003-5, HBV vaccines were given as separate injections at 2, 4 and 11 months of age.[8] Since January 2006, the HBV vaccine is administered as part of a combination vaccine with diphtheriaetetanusepertussis, inactivated poliomyelitis, and Haemophilus influenza type b (DPT-IPV-HBV/Hib; Infanrix hexa) and given at 2, 3, 4 and 11 months of age.

Evaluation of vaccination programmes is vital to their success. The question of whether a risk group approach for childhood HBV vaccinations is an effective way to reduce the burden of HBV-related disease is under continuous debate.[7] Therefore, we aimed to evaluate the effectiveness of the current Dutch HBV vaccination programme for children born to first-generation immigrants.

METHODS

Coverage

We used data from the Dutch vaccination registration database 'Praeventis' for birth cohorts in 2003-5. Data were extracted on 7 December 2008. Children without a valid municipal registration number (GBA) and children with a recorded date of death or emigration were excluded. We defined children with at least one parent born in an intermediate or high-endemic country for hepatitis B as second-generation migrants (SGM). We excluded SGM born to HBsAg-positive mothers from coverage estimates. We defined HBV vaccine coverage as the proportion of SGM who had received at least three HBV vaccinations at age 12 months. We defined DPT-IPV-Hib coverage as the percentage of all children who completed the primary series of at least three injections at 12 months of age. We compared the observed coverage with the target in The Netherlands (90% for all vaccinations, including HBV).[14] We used χ^2 tests for trend to assess changes in vaccine coverage over time. All analysis were done using SAS version 8.0.

Impact

Since 1976, all physicians are obliged to notify newly diagnosed HBV infections to the Municipal Health Service within 24 h after diagnosis. Before 1999, only the notification of acute (symptomatic) HBV infection was compulsory.[15] Since 1999, all newly diagnosed HBV infections are notifiable, including asymptomatic and chronic infections when first diagnosed. The Municipal Health Service forwards the notification to the National Institute of Public Health, where they are recorded in a database. Additional information such as date of birth, date of notification and diagnosis, type of infection (chronic or acute) and most likely route of transmission is collected. We included in our analyses all HBV notifications between 1990 and 2007 in children aged 0e4 years who were recorded as 'acute HBV infection', except those in whom it was stated that the infection was the result of vertical transmission. Only children up to 4 years of age were included, as only in those can the effects of the vaccination policy introduced in 2003 be expected using data up to 2007.

For notified cases of hepatitis B, the country of birth of the parents and child are rarely recorded. We were thus unable to determine whether cases of hepatitis B occurred in children eligible for HBV vaccination. Therefore, we stratified notifications into a pre-vaccination (1990-2002) and post-vaccination (2003-7) cohort, based on the year of birth. Because the introduction of notification for chronic hepatitis B in 1999 could interfere with our results, we also compared the 1999-2002 cohort with the

post-vaccination cohort. Population denominators (person-years at risk) were obtained from Statistics Netherlands.[11]

We calculated the cumulative age-specific number of notifications in the pre and post-vaccination cohort and divided this by the corresponding number of person-years to estimate the incidence. We compared the incidences by calculating an incidence rate ratio (IRR). P Values were calculated using Poisson regression analysis.

Asymptomatic or subclinical HBV infections are very common in children and the age of infection is the strongest determinant of whether children will develop symptoms following infection.[1] We used a previously described logistic function to adjust for the proportion of infections that is asymptomatic.[16 17] In addition, we assumed that 75% of all acute HBV infections are reported (based on estimates from the UK)[16 18] and adjusted the number of new acute HBV cases by a factor of 1.33 in order to correct for this underreporting. We estimated the number of acute infections that progress to chronic infections using a previously described method[16] that is based on a meta-analysis of observational studies. These adjustments resulted in estimates for the annual incidence of acute and chronic cases in the age group 0-4 years. Based on these estimates we estimated the annual number of new infections and new chronic infections prevented by the current vaccination programme.

All analyses were done using SAS version 8.0.

RESULTS

Vaccine coverage

The number of children born in 2003, 2004 and 2005 who were offered vaccinations in the national immunisation programme was 600 332. Of these, we excluded 20 116 (3.4%) children with a recorded date of emigration, 1220 (0.2%) children with a recorded date of death and 133 (0.02%) children without a registered GBA number. The final dataset comprised 578 863 children, of which 108 717 (18.6%) were SGM who were offered HBV vaccination. Of these, 1379 (1.3%) SGM were born to HBsAg-positive mothers and were excluded from further analysis.

In total, 96 186 (89.6%) SGM born in 2003-5 were fully vaccinated for HBV (table 1). There was a significant increasing trend in the HBV vaccine coverage over the birth cohorts 2003-5 (87.9%, 89.9% and 91.1%, respectively; p<0.0001; χ^2 tests for trend). The HBV vaccine coverage in SGM was significantly lower than the DPT-IPV-Hib coverage (89.6% vs 97.7%; p<0.0001). Although the DPT-IPV-Hib coverage was significantly higher in SGM than in children from parents born in The Netherlands, the difference was not epidemiologically relevant (97.7% vs 97.2%; p<0.0001). Among SGM, the HBV vaccine coverage was lower for SGM from Surinamese/Dutch Antillean origin than the HBV coverage in those of Moroccan and Turkish origin (89.4%, 95.4% and 95.4%, respectively, p<0.0001). The lowest HBV vaccine coverage was found in the 'other ' category (85.8%, p<0.0001). Although the DPT-IPV-Hib vaccine coverage differed significantly between Surinamese/Dutch Antillean, Turkish and Moroccan and SGM (98.9%, 99.4% and 99.2%, respectively, p<0.0001), the differences were not epidemiologically relevant. HBV vaccine coverage was lower in those living in rural regions compared with urban regions for Surinamese/ Dutch Antillean SGM (81.1% and 90.1%, respectively, p<0.0001) and SGM in the 'other ' category (79.4% and 86.3% respectively, p<0.0001).

Table 1 HBV and DPT-IPV-Hib vaccine coverage by country of birth of at least one of the parents, The Netherlands, birth cohorts 2003—2005*

Country of birth of parents	No of children born in 2003—5	HBV vaccine coverage	DPTP vaccine coverage
Both parents born in The Netherlands	469538	NA	97.2%
At least one parent born in HBV-endemic country	107338	89.6%	97.7%
Morocco	23320	95.4%	99.4%
Turkey	18563	95.4%	99.2%
Suriname/Antilles	15638	89.4%	98.9%
Other	48075	85.8%	96.9%

*Data as of 7 December 2008.

Impact

Between 1990 and 2007, 121 HBV infections were notified in children aged 0-4 years who were born in 1990-2007. Of these, 38 (31.4%) were labelled 'acute', 78 (64.5%) as 'chronic' and five (4.1%) as 'unknown'. Sixty cases (49.6%) were reported as the result of vertical transmission and were excluded from further analysis. Table 2 shows the number of notifications and the agespecific incidence of acute HBV infections by birth cohort. Of the 38 acute HBV infections notified in children aged 0-4 years, 37 were in children born in the pre-vaccination cohorts (1990-2002) of which five were born in 1999-2002. There was one notification of an infected child born in the cohort that was offered vaccination (2003-7). The latter child was not vaccinated against HBV, but information on the country of birth of his/her parents was not available.

Figure 1 shows the cumulative incidence of HBV notifications per 1 000 000 person-years in children aged 0-4 years before and after the introduction of HBV vaccinations in SGM in The Netherlands. The incidence of HBV notifications in 0-4-year-old children was significantly higher in the pre-vaccination cohort compared with the vaccinated cohort (2.9 and 0.3 per 1 000 000 person-years, respectively, IRR 0.12; 95% CI 0.02 to 0.87; p=0.04). For the period 1999-2002 the cumulative incidence was also higher in the pre-vaccination cohort compared with the vaccination cohort (1.2 and 0.3 per 1 000 000 person-years) but the difference was smaller and was not statistically significantly (IRR 0.28; p=0.24).

Figure 2 shows the incidence of notified acute and chronic HBV infections in children aged 0-4 years by year of notification over the period 1990-2007. In 1999 the number of notified acute infections decreases as compulsory notification for chronic infection was introduced. After 2003, the incidence of both chronic and acute HBV notifications drops to near zero.

We estimated that the programme of HBV vaccination of SGM, 5 years after its implementation, prevented seven (95% CI 1 to 8) notifications of acute HBV in children aged 0-4 years born in 2003-7, reflecting 123 new HBV infections and 117 new chronic infections.

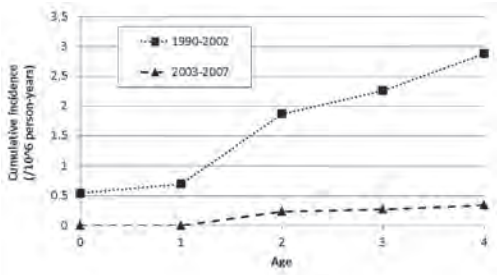

Figure 1 Age-specific cumulative incidence of hepatitis B virus (HBV) in children aged 0—4 years born before (1990—2002) and after (2003—7) the introduction of childhood HBV vaccination of second-generation migrants in The Netherlands, excluding cases reported as the result of vertical transmission.

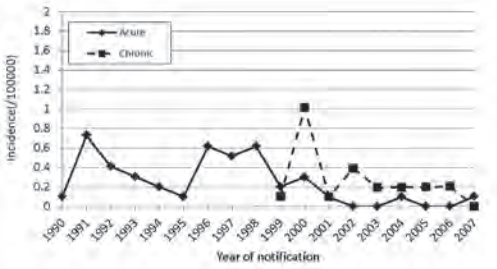

Figure 2 Incidence of hepatitis B virus notifications in children aged 0—4 years by year of notification, 1990—2007, The Netherlands. Cases reported as the result of vertical transmission were excluded.

DISCUSSION

We found that the incidence of HBV notifications in children aged 0-4 years decreased significantly after the introduction of HBV vaccination for SGM in The Netherlands, suggesting that this targeted HBV vaccination programme may be effective. This is consistent with the observed high HBV vaccine coverage in this group of 89.6%; even though it is 0.4% lower than the target (90.0%).

Our study had several limitations. The total number of notifications of HBV infections was low, and a change in notification protocols in 1999 appears to have had an effect on the notification behaviour of physicians. The incidence of HBV notifications in children declined in 1999, when chronic infections became notifiable (figure 2). As no interventions to prevent HBV were introduced in 1999, this suggests

Table 2 Number and incidence rate of notified acute HBV infections in children aged 0—4 years by birth cohort in The Netherlands, excluding cases reported as a result of vertical transmission

	Age at infection	No of notified cases	Cohort size (person-years)	Incidence (/10^6 person-years)
Pre-vaccination birth cohorts 1990—2002	0	7	2568358	2.7
	1	9	2568358	3.5
	2	8	2568358	3.1
	3	5	2568358	1.9
	4	8	2568358	3.1
Post-vaccination birth cohorts 2003—2007*	0	0	948607	0.0
	1	0	767271	0.0
	2	1	582214	1.7
	3	0	394304	0.0
	4	0	200297	0.0

*Cohort size in person-years declines because not all children born in 2003—2007 could have reached the age of 4 years at the time of this study.

What is already known on this topic

- The Netherlands is a low-endemic country for HBV infections.
- Since 2003, children with at least one parent born in an intermediate or high-endemic country for HBV are offered HBV vaccinations in the national immunisation programme in The Netherlands.
- The effectiveness and impact of targeted HBV vaccinations for children with at least one parent born in an intermediate or high-endemic country for HBV have never been determined.

What this study adds

- It is feasible to target SGM for HBV vaccination within the Dutch national immunisation programme.
- Surveillance data suggest that targeted HBV vaccination for children with at least one parent born in an intermediate or high-endemic country has reduced the incidence of childhood HBV infection in The Netherlands.

that before 1999, some chronic infections were reported as 'acute'. This could have resulted in an overestimation of the difference between the 1990-2002 and 2003-7 birth cohorts. However, although not significant, also when comparing 1999-2002 with 2003-7 the incidence in the post-vaccination period is much lower than in the pre-vaccination cohort (IRR 0.28).

Using person-years as a denominator could have led to a small overestimation of the decrease in incidence between the post and pre-vaccination cohort, as the risk of developing an acute HBV infection increases slightly with age, and older children were less represented in the post-vaccination cohort.

Acute and chronic infections are difficult to differentiate, and current case definitions are not very specific.[15] In the absence of a clear case definition, physicians may be more likely to report infections as chronic or asymptomatic infections.[15] The peak in the incidence of notified chronic infections in 2000 could be an artificial peak caused by delayed notifications of earlier diagnosed infections.

These limitations of surveillance data make it difficult to interpret whether the decline in the incidence of acute HBV notifications is a result of the vaccination campaign. In addition, information on country of birth of the patient or the parents was limited, so we were unable to determine whether the patients before 2003 would have been targeted by the vaccination programme. This emphasises the need for a more complete and comprehensive surveillance system in order to evaluate the impact of public health interventions adequately.

Groups of first-generation migrants from different countries might also have a different prevalence of HBV infection. As migration patterns and the age distribution of migrants may have changed over time, this could also influence the incidence of HBV in SGM. However, as the country of birth of notified patients is not recorded, we could not identify whether this was the case.

Our finding that a vaccination programme based on the country of birth of parents can be effective is consistent with a study by Sonder et al,[19] which showed that vaccination against the hepatitis A virus targeted at migrant children is an effective strategy to reduce the transmission of hepatitis A in The Netherlands.

These findings contrast with results from the USA, where it was concluded that vaccination programmes targeted at SGM were not effective in reducing the incidence of HBV, because it was difficult to assess and vaccinate children from immigrants.[20 21] An important difference between The Netherlands and the USA is that the latter had much lower vaccine coverage, especially in lower socioeconomic groups and ethnic minorities.[22]

We found that HBV vaccine coverage for children born to Surinamese or Antillean parents was lower compared with children from parents of Turkish or Moroccan origin, whereas DPT-IPV-Hib vaccine coverage was comparable for the three groups. We also found that HBV vaccine coverage was significantly lower for children born to Surinamese or Antillean parents living in rural areas compared with those living in urban areas. This might indicate that there is a difference in the perception of necessity for HBV vaccination for Surinamese/ Antillean SGM between those living in urban and rural areas, either among parents or among healthcare providers. The reasons for a lower HBV vaccination rate in children from Surinamese or Antillean parents need further study.

We furthermore found that the DPT-IPV-Hib coverage among SGM was higher than vaccine coverage among children from Dutch parents. This contrasts with Dutch studies from the 1990s, which suggested that vaccine coverage among Turkish children was comparable with the Dutch population, whereas vaccine coverage was much lower for Moroccan children.[19 23]

This might indicate that vaccine coverage in SGM has been increasing over the last decades, especially among Moroccan children. This is further supported by a study in Amsterdam, which showed that vac-

cine coverage for SGM of Turkish, Moroccan, or Surinamese origin was comparable to vaccine coverage among Dutch children.[24]

Vaccine coverage for both DPT-IPV-Hib and HBV was the lowest in the 'other' group. As 45% of all SGM were in this category, their contribution to overall vaccine coverage is important. The category is, however, a mix of numerous nationalities and origins, which makes it difficult to identify determinants.

Our finding that it is feasible to reach SGM within a national immunisation programme and that it has prevented a considerable number of new HBV infections is important for policy making on vaccination in The Netherlands. It is also particularly relevant for other countries that do not have universal HBV vaccination in childhood and countries that do not achieve sufficient coverage with their programmes.

Although the HBV vaccine is currently being offered in a combination vaccine (DTP-IPV-HBV/Hib; infanrix hexa), we still think that our findings are relevant for policy making. The introduction of a new vaccine in The Netherlands could create opposition towards the national immunisation programme among the general population, even when introduced in a combination vaccine.[25] This could have an effect on the general coverage of the national immunisation programme, and careful planning should also involve the option of targeting a vaccine only at high-risk groups.

We found that there are several limitations in the current surveillance systems that make impact studies difficult to conduct. Therefore, findings from the national seroprevalence study (PIENTER II) that became available in 2009 will be important to study further the effects of the targeted HBV vaccination programme.26 A comparison between the first national seroprevalence study (PIENTER I), conducted in 1996, could give more insight into a possible decline in seroprevalence of HBV infections in children before and after the start of the vaccination programme.

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Competing interests None.

Contributors JACH performed all analysis and was the principal writer of the manuscript. SH originally designed the study and was intensively involved in data analysis and interpretation as well as the writing of the manuscript. FHDK and HEM assisted in interpreting results and writing the manuscript. All authors approved the publication and take responsibility for the integrity of the data.

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Chapter 2.7

Parental attitude towards childhood HBV vaccination in The Netherlands

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Parental attitude towards childhood HBV vaccination in The Netherlands

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In The Netherlands, children with at least one parent born in a hepatitis B virus (HBV) endemic country are offered HBV vaccination within the National Immunization Programme (NIP) since 2003. However, in the eligible group the HBV vaccine coverage is lower than the DPT-IPV-Hib coverage. We therefore conducted a questionnaire survey in order to determine the acceptance of HBV vaccination among parents of eligible children. Given the possibility that universal HBV vaccination will be introduced in the Netherlands, we also assessed the attitude towards universal HBV vaccination among parents whose children are currently not eligible for HBV vaccination. Participants were selected based on the registered vaccination status of their child. Only 13 of 83 parents (16%) within the HBV-eligible group whose child was registered as 'incompletely vaccinated' for HBV reported that they refused a vaccine for their child. Risk factors for HBV refusal were a low risk perception of HBV, a high socioeconomic status and one parent born in The Netherlands. Within the non-eligible group, we found that 9% (95% CI: 3–22%) of the parents whose child was fully vaccinated with DPT-IPV-Hib had a negative attitude towards universal HBV vaccination. Considering the recent recommendation of the Dutch Health Council to introduce universal HBV vaccination, this resistance deserves further attention.

Keywords: HBV Vaccinations, Vaccine acceptation, Hepatitis B

1. INTRODUCTION

In The Netherlands, parents are given the opportunity to have their children vaccinated in the National Immunization Programme (NIP), which is a free, non-mandatory programme that started in 1957. Currently, the NIP includes vaccination against the following eleven diseases: diphtheria, pertussis, tetanus, poliomyelitis, Haemophilis influenza type b (DPT-IPV-Hib); mumps, measles, rubella (MMR); meningococcal serogroup C disease, pneumococcal disease and hepatitis B. The hepatitis B vaccine is the only vaccine that is targeted at risk groups only, including children born to HBV positive mothers. Since 2003, newborns with at least one parent born in an intermediateor high-endemic country for HBV are offered HBV vaccination within the NIP [1]. The rationale for this is that these children are at increased risk of contact with HBV carriers, either within the family or their social environment, as the prevalence of carriers is higher among first generation migrants (FGMs). The epidemiology in these groups hence reflects the patterns observed in their country of origin [2,3]. The largest groups of FGMs in The Netherlands originate from three intermediate endemic countries: Morocco, Turkey, and Suriname [4,5]. From March 2003 up to May 2006 HBV vaccine was offered as separate injections at 2, 4, and 11 months of age. From 1st June 2006 onwards, it is given as part of a combination vaccine (Infanrix hexa), given at 2, 3, 4, and 11 months of age [6].

The target for vaccine coverage in the Netherlands is 90% for all vaccines [1]. We recently found that the HBV vaccine coverage among children from FGMs in birth cohorts 2003–2005 was 8.1% lower than their DPT-IPV-Hib coverage (89.6% and 97.7%, respectively). In addition, HBV vaccine coverage was lower for children from parents born in Surinam or the Dutch Antilles compared to children from Turkish and Moroccan parents (89.4% and 95.4%, respectively) [7]

Research into the reasons for a lower vaccine coverage for HBV could help to identify determinants for DPT-IPV-HIB and HBV vaccination. Little is

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known about the acceptance of HBV vaccination among parents from Moroccan, Turkish or Surinamese/Antillean origin.

Recently the Health Council advised to introduce universal hepatitis B vaccination [8]. One study among highly educated people suggests that that the willingness to accept new vaccinations in the NIP might be lower than the current vaccination coverage [9]. It is therefore important to determine the attitude towards universal HBV vaccination in the general population.

We aimed to identify determinants of HBV vaccine acceptance and explain the large difference between DPT-IPV-Hib and HBV vaccine coverage among the population eligible for HBV vaccination in the NIP. In addition, we aimed to determine the attitude towards universal HBV vaccination among parents of HBV-eligible and non-HBV-eligible children.

2. METHODS

We carried out a questionnaire survey to determine the attitude of parents towards childhood HBV vaccinations in the NIP. We defined children as 'incompletely vaccinated' when they received less than the recommended number of doses for the selected vaccines. We were unable to make a distinction between partially and fully unvaccinated children. Children who were eligible for HBV vaccination based on the country of birth of at least one of the parents were defined as 'HBV-eligible children' and children who were not eligible for HBV vaccination as 'non-HBV-eligible children'

2.1. Participants

All newborn children in the Netherlands need to be registered at the municipal of residence of the parents. This birth notification is also entered in 'Praeventis', which is a national vaccination registration database that contains information about the number of vaccines received and the demographic information of all newborns registered in The Netherlands. This database is used to send invitations to participate in the NIP and to register vaccine coverage in The Netherlands.

Parents of children born in 2003–2005 and registered in the national vaccination registration database 'Praeventis' were eligible for participation in the study. We excluded children that had a known date of emigration and children with a recorded date of death. We further excluded children born to HBsAg positive mothers since their motivation to have their child vaccinated for HBV in the NIP will differ from parents who are HBsAg negative. Also, children who had incomplete demographic information were excluded.

Parents of HBV-eligible children were randomly sampled within three strata: having a child that was registered as: (i) vaccinated with the HBV and DPT-IPV-Hib vaccine (4 doses); (ii) vaccinated with DPT-IPV-Hib vaccine (4 doses) but incompletely vaccinated with the HBV vaccine; and (iii) incompletely vaccinated with the DPT-IPV-Hib and HBV vaccines. Parents of non-HBV-eligible children were randomly sampled in two strata: having a child that was registered as: (i) vaccinated with the DPT-IPV-Hib vaccine and (ii) incompletely vaccinated with the DPT-IPV-Hib vaccine (Fig. 1).

The number of parents that we invited to participate in this study was based on power calculations. We aimed to identify factors for which the prevalence differed at least 30% between children who are completely vaccinated and incompletely vaccinated for HBV. The number of participants needed in order to find these factors was determined at 50 for each of the three strata for FGMs. With an expected response of 25%, we determined the number of parents needed to invite at 200 for each of the three strata of parents of HBV-eligible children. In addition, 100 parents of non-HBV-eligible children were randomly sampled within each of the two strata. The total number of parents we invited was thus 800.

2.2. Questionnaire

A theoretical framework of proximal and distal determinants of parents' vaccination behaviour developed by Paulussen et al. [10] was used to develop the questionnaire (Fig. 2). Distal determinants (knowledge, experience, information processing and socio-demographics) are thought to influence proximal determinants (outcome expectation, risk perception, anticipated regret, social norms, and self-efficacy). These proximal determinants, which can be seen as 'mediators', are related to vaccination intention and behaviour. Questions on all determinants were included in the questionnaire.

The questionnaires contained questions on the following items: (i) knowledge of the NIP and HBV vaccinations; (ii) attitude towards childhood vaccinations in general; (iii) attitude towards HBV vaccinations; (iv) demographics.

The attitude towards childhood vaccination in

general contained 15 statements about vaccination acceptance with answers on a five-point Likert scale (from strongly agree to strongly disagree). In addition, questions were asked about the perceived risk of getting infected with any vaccine preventable disease in case of vaccination and non-vaccination. We also asked for the most important reason for refusing vaccination, and about vaccination behaviour of other parents in the participant's social network.

The questions about the attitude towards HBV vaccinations contained eight statements about attitude and beliefs. Furthermore, we asked for the opinion on current vaccination strategies and the attitude towards universal HBV vaccination. Also, we asked for the perceived risk of HBV infection for their child in case of vaccination and non-vaccination.

Questionnaires were translated in Arabic and Turkish. Parents of Moroccan origin received a Dutch and an Arabic version of the questionnaires. Parents of Turkish origin received a Turkish and a Dutch questionnaire. Questionnaires can be obtained from the authors on request.

2.3. Analysis

Double, manual entry was used to enter the questionnaires in a database. Data were cleaned and checked for inconsistencies. Responses to the various statements were dichotomized as 'agree' (score 1 and 2) and 'disagree' (score 3–5). We made a new dichotomous variable on risk perception based on three questions concerning the perceived risk of an HBV infection.

We defined HBV vaccine refusers as participants whose child was eligible for HBV vaccination but remained incompletely vaccinated for HBV and who reported that they ever refused a vaccination for their child. DPT-IPV-Hib vaccine refusers were defined as parents of children who were incompletely vaccinated for DPT-IPV-Hib and said that they ever refused a vaccine for their child.

Descriptive analyses of the study groups were done using x^2 tests. Next, binary logistic regression was used to identify proximal and distal determinants that were associated with HBV vaccine refusal. Determinants were reported to be significantly associated when they had a p-value of 0.05 or lower. The low number of HBV refusers in our study did not allow multivariate logistic regression analysis.

3. RESULTS

3.1. Response

We sent out 803 invitations and questionnaires. Seven of these were not delivered because of a wrong address. A total of 203 questionnaires were returned, of which five were incomplete and were excluded. Hence, the response rate was 25%, ranging from 9% to 44% by study group (Fig. 1). Among parents of HBV-eligible children, the response rate was significantly lower among Moroccan (13%) compared to Turkish (24%) and Surinamese/Antillean parents (29%) (p = 0.001). None of the Moroccan partici-

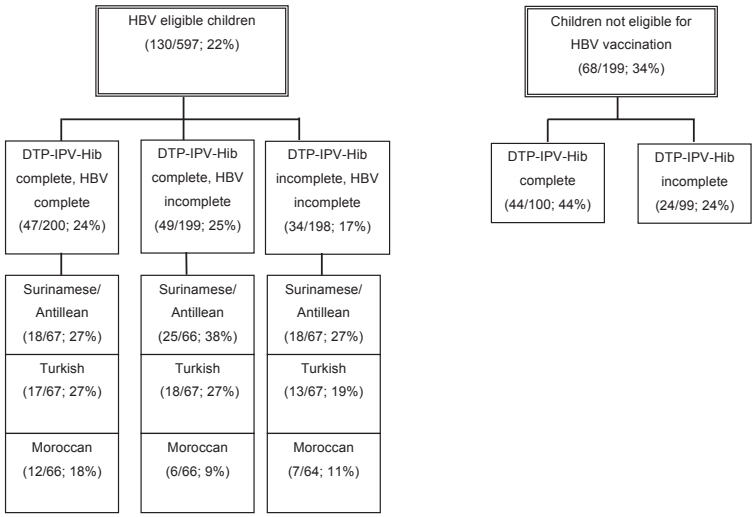

Fig. 1. Participants' characteristics and response rates among parents of children born in 2003–2005 in the Netherlands that were invited to participate in this questionnaire survey.

pants returned the translated version of the questionnaire, while 21 (44%) of the Turkish respondents responded with a Turkish questionnaire. Among parents of non-HBV-eligible children, the response rate was significantly lower for those whose children were incompletely vaccinated compared to those whose children were completely vaccinated children for DPT-IPV-Hib (44% and 24%, respectively, p = 0.05).

3.2. Demographics of participating parents

Table 1 shows the baseline characteristics of the participants. The proportion of families in our study with at least one parent that completed higher or academic education was 40% (95% CI: 21–61%) in Moroccan, 46% (95% CI: 31–61%) in Turkish, 61% (95% CI: 48–74%) in Surinamese/Antillean parents and 59% (95% CI: 46–71%) for parents of non-HBV-eligible children. The average household income among parents of HBV-eligible children was lower compared parents of non-HBV-eligible children. The proportion of parents of HBV-eligible children in our study that had an income of D 1750 or less per month was 46% (95% CI: 36–57%), compared to 9% (95% CI: 3–18%) of the parents of non-HBV-eligible children. Information on household income was missing in 25% of the respondents.

Also, the distribution of religious beliefs differed between parents of HBV-eligible and non-HBV-eligible children. Muslim religion was more prevalent among parents of HBV-eligible children (49% vs. 0%), while a Protestant Christian religion was more prevalent among parents of non-HBV-eligible children (34% vs. 8%).

Within the HBV-eligible group, there were no significant differences in baseline characteristics between parents whose child was registered as vaccinated for HBV compared to those registered as incompletely vaccinated for HBV. Parents of non-HBV-eligible children were more likely to be of Protestant Christian religion when their child is incompletely vaccinated for DPT-IPV-Hib ($p < 0.0001$), while they were more likely to be of Roman Catholic religion when their child is registered as fully vaccinated ($p = 0.01$) (Table 1).

Of the 83 parents of HBV-eligible children that were recorded to be incompletely vaccinated for HBV, 13 (16%) reported that they ever refused a vaccine for their child. This proportion was significantly higher in Surinamese/Antillean parents (22%) compared to Turkish or Moroccan parents (2% and 0%, respectively) ($p = 0.001$; χ^2 -test). All parents of non-HBV-eligible children that were registered as incompletely vaccinated for DTP-IPV-Hib also reported to have refused a vaccine for their child.

3.3. Determinants of HBV vaccine refusal in parents of HBV-eligible children

Univariate ORs for proximal and distal determinants that were significantly associated with HBV refusal are shown in Table 2. Risk perception was the most significantly associated with HBV vaccine refusal (OR = 758.7; $p < 0.0001$). The prevalence of a low risk perception was higher in parents from Surinamese/Antillilean origin (46%) compared to parents form Moroccan (29%) and Turkish (21%) origin ($p = 0.02$; χ^2 -test)

Also, the belief that vaccination has a negative influence on the child's immune system was signifi-

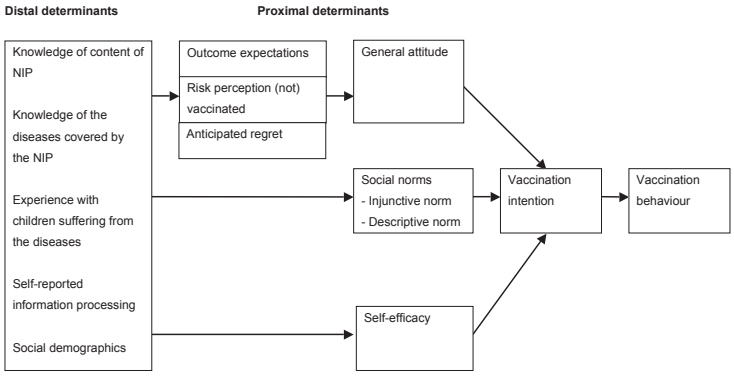

Fig. 2. Theoretical model of determinants of vaccination behaviour [10].

Table 1
Demographics of parents of children born in 2003–2005 in The Netherlands that participated in the questionnaire survey.

	Parents of HBV-eligible children				Parents of non-HBV-eligible children				Total (n = 198)
	HBV vaccination status				DPT-IPV-Hib vaccination status				
	Vaccinated (N = 47)	Percentage (95% CI)	Incompletely vaccinated (N = 83)	Percentage (95% CI)	Vaccinated (N = 44)	Percentage (95% CI)	Incompletely vaccinated (N = 24)	Percentage (95% CI)	
Education[a]									
High school or lower	8/46	17% (8–31%)	13/82	16% (9–26%)	3/43	7% (1–19%)	1/24	4 (0–21%)	25/195 (13%)
Vocational education	17/46	37% (23–52%)	23/82	28% (19–39%)	15/43	35% (21–51%)	8/24	33% (16–55%)	63/195 (32%)
Higher education	12/46	26% (14–41%)	30/82	37% (26–48%)	17/43	40% (25–56%)	11/24	46% (26–67%)	70/195 (36%)
Academic	16/46	35% (21–50%)	16/82	20% (12–30%)	8/43	19% (8–33%)	4/24	17% (5–37%)	37/195 (19%)
Income									
Less than €1750/month	13/32	41% (24–59%)	32/65	49% (37–62%)	3/32	9% (2–25%)	3/19	16% (3–40%)	51/148 (34%)
Between €1751 and 3050/month	12/32	38% (21–56%)	18/65	28% (17–40%)	14/32	44% (26–62%)	10/19	53% (29–76%)	54/148 (36%)
More than €3051/month	7/32	22% (9–40%)	15/65	23% (14–35%)	15/32	47% (29–65%)	6/19	32% (13–57%)	43/148 (29%)
Religion									
Protestant	4/47	9% (2–20%)	9/83	11% (5–20%)	7/44	16% (7–30%)	16/24	67% (45–84%)	36/198 (18%)
Catholic	6/47	13% (5–26%)	16/83	19% (11–29%)	15/44	34% (20–50%)	1/24	4% (0–21%)	38/198 (19%)
Muslim	28/47	60% (44–74%)	36/83	44% (33–55%)	0/44	0% (0–8%)	0/24	0% (0–14%)	64/198 (32%)
Other	2/47	4% (1–15%)	6/83	7% (3–15%)	0/44	0% (0–8%)	1/24	4% (0–21%)	9/198 (5%)
No religion	7/47	15% (6–28%)	16/83	19% (11–29%)	22/44	50% (35–65%)	6/24	25% (10–47%)	51/198 (26%)

p-Values were calculated using the χ^2-test, n/a = not available.
[a] Based on the parent with the highest educational level.

cantly associated with HBV vaccine refusal (OR = 13.5; p = 0.01). However, fears for side effects of the HBV vaccine was not significantly associated with HBV refusal (OR = 2.3; p = 0.34). We found a negative association between the presence of anticipated regret and HBV vaccine refusal (OR = 0.1; p = 0.01).

Distal determinants that were significantly associated with refusal of HBV vaccination were: One parent born in the Netherlands; high or academic education; monthly household income of D 3051/ month or more; knowledge of the HBV vaccination programme in The Netherlands; and country of birth of at least one of the parents (Table 2). Religious beliefs were not an important determinant in HBV vaccine refusal (OR = 4.6; p = 0.22). Only one parent of an HBV-eligible child that refused a HBV vaccination marked religious beliefs as an important reason to refuse vaccination (8%), compared to two (2%) in parents of HBV-eligible children that did not refuse any vaccination for their child.

3.4. Attitude towards universal vaccination

A negative attitude towards universal HBV vaccination was found among four of the 44 parents of non-HBV-eligible children that had their child fully vaccinated for DPT-IPV-Hib 9% (95% CI: 3–22%). This was significantly higher for parents that refused DPTIPV-Hib vaccination for their child (43.5%; p < 0.0001).

Ten percent (95% CI: 6–17%) of parents of HBV-eligible children reported a negative attitude towards universal HBV vaccination. This was significantly associated with HBV vaccination refusal (64% among refusers, 4% among non-refusers; p < 0.0001). Fifteen percent (95% CI: 9–21%) of parents of HBV-eligible children responded that they found the current HBV vaccination programme discriminating. This was, however, not significantly related to HBV refusal (19% vs. 12%; p = 0.24).

4. DISCUSSION

We found that parents' refusal of HBV vaccination for their children explains only a small proportion of the lower vaccine coverage for HBV compared to DPT-IPV-Hib among HBV-eligible children. A possible explanation for this is that the child is not offered HBV vaccination because the nurse administrating the vaccinations forgot the HBV vaccine or deemed the HBV vaccination unnecessary. Research is needed in order to determine the reasons why these children are not offered HBV vaccination and whether more extensive education of health care workers involved is needed. There are other examples of targeted vaccinations programmes for children in which the vaccine coverage is lower compared to the general vaccination scheme [11], however, these are not incorporated within the NIP. The combination of a targeted vaccine strategy within the general childhood vaccination campaign makes the current HBV vaccination strategy relatively unique.

Table 2
Determinants of HBV vaccination refusal among parents of HBV-eligible children born in 2003–2005 in The Netherlands. Only crude ORs of proximal and distal determinants that were significantly associated with HBV refusal in parents are shown.

	Prevalence of HBV vaccine refusal	Univariate OR (95% CI)	*p*-value
Proximal determinants			
Immunizations have a negative influence on the immune system			
Yes	4/13 (31%)	5.1 (1.3–19.8)	0.02
No	9/112 (8%)	1.0	
Anticipated regret			
Yes	5/100 (5%)	0.1 (0.03–0.38)	0.001
No	8/25 (32%)	1.0	
Perceived risk of HBV when not vaccinated			
Low	7/8 (88%)	130.7 (13.2 to >999.9)	<0.0001
High	6/118 (5%)	1.0	
Distal determinants			
Duration of residence in The Netherlands[a]			
Less than five years	0/4 (0%)	n/a	
More than five years	3/68 (4%)	1.0	
Since birth	9/49 (18%)	4.8 (1.2–19.1)	0.02
Education[b]			
Vocational education or lower	2/60 (3%)	1.0	
Higher education	5/40 (13%)	4.3 (0.8–23.3)	0.09
Academic	6/24 (25%)	10.0 (1.9–53.9)	0.007
Income			
Less than €1750/month	0/45 (2%)	n/a	
Between €1751 and €3050/month	3/29 (10%)	1.0	
More than €3051/month	7/21 (33%)	4.3 (1.0–19.4)	0.06
Knowledge of the HBV vaccination policy in the Netherlands			
Yes	4/12 (33%)	5.8 (1.5–23.2)	0.01
No	9/114 (8%)	1.0	
Country of birth[c]			
Morocco	0/24 (0%)	n/a	
Turkey	1/47 (2%)	1.0	
Surinam/Antilles	12/55 (23%)	12.8 (1.6–102.9)	0.02
Religion			
Protestant	4/13 (31%)	1.1 (0.2–5.0)	0.89
Catholic	2/22 (9%)	0.3 (0.04–1.42)	0.11
Muslim	0/62 (0%)	n/a	
Other	1/8 (13%)	0.4 (0.04–3.56)	0.38
None	6/21 (29%)	1.0	

OR = odds ratio, 95% CI = 95% confidence interval, n/a = not available.
[a] Duration of residence is based on the parent that has the longest period of residence in The Netherlands. Since birth means that only one parent was born in an intermediate- or high-endemic country for HBV.
[b] Based on the parent with the highest educational level.
[c] Country of birth of at least one of the parents.

We found that the proportion of HBV vaccination refusers in parents of HBV-eligible children was significantly higher among parents born in Surinam or the Dutch Antilles compared to those born in Morocco or Turkey. The few available data suggests that that the prevalence of HBV carriers in Surinam is comparable to the prevalence in Morocco and Turkey [5]. Nevertheless, we found that risk perception for HBV is lower among Surinamese/Antillean parents compared to Moroccan or Turkish parents (prevalence of low risk perception: 46% vs. 29% and 21%, respectively). This suggests that risk perception is not strongly associated with the prevalence of HBV in the country of birth.

4.1. Universal vaccination

Our data suggests that a considerable part of the general Dutch population has a negative attitude towards universal HBV vaccinations. Although a negative attitude towards universal HBV vaccination was strongly associated with DPT-IPV-Hib vaccination status, 9.1% of the Dutch parents of children registered as DPT-IPVHib fully vaccinated reported a negative attitude towards universal HBV vaccination. This is consistent with research from Sweden, a country with a HBV vaccination strategy comparable to the Netherlands. Here, a negative attitude towards universal HBV vaccination was significantly related to whether the child did not receive all recommended vaccinations (OR = 2.51; p = 0.001) and to a high educational level (OR = 1.37; p = 0.006) [12].

Previously, Hak et al. showed that the unwillingness to comply with new vaccinations such as HBV was 11% among highly educated people and health care workers [9]. These results and our result need further study, whereby it needs consideration that the HBV vaccine will be offered in a combination vaccine (DPT-IPV-HBV/Hib) for infants and as separate injections to adolescents [8].

4.2. Determinants of vaccination behaviour

Distal determinants that were associated with HBV vaccination refusal were socioeconomic variables, where a higher level of education or income was associated with HBV vaccine refusal. In addition, the time since migration to the Netherlands was an important distal determinant associated with HBV vaccination refusal. Proximal determinants that were significantly associated with HBV refusal were low risk perception, no anticipated regret, and a fear of a negative influence on the immune system (Table 2). This is consistent with other studies [10,13–17]. It suggests that health promotion interventions aimed at increasing vaccination coverage should focus on improving appropriate risk perception.

Many studies have concentrated on socioeconomic variables and vaccine behaviour, however Paulussen et al. [10] and Prislin et al. [16] found that these factors were not associated with vaccination behaviour. Important differences between these studies and our study are that we concentrated on a specific and targeted vaccine, whereas the other studies concentrated on vaccination behaviour in general.

We were unable to find socioeconomic determinants that were significantly associated with HBV-under vaccination (whether consciously refused or not). It has previously been shown that low HBV vaccine coverage in The Netherlands is more prevalent among HBV-eligible children living in rural areas, especially among HBV-eligible children of Surinamese/Antillean parents [7].

4.3. Limitations

Our study had several limitations. The possibility of selection bias is high in our study. Although we were unable to compare non-responders with responders with respect to their socioeconomic background and duration of residence in The Netherlands, it would seem fair to assume that response rates are higher among parents that are highly educated and more integrated in the Dutch community.

Response rates among FGMs were low, especially in FGMs from Morocco. Our efforts to improve response rates in this group by sending questionnaires translated to Arabic (official language of Morocco) did not seem to be effective because no translated questionnaire was returned. Furthermore, the number of HBV vaccine refusers was lower than the number of children registered as incompletely vaccinated for HBV. This limited the power of our study since only HBV refusers could be used to determine factors associated with HBV vaccine refusal. We were unable to perform multivariate analysis due to a limited number of HBV refusers.

All five-point answers were dichotomised in order to enhance analysis. However, this reduction in the number of categories could have distorted our results since the 'disagree' category also contained the middle category (score = 3). Therefore, the number of people disagreeing with a given statement might be overestimated. Furthermore, we were unable to determine whether children registered as incompletely vaccinated in 'Praeventis' were completely unvaccinated or partially vaccinated. This could have influenced our results, since determinants of partial vaccination have been shown to differ from non-vaccination [18].

We found that a higher income was significantly associated with vaccine refusal. However, 25% of the respondents did not answer the question on household income, which could have biased our results.

4.4. Conclusions

In conclusion, our data suggest that HBV vaccine refusal does not explain the relatively low HBV vaccine coverage in eligible children. We found that HBV vaccine refusal is more prevalent among parents of HBV-eligible children from Surinamese/Antillean origin, probably due to a lower risk perception for HBV among these parents. Among study participants, a considerable proportion of parents born in the Netherlands who participate in the NIP had a negative attitude towards universal HBV vaccination.

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Chapter 2.8

Immunogenicity of a hexavalent vaccine co-administered with 7-valent pneumococcal conjugate vaccine Findings from the national immunization program in the Netherlands

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Immunogenicity of a hexavalent vaccine co-administered with 7-valent pneumococcal conjugate vaccine Findings from the national immunization program in the Netherlands

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The hexavalent vaccine Infanrix hexa was introduced into the national childhood vaccination schedule in the Netherlands in 2006. It is offered, concomitantly with pneumococcal vaccine (prevenar), to children at increased risk of hepatitis B, administered in a 4-dose schedule at 2, 3, 4 and 11 mo of age. We assessed the immunogenicity of the HBV component of Infanrix hexa co-administered with prevenar, and compared pertussis and Hib components in Infanrix hexa with the standard Infanrix-IpV + Hib vaccine. Target thresholds for immune responses were achieved for all antigens studied. Over 99% (163/164) of children vaccinated with Infanrix hexa achieved an adequate immune response (≥10 mIU/ml) to the HBV component and peak anti-HBs geometric mean concentration (GMc) was 2,264 mIU/ml (95% cI: 1,850–2,771 mIU/ ml). The GMc of a pertussis component, filamentous hemagglutinin (FHa), of Infanrix-hexa was significantly lower in children vaccinated with Infanrix hexa and prevenar than in children vaccinated with Infanrix-IpV + Hib. Universal infant HBV vaccination using Infanrix hexa was introduced in the Netherlands in 2011. Despite very high rates of seroconversion for the HBV component of Infanrix hexa, its long-term immunogenicity and effectiveness should be monitored after concomitant vaccination.

Abbreviations: IH, infanrix hexa; GMC, geometric mean concentration; HBV, hepatitis B virus; FHA, filamentous hemagglutinin; D, diphtheria; T, tetanus; Pa, acellular pertussis; IPV, poliomyelitis; *Hib*, *Haemophilus influenzae* type b; EMEA, european medicines agency

INTRODUCTION

Combination vaccines are used in national immunization programs for children worldwide. Simultaneous administration with newer vaccines against meningococcal or pneumococcal infection is thought to be more convenient, cost-effective and efficient for health care workers, and more acceptable to parents.[1,2] However, the composition of combination vaccines has become increasingly complex and concerns have been expressed that co-administration of multicomponent and mono-multivalent vaccines may lead to a suboptimal induced immune response.[3] Reduced clinical efficacy was shown in the UK with the *Hemophilus influenzae* type b (Hib) component when given in a combined, acellular pertussis containing vaccine (DTaP-Hib),[4] and detailed assessment of potential antigenic interaction is advocated as new vaccines are introduced into national immunization programs.[3,4]

The hexavalent vaccine, Infanrix hexa (GSK), was introduced into the national childhood vaccination schedule in the Netherlands on 1 June 2006 for children born to hepatitis B infected mothers and children of whom either parent was born in middleor high-endemic countries for hepatitis B (prevalence ≥2%). This corresponds to approximately 18% of the total birth cohort in the Netherlands. Infanrix hexa contains recombinant surface antigen of the hepatitis B virus (HBV) in addition to vaccine components against diphtheria (D), tetanus (T), pertussis (Pa), poliomyelitis (IPV) and *Hemophilus influenzae* type b (Hib).[5] It is administered by a single injection given

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at the same time as the pneumococcal vaccine, Prevenar (Pfizer Pharmaceuticals), added to the schedule in 2006. This limits the number of injections to two at 2, 3, 4 and 11 mo of age, respectively. For children who are not in the target group for HBV vaccination, the pentavalent Infanrix-IPV + Hib (DTPa IPV/Hib), was given in 2005–2006. An alternative vaccine, Pediacel (Sanofi Pasteur, MSD) was used in 2007, while Infanrix-IPV + Hib was reintroduced from 2008 onwards and is given concomitantly with Prevenar. Universal infant HBV vaccination using Infanrix hexa will be introduced in September 2011.

Hexavalent vaccines were first licensed by the European Medicines Agency (EMEA) in 2000. In September 2005, authorization of one such vaccine, Hexavac (Sanofi Pasteur, MSD), was suspended by the EMEA: this was due to concerns about long-term immunogenicity of the hepatitis B component when co-administered with meningococcal or pneumococcal vaccines, and reduced boostability post-priming with Hexavac in infants with a low initial immune response.[6] The WHO target threshold of ≥95% of vaccine recipients achieving a peak geometric mean concentration ≥10 IU/l has consistently been achieved in other research.[7-9] However, reductions (albeit non-significant) have been observed in the seroconversion rate[8] and geometric mean concentration (GMC),[8,9] of HBV antibodies following vaccination with Infanrix hexa concomitantly with pneumococcal vaccine when compared with that of Infanrix hexa administered alone. Reduced Hib immunogenicity has similarly been reported in combination vaccines co-administered with meningococcal and pneumococcal vaccines conjugated to the CRM_{197} carrier protein.[10,11] Long-term immunogenicity of the HBV component[12,13] and of all components (with the exception of PT),[14] of Infanrix hexa administered alone have been shown. This is irrespective of peak GMCs post primary vaccination, because they were not determined in these studies. The effectiveness of Prevenar and the Hib component of Infanrix hexa have also been demonstrated.[15,16] There is as yet however, no evidence/data of the long-term immunogenicity or effectiveness of Infanrix hexa co-administered with Prevenar. In the context of the National Immunisation Programme (NIP) in the Netherlands, we assessed whether the immune response to HBV vaccination within the Dutch NIP was sufficient according to WHO standards. Our secondary objective was to compare the immunogenicity of the Hib and pertussis components between Infanrix hexa co-administered with Prevenar with that of the standard vaccine, Infanrix-IPV + Hib administered alone.

RESULTS

In total, 478 children were eligible to participate in group 1 and 1,181 in group 2. The proportion of respondents was 34% (n = 164) and 8% (n = 92) respectively. In group 1, 100% of children were born in the Netherlands, with only 26% of fathers and 23% of mothers themselves born in the Netherlands. Parents were of mixed ethnicities. Most commonly reported ethnicities were: Turkish (15%), Moroccan (12%), Antillian (4%) and Surinamese (3%). The mean gestational age in group 1 was 39 weeks (range 33–42 weeks) and the mean birth weight was 3,275 g (range: 1,870 g–4,760 g); 49% (n = 72/146) were male.

At the time of booster vaccination, children were a median age of 11.1 mo in group 1 (range: 9.6 to 13.4) and 11.7 mo in group 2 (range: 10.1 to 13.5), $p < 0.001$. The median time between last vaccination and blood sampling was 5.3 weeks in group 1 (range: 1.6 to 19.8) and 4.0 weeks in group 2 (range: 3.3 to 9.3 weeks), $p < 0.001$. Proportions achieving seroprotection and geometric mean concentrations (GMCs) are presented in **Table 1**.

Of children in group 1, 99.4% (163/164) achieved an adequate immune response (≥10 mIU/ml) to the HBV component of Infanrix hexa. The geometric mean concentration was 2,264 mIU/ml (95% CI: 1,850–2,771 mIU/ml). Male infants had a lower anti-HBs concentration (1,830 mIU/ml, 95% CI: 1,357– 2,469 mIU/ml) than females (2,898 mIU/ml, 95% CI: 2,110– 3,980 mIU/ml, $t = -2.1$, $p = 0.03$). This difference persisted after controlling for ethnicity of parents (dichotomised as any-Dutch vs. no-Dutch parent), birth weight and gestational age and explained 5% of the variance in anti-HBs (adjusted r-squared = 0.05, $p = 0.002$).

For both groups 1 and 2, no statistically significant differences in the proportions achieving seroprotection were seen between Infanrix hexa and Infanrix-IPV + Hib for Hib or pertussis. An adequate immune response was consistently achieved in over 98% of samples to all antigens tested. Five different children did not reach the seroprotective level in one component only, but each child responded satisfactorily to all the other components in the vaccines (Table 1). In relation to Prevenar, seven children did not achieve the seroprotective threshold of one or more components. In 2 cases, children did

not respond to several subtypes (case 1, to subtypes 4, 9, 18c and 23f, and case 2 to subtypes 4 6b 9v 18c and 23f); five children showed an inadequate response to one component only (subtypes 6b (n = 2), 14, 18c and 23f (n = 1 each)). The proportion achieving pneumococcal antibody concentrations of ≥0.35 µg/ml ranged from 98.2% (subtypes 6B, 18C and 23F) to 100.0% (subtype 19F). The pneumococcal antibody GMCs ranged from 2.6 µg/ml (subtype 18C) to 11.0 µg/ml (subtype 14) (**Table 1**).

When comparing GMCs between groups 1 and 2 (**Fig. 1**), the titers against the pertussis FHA component of Infanrix hexa was significantly lower than in Infanrix-IPV + Hib (302 EU/ml vs. 422 EU/ml; mean difference, 120 EU/ml; p = 0.001). There were no other statistically significant differences between antibody GMCs of other analyzed vaccine components.

DISCUSSION

The HBV component of Infanrix hexa co-administered with Prevenar adhered to the WHO guideline for an adequate immune response (≥95%),[17] achieving seropositivity in ≥99% of cases. We found an anti-HBs GMC of 2,264 mIU/ml (95% CI: 1,850–2,771), higher in girls than in boys. The anti-HBs GMC reported here is lower than has been reported elsewhere one month post-booster dose (given at 12–15 mo) after the same primary vaccination schedule and using the same standard immune

Table 1. Proportion of samples achieving seroprotection (%), and peak antibody GMC levels of Infanrix hexa + Prevenar (Group 1) and Infanrix-IPV + Hib (Group 2) vaccines

Vaccine Component	Immunogen	Target concentration	% Achieving seroprotection				Geometric Mean Concentrations			
			Group 1 (n = 164)		Group 2 (n = 92)		Group 1 (n = 164)		Group 2 (n = 92)	
			Infanrix hexa + Prevenar[a]		Infanrix IPV/Hib		Infanrix hexa + Prevenar		Infanrix IPV/Hib	
			% (n)	[95% CI[c]]	% (n)	[95% CI]	GMC	[95% CI]	GMC	[95% CI]
Hemophilus Influenza B	Anti-PRP	≥0.15 µg/ml	98.7 (162)	[95.9–99.9]	98.9 (88[b])	[93.9–99.9]	7.3	[5.9–9.0]	9.8	[7.0–13.9]
Pertussis	PT	>25 EU/ml[d]	98.2 (161)	[95.0–99.6]	100.0 (92)	[96.7–100]	148.1	[130.3–168.2]	134.1	[118.7–151.4]
	FHA[e]	>25 EU/ml	100.0 (164)	[97.9–100.0]	100.0 (92)	[96.7–100]	302.2	[274.5–332.7]	422.0	[367.1–485.0]
	PRN	>25 EU/ml	99.4 (163)	[96.8–99.9]	100.0 (92)	[96.7–100]	380.3	[331.1–436.8]	410.1	[343.7–489.2]
Hepatitis B	Anti HBS	≥10 mIU/ml[f]	99.4 (163)	[96.8–99.9]			2264.1	[1849.7–2771.3]		
Pneumococcus serotypes	4	≥0.35 µg/ml for all subtypes	98.8 (161)	[95.9–99.9]	N/A[g]		3.7	[3.2–4.2]	N/A	
	6b		98.2 (160)	[95.0–99.6]			5.2	[4.4–6.0]		
	9v		98.8 (161)	[95.9–99.9]			3.4	[3.0–3.8]		
	14		99.4 (162)	[96.8–99.9]			11.1	[9.6–12.8]		
	18c		98.2 (160)	[95.0–99.6]			2.7	[2.3–3.0]		
	19f		100.0 (163)	[97.9–100.0]			3.7	[3.3–4.2]		
	23f		98.2 (160)	[95.0–99.6]			4.0	[3.4–4.8]		

[a]Prevenar, Ntotal=163; [b]Due to insufficient samples, results for anti-PRP were available for n=89 samples; [c]Exact confidence intervals for a proportion; [d]EU/ml: ELISA units per millilitre; [e]Difference between the geometric mean titres was significant, p<0.001 ; [f]IU/ml: International Units per millilitre; [g]Not applicable

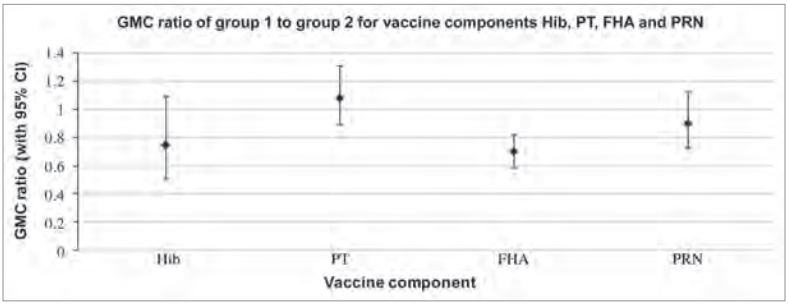

Figure 1. GMC ratio of group 1 to group 2 for vaccine components Hib, PT, FHA and PRN.

assays.[8,9] Anti-HBS GMCs 2.6 to 3.4 times higher than those observed here are also reported where Infanrix hexa was administered alone (5,754 mIU/ml,[8] 6,539 mIU/ml[9] and 7,517 mIU/ml[18] respectively, though in the latter study, the vaccination schedule differed: 2, 4, 6 mo with a booster at 12–19 mo). Peak GMCs achieved post-vaccination are thought to be important because they are associated with the duration that concentrations remain above accepted protective thresholds. Although these studies are not directly comparable, such differences might be explained by the fact that children were boosted at 11 mo here rather than at 12–19 mo in the studies referenced. Second, there was a slightly longer period between the last vaccination and serum collection here (5.3 weeks), vs. 4 weeks in Tichmann-Schumann et al. Third, children in our study who received HBV vaccination (group 1) all had parents of mixed ethnicities whereas the studies referenced[8,9] included samples of the general population. Ethnic differences have been implicated in reduced serological response in other research.[19] Fourthly, it is possible that the maternal anti-HBs concentrations at the time of immunization and sampling influenced infant anti-HBs, though the effect of this on the immune response to the vaccination series is uncertain. We could not study the effect of maternal antibody concentration. Finally, gender-linked differences in vaccine immune response are not uncommon:[20] females have been reported to develop a more robust immune response to rubella and mumps vaccines for example.[21,22] Although there are still uncertainties about the long-term duration of protection after vaccination against hepatitis B,[23,24] it is clear that immunological memory with anamnestic response continues to protect children against acute disease despite undetectable antibodies.[13,25,26] Infanrix hexa was introduced into the universal childhood vaccine schedule in the Netherlands in September 2011 and protection against Hepatitis B will therefore be necessary for decades.[27]

In relation to the antibody response to Prevenar when coadministered with Infanrix hexa, seroprotective thresholds to all seven serotypes included in the vaccine were achieved. GMCs for all components of Prevenar were similar to those reported in previous studies.[8,9] On comparing Infanrix hexa co-administered with Prevenar and Infanrix-IPV + Hib (administered alone). There was no difference in the proportions seroprotected for Hib and pertussis. These findings are again broadly similar to those in other research.[8,9,28] Co-administration of Infanrix hexa with Prevenar did not affect GMCs for Hib, PRN or PT—but the FHA component in Infanrix hexa was significantly lower. Though speculative, it is hypothesized that FHA could play a role in modulating the protective immune response in combination vaccine formulations.[29]

There were a number of study limitations. We studied a nonrandomized sample of two groups of healthy infants recruited in

2006 and 2009. Different clinics participated in 2009 (group 1) and in 2006 (group 2) but regional variation in the delivery of the vaccination program over time, including vaccine storage and administration is unlikely. National immunization coverage in the Netherlands consistently exceeded 94% for DT-Pa-IPV/Hib in the general population since 2006. In 2009, uptake of Infanrix hexa in the risk group was 92.9%.[30] The time of the 4th vaccination and sampling of children was statistically different between the groups (persisting when outliers were excluded i.e., those beyond ±2* standard deviations of the mean). In absolute terms the difference was small and both vaccination and sampling were timely. Finally, the proportion of respondents from each clinic varied significantly by study group. This is probably explained by greater staff vigilance and a higher intensity of recruitment in the minority group who were administered a new vaccine. Other differences such as socioeconomic background were not recorded, although, given that only healthy babies were included, it is unlikely that there were systematic differences between respondents and non-respondents that would influence the immunogenicity of the vaccines.

In conclusion, more than 99% of Infanrix hexa recipients vaccinated at 2, 3, 4 and 11 mo of age and measured one month after booster vaccination, achieved an adequate immune response to the HBV component. For all components of both vaccines, standard seroprotective thresholds were achieved. The GMC for the anti-HBs component of Infanrix hexa co-administered with Prevenar reported here was robust, though it was lower than has been reported elsewhere. When compared with Infanrix-IPV + Hib vaccine, a significant reduction in peak GMC was observed for the FHA component of Infanrix-hexa. Antibodies to other components (Hib, PRN, PT) were similar. Despite very high rates of seroconversion, there is a lack of long-term immunogenicity and effectiveness data for Infanrix hexa simultaneously administered with Prevenar. Long-term monitoring of children by immunoand disease surveillance is indicated.

MATERIALS AND METHODS

Study design. A sample of children vaccinated at 2, 3, 4 and 11 mo of age who attended healthy baby clinics in 10 municipalities and who had an indication for hepatitis B vaccination, was invited to participate in the study. Children with chronic disease or Down syndrome and children of HBsAg positive mothers were excluded. This group, Group 1, were children born after 1 June 2006 and were vaccinated with Infanrix hexa co-administered with Prevenar. For children in group 1, information about the ethnicity of parents and their children, gestational age, birth weight and gender was also recorded.

To meet the second objective, a second group of children not considered at risk of HBV, Group 2, born from 1 November 2004 through 31 March 2005 and vaccinated with InfanrixIPV + Hib, were recruited. Parents with children aged 11 mo in group 1 were invited in person to participate at the healthy baby clinic and those in group 2 received an information letter at home. Both groups were asked to return to the clinic 4 to 6 weeks after booster vaccination. Written informed consent was obtained from the parents and a blood sample was taken from the child. The study protocol was approved by the Medical Ethics Review Committee (METC) of UMC Utrecht (group 1) and by the Central Committee on Research in Human Subjects (CCMO) in the Hague (group 2).

Laboratory methods. Venous samples were collected from infants 4 to 6 weeks post-vaccination after the 4th vaccine dose. Serological testing was performed at the Dutch National Institute for Public Health and the Environment (RIVM). Hib, pertussis and pneumococcal specific antibodies were measured using an ELISA with a 2-fold dilution series of samples. On each plate an in-house reference serum and a control serum were included. The in-house reference sera were calibrated to international reference sera[31,32] as follows: Hib IgG antibody concentrations (μg/ ml) with reference serum of CBER, FDA (Lot 1983);[33] pertussis IgG antibody concentrations in ELISA Units/ml (EU/ml) against pertussis antigens Ptx and FHA with lot 3 and PRN with lot 4 FDA;[31,32] and pneumococcal IgG antibody concentrations (μg/ml) with 89S-reference serum.[11,34] Hepatitis B markers included anti-HBs and HBsAg. Anti-HBs was determined using ELISA (Axsym, Abbott). HBsAg was determined only in children with an anti-HBs titer of <1,000 IU/ml. The proportion of respondents achieving seroprotection was estimated according to the relative frequency of vaccinees achieving antibody concentrations above the pathogen specific cut-off levels: 0.15 μg/ml for Hib-PRP[35,36] and 0.35 μg/ml for pneumococcus.[11] There are no internationally accepted standards for pertussis. Field studies have shown however, that antibodies directed against virulence factors pertactin (prn), fimbriae 2/3 (Fim) and pertussis toxin (ptx) are protective.[37,38] For filamentous hemagglutinin (FHA), this is more complex.[39] We used the arbitrary industry standard of 25 EU/ml.

Power calculation. We determined the sample size by considering only the primary objective regarding HBV immunogenicity in group 1. The WHO standard for an adequate immune response requires ≥95% of the vaccinees to achieve an anti-HBs titer ≥10 IU/ml. We expected 98% of vaccines to achieve this. At a precision of 2.5%, with a confidence level of 95%, 120 children were required in group 1. Based on a response of 32% in a recent cross-sectional population-based Dutch national sero-survey,[40] it was estimated that a minimum of 480 children should be invited in group 1.

Statistical analysis. Data were entered and analyzed using Microsoft Access and STATA 11. The proportion of respondents achieving seroprotection with its confidence limits was calculated using exact methods.[41] The geometric mean concentration (GMC) was calculated using the antilog of the mean of the logarithmically transformed antibody concentrations. Given the non-normal distribution of the outcome variables, categorical associations were tested using the Kruskal-Wallis (KW) test. Based on Tukey's ladder of powers,[42] the square-root transformed outcome variable was used to conduct linear regression to determine how much of the variance in anti-HBs could be explained by predictor variables. To detect significant differences between the GMCs of groups 1 and 2, Student's t-test was conducted (independent groups, two-tailed).

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Chapter 3

Hepatitis B virus in England and Wales

Chapter 3.1

Incidence and routes of transmission of hepatitis B virus in England and Wales, 1995–2000: implications for immunisation policy

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Incidence and routes of transmission of hepatitis B virus in England and Wales, 1995–2000: implications for immunisation policy

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Background: The incidence of hepatitis B virus (HBV) infection in the UK is low. Since the infection can have serious sequelae, there is a continuing need to examine its epidemiology so as to inform control measures. *Objectives:* We aimed to describe the current HBV incidence and patterns of transmission in the UK, to estimate the rate of new carrier infections, and to discuss implications for the control of HBV through immunisation. *Study design:* We analysed routine England and Wales laboratory surveillance data of acute HBV infection (1995–2000) and data on migration and global HBsAg prevalence. *Results:* The estimated annual incidence of HBV infection in England and Wales was 7.4 per 100,000. Injecting drug use was the most frequently reported route of transmission. The number of cases attributed to heterosexual contact was fairly stable, whereas the number of cases in men having sex with men decreased. These observations continue trends reported for the early 1990s. Transmission during childhood was rarely reported, but was more frequent among South Asians. The incidence in South Asians is relatively high, and their main risk factors are medical treatment overseas and heterosexual contact. For about a third of cases of acute HBV infection no route of transmission is reported, but analysis of secular trends and age distribution suggest that many of these may be related to injecting drug use. Endemic transmission gives rise to only a small proportion of all new chronic infections, with the vast majority arising from immigration of established HBV carriers. *Conclusions:* The incidence of acute HBV infection in England and Wales has remained low, with a similar pattern of reported routes of transmission compared to the early 1990s. The UK prevalence of HBV infection is dependant on global rather than national immunisation policy. Endemic transmission may be reduced by improving immunisation coverage among injecting drug users, which is expected to also reduce the number of cases without a risk factor reported. In addition, immunisation options that better suit the needs of ethnic minorities need to be explored.

Keywords: Hepatitis B virus; HBV; Epidemiology; Vaccination policy; Injecting drug use

Abbreviations: HBV, hepatitis B virus; HPA-CDSC, Health Protection Agency-Communicable Disease Surveillance Centre; MSM, men who have sex with men; CI, confidence interval

1. INTRODUCTION

The incidence of hepatitis B virus (HBV) infection varies widely between countries, with the estimated life time risk of infection ranging from 0.4% in the UK to over 90% in East Asia (Ramsay et al., 1998; Kane et al., 1993). A likely explanation for this marked difference is that a higher incidence is linked to a lower average age at infection, which has a much higher probability of development of HBV carriage, and therefore of onward transmissions (Edmunds et

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al., 1996a; Medley et al., 2001).

Although the incidence of infection in England and Wales is amongst the lowest in the world, its potential to cause chronic infection with serious sequelae such as cirrhosis and cancer of the liver means that control of HBV infection is still a public health priority.

Safe and effective vaccines to prevent HBV infection have been available since 1982, and the World Health Organization (WHO) recommended that all countries should have implemented universal immunisation programmes by 1997 (WHO, 1992). However, the current HBV control programme in the UK is based upon selective immunisation of those at highest risk of infection and universal antenatal screening and immunisation of babies born to infected mothers (NHS, 1998; Salisbury and Begg, 1996). The UK strategy is based on the observation that overall incidence of HBV is low, that the risk of infection is distributed heterogeneously in the population (Mortimer and Miller, 1997), and that it is determined mainly by country of birth, ethnicity and adult risk behaviours such as injecting drug use (Soldan et al., 2000; Aweis et al., 2001; Department of Health, 2000). There is a continuing need to examine the effectiveness of the UK's HBV vaccination strategy. This is done by analysing current incidence and patterns of transmission. The main source of data for this is the laboratory surveillance of acute HBV infection in England and Wales, complemented by prevalence surveys among groups at different risk of infection. The surveys allow quantification of the burden of infection at a population level, including the many chronic infections acquired prior to immigration into the UK. These latter infections are not preventable by any UK programme, and they limit the potential impact of
vaccination on the overall burden of HBV infection.

We have analysed recent routine surveillance data on acute HBV infection in England and Wales, estimated the number of infections imported through immigration, and discuss implications for the control of HBV through immunisation.

2. MATERIALS

2.1. Patients and samples

Laboratories in England and Wales routinely report acute HBV infections and associated risk factors to the Health Protection Agency-Communicable Disease Surveillance Centre (HPA-CDSC). A case of acute HBV infection is normally defined by positive serology for HBsAg and anti-HBc IgM, or seroconversion to anti-HBc detected during epidemiological investigation in a person with or without a compatible illness. Cases reported between 1 January 1995 and 31 December 2000 were included in the analyses. The majority of case reports include information on age, sex, most likely route of transmission and country of exposure. These countries were grouped into low, intermediate and high endemicity for HBV, according to WHO criteria. Duration of residence in, or travel to the country of exposure was not recorded. Infection was considered to have been endemically acquired (in the UK) when no travel was reported to a high or mid endemic country during the incubation period. Information on country of birth or ethnicity is not reported and therefore 'Nam Pehchan' software (Bradford Health Authority, 1995) was used to assign South Asian ethnicity based on names. The results of 'Nam Pehchan' correlate well with self assigned ethnicity (Harding et al., 1999), with South Asian referring to the Indian subcontinent. Names are temporarily held on the laboratory surveillance database in agreement with guidance on confidential patient information (http://www.doh.gov.uk/ipu/confiden/index.htm).

To estimate the incidence of infection, the number of laboratory reports was adjusted to take into account underreporting and asymptomatic infections. The proportion of under-reporting is assumed to be 25% (Ramsay et al., 1998). The proportion of infections that is symptomatic (and may therefore lead to diagnosis and reporting) is age-dependent. From a review of literature a model was fitted by assuming a logistic function with three parameters (Appendix A). The parameters were estimated by the maximum likelihood method.

Mid-1997 population estimates from the Office of National Statistics (ONS) were used to calculate incidence rates. Mid-2000 population estimates from the ONS's Labour Force Survey were used to calculate the incidence rate ratio by ethnicity.

To assess the validity of our incidence estimate, we compared the resulting cumulative incidence by age with the prevalence of infection (anti-HBc prevalence) found in population surveys (Gay et al., 1999). This prevalence will reflect incidence from both during and before the period of surveillance analysed here.

Incidence estimates were subsequently used to estimate the annual number of new chronic infections resulting from infection in residents of England and

Wales. Up to 32 years of age, an age specific risk of chronic infection was calculated by a formula based on a meta-analysis of observational studies (Appendix A) (Edmunds et al., 1993). For infections acquired over 32 years of age, a 4% risk of chronicity following infection was applied (Hyams, 1995).

In order to quantify the additional impact of universal immunisation over and above that potentially achievable with the current selective strategy, cases were divided into two groups: Firstly those due to transmission that was potentially preventable by selective immunisation, and secondly those due to transmission that could only be interrupted by universal infant immunisation.

2.2. Cases of chronic HBV infection imported by immigration

The net annual migration of chronically HBV infected persons into England and Wales was estimated by subtracting the number of emigrants from the number of immigrants, taking into account the prevalence of HBsAg in their country of birth and their country of last residence, respectively. Data was used from the ONS International Passenger Survey (1996–2000), combined with country specific HBsAg prevalence estimates (WHO).

2.3. Statistical tests

Data were handled in Excel 2000 and Access 2000. Analysis were done with Stata statistical software, version 8.1 (StataCorp, College Station, TX, USA). Proportions were tested with the Chi-square statistic. The command 'nptrend' in Stata was used to test for the presence of trends in median ages. Details of parameter estimation using maximum likelihood are given in Appendix A.

3. RESULTS

3.1. Incidence of acute HBV infection and demographics

Between 1995 and 2000, on average 673 cases of laboratory confirmed acute HBV infection were reported to the HPA-CDSC each year, with a peak of 840 cases in 1998 (Fig. 1). The average incidence of laboratory reports was 1.3 per 100,000 per year, with an incidence rate of 1.6 per 100,000 per year among those aged 15 years or older. The number of reports represents an estimated total of 3,780 infections per year, resulting in an estimated 269 new chronic infections per year. From this, the true incidence of HBV infection was estimated to be 7.4 per 100,000 persons per year. At this incidence, the lifetime risk of infection was 0.55% and the lifetime risk of chronic infection was 0.04%. At median population age (36 years), the cumulative risk of infection was 0.45%. This is similar to the prevalence of anti-HBc found in UK born blood donors in 1995 (0.49%) (Soldan et al., 2000), but lower than the 3.9% prevalence of anti-HBc found in a population sample of adults in 1996 (Gay et al., 1999).

Of all laboratory reported cases, 68% were in males and 30% in females (ratio M:F = 2.3:1); for 2%, the sex was not reported. The median age of infection (range 0–91 years) was 31 years for males (95% confidence interval (CI): 30–31 years), and 27 years for females (95% CI: 26–28 years). The majority of acute infections occurred in young adults, with the

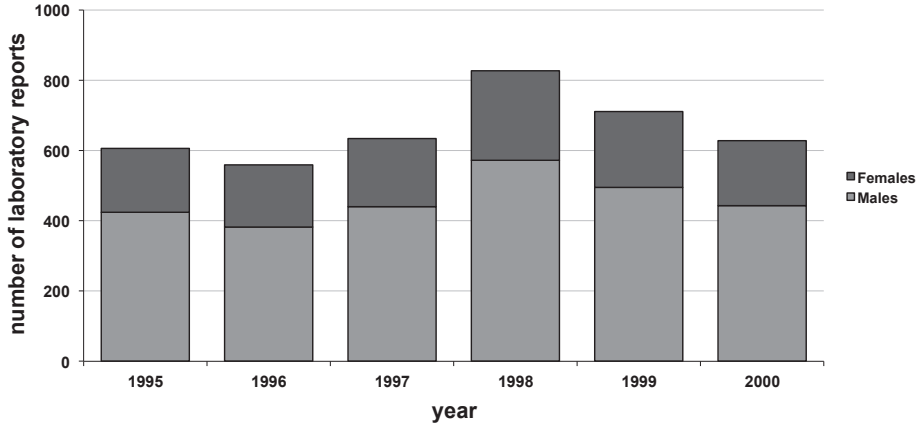

Fig. 1. Number of laboratory reports of acute HBV infection by year and sex, England and Wales, 1995–2000 (for on average 13 cases per year the sex was not recorded).

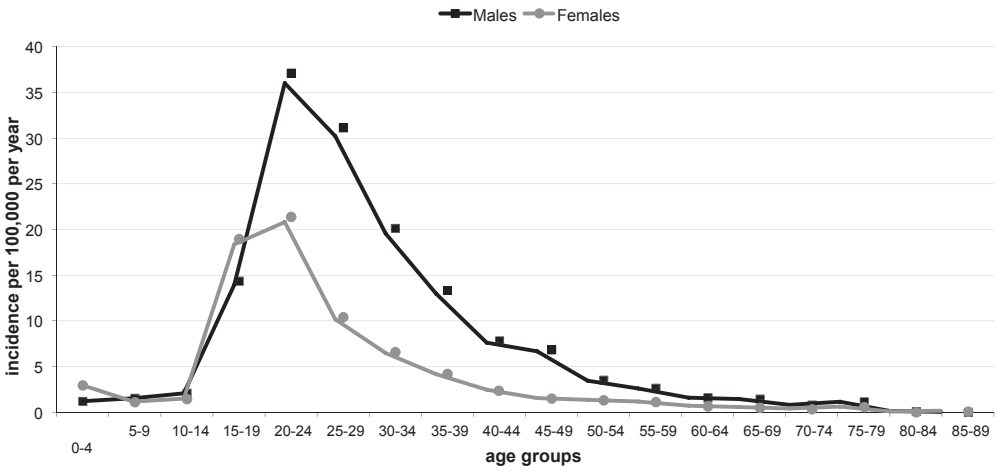

Fig. 2. Estimated incidence of HBV infection (per 100,000 persons per year) by age group and sex, England and Wales, 1995–2000.

estimated age specific incidence of infection highest among those between 15–24 years of age (Fig. 2).

3.2. Risk factors in those ≥15 years of age

Of the 4,040 acute HBV infections reported between 1995 and 2000, 3,905 (97%) were in individuals of 15 years or older ('adults'). For 2,455 (63%) of these, the report included information on the most likely route of acquisition of the infection. Injecting drug use was the most frequently reported route among both male and female adult endemic cases (males: 843 cases (55% of endemic male cases with a risk factor reported); females: 301 cases (43%)). The annual number of cases attributed to injecting drug use peaked in 1998 (Fig. 3). The median age of infection among injecting drug users remained constant over time at around 27 years (Fig. 4).

Homosexual intercourse was the second most frequently reported risk factor among endemic male cases in adults (n = 327 cases, 21% of male cases with a risk factor reported). The total number of cases attributed to male homosexual contact decreased

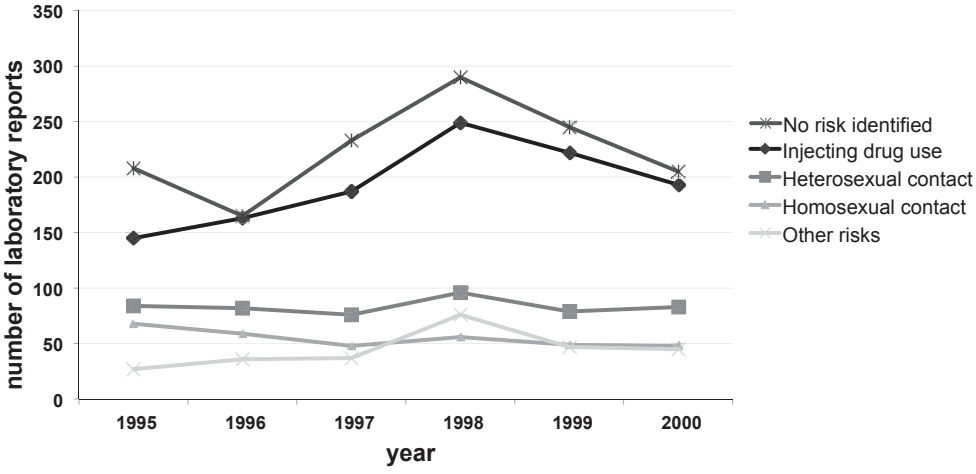

Fig. 3. Number of laboratory reports of acute, endemically acquired HBV disease, by most likely route of acquisition, persons 15 years of age and older,England and Wales, 1995–2000.

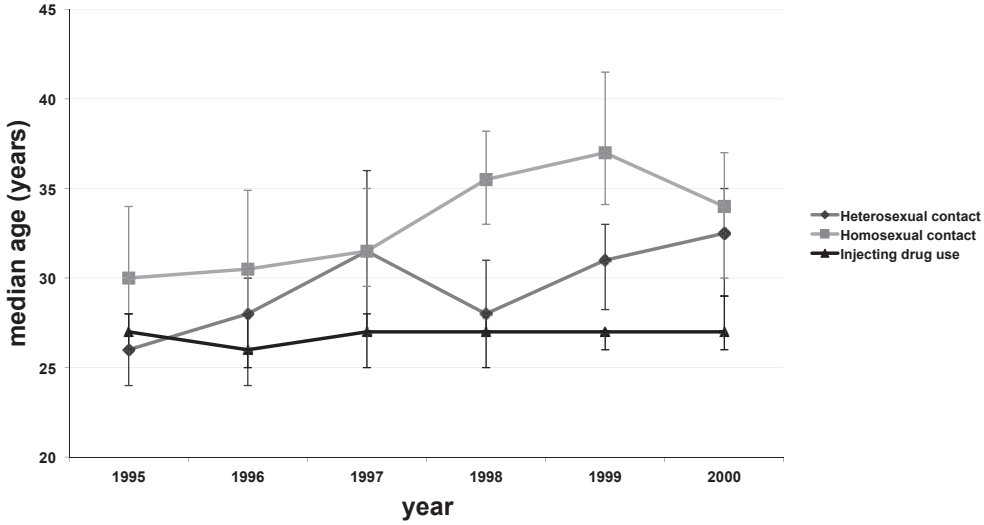

Fig. 4. Median age, with 95% confidence interval, of laboratory reported cases of acute HBV infection by most likely route of acquisition and year, England and Wales, 1995–2000.

from 70 in 1995 to 55 in 2000. The median age of infection among men who had sex with men (MSM) increased from 30 in 1995 to 34 in 2000 (*P*-value for trend 0.01, Fig. 4). The proportion of acute infections with travel to an intermediate or high endemicity area during the incubation period, was significantly lower for homosexually compared to heterosexually acquired infections (6% versus 17%, $P < 0.001$).

Among endemic female cases of 15 years or older, the second most frequently reported route of transmission was heterosexual transmission (295 cases, 42% of cases with a risk factor reported).

The overall number of cases attributed to heterosexual contact remained stable at about 100 per year during the period under study, though with a slight increase to 120 in 1998. The median age of cases attributed to heterosexual contact increased steadily from 26 in 1995 to 33 in 2000(*P*-value for trend <0.001), with exception of 1998 when the median age of infection was 28 years (Fig. 4).

3.3. Travel

A total of 482 (12%) cases were in people who reported overseas travel during the incubation period. Of these, 173 (45% of those with information on country of destination ($n = 384$)) had travelled to a country of intermediate endemicity, and 147 (38%) to a high endemicity country.

Among those who travelled to intermediate endemic countries, medical treatment and heterosexual sex (36 and 34%, respectively) were the most frequently reported routes of transmission. For those

Table 1
Average annual number of laboratory reports of acute HBV infection, estimated annual number of acute HBV infections and estimated annual number of infections leading to carriage by age group for (a) total population and (b) South Asian ethnic groups, England and Wales, 1995–2000

Age group	Laboratory reports		Estimated number of infections		Estimated number of chronic infections	
(a) Over all						
Perinatal (≤2 years)	3	0.4%	48	1.3%	32	11.9%
Childhood (3–14 years)	10	1.5%	122	3.2%	23	8.6%
Adult (≥15 years)	660	98.1%	3610	95.5%	214	79.6%
Total	673		3780		269	
(b) South Asians						
Perinatal (≤2 years)	1	2.7%	15	7.2%	11	42.3%
Childhood (3–14 years)	2	5.4%	30	14.4%	6	23.1%
Adult (≥15 years)	34	91.9%	164	78.5%	9	34.6%
Total	37		209		26	

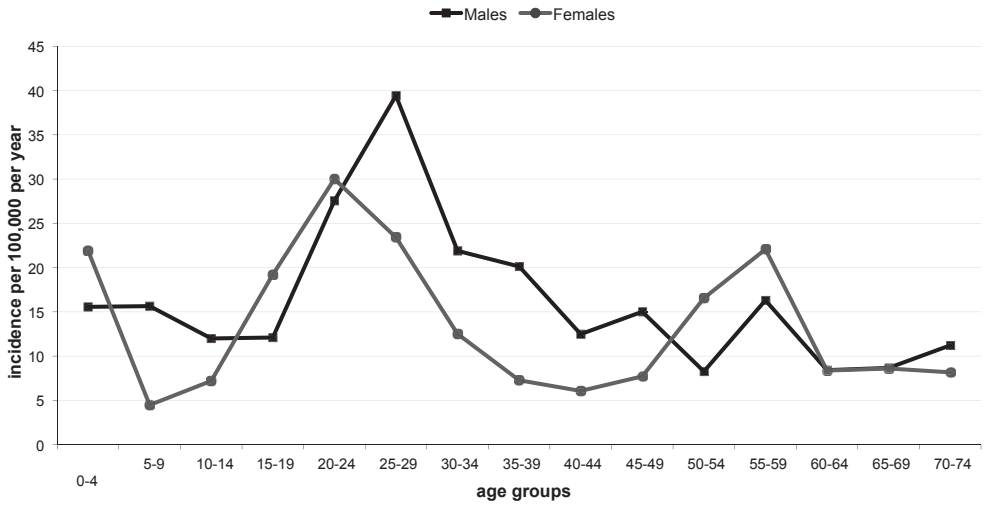

Fig. 5. Estimated incidence of HBV infection (per 100,000 persons per year) by age group and sex, South Asians, England and Wales, 1995–2000.

who had travelled to high endemicity countries, heterosexual sex was the most frequently reported route of transmission reported (68 cases, 70% of cases with information on risk factors). The Indian subcontinent was the most common destination (99 cases, 26% of cases in travellers with information on the country of destination), and medical treatment was the most frequently reported risk factor there (32 cases, 52% of those with a risk factor reported).

3.4. South Asian ethnic minority

Names were included in 68% (2,763) of all laboratory reports, and of these 8% (222 cases) were identified as South Asian. The male to female ratio among South Asians was 1.3 to 1. Laboratory reports of infection were relatively more common in South Asian children and adults over 40 years of age compared to the overall distribution by age group (Table 1, Fig. 5). The estimated overall incidence of infection in South Asians was 16.4 per 100,000 per year, which is 2.2

Table 2
Number of laboratory reports of acute HBV infection, estimated number of acute HBV infections and estimated number of infections leading to carriage, by reported route of transmission for (a) risk factors targeted in the current programme and (b) risk factors not targeted in the current programme, England and Wales, 1995–2000

Risk factor reported	Laboratory reports (% of all)	Infections (% of all)	Chronic infections (% of all)
(a) Targeted in current programme			
Injecting drug use	1,171	7,239	456
Vertical transmission	8	142	97
Male homosexual contact	331	1,676	90
Travel to high endemicity areas	147	736	50
Institutionalisation	28	152	9
Other occupational risk	6	19	1
Health care work	0	0	0
Total	1,691 (42%)	9,964 (44%)	703 (43%)
(b) Not targeted in current programme			
No risk identified	1,415	7,436	503
Heterosexual contact	504	2,967	191
Travel to intermediate endemicity area	173	967	105
Family/household	55	340	52
Other	70	374	26
Medical treatment/transfusion/dentistry	81	336	19
Unspecified sexual contact	30	162	9
Tattoo/skin piercing	21	132	9
Total	2,349 (58%)	12,714 (56%)	914 (57%)
Total	4,040	22,678	1,617

Table 3
Annual average number of persons and estimated number of HBV-carriers immigrating to and emigrating from England and Wales, 1996–2000 (ONS, WHO)

	To England and Wales	From England and Wales	Net immigration
Migrating persons	300,820	210,600	90,220
Estimated number of migrating persons with chronic HBV infection	9,922	3,351	6,571

times (95% CI: 1.9–2.6 years) the estimated overall incidence of acute HBV in England and Wales.

Among South Asians, 37% of infections (82 cases) were in individuals who were reported to have travelled to an intermediate or high endemicity country during the incubation period. Of these, 83% (68/82) had travelled to South Asia, of whom the majority (57% of those with a risk factor) reported medical treatment as the most likely route of transmission.

The most frequently reported risk factor among endemically (UK) acquired South Asian cases of 15 years or older was heterosexual contact. Injecting drug use and homosexual contact were less frequently reported in them compared to in the equivalent non-South Asian population (6 and 1% of adult South Asian endemic cases compared to 34 and 9% of adult endemic non-South Asian cases, respectively).

3.5. Childhood infection

The number of infections in children reported between 1995 and 2000 (75; 13 per year) was similar to that reported between 1985 and 1996 (on average 14 per year, Balogun et al., 1999). The incidence of laboratory reports among children of 1–9 years of age was 0.10 per 100,000 per year. Childhood infection contributed to 2% of all laboratory reports, and to an estimated 21% of all new chronic infections (Table 1a). Among South Asians, infections in infants were relatively more common with infection in childhood contributing to 65% of new chronic infections (Table 1b, Fig. 5). Overall, the most frequently reported route of transmission in childhood was through contact with a carrier in the family (13/75 (17%)). Mother to infant transmission was reported for 10 (13% of all). Travel to a intermediate or high endemicity country during the incubation period was reported for 13 (17%) children, of which 8 were of South Asian origin. For 38 (51%) cases childhood no risk factor information was reported.

3.6. Infections potentially preventable

An estimated 44% of infections and 43% of new chronic infections were potentially preventable by the current selective programme (Table 2). This assumes that cases in which no risk factor was recorded would not be prevented by a targeted programme. Also, it does not take indirect protective effects into account.

3.7. Cases of chronic HBV infection associated with immigration

During 1996 to 2000 inclusive, the total annual net legal immigration to England and Wales was 90,220 persons (Table 3). The net immigration of persons chronically infected with HBV was estimated to be 6,571 per year. Chronic HBV infection as a result of infection in residents of England and Wales (269 per year, Table 1) is therefore estimated to account for only 3.9% of the total annual incidence of chronic infections ($(269/(269 + 6571)) \times 100$). The estimated average prevalence of HBV carriage among immigrants between 1996 and 2000 was 3.3% (9922/300,820). Even allowing for a substantially lower prevalence of chronic infection than the WHO estimates, infection in residents of England and Wales would account for a small minority of all new chronic infections.

4. DISCUSSION

Our analyses of laboratory surveillance of acute HBV infection in England and Wales between 1995 and 2000 suggest that the incidence of HBV has remained at a low level, though with a modest increase in 1998. This increase was mainly due to an increase in the number of cases reported to have resulted from injecting drug use, and a single outbreak linked to autohaemotherapy (Webster et al., 2000).

The cumulative risk of infection at adult age based on our current incidence estimate is similar to the prevalence of anti-HBc found in UK born blood donors (Soldan et al., 2000). Blood donors are a group selected to be at low risk of blood borne infections, and so the incidence we derived may be an underestimate of the incidence in the general population. Surveillance data suggest, however, that the incidence has declined since the early 1980s (Balogun et al., 1999) and so we would expect prevalence studies in adults to reflect a higher incidence than that suggested by the current analysis. Our cumulative incidence estimate is substantially lower than the prevalence in the population based sample in 1996 (Gay et al., 1999). However, within this sample, prev-

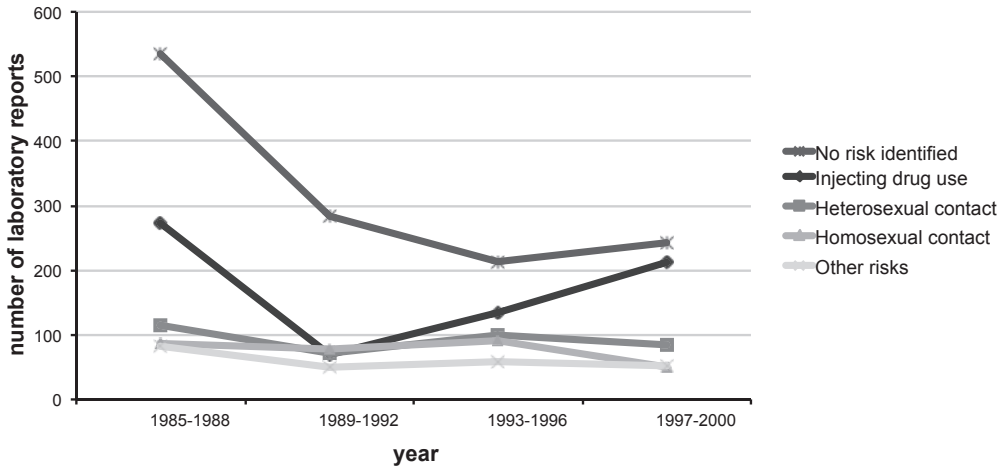

Fig. 6. Number of laboratory reports of acute HBV infection by most likely route of acquisition, persons 15 years of age and older, England and Wales, 1985–2000.

alence in areas with a low proportion of people born in Africa or Asia was lower, suggesting that many of the infections in the population based sample may have been acquired prior to immigration.

4.1. Injecting drug use

Between 1995 and 2000, injecting drug use was the most frequently reported route of transmission for both male and female endemic cases, although the absolute number of infections in injecting drug users was far less than during the mid-eighties (Balogun et al., 1999). The proportion of all cases reporting injecting drug use was increased compared to the mid-eighties, indicating that control of HBV infection in injecting drug users had lagged behind control in other at-risk groups.

Both the number of cases reported to be acquired through heterosexual contact and the number without a risk factor identified show a similar secular trend to the number of cases attributed to injecting drug use. This was apparent in the period under study as well as between 1985 and 1996 (Fig. 6) (Balogun et al., 1999). This parallel trend could result from infections in injecting drug users being wrongly attributed, but is also consistent with injecting drug users being a source of infection among heterosexuals and those without identified risks. High-risk behaviour associated with injecting drug use (such as prostitution) makes injecting drug users a likely source of many secondary transmissions. A molecular epidemiological study in The Netherlands supports this hypothesis (van Steenbergen et al., 2002). Immunisation among injecting drug users therefore has the potential to be highly efficient in reducing HBV incidence both by direct protection of individuals who inject drugs and indirectly through protecting their contacts.

We found that the median age of infection of injecting drug users is 27 years. Considering that the median age of starting an injecting career is estimated to be 23 years (A. Sutton, personal communication), this would potentially allow sufficient time to achieve high pre-exposure vaccination coverage in this group. This is corroborated by a survey in 2000 among injecting drug users attending services who started injecting within the past 3 years. This showed an anti-HBc prevalence of 7%, indicating that 93% of injecting drug users were susceptible (Department of Health, 2000). A high coverage is needed among injecting drug users and this would require targeting the group at every available opportunity (CDC, 2002), ensuring coordination between specialist drug services and primary care, and improving vaccine supply and financial arrangements. A high proportion of injecting drug users has a history of having been in prison (59% in 2000 (Department of Health, 2000)), and the current programme to immunise prisoners might therefore contribute substantially to control in this population ( ). The recent increase in cases of hepatitis A virus (HAV) among injecting drug users suggests that using a combined HBV/HAV vaccine should also be considered (Perrett et al., 2003).

4.2. Homosexual transmission

Homosexual contact was the second most frequently reported risk factor among male cases. We observed a decrease in the number of cases in MSM between 1995 and 2000, which is a continuation of the decreasing trend since the early nineties (Balogun et al., 1999). The median age of infection among MSM increased by nearly 10 years between 1985 and 2000. Both observations suggest that the transmission rate through male homosexual contact is decreasing, and contrasts with the current increase in high risk sexual behaviour and outbreaks of other sexually transmitted infections among MSM (PHLS, 2002). The decreased incidence of HBV in MSM is likely to be attributable to increased HBV vaccine coverage in this group, which is consistent with information from surveys among MSM attending GUM clinics (Gilson et al., 1998). Coverage among MSM is being improved by offering HBV vaccine to all MSM attending GUM clinics, with central purchase and supply of vaccine, and clear coverage targets being set (Department of Health, 2001).

4.3. Childhood infection

The incidence of acute HBV infection in children has remained at a constant low level since 1985 (Balogun et al., 1999). Our estimated incidence rate of laboratory reports of acute HBV infection in 1–9 year olds (0.10 per 100,000 per year) is lower than the equivalent incidence reported from the USA, where infant HBV vaccination coverage is reported to be 90% (CDC, 2002). Mother to infant transmission was the most frequently reported route for childhood infections in the late 1980s and early 1990s (Balogun et al., 1999), but in the late 1990s this has been replaced by transmission within the household. Although the number of reports of HBV infections in children is very low, infection during childhood contributes to an estimated 20–65% of chronic infections, depending on ethnicity (Table 1). An immunisation programme based on adolescent immunisation is therefore likely to be less effective than a childhood programme in preventing the long term sequelae of HBV infection, and would disadvantage ethnic minorities.

4.4. Ethnic minorities

Laboratory surveillance does not at present allow direct estimation of HBV incidence among ethnic minorities. However, our analysis of cases with South Asian names suggests that the incidence among South Asian residents of England and Wales is more than double the overall incidence. The pattern of reported routes of transmission among South Asian cases differs from other cases: heterosexual exposure was the most frequently reported source of infection, with relatively more infections in females. Injecting drug use and homosexual exposure are rarely reported. A high proportion of the infections in South Asians was acquired abroad, often through medical treatment in the Indian subcontinent. This suggests that travel to the country of (ethnic) origin carries a higher risk of acquiring HBV than other travel. This observation may be due to the nature or the duration of travel by this group.

The current recommendation is that only travellers to high endemicity countries should consider HBV immunisation (Salisbury and Begg, 1996). The number of cases associated with travel to intermediate endemicity countries suggests that these travellers should also be considered for HBV immunisation. More information on specific risk factors during travel, however, might allow more specific targeting.

Childhood infections are relatively more frequent in South Asians than in others, resulting in a higher risk of chronic infection in South Asians. Part of the difference in age distribution and incidence among South Asians could be explained by misclassification of chronic HBV infection as acute, since the prevalence of chronic infection is higher among South Asians (Boxall et al., 1994), and reactivation of HBV infection can lead to symptoms and anti-HBc-IgM levels similar to these in acute disease (Perillo, 2001). However, the increased incidence we found among South Asians is consistent with the increased prevalence of past infection among UK born South Asian children in the North West (CDSC, unpublished data). Results from a recent prevalence survey among UK born Somali children in Liverpool suggest that significant transmission during childhood is also occurring among other ethnic minorities resident in the UK (Aweis et al., 2001). Studies from the USA have similarly demonstrated that increased risk of HBV infection among ethnic minorities continues after immigration (Mahoney et al., 1995). Currently, ethnic minorities are not targeted for HBV vaccination, unless there is known to be a chronically infected individual in the household (Salisbury and Begg, 1996). The opportunities for and cost-effectiveness of offering better protection to ethnic minorities against HBV infection may therefore need to be investigated further.

4.5. Over-all implications for control through immunisation

Acute infections in residents of England and Wales give rise to only a small proportion of all new chronic infections, with the vast majority of the latter being attributable to immigration of carriers. As a consequence, the prevalence of HBV infection in England and Wales reflects global rather than national control policies (Gay and Edmunds, 1998). Because of the incompleteness of global control programmes, complications related to chronic HBV infection will continue to be a UK health problem, particularly since immigrating HBV carriers are likely to have acquired the infection at an earlier age compared to those in UK residents. UK vaccination policy will not be able to protect existing or newly immigrating HBV carriers, although there may be opportunities to protect their contacts.

The influx of new carriers will add to the far greater pool of existing carriers in the UK and our review suggests that the increase in immigration since the early 1990s (National Statistics, 2003) had little impact on the epidemiology of acute HBV infection in England and Wales. The over-all incidence has remained at a low level and no increase was observed in heterosexual or childhood transmission. We estimated that nearly half of the number of new HBV infections, and probably many more through indirect effects, could potentially have been prevented by the current programme (Table 2). This highlights the need to achieve higher coverage in those currently targeted for HBV immunisation (notably young injecting drug users in and out of prison). In addition, there is a need to identify and protect those at risk, particularly children, in ethnic minorities with origins in high prevalence countries. Continued surveillance of acute HBV infection is necessary to inform future immunisation strategies.

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APPENDIX A

A.1. Adjustment for asymptomatic infections

The proportion of infections that is symptomatic (and may therefore lead to diagnosis and reporting), $q(a)$, is agedependent. A model was fitted to data from literature review (Edmunds et al., 1996b) by assuming a logistic function with three parameters. The best fit (deviance 6.1, df = 6) function was

$$q(a) = \frac{0.07 \times 0.71}{(0.07 + ((0.71 - 0.07)\exp(-0.05 \times \text{age})))}$$

where age is given in years. To adjust for the proportion of infections that is asymptomatic, the number of laboratory reports by age was multiplied by $1/q(a)$.

A.2. Adjustment for the proportion of infections that become chronic

A function to describe the relation between age and development of carrier state $p(a)$ based on modelling data from a literature review was used:

$p(a) = \exp(-0.645a^{0.455})$

For $a < 1$, $P = 0.885$ was used.

For $a > 32$, $P = 0.04$ was used (Hyams, 1995).

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Chapter 3.2

Hepatitis B incidence among South Asian children in England and Wales: implications for immunisation policy

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Arch Dis Child 2003;88:1082–1083

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Hepatitis B incidence among South Asian children in England and Wales: implications for immunisation policy

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The incidence of acute hepatitis B virus (HBV) infection is higher among South Asian than among non-South Asian UK residents, and infections in South Asians occur more often during childhood. The UK's immunisation policy should be changed to protect ethnic minority children against HBV infection.

In the UK, hepatitis B virus (HBV) infection is more prevalent among minority ethnic groups than others, particularly in people born abroad.[1] This has limited implications for UK control policy, as the prevalence of HBV infection in ethnic minorities reflects infection risks both prior to and since immigration. The main source of data on the incidence of HBV infection in England and Wales is the routine laboratory surveillance of acute hepatitis, but direct estimates of the incidence among minority ethnic groups resident in the UK are not available. Using a validated method based on names to assign South Asian ethnicity we have compared the epidemiology of acute HBV infection in South Asian and non-South Asian ethnic groups. In addition, we compared the incidence of new HBV infections in blood donors of South Asian and other ethnicity.

METHODS

Acute HBV infections and associated risk factors reported by laboratories in England and Wales between 1 January 1988 and 31 December 2000 were studied. Reported names are held temporarily on the laboratory surveillance database in agreement with guidance on confidential patient information (http://www.doh.gov.uk/ipu/confiden/index.htm). "Nam Pehchan" software[2] was used to assign South Asian ethnicity based on names. As previously described,[3] to calculate incidence of infection from laboratory reports, infections were assumed to be symptomatic in 10% of cases under 15 years of age and in 33% of cases of 15 years and older. It was assumed that 75% of all symptomatic infections were reported and, conservatively, all infections reported in cases aged (2 years were regarded as perinatally acquired and so excluded from incidence and cumulative incidence estimates. (Perinatal transmission has been largely preventable in the UK since April 2000 by the introduction of universal antenatal screening and vaccination of infants at risk.) The number of new infections that became chronic was estimated by applying an age dependent risk of developing chronic infection[4] for cases up to age 20, and a risk of 4% for older cases. Denominators were obtained from mid-1997 population estimates (Office for National Statistics).

The number of new HBV infections (acute infections and seroconversions) among blood donors in England and Wales tested between 1 October 1995 and 30 June 2000 were grouped by reported ethnicity. The proportion of South Asian and non-South Asian donors among all tested donors was estimated using "Nam Pehchan" software on a sample of 26 970 named donors, selected to be representative of UK blood donors.

RESULTS

Between 1988 and 2000, an annual average of 635 cases of laboratory confirmed acute HBV infection was reported, of which 79% included names. Of named records, 8.5% (555) were identified as South Asian. The adjusted HBV incidence was 3.1 times higher in South Asians than in non-South Asians (14.9 and 4.8 per 100 000 person years, respectively; 95% confidence interval (CI) incidence ratio 2.8 to 3.4). The estimated lifetime risk of infection was 1.4% in South Asians and 0.4% in non-South Asians, and of chronic infection was 0.08% in South Asians and 0.02% in non-South Asians (fig 1). In South Asian blood donors the frequency of new HBV infections was 4.3 times higher than in non-South Asian blood donors (95% CI 1.1 to 11.8).

Nine per cent (51 cases) of reported acute HBV

infections in South Asians were children (age ,15 years), significantly higher than this proportion in non-South Asians (1.7%; 99 cases; p,0.0001). Of South Asian cases in children, 22% (11 cases) were 2 years of age or less, compared to 30% of non South Asian cases in children (30 cases; p = 0.3). After excluding all cases (2 years of age, the adjusted incidence of acute HBV infection was 10 times higher in South Asian children than in non-South Asian children (10.5 and 1.0 per 100 000 per year, respectively; 95% CI incidence ratio 6.9 to 15.4). The most frequent source of infection for South Asian children .2 years of age was within the household (11 cases; 61% of cases with a risk factor reported); 45% of infections (18 cases) in South Asian children .2 years of age were reported to have been acquired overseas.

DISCUSSION

Our study shows that South Asians are at higher risk of HBV infection than non-South Asians while resident in England and Wales, particularly in childhood. A high incidence of HBV infection has also been shown among UK born children of Somali ethnicity resident in the UK,[5] suggesting that significant transmission during childhood may occur among UK ethnic minorities who originate in high or intermediate prevalence countries.

Preventing HBV infections in children needs special emphasis, as the risk of developing chronic infection is higher in children than adults.[4] Chronic HBV infection sustains HBV transmission in the population and its sequelae constitute most of the associated burden of disease. For nonSouth Asians, HBV infection in childhood is rare. By contrast, we found that HBV infection among South Asian children in England and Wales is more common, with transmission within the household (and while travelling) frequently reported. Both are unlikely to be prevented by the current UK selective vaccination policy.

Our data suggest that infant immunisation targeted at UK resident ethnic minorities originating from HBV endemic areas could significantly reduce HBV transmission. Such a policy was recommended in 1990 in the United States,[6] and started in the Netherlands in 2003 (http://www.gr.nl/adviezen.php?ID=730). In the USA, coverage achieved with this policy was limited, possibly due to difficulties targeting vaccination based on ethnicity.[6]

In the UK, universal infant HBV immunisation in geographical areas with a high proportion of ethnic minorities may be more acceptable and feasible than targeting individual families on the basis of ethnic status. Such a policy would be similar to the universal neonatal BCG immunisation programme currently used in some areas. To avoid additional visits or additional injections, the vaccine could be given in combination with other routine childhood immunisations, and has the potential to achieve high coverage in ethnic minority children at risk of hepatitis B.

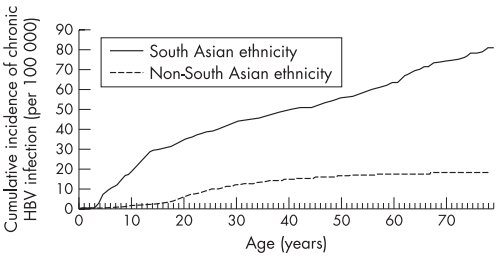

Figure 1 Estimated cumulative incidence of chronic HBV infection per 100 000 population by ethnicity, based on laboratory surveillance of acute HBV infection in England and Wales, 1988–2000, adjusted for asymptomatic infections and under-reporting and excluding infections in children ⩽2 years of age.

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Chapter 4

Hepatitis B virus in Europe

Chapter 4.1

Infection with hepatitis B and C virus in Europe: a systematic review of prevalence and cost-effectiveness of screening

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Infection with hepatitis B and C virus in Europe: a systematic review of prevalence and cost-effectiveness of screening

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ABSTRACT

Background Treatment for chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection is improving but not benefiting individuals unaware to be infected. To inform screening policies we assessed (1) the hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus antibodies (anti-HCV-Ab) prevalence for 34 European countries; and (2) the cost-effectiveness of screening for chronic HBV and HCV infection.

Methods We searched peer-reviewed literature for data on HBsAg and anti-HCV-Ab prevalence and cost-effectiveness of screening of the general population and five subgroups, and used data from two European organizations for injecting drug users (IDUs) and blood donors. Of 1759 and 384 papers found in the prevalence and cost-effectiveness search respectively, we included 124 and 22 papers after checking the paper's quality. We used decision rules to calculate weighted prevalence estimates by country.

Results The HBsAg and anti-HCV-Ab prevalence in the general population ranged from 0.1%-5.6% and 0.4%-5.2% respectively, by country. For IDUs, men who have sex with men and migrants, the prevalence of HBsAg and anti-HCV-Ab was higher than the prevalence in the general population in all but 3 countries. There is evidence that HCV screening of IDUs and HBsAg screening of pregnant women is cost-effective. HBsAg screening of migrants is likely to be cost-effective.

Conclusion The prevalence of chronic HBV and HCV infection varies widely between European countries. Anti-HCV-Ab screening of IDUs and HBsAg screening of pregnant women are European public health priorities. HBsAg screening of migrants is likely to be cost-effective. Cost-effectiveness analyses may need to take effect of antiviral treatment on preventing HBV and HCV transmission into account.

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Keywords Hepatitis B virus, hepatitis C virus, Europe, prevalence, HBsAg, anti-HCV-Ab, cost-effectiveness analyses.

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BACKGROUND

Hepatitis B and C virus (HBV and HCV) infect the liver and can lead to a broad spectrum of disease outcomes. Between 15% and 40% of those chronically infected with HBV or HCV will in their lifetimes develop serious liver disease due to cirrhosis and/

or hepatocellular cancer (HCC).[1,2] People with chronic infection with HBV or HCV can remain infectious to others. Both HBV and HCV are widely present with broad variation in prevalence by country [3]. Worldwide, between 350 to 400 million people are infected with HBV, accounting for 1 million deaths per year [4,5]. Between 130 and 170 million people are infected with HCV, causing over 350,000 deaths per year [6].

A safe and effective vaccine for HBV has been available since 1982, whereas no vaccine for HCV exists [7]. Treatment options are advancing rapidly, and several new antiviral drugs have become available in the past decade. Evidence is accumulating that these therapies provide a cost-effective means to reduce the morbidity and mortality associated with chronic infection with HBV and HCV [8-10]. European treatment guidelines for chronic HBV and HCV infection are available [11,12]. In addition to improving the outcome of chronic hepatitis, antiviral treatment is likely to reduce transmission by reducing the viral load and therefore infectivity of chronic carriers, similar to what has been documented for HIV [13-15]. For HBV, vaccination of susceptible contacts of identified carriers can prevent new infections. Nonpharmaceutical interventions, such as the advice to limit alcohol intake and cease smoking, can improve outcomes for people living with chronic viral hepatitis [16,17].

Since the acquisition of HBV and HCV is often asymptomatic or subclinical, and sequelae take several decades to develop, between 40% and 80% of people with chronic hepatitis are unaware of their infection [18-25]. Therefore, screening programmes for chronic HBV and HCV infection have the potential to contribute considerably to primary and secondary prevention of these infections. However, existing HBV and HCV screening programmes in Europe stem from an era when treatment options for were limited. Hence they are mainly aimed at primary prevention, targeting blood donors, pregnant women, and behavioral high-risk groups [26]. Now that secondary prevention of HBV and HCV is possible, there is an urgent need to identify chronic carriers who may benefit from treatment.

For policy development in this area data on the size and characteristics of the population with chronic hepatitis and the evidence for cost-effectiveness of screening are needed. The most recent HBsAg prevalence review including data on European countries was from 2004, and reported findings from only 11 European countries [27]. For HCV, a review of the burden of disease in Europe was published in 2009 [28]. In this review, however expert opinion was a main source of data, which makes the validity of conclusions difficult to ascertain. Esteban reviewed the HCV prevalence in Europe in 2008 [29], but studies on blood donors were included, limiting the representatives of the estimates for the general population. Regarding the cost-effectiveness of screening for HCV, an earlier review included studies published up to March 2007 and was partly sponsored by the pharmaceutical industry [30]. It concluded that HCV screening of former or current IDUs was cost-effective. Systematic reviews of cost-effectiveness of screening for HBV infections have not been published.

To address the missing information we performed a systematic literature review of the prevalence of hepatitis C virus antibodies (anti-HCV-Ab) and hepatitis B surface antigen (HBsAg) as well as the cost-effectiveness of screening for these markers. Our review included the general population and population subgroups (pregnant women, first-time blood donors, IDUs, men who have sex with men [MSM], and migrants) for 34 European countries.* We subsequently used our prevalence estimates to assess the total number of people living with chronic HBV and HCV infection by country.

METHODS

To find studies that describe prevalence of HBsAg and anti-HCV-Ab (the serological markers used as proxies for chronic infection in this study) we searched Medline, Embase, and SciSearch for English-language, peer-reviewed literature published between 1 January 2000 and 27 July 2009. Reference lists of included studies were hand searched. Studies were eligible for inclusion in the review if they reported the anti-HCV-Ab and/or HBsAg prevalence in the general population or among pregnant women, first-time blood donors, MSM, or migrants. Studies that reported on children only were not included. We only used the most recent estimate when more than 1 regional estimate was available based on studies performed 5 or more years apart. When several estimates were available for a specific country, an average weighted by study size was calculated. For first-time blood donors, we used data from a recent report for the Council of Europe in addi-

* All 27 EU member states, 4 EEA/EFTA countries (Norway, Iceland, Liechtenstein and Switzerland) and 3 EU enlargement countries (Croatia, the former Yugoslav Republic of Macedonia and Turkey).

tion to data from the published literature [31]. Anti-HCV-Ab and HBsAg prevalence estimates among IDUs were obtained from 2 sources: the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [32] and from a recent review on HBV and HCV prevalence among IDUs [33]. The source with the most recent national prevalence estimate was used. We excluded estimates from studies performed before 2000, with fewer than 50 participants, and where IDU status was unknown.

For the systematic literature review of the evidence for cost-effectiveness of screening for chronic HBV and/or HCV infection we searched Medline, Scopus, and the NHS Economic Evaluation Database (EED) for studies published in the English-language, peer-reviewed literature between 1 January 2000 and 2 May 2011. Studies reporting only on screening of transfusion recipients and/or of patients treated by infected health care workers (look-back studies) were excluded. Studies were only eligible when reporting estimated costs per additional chronic infection identified and/or costs per life year (LY) gained (quality or disability adjusted). Cost estimates were converted into 2010 Euros using information from Eurostat and OECD [34,35]. For both systematic literature searches, data were extracted using a data-extraction form by two authors (SH and IV). For the prevalence search, the form included year, country population of the study, the sampling method, laboratory test used, participation rate, number of participants, and HBsAg and anti-HCV-Ab results. For the cost-effectiveness search, the form included year and country of study, target population for screening, screening scenario, type of model used, outcome measure(s) used, monetary value and year, discounting percentage (costs/effects), results, and conclusions. The quality of the prevalence studies was assessed by reviewing the representativeness of sampling (eg, random vs convenience sampling) and, for the general population, whether estimates were standardized by age and sex.

Prevalence estimates were summarized by country. When multiple general population prevalence estimates were available for one country, we used the estimate that was most representative for the entire country regarding demographic coverage. In case multiple representative general population prevalence estimates for one country were found, the average prevalence was calculated weighted by study size. Where estimates for 3 or more regions in a country were available, regional estimates were presented only when the difference between regions was more than 0.5%. Countries were grouped into low, intermediate, and high HBsAg and anti-HCV-Ab prevalence using cut offs of ≤ 1%, > 1% to ≤ 2%, and > 2%. On the basis of prevalence estimates in the general population for infection with HBV and HCV and 2009 population size [36], we estimated the total number of people in that country who would likely test positive for HBsAg or anti-HCV-Ab. Search terms used for both searches are available in supporting information (S1). The methods of our systematic literature reviews and their reporting are consistent with those recommended by the PRISMA statement and specified in advance in a protocol that is available from the corresponding author on request [37].

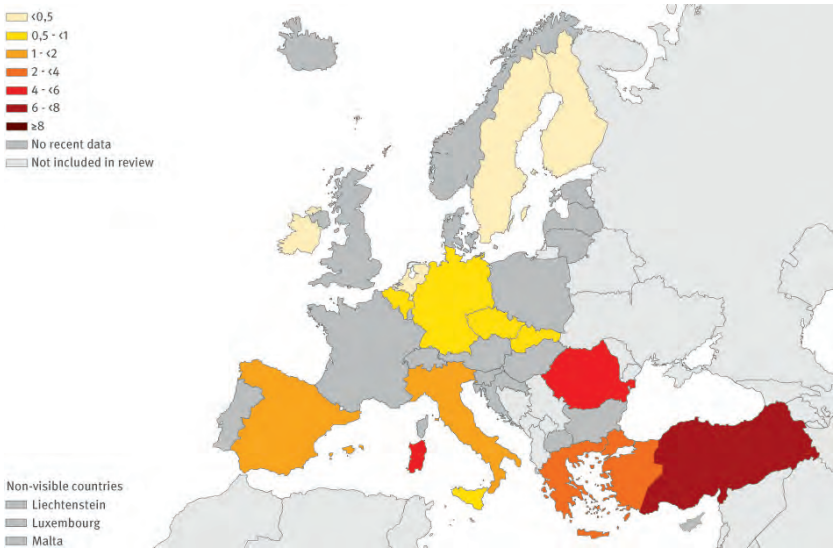
RESULTS

Seroprevalence of HBsAg and anti-HCV-Ab

The search for data on the HBsAg and/or anti-HCV-Ab prevalence in the general population and 5 subgroups identified 1759 citations, from which the full-text publication of 236 (13%) was retrieved. From the reference lists of included studies, an additional 8 potentially relevant citations were identified. After review of the full text of these 244 papers, 53 publications were considered not relevant. Furthermore, 67 publications on IDUs were excluded, since prevalence estimates from IDUs were obtained from the EMCDDA and a recent literature review [33]. Finally, 124 publications were included in the review of prevalence data, with 81 publications used for the prevalence estimate for the general population or population subgroups. A flow diagram depicting the inclusion of studies is available in the supporting information S2.

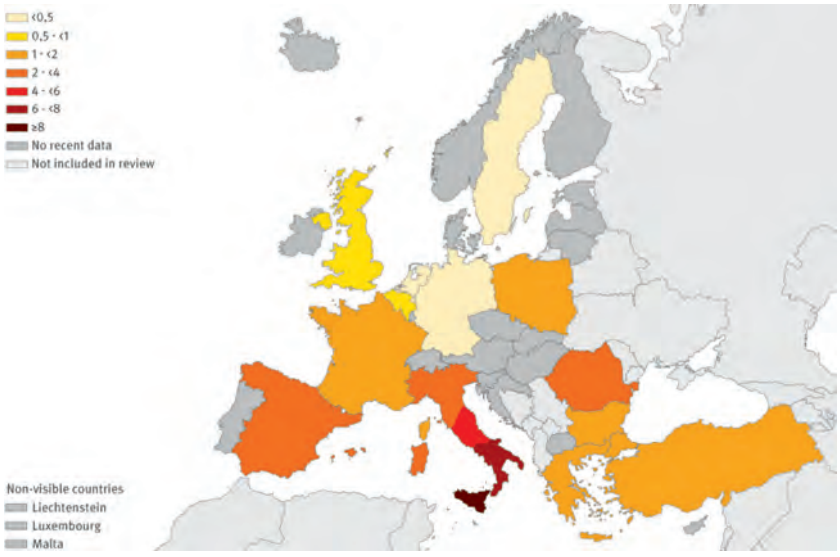
HBsAg general population prevalence estimates were found for 13 of the 34 countries in our review, ranging from 0.1% to 5.6% by country (Figure 1a, Table 1a). The estimated number of people with chronic HBV infection ranged from 3,718,889 in Turkey to 4,466 in Ireland (Table 1a). Prevalence estimates of anti-HCV-Ab in the general population were found for 13 of the 34 countries in our review, ranging from 0.4% to 5.2% by country (Figure 2a, Table 2a). The estimated number of people who were anti-HCV-Ab positive ranged from 3,122,779 in Italy to 37,025 in Sweden (Table 1b). For only a minority of countries (9/34) information was available on both es-

Figure 1a. Hepatitis B (HBsAg)* prevalence (%) in the general population by country, Europe, 2000-2009.



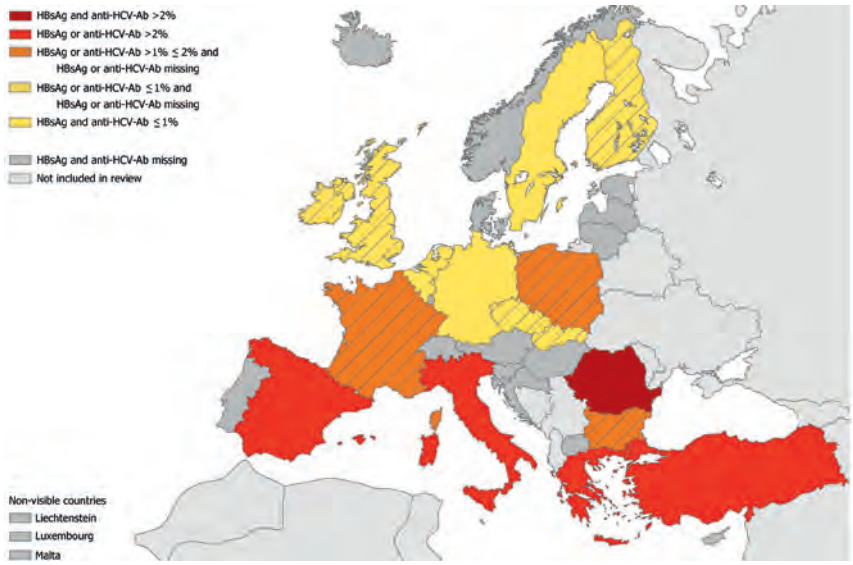
* Hepatitis B surface antigen

Figure 1b. Hepatitis C (anti-HCV-Ab)* prevalence (%) in the general population by country, Europe, 2000-2009.



* anti-HCV antibody

Figure 2. Summary of HBsAg and anti-HCV-Ab prevalence profiles in Europe, 2000-2009.



timates. Countries in the north-western part of Europe had a low prevalence for both infections whilst those in the south and south-east had an intermediate to high prevalence (Figure 2).

HBsAg prevalence estimates for first-time blood donors were found for 24 countries, ranging from 0.0% to 5.2% (Figure S3.1a, Table S3.1). Anti-HCV-Ab prevalence estimates for firsttime blood donors were available for 23 countries, ranging from 0.02% to 3.3% (Figure S3.1b, Table S3.1). The prevalence of HBsAg and anti-HCV-Ab in first-time blood donors was on average respectively 3 and 4 times lower than the corresponding prevalence for the general population in countries that had both estimates available (12 countries for HBsAg and 11 for anti-HCV-Ab).

Estimates of antenatal HBsAg prevalence were found for 11 countries, ranging from 0.1% to 4.4% (Figure S3.2a, Table S3.2). Estimates of antenatal anti-HCV-Ab prevalence were found for 6 countries, ranging from 0% to 1.7% (Figure S3.2b, Table S3.2). The antenatal HBsAg prevalence was on average 3 times higher than the general population prevalence in 6 of the 7 countries that had both estimates available. The country where it was lower was Spain (based on regional data from Catalonia), likely reflecting the effect of the HBV vaccination programme for adolescents. In Italy and the United Kingdom, the antenatal anti-HCV-Ab prevalence was lower than the general population prevalence. In Germany and Greece, it was higher.

An estimate of HBsAg prevalence in IDUs was available for 21 of the 34 countries in this review, ranging from 0% to 21.3% (Figure S3.3a, Table S3.3). An estimate of anti-HCV-Ab prevalence in IDUs was available for 29 of the 34 countries, ranging from 5.3% to 90% (Figure S3.3b, Table S3.3). The HBsAg prevalence in IDUs was on average 9 times higher than that in the general population (in 6 of the 8 countries that had both estimates available). In Romania and Ireland, the general population HBsAg estimate was higher. The estimate of anti-HCV-Ab prevalence in IDUs was on average 47 times higher than that in the general population (in 13 countries that had both estimates available).

Estimates of HBsAg prevalence in migrants were found for 5 countries. The HBsAg prevalence in migrants ranged from 1.0% to 15.4% (Table S3.4). Estimates of anti-HCV-Ab prevalence in migrants were found for 5 countries, ranging from 0% to 23.4% (Table S3.5). The estimate of HBsAg and anti-HCV-Ab prevalence in migrants was on average respectively 6 and 2 times higher than that in the general population in all countries that had both estimates available (4 countries for HBsAg and 4 for anti-HCV-Ab), except for Italy, where the estimate of anti-HCV-Ab prevalence in migrants was lower than that in the general population. Estimates of HBsAg prevalence

Table 1a. Estimates of general population HBsAg prevalence, and number of HBsAg positive people in the general population, by country, Europe, 2000-2009.

Country* (Reference)	Period	Area	Region	Sampling	N	%	(95% CI)**	Remarks	Population size [80]	Number of HBsAg-positive inhabitants
Belgium[81]	2003	regional	Flanders	random	1.834	0,7	(0,5-0,8)&	Oral fluid	10.754.528	75.282
Czech Republic [82]	2001	nationwide	-	random	2.658	0,6	(0,3-1,0)$	Standardized	10.467.542	62.805
Finland [83]	1997-1998	nationwide	-	residual	3.083	0,2	(0,1-0,4) $		5.326.314	10.653
Germany [84]	1993-1996	nationwide	-	random	5.305	0,6	(0,4-0,8) &		82.050.000	492.300
Germany [85]	1998	nationwide	-	random	6.748	0,6	(0,4-0,8) &			
Greece [86]	1997-1998	regional	Peloponnesos	random	1.500	2,1	(1,5-3,0) &		11.257.285	236.403
Ireland [83]	2003	nationwide	-	residual	2.535	0,1	(0,0-0,3) $		4.465.540	4.466
Italy [83]	1996	nationwide	-	residual	3.522	0,6	(0,4-1,0) $		60.053.442	840.748
Italy [67]	2002	regional	North	convenience	956	1,0	(0,5-1,9) $			
Italy [87]	1997	regional	Central	random	250	1,2	(0,3-3,5) $			
Italy [88]	1997	regional	South	random	488	0,2	(0,0-3,5) $			
Italy [89]	2002-2003	regional	South	random	1.645	1,8	(0,4-1,2) &			
Italy [90]	1994-1994	regional	Sardinia	convenience	3.324	4,3	(3,6-5,1) $			
Italy [91]	1999-2000	regional	Sicily	random	721	0,7	(0,2-1,6) $			
Netherlands [83]	1995-1996	nationwide	-	random	6.750	0,1	(0,0-0,2) $		16.486.587	16.487
Romania [83]	2002	nationwide	-	residual	1.259	5,6	(4,4-7,0) $		21.498.616	1.203.922
Slovakia [83]	2002	nationwide	-	random	3.569	0,6	(0,4-0,9) $		5.412.254	32.474
Spain [92]	1996	regional	Catalonia	random	2.142	1,2	(0,7-1,7) &	Standardized	45.828.172	458.282
Spain [93]	2002	regional	Catalonia	random	2.620	0,7	(0,4-1,0) &			
Sweden [94]	1991-1994	regional	Malmö	random	5.533	0,2	(0,1-0,4) $		9.256.347	18.513
Turkey [95]	2006-2007	regional	West	random	2.852	2,5	(2,0-3,1) $		71.517.100	3.718.889
Turkey [96]	2002-2004	regional	Central	convenience	1.320	6,6	(5,3-8,1) $			
Turkey [97]	1996	regional	Central	convenience	571	6,7	(4,8-9,1) $			
Turkey [98]	Not reported	regional	Central	random	1.095	5,5	(4,2-7,0) $			
Turkey [99]	1997-1999	regional	East	convenience	400	9,0	(6,4-12,2) $	32-year-olds		
Turkey [100]	2003	regional	East	random	2.888	7,0	(6,1-8,0) $			

* No estimate of HBsAg general population prevalence was found for Austria, Bulgaria, Croatia, Denmark, Estonia, Former Yugoslav Republic of Macedonia, France, Hungary, Iceland, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Norway, Poland, Portugal, Slovenia, Switzerland and the United Kingdom.

** CI Confidence interval

& CI provided in the original paper

$ CI estimated by the exact method

Table 1b. Estimates of general population anti-HCV-Ab prevalence, and number of anti-HCV positive people in the general population, by country, Europe, 2000-2009.

Country* (Reference)	Period	Area	Region	Sampling	N	%	95% CI**	Remarks	Population size [80]	Number of inhabitants who are anti-HCV-Ab positive
Belgium [81]	2003	regional	Flanders	random	1.834	0,1	(0.1-0.4)&	Oral fluid	10.754.528	64.527
Belgium [102]	1993-1994	regional	Flanders	random	4.055	0,9	(0.5-1.1) &			
Bulgaria [103]	1999-2000	regional	South-Central	convenience	2.211	1,3	(1.2-1.4) &	Standardized	7.606.551	98.885
Czech Republic [82]	2001	nationwide	-	random	2.658	0,2	(0.1-0.4)$		10.467.542	20.935
France [104]	1997	regional	South	convenience	11.804	1,3	(1.1-1.5) &		64.351.000	836563
Germany [85]	1998	nationwide	-	random	6.748	0,4	(0.2-0.5) &		82.050.000	328200
Greece [86]	1997-1998	regional	Peloponnesos	random	1.500	0,5	(0.2-1.1) &		11.257.285	112.573
Greece [105]	1997	regional	Zakinthos	random	718	1,3	(0.6-2.4) $			
Italy [67]	2002	regional	North	convenience	956	2,6	(1.7-3.8)$			
Italy [106]	1994-1995	regional	North	convenience	2.154	3,3	(2.6-4.1) &			
Italy [107]	Not reported	regional	North	convenience	4.820	2,4	(2.0-2.8) &			
Italy [108]	Not reported	regional	Central	convenience	300	16,3	(12.0-20.6) &			
Italy [87]	1997	regional	Central	random	250	22,4	(20.8-24.1) &			
Italy [88]	Not reported	regional	South	random	488	16,2	(13.0-19.8) $			
Italy [109]	2000-2002	regional	South	convenience	2.753	7,9	(6.9-9.0) $			
Italy [89]	2002-2003	regional	South	random	1.645	6,5	(5.3-7.7) &			
Italy [90]	1994-1995	regional	Sardinia	convenience	3.324	3,2	(2,6-3,8) $			
Italy [91]	1999-2000	regional	Sicily	random	721	10,4	(8,2-12,9) $			
Netherlands [41]	2004	regional	Amsterdam	random	1.364	0,6	(0.1-1.1) &	Standardized	16.486.587	65.946
Netherlands [110]	2006	regional	East	convenience	2.200	0,2	(0.1-0.5) $			
Poland [111]	1999	regional	North	convenience	2.561	1,9	(1.4-2.5) $		38.135.876	724.582
Romania [112]	2006-2008	nationwide	-	random	8.039	3,5	(3.1-3.9) &		21.498.616	752.452
Spain [113]	1996	regional	Catalonia	random	2.142	2,5	(1.8-3.2) &	Standardized	45.828.172	916.563
Spain [114]	1997-1998	regional	North	random	1.170	1,6	(1.0-2.6) &			
Sweden [94]	1991-1994	regional	Malmö	random	5.533	0,4	(0,3-0,6) $		9.256.347	37.025
Turkey [95]	2006-2007	regional	South West	random	2.852	1,0	(0,7-1,4) $		71.517.100	1.072.757
Turkey [96]	2002-2004	regional	Central	convenience	1.320	2,2	(1,5-3,1) $			
Turkey [98]	Not reported	regional	Central	random	1.095	2,1	(1,3-3,1) $			
United Kingdom [115]	1996	regional	England&Wales	residual	6.401	0,7	(0,1-0,5) $		61634599	431442

* No estimate of anti-HCV general population prevalence was found for Austria, Croatia, Cyprus, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, Hungary, Iceland, Ireland, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Norway, Portugal, Slovakia, Slovenia and Switzerland.
** CI Confidence interval
& CI provided in the original paper
$ CI estimated by the exact method

Table 2. Overview of publications included in the cost-effectiveness review (n=22)

First Author	Year of publication	Target group	Setting	Country	Infection	Model	Data
Thomas[54]	1990	Pregnant women	Antenatal care	Australia	HBV	None	Actual screening
Audet[53]	1991	Pregnant women	Antenatal care	Canada	HBV	None	Hypothetical cohort
Tormans[50]	1993	Pregnant women	Antenatal care	Belgium	HBV	Markov	Hypothetical cohort
Dwyer[52]	1996	Pregnant women	Antenatal care	UK	HBV	Markov	Hypothetical cohort
Jordan[51]	1997	Pregnant women	Antenatal care	Britain	HBV	Markov	Hypothetical cohort
Plunkett[55]	2005	Pregnant women	Antenatal care	USA	HCV	Markov	Hypothetical cohort
Singer[48]	2001	General population	Not specified	USA	HCV	Markov	Hypothetical cohort
Nakamura[49]	2008	General population & risk groups	Not specified	Japan	HCV	Markov	Actual screening
Loubiere[47]	2003	General population, IDUs & other risk groups	Not specified	France	HCV	Markov	Hypothetical cohort
Hutton[63]	2007	Migrants	Not specified	USA	HBV	Markov	Hypothetical cohort
Veldhuijzen[64]	2010	Migrants	Population based	Netherlands	HBV	Markov	Hypothetical cohort
Leal[56]	1999	IDUs	Drug services	UK	HCV	Markov	Hypothetical cohort
Castelnuovo[57]	2006	IDUs	Various	UK	HCV	Markov	Hypothetical cohort
Thompson Coon[58]	2006	IDUs	Primary care	UK	HCV	Markov	Hypothetical cohort
Kerr[43]	2009	IDUs & MSM	STD clinic	Scotland	HCV	None	Actual screening
Josset[59]	2004	IDUs & other risk groups	Primary care	France	HCV	None	Actual screening
Stein[44]	2004	IDUs & other risk groups	STD clinic/ drug services	UK	HCV	Markov	Hypothetical cohort
Honeycutt[60]	2007	IDUs & other risk groups	STD clinic	USA	HCV	None	Hypothetical cohort
Tramarin[61]	2008	IDUs & other risk groups	Not specified	Italy	HCV	Markov	Hypothetical cohort
Helsper[62]	2012	IDUs & other risk groups	Primary care/ drug services	Netherlands	HCV	Markov	Actual screening
Sutton[65]	2006	Prisoners	Prison	UK	HCV	Markov	Hypothetical cohort
Sutton[66]	2008	Prisoners	Prison	UK	HCV	Markov	Hypothetical cohort

* Results of most favorable scenario reported here.
** Where the paper did not quote year of monetary value the year of publication was used to convert the results into 2010 Euros [34].
n.a. Not available
*** Quality adjusted life year

First Author	Indicator	Result*	Year of monetary value	Result in Euro 2010**	Cost-effective?
Thomas[54]	Cost per case detected	$354 (AU]	1988	€410	Yes
Audet[53]	Cost per case detected/ infant carrier prevented	$1,693/$8,915 (CA]	1988	€1,880/€9,900	Yes, probably
Tormans[50]	Cost per life year gained	BEF 583,581	1991	€22,095	Yes
Dwyer[52]	Cost per carrier prevented / life year saved	£2,437 / £ 16,450	Not mentioned	€3,879/€26,181	Yes
Jordan[51]	Cost per life year saved	£1,300	Not mentioned	€2,032	Yes
Plunkett[55]	Cost per QALY***	No screening dominant	2003	n.a.	No
Singer[48]	Cost per QALY	No screening dominant	2001	n.a.	No
Nakamura[49]	Cost per life year gained	$848 $4,825 (US]	2007	€726/€4,130	Yes
Loubiere[47]	Cost per life year gained	$4,513	1998	€4,856	Yes
Hutton[63]	Cost per QALY	$36.088 (US]	2006	€31,692	Yes
Veldhuijzen[64]	Cost per QALY	€ 8.966	2009	€9,047	Yes
Leal[56]	Cost per QALY	£9.300	1997	€14,540	Yes
Castelnuovo[57]	Cost per QALY	£15.493-£20,083	2004	€22,172/€28,741	Yes
Thompson Coon[58]	Cost per QALY	£16,493	2002/2003	€24,245	Yes
Kerr[43]	Cost per case detected	£170 (IDUs]/ £15,000 (MSM]	Not mentioned	€215/€18,975	No (MSM]/Yes (IDUs]
Josset[59]	Cost per case detected	n.a.	Not mentioned	n.a.	Not stated
Stein[44]	Cost per QALY	£28.120 (IDUs]/ £84,570 (GUM]	2001	€41,874/€125,933	Yes (IDUs]/No (GUM]
Honeycutt[60]	Cost per case detected	$54 (US]	2006	€47	Yes
Tramarin[61]	Cost per QALY	-€ 3,132	2007?	-€3,320	Yes
Helsper[62]	Cost per QALY	€ 7,321	2007	€7,625	Yes
Sutton[65]	Cost per case detected	£2,102 £3,107	2004	€3,008/€4,446	Yes
Sutton[66]	Cost per QALY	£54,852	2004	€78,498	No

for MSM were found for 3 countries, ranging from <1% to 4% [38-41]. Estimates of anti-HCV-Ab prevalence among MSM were available for 3 countries, ranging from 0.07% to 2.9% [41-43]. The HBsAg and anti-HCV-Ab prevalence in MSM was on average respectively 22 and 3 times higher than that for the general population in all countries that had both estimates available (2 countries for HBsAg and 1 for anti-HCV-Ab).

Cost-effectiveness of HBV and/or HCV screening

The search for evidence on cost-effectiveness of HBV and/or HCV screening identified 384 publications. We retrieved the full text for 31 publications (8%). From the reference lists of included studies 3 additional potentially relevant citations were identified. Of these 34 papers, 10 were considered not relevant following full text review. Two additional publications were excluded [44,45]. because they reported on data that were more extensively presented in a third publication.[46] Finally, 22 publications were included in the review of cost-effectiveness of screening (flow chart S2, table 2). No paper studied combined screening for HBV and HCV. Of the 22 papers, 17 used a Markov model (15 with hypothetical data and 2 presented actual screening results). Five other studies did not use a model and presented costs per case identified or cost per infection prevented. None of the studies included dynamic modeling to take into account effects of reducing transmission by lowering viral load through treatment, behavior change, or HBV vaccination.

No economic analyses of HBsAg screening of the general population were found. Three economic analyses reported on HCV screening and subsequent treatment of HCV positives of the general population [47-49]. Two of these reported costs per LY gained.[47,49] Of these, 1 study assuming a 1.2% HCV prevalence in the general French population concluded that HCV general population screening was cost-effective (cost per LY saved €4856) [47]. The other study reported on an actual screening programme performed between 2003 and 2006 in one region of Japan. In the target population aged 40 to 70 years, an anti-HCV-Ab prevalence of 0.4% was found [49]. Estimated costs per LY gained, ranging between €726 and €4,130 by age-group, all of which were considered cost-effective. A US group studied costs per quality-adjusted LY gained in relation to a HCV screening and treatment programme for 35-year-olds from the general population of whom 2.9% were HCV-RNA positive [48]. They found that this was not cost-effective. In this study, incomplete treatment adherence and more cost items were taken into account compared to the other 2 studies, which may partly explain the less favorable outcome for screening.

Five economic analyses reported on antenatal HBsAg screening [50-54], presenting estimated costs per LY gained [50-52], costs per case detected and per infant carrier prevented [53] and costs per case detected [54]. The 3 studies presenting costs per LY gained studied the scenario of universal screening of all pregnant women, with vaccination of infants born to HBsAg positive mothers. Studies were published between 1993 and 1997, and none considered antiviral treatment. Incremental cost-effectiveness ratios (ICERs) ranged from €2,032 to €26,181 per LY gained. All studies concluded that universal antenatal HBV screening is cost-effective considering the respective thresholds used. One economic analysis of antenatal HCV screening was found, which considered universal antenatal HCV screening and treatment of HCV infection with or without elective cesarean delivery [55]. Neither of these scenarios was considered cost-effective. No economic analysis of HBsAg screening of IDUs was found. Ten studies reported on cost-effectiveness of HCV screening and treatment of IDUs [43,46,47,56-62]. Seven of these reported estimated costs per quality-adjusted LY. These studies varied widely, including different screening settings, treatments considered, and discount rates. Nevertheless, all 7 studies concluded that HCV screening of IDUs was likely to be cost-effective considering the respective thresholds used, with ICERs ranging from – €3.320 to €41,874 per quality-adjusted LY.

Two economic analyses of screening migrants for HBsAg were found, both presenting cost per quality-adjusted LY [63,64]. Target populations and the interventions studied varied. Both studies concluded that all scenarios in the analysis were cost-effective, with ICERs ranging from €9,047 [64] to €31,692 [63]. One economic analysis of HCV screening of migrants was found [62]. In this study, the target group for screening included migrants from countries with a HCV prevalence >10%, as well as from other population subgroups. Separate estimates of cost-effectiveness of screening migrants were, however, not presented.

We found no economic analysis of HBsAg screening of MSM. One economic analysis of HCV screening of MSM at sexually transmitted disease (STD) clinics concluded that HCV screening of MSM in this

setting was not cost-effective [43].
We did not find any economic analyses of HBsAg screening of STD-clinic attendees, but 2 of HCV screening of STD-clinic attendees [46,60]. Universal screening and treatment of UK STD-clinic attendees was assessed as not cost-effective (ICER €125,933/ quality-adjusted LY) [46,60]. Among STD-clinic attendees in the US, HCV screening of non-IDUs was only cost-effective when restricted to men with >100 lifetime sex partners.60
No economic analyses of HBsAg screening of prisoners were found. Regarding HCV screening of prisoners, we found two studies both from England and Wales [65,66]. The first study found that asking prisoners about their HCV and injecting status prior to laboratory testing can considerably reduce the cost per case detected [65]. The second paper found that HCV screening and treatment of prisoners was not cost-effective (cost per quality-adjusted LY €78,498) [66].
Five economic analyses of HCV screening of other population subgroups were found [47,49,59,61,62,67]. Both studies that considered screening programmes targeting several population subgroups concluded that the specific programmes considered were potentially cost-effective [49,62]. Josset et al reported estimated costs per positive test result for 6 screening scenarios, which varied regarding population subgroups targeted [59]. Analyses of HCV screening of people transfused before 1991 in France and of people with a history of surgery in Italy both concluded this was not cost-effective [47,61].

DISCUSSION AND CONCLUSIONS

The general population prevalence of chronic HBV and HCV infection varies widely between European countries, with those in the south and east of the European Union and in Turkey having a much higher prevalence than those in northwestern Europe. Among countries for which data were available for both infections, Romania stands out, with high prevalence for both HBV and HCV. In contrast, Belgium, Sweden, Germany, and The Netherlands have low-population prevalence for both infections. Results from a study published after our literature search was completed suggest that France belongs to this latter category as well [24]. For HCV, Italy had the highest estimated population prevalence, much higher than its estimated HBV prevalence. Epidemiologic and phylogenetic assessments suggest that this phenomenon in Italy may have been caused by a period of frequent iatrogenic transmission that took place around the 1950s [68]. Without screening and early treatment, these infections will lead to a considerable disease burden and many deaths due to liver disease in the coming decades. Given that HBV and HCV disproportionately affect disadvantaged groups and less affluent countries in Europe, these infections will also contribute to increasing inequalities in health.
For the majority of countries, data on the general population prevalence of HBV or HCV were lacking. Availability of sufficiently recent estimates is necessary to be able to prioritize primary and secondary prevention of HBV and HCV among other public health interventions, to evaluate control measures, and for health care planning. Estimates of prevalence obtained from blood-donor and antenatal screening were found to differ substantially from general population estimates, and in addition usually do not include vulnerable groups at high risk of infection. Within countries, the prevalence of HBsAg and anti-HCV-Ab among IDUs, MSM, and migrants was much higher than the corresponding prevalence in the general population, with only a few exceptions. Of the high-risk groups considered, IDUs had the highest prevalence, particularly for HCV.
Regarding cost-effectiveness of screening, we found evidence that HCV screening of IDUs and HBsAg screening of pregnant women are cost-effective interventions to reduce the burden of disease due to HBV and HCV infection. HCV screening of pregnant women and comprehensive screening of all STD-clinic attendees is probably not cost-effective, although there may be exceptions for some specific local or subpopulation conditions. For other programs, including HBV screening of IDUs, HCV screening of the general population and migrants, HBV and HCV screening of prisoners and MSM, the evidence found in this systematic review was insufficient to draw conclusions.
The strongest evidence regarding cost-effectiveness was available for HCV screening of IDUs. It is unclear, however, to what extent IDUs in Europe are offered HCV screening and are successfully referred once found to be positive. HCV screening programmes for IDUs exist in only 16 of the 29 European Union/European Economic Area countries reviewed in 2009 [26] whereby testing coverage and referral to treatment often remain poor [69]. On the other hand, several countries without screening programmes report adequate HCV testing of IDUs [70]. Optimizing implementation of IDU testing guide-

lines and monitoring of this are priorities [71,72].
Regarding HBsAg screening of pregnant women it is likely that it would be even more economically favourable if antiviral treatment of the mother was considered. European countries that currently have selective or no antenatal HBsAg screening programmes, including Lithuania, Luxembourg, Romania, and Norway, should consider implementing universal antenatal screening.[26] This holds also for countries with a universal infant HBV-vaccination programme with an at-birth dose of vaccine, since prevention of perinatal HBV transmission requires the first dose of vaccine to be given within 24 hours, and providing hepatitis B immunoglobulin is of additional effectiveness [73,74].
The two publications examining HBsAg screening of migrants born in endemic countries (HBsAg prevalence ≥ 2%) suggest this is cost-effective, consistent with a recent additional study from the United States [75]. Main determinants of ICER were the proportion of eligible people starting treatment, disease progression rates with and without treatment, and costs of treatment [63,64]. Further research should focus on these areas of uncertainty, as well as on how to optimize participation in screening and referral pathways [76]. Given that HCV could be tested using the same blood sample and that migrants generally have higher HCV prevalence than the indigenous population in European countries, an economic assessment of combined HBV/HCV screening for migrants is a priority [77,78].
There was lack of evidence of cost-effectiveness of screening for HBsAg and/or anti-HCV-Ab in the general population or population subgroups other than pregnant women, IDUs, and migrants. Of importance, no economic analyses were found of HBV and/or HCV screening in the general population in countries that have an intermediate to high prevalence (Greece, Italy, Romania, Spain and Turkey). There is an urgent need to study cost-effectiveness and feasibility of general population screening in these mid-to-high level endemic countries.
The main limitation of our review is regarding the comparability of the estimates found. First this is limited since different laboratory tests were used, particularly for HCV where antibody assay validity has improved in recent years. Second, prevalence estimates were not always standardized by age and sex. Lastly, the definition and sampling of the high risk population groups are likely to influence prevalence estimates found. A limitation of the cost-effectiveness studies is that most analyses used Markov modeling, necessary since the disease outcomes of chronic HBV and HCV infection take several decades to develop. These models do not allow quantifying the effect of reduced transmission by lowering viral load due to antiviral treatment and due to potential behavior change. Since these effects can be considerable [13-15], dynamic models assessing the effects of screening and treatment need to be developed. This is likely to be of particular relevance for population subgroups where not only the prevalence, but also the incidence, is increased compared to the general population, such as MSM and IDUs. Lastly, methods, assumptions, and quality varied between studies, making it difficult to compare results and limiting the possibilities of carrying out meta-analyses. Guidelines such as those developed for economic analyses of vaccination programmes may be helpful to improve the quality of studies [79].
In conclusion, we demonstrated the wide variation in prevalence of chronic HBV and HCV infection between countries in Europe. Countries in the south and east of the European Union and in Turkey have a much higher prevalence for chronic HBV and HCV than countries in northwestern Europe. For the majority of countries data on the general population prevalence of HBV or HCV were lacking. Within countries, the prevalence of HBsAg and anti-HCV-Ab among IDUs, MSM, and migrants was generally much higher than the general population prevalence. Considerable health benefits can be gained cost-effectively by anti-HCV-Ab screening of IDUs and HBsAg screening of pregnant women. HBsAg screening of migrants is likely to be cost-effective. Appraisals of the evidence for screening the general population in midand highly endemic countries and of combined HBV/HCV screening are needed. Future cost-effectiveness analyses may need to take effect of antiviral treatment on preventing HBV and HCV transmission into account.

Competing interests
No authors have any competing interests.

Authors' contributions
SH, IV, ML and MS designed the study. SH and IV carried out the systematic review and wrote the manuscript. LW provided and interpreted the data on IDUs. TL assisted with the economic analyses. All authors contributed to interpreting the data and writing the manuscript.

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Ethics statement
An ethics statement was not required for this work.

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Supporting information 1: Search strategy

S1.1 Prevalence studies
General population, 34 countries European region

Ovid-MEDLINE	
1	(hepatitis B or hbv or hepatitis c or hcv).ti.
2	exp hepatitis b/ or exp hepatitis c/
3	1 or 2
4	(prevalence or seroprevalence or seroepidemiolog* or serologic* markers or serology or residual sera or hbsag or hbs ag or hepatitis b surface antigen* or hbv surface antigen* or anti-hcv or anti hcv or hcv rna or carrier*).tw.
5	prevalence/ or seroepidemiological studies/ or hepatitis b surface antigens/ or hepatitis c antibodies/ or carrier state/
6	3 and (4 or 5)
7	(population or community or child* or adolesc* or adults or elder* or older or surveillance or serosurveillance or survey*).tw.
8	population surveillance/ or health surveys/
9	6 and (7 or 8)
10	("european populations" or europ* or iceland or norway or sweden or finland or denmark or "great britain" or england or scotland or wales or ireland or netherlands or belgium or france or luxemburg or spain or portugal or italy or switzerland or austria or germany or poland or hungary or czech or croatia or slovakia or slovenia or romania or bulgaria or lithuania or latvia or estonia or estland or greece or turkey or macedonia or cyprus or malta).tw.
11	exp europe/ or european union/
12	9 and (10 or 11)
13	limit 12 to yr=2000-2009
14	13 and english.lg.

DIMDI (sbas me90;em90;is74;rd=01.01.2000-27.07.2009)	
1	c=me90; em90; is74
2	s=ft=(hepatitis b;hbv;hepatitis c;hcv)/ti
3	ct d (hepatitis b;hepatitis c)
4	2 or 3
5	ft=(prevalence;seroprevalence;seroepidemiolog?;serologic ? markers;serology;residual sera;hbsag;hbs ag;hepatitis b surface antigen?;hbv surface antigen?;anti-hcv;anti hcv;hcv rna;carrier?)/(ti;ab)
6	ct=(prevalence;seroepidemiological studies;seroepidemiology;hepatitis b surface antigens;hepatitis b surface antigen;hepatitis c antibodies;carrier state)
7	4 and (5 or 6)
8	ft=(population;community;child?;adolesc?;adults;elder?; older;surveillance;serosurveillance;survey?)/(ti;ab)
9	ct=(population surveillance;population research;population exposure;health surveys;health survey)
10	7 and (8 or 9)
11	ft=(european populations;europ?;iceland;norway; sweden;finland; denmark; great britain; england;scotland;wales;ireland; netherlands; belgium; france;luxemburg;spain;portugal;italy;switzerland;austria; ;germany)/(ti;ab)
12	ft=(poland;hungary;czech;croatia;slovakia;slovenia;romania;

	bulgaria;lithuania;latvia;estonia;estland;greece;turk
	ey;macedonia;cyprus;malta)/(ti;ab)
13	ct d europe or ct=(european union;european economic
	community)
14	10 and (11 or 12 or 13)
15	14 and py>1999
16	15 and la=english
17	check duplicates: unique in s=16
18	17 and base=me90
19	17 not 18

Prevalence in 5 specific population subgroups, 34 countries European Region

Ovid-Medline	
1	(hepatitis B or hbv or hepatitis c or hcv).ti.
2	exp hepatitis b/ or exp hepatitis c/
3	1 or 2
4	(prevalence or seroprevalence or seroepidemiolog* or serologic* markers or serology or residual sera or hbsag or hbs ag or hepatitis b surface antigen* or hbv surface antigen* or anti-hcv or anti hcv or hcv rna or carrier*).tw.
5	prevalence/ or seroepidemiological studies/ or hepatitis b surface antigens/ or hepatitis c antibodies/ or carrier state/
6	3 and (4 or 5)
7	(blood donor* or blood-donor* or idu or injecting drug users or intravenous drug users or substance abuse* or drug abuse* or drug users).tw.
8	(msm or (men adj3 sex adj3 men) or homosex* or (homo adj3 sexual) or gay men or migrant* or immigrant* or minorit* or pregnan* or antenatal or prenatal).tw.
9	blood donors/ or intravenous substance abuse/ or drug users/ or male homosexuality/ or "transients and migrants"/ or "emigrants and immigrants"/ or minority groups/ or pregnancy/
10	6 and (7 or 8 or 9)
11	("european populations" or europ* or iceland or norway or sweden or finland or denmark or "great britain" or england or scotland or wales or ireland or netherlands or belgium or france or luxemburg or spain or portugal or italy or switzerland or austria or germany or poland or hungary or czech or croatia or slovakia or slovenia or romania or bulgaria or lithuania or latvia or estonia or estland or greece or turkey or macedonia or cyprus or malta).tw.
12	exp europe/ or european union/
13	10 and (11 or 12)
14	limit 13 to yr=2000-2009
15	14 and english.lg.

DIMDI (sbas me90;em90;is74;rd=01.01.2000-27.07.2009)	
1	c=1 me90; em90; is74
2	s=2 ft=(hepatitis b;hbv;hepatitis c;hcv)/ti
3	ct d (hepatitis b;hepatitis c)
4	2 or 3
5	ft=(prevalence;seroprevalence;seroepidemiolog?;serologic
	? markers;serology;residual sera;hbsag;hbs ag;hepatitis
	b surface antigen?;hbv surface antigen?;anti-hcv;anti
	hcv;hcv rna;carrier?)/(ti;ab)
6	ct=(prevalence;seroepidemiological
	studies;seroepidemiology;hepatitis b surface
	antigens;hepatitis b surface antigen;hepatitis c

	antibodies;carrier state)
7	4 and (5 or 6)
8	ft=(blood donor?;blood-donor?;idu;injecting drug users;intravenous drug users;substance abuse?;drug abuse?;drug users)/(ti;ab)
9	ft=(msm;men # # # sex # # # men;homosex?;homo # # # sexual;gay men;migrant?;immigrant?; minorit?;pregnan?;antenatal;pren atal)/(ti;ab)
10	ct=(blood donors;blood donor;intravenous substance abuse;intravenous drug abuse;drug users;drug use;drug abuse;male homosexuality;homosexuality;transients and migrants;emigrants and immigrants;immigrant;minority groups;minority group;pregnancy)
11	7 and (8 or 9 or 10)
12	ft=(european populations;europ?;iceland; norway;sweden;finland;denmark; great britain;england;scotland;wales;ireland; netherlands;belgium;france;luxemburg;spain;portugal;italy;switzerland;aus tria; germany)/(ti;ab)
13	ft=(poland;hungary;czech;croatia;slovakia;slovenia;roman ia; bulgaria;lithuania;latvia;estonia;estland;greece;turkey; macedonia;cyprus;malta)/(ti;ab)
14	ct d europe or ct=(european union;european economic community)
15	11 and (12 or 13 or 14)
16	15 and py>1999
17	16 and la=english
18	check duplicates: unique in s=17
19	18 and base=me90
20	18 not 19

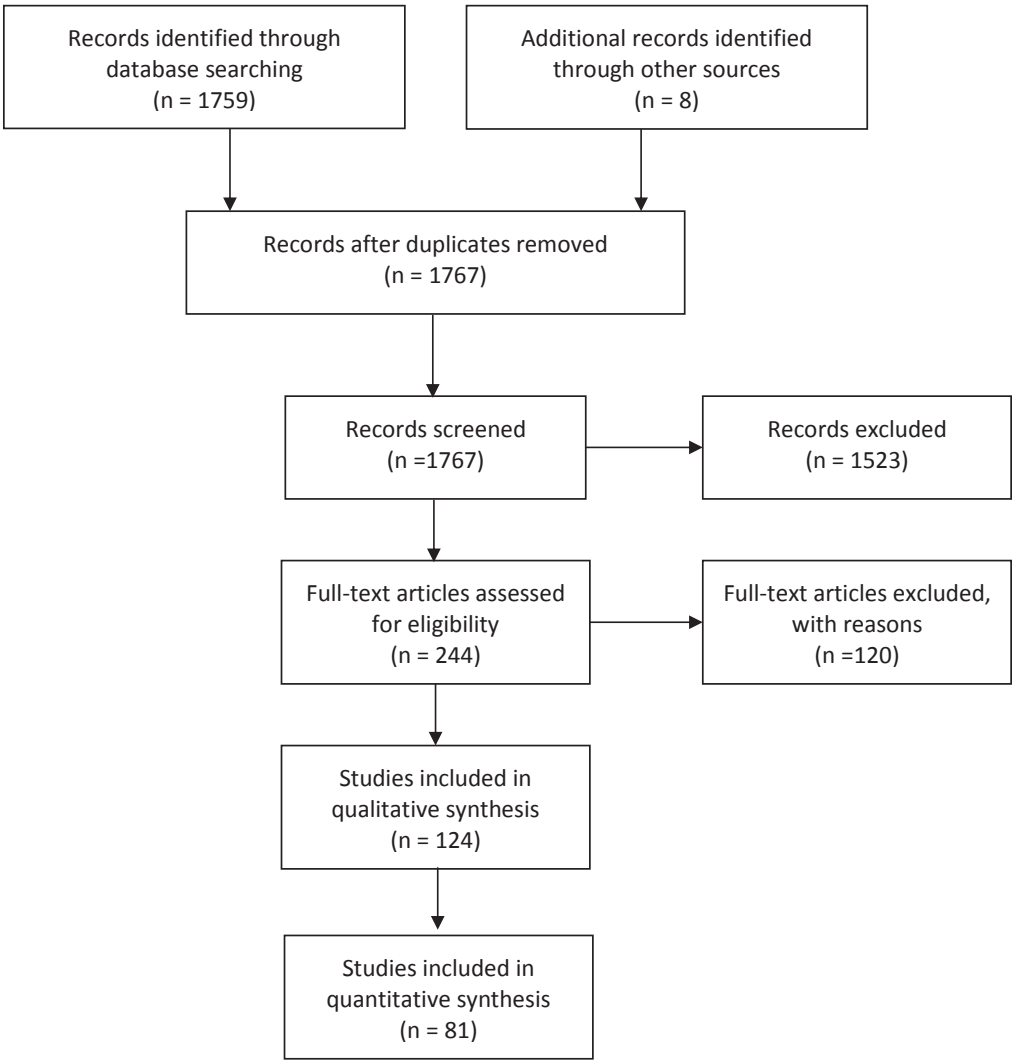
S1.2 Cost-effectiveness studies

Ovid MEDLINE(R), Ovid MEDLINE(R) In-Process	
1	(hepatitis B or hbv or hepatitis c or hcv or hbsag or hbs ag or hcv-rna or anti-hcv).ti.
2	exp hepatitis b/ or exp hepatitis c/ or hepatitis b surface antigens/ or hepatitis c antibodies/
3	1 or 2
4	(screening or testing).tw.
5	mass screening/
6	3 and (4 or 5)
7	cost-benefit analysis/ or "costs and cost analysis"/ or mass screening/ec or quality-adjusted life years/
8	(cost benefit* or cost effect* or cost utilit* or cost efficien* or econom* or quality adjusted or disability adjusted or qaly* or daly* or icer).ti.
9	6 and (7 or 8)
10	(blood donors or blood recipients or blood donations or transfusion* or posttransfusion or donor* or donation* or blood screening or blood supply or blood product* or plasma product* or plasma or postdonation or coagulation factor concentrates or blood bank or transplantation or hepatocellular carcinoma or HCC).ti.
11	9 not 10
12	11 and english.lg.
13	limit 12 to yr=1990-2011
14	remove duplicates from 13

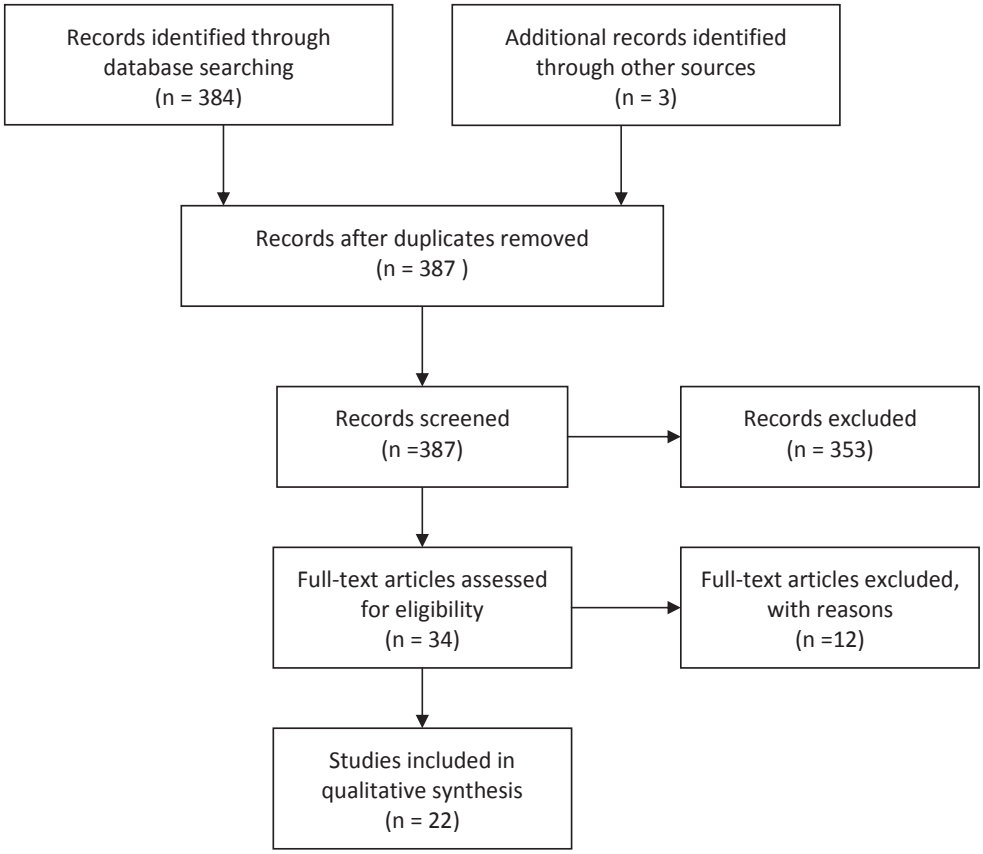
Scopus
(((TITLE((hepatitis-b) OR hbv OR (hepatitis-c) OR hcv OR hbsag OR (hbs-ag) OR (hcv-rna) OR (anti-hcv)) OR KEY((hepatitis-b) OR hbv OR (hepatitis-c) OR hcv OR hbsag OR (hbs-ag) OR (hcv-rna) OR (anti-hcv))) AND (TITLE(screening OR testing) OR KEY(screening OR testing)) AND (TITLE((cost-benefit*) OR (cost-effect*) OR (cost-utilit*) OR (cost-efficien*) OR econom* OR (quality-adjusted) OR (disability-adjusted) OR qaly* OR daly* OR icer) OR KEY((cost-benefit*) OR (cost-effect*) OR (cost-utilit*) OR (cost-efficien*) OR econom* OR (quality-adjusted) OR (disability-adjusted) OR qaly* OR daly* OR icer))) AND NOT (TITLE((blood-donors) OR (blood-recipients) OR (blood-donations) OR transfusion* OR posttransfusion OR donor* OR donation* OR (blood-screening) OR (blood-supply) OR (blood-product*) OR (plasma-product*) OR plasma OR postdonation OR (coagulation-factor-concentrates) OR (blood-bank) OR transplantation OR (hepatocellular-carcinoma) or HCC))) AND (LANGUAGE(english) AND PUBYEAR AFT 1989)

Supporting information 2: PRISMA flow diagrams[116]

S2.1 Systematic review of seroprevalence of HBsAg and anti-HCV-Ab



S2.2 Systematic review of cost-effectiveness of screening for chronic HBV and HCV infection



Supporting information 3: Figures: HBsAg and anti-HCV-Ab prevalence estimates in population subgroups, by country, European neighbourhood.

Figure S3.1a First-time blood donors: HBsAg prevalence (%) by country, Europe, 2000-2009.

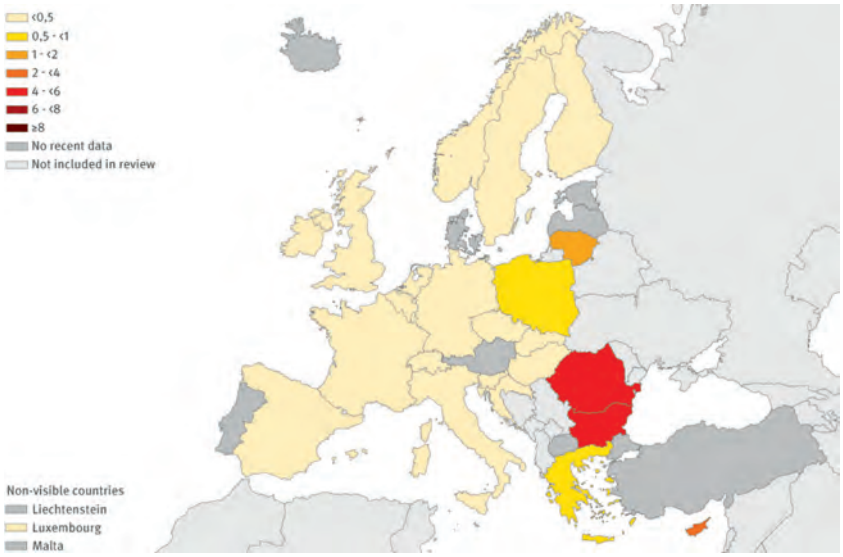


Figure S3.1b First-time blood donors: anti-HCV-Ab prevalence (%) by country, Europe, 20002009.

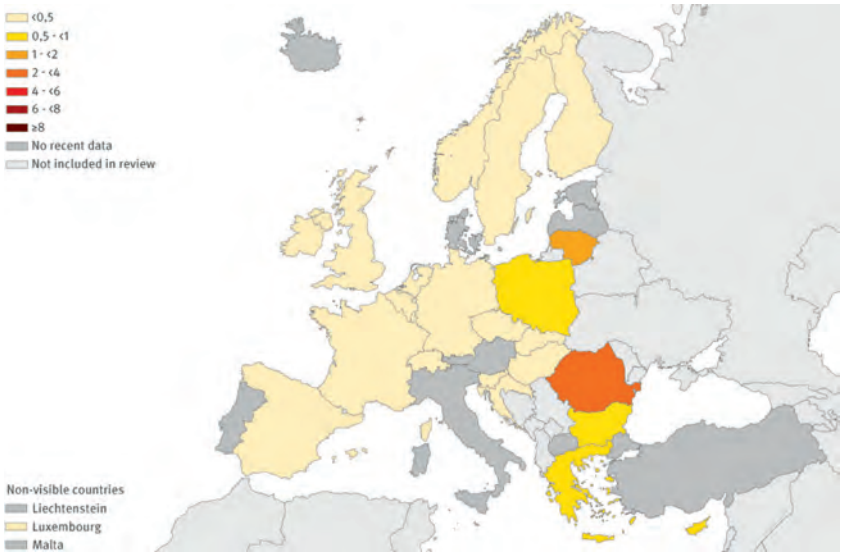


Figure S3.2a Pregnant women: HBsAg prevalence (%) by country, Europe, 2000-2009.

Figure S3.2b Pregnant women: anti-HCV-Ab prevalence (%) by country, Europe, 2000-2009.

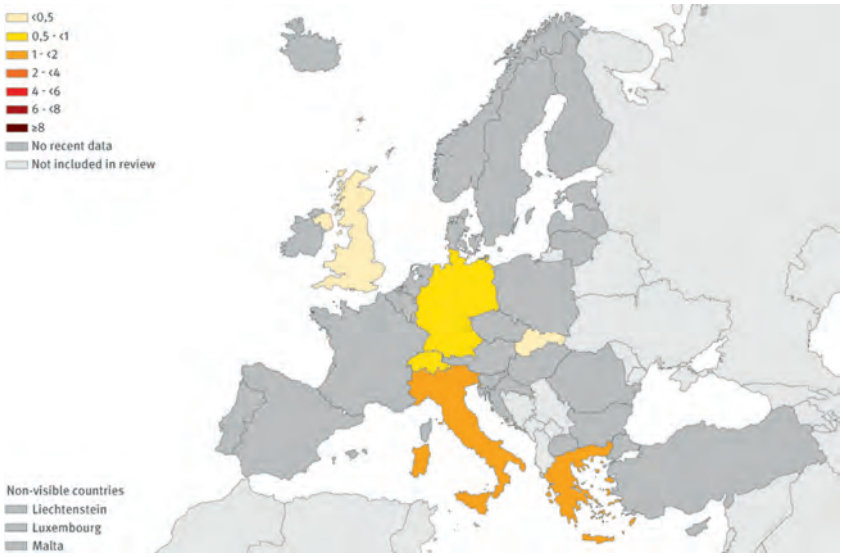


Figure S3.3a IDUs: Prevalence of HBsAg among IDUs (%), Europe, 2000-2009.

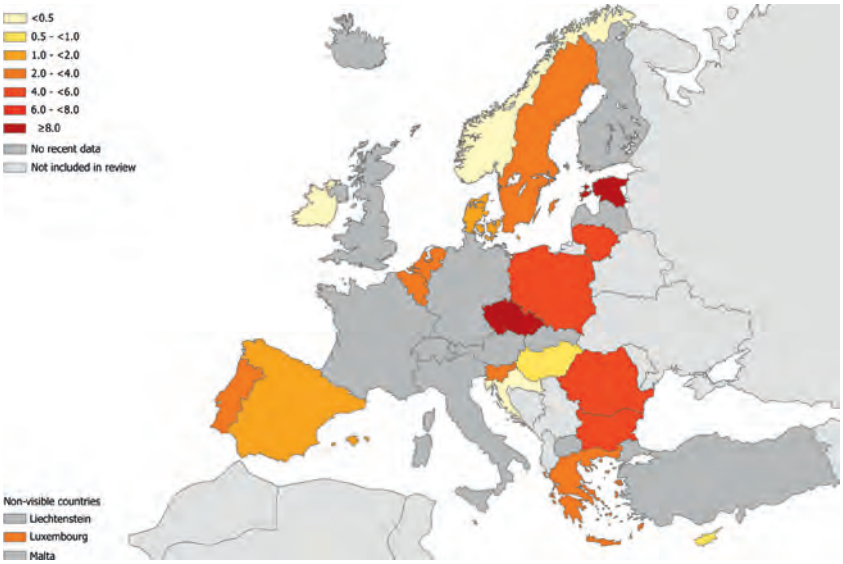


Figure S3.3b IDUs: Prevalence of anti-HCV-Ab among IDUs (%), Europe, 2000-2009.

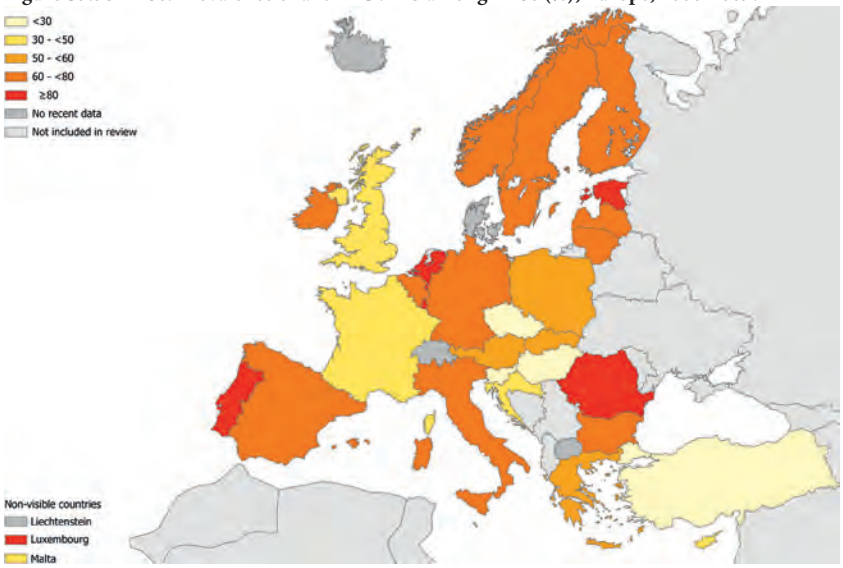


Table S3.1a First-time blood donors: HBsAg prevalence (%), Europe, 2000-2009.

Country (Reference)	Period	Prevalence (%)
Belgium (31)	2005	0.06
Bulgaria (31)	2005	5.2
Croatia (31)	2005	0.2
Cyprus (117)	Not reported	3.0
Czech Republic (31)	2005	0.07
Finland (31)	2005	0.04
France (31)	2005	0.1
Germany (118)	1997-2002	0.2
Greece (119)	1995-1997	0.9
Hungary (31)	2005	0.00
Ireland (31)	2005	0.02
Italy (120)	2005	0.4
Lithuania (121)	2005-2006	1.7
Luxembourg (31)	2005	0.1
Netherlands (31)	2005	0.09
Norway (31)	2005	0.02
Poland (122)	1998-2000	0.9
Romania (31)	2005	4.3
Slovakia (31)	2005	0.2
Slovenia (31)	2005	0.09
Spain (31)	2005	0.1
Sweden (31)	2005	0.06
Switzerland (31)	2005	0.1
United Kingdom (31)	2005	0.04

Table S3.1b First-time blood donors: anti-HCV-Ab prevalence (%), Europe, 2000-2009.

Country (Reference)	Period	Prevalence (%)
Belgium (31)	2005	0.06
Bulgaria (31)	2005	0.9
Croatia (31)	2005	0.06
Cyprus (117)	2005	0.5
Czech Republic (31)	2005	0.1
Finland (31)	2005	0.04
France (31)	2005	0.06
Germany (31)	2005	0.08
Germany (118)	1997-2002	0.1
Greece (31)	2005	0.6
Hungary (31)	2005	0.3
Ireland (31)	2005	0.02
Lithuania (121)	2005-2006	1.7
Luxembourg (31)	2005	0.06
Netherlands (31)	2005	0.03
Norway (31)	2005	0.06

Poland (122)	1998-2000	0.6
Romania (31)	2005	3.3
Slovakia (31)	2005	0.06
Slovenia (31)	2005	0.02
Spain (123)	1999-2001	0.2
Sweden (31)	2005	0.1
Switzerland (31)	2005	0.08
United Kingdom (31)	2005	0.04

Table S3.2a Pregnant women: HBsAg prevalence (%), Europe, 2000-2009.

Country (Reference)	Period	Area	Region	N	Prevalence (%)	95% CI
Denmark (124)	2005-2006	Nationwide		29,708	0.3	
France (125)	1984-1998	Regional	Limoges	22,859	0.7	
Germany (126)	1996-2005	Regional	Heidelberg	5,518	1.6	
Greece (127)	2003	Nationwide		3,384	2.9	2.3-3.4
Ireland (128)	1998-2000	Regional	Dublin	16,222	0.4	
Italy (129)	2001	Nationwide		10,881	1.7	1.4-1.9
Netherlands (130)	1993-1998	Regional	Amsterdam	56,756	1.2	
Slovakia (131)	2000-2004	Regional	Bratislava	90	4.4	
Spain (132)	2004	Regional	Catalonia	1,534	0.1	0.0-0.3
Switzerland (133)	2001	Regional	Basel	1,503	1.2	0.7-1.8
United Kingdom (134)	2002	Regional	London	110,621	1.0	

Table S3.2b Pregnant women: anti-HCV-Ab prevalence (%), Europe, 2000-2009.

Country (Reference)	Period	Area	Region	N	Prevalence (%)	95% CI
Germany (135)	1992-1996	regional	Munich	3,712	0.9	
Greece (136)	1994-2002	regional	Athens (Piraeus)	5,497	0.8	
Greece (137)	1996-1997	regional	North	2,408	2.0	
Italy (138)	1996	regional	North	2,059	1.9	
Italy (139)	1995-1998	regional	North	15,25	2.4	
Italy (140)	1996-2001	regional	North	13,025	0.8	
Slovakia (131)	2000-2004	regional	Bratislava	90	0.0	
Switzerland (141)	1990-1991	nationwide		9,057	0.7	
United Kingdom (142)	1997-1998	nationwide		126,009	0.2	0.1-0.3
United Kingdom (143)	1996	regional	Northern and Yorkshire	16,675	0.2	0.1-0.3
United Kingdom (143)	1996	regional	London	25,94	0.4	0.3-0.5
United Kingdom (144)	1997-1999	regional	London	4,729	0.8	0.6-1.0
United Kingdom (145)	1997	regional	Scotland	3,548	0.6	0.4-1.0
United Kingdom (146)	2000	regional	Scotland	30,259	0.3	

Table S3.3a IDUs: Prevalence of HBsAg among IDUs (%), Europe, 2000-2009.

Country* (Reference)	Period	Area	Region	N	Prevalence (%)	95% CI*
Belgium (32)	2008-2009	Subnational	Antwerp, Flemish community	434	3.5	2.0-5.6
Bulgaria (32)	2009	Subnational	Sofia	941	5.8	4.4-7.5
Croatia (32)	2007	National		200	0.5	0.0-2.8
Cyprus (32)	2009	National	-	115	0.9	0.0-4.8
Czech Rep. (33)	2010	Not reported		575	15·1	12.3-18.3
Denmark (33)	2007	Subnational	Funen	239	1·3	0.3-3.6
Estonia (33)	2004	Subnational	Tallinn	155	21·3	15.1-28.6
Greece (32)	2009	National		1814	2.6	1.9-3.4
Hungary (32)	2009	National	-	676	0.7	0.2-1.7
Ireland (32)	2003	Subnational	Dublin	63	0.0	0.0-5.7
Lithuania (32)	2005-2006	Subnational	Alytus city, Vilnius	517	4.4	2.8-6.6
Luxembourg (32)	2005	National	-	255	3.9	1.9-7.1
Netherlands (32)	2000	Subnational	The Hague	199	3.0	1.1-6.5
Norway (32)	2009	Subnational	Oslo	179	0.0	0.0-2.0
Poland (32)	2002-2009	Subnational	Eight cities	952	4.2	3.0-5.7
Portugal (32)	2009	National	-	838	2.9	1.8-4.2
Romania (32)	2009	Subnational	Bucharest	447	4.7	2.9-7.1
Slovenia (32)	2002	National	-	564	3.4	2.0-5.2
Spain (33)	2006	Subnational	Barcelona	166	1·8	0.4-5.2
Sweden (33)	2006	Subnational	Stockholm	310	2·6	1.1-5.0

*CI Confidence Interval
No estimate of HBsAg prevalence for IDUs was available for Austria, Finland, the FYR Macedonia, France, Germany, Iceland, Italy, Latvia, Liechtenstein, Malta, Slovakia, Switzerland, Turkey and the United Kingdom.
For EMCDDA data (32) original sources are available online: http://www.emcdda.europa.eu/stats11/inftab0

Table S3.3b IDUs: Prevalence of anti-HCV-Ab among IDUs, Europe, 2000-2009.

Country (Reference)	Period	Area	Region	N	Prevalence (%)	95% CI*
Austria (32)	2009	National		511	53.2	48.8 57.6
Belgium (32)	2008-2009	Subnational	Antwerp, Flemish community	454	68.3	63.9 72.5
Bulgaria (32)	2009	Subnational	Sofia	955	61.2	58.0 64.3
Croatia (32)	2007	National	-	200	44.0	37.0 51.2
Cyprus (32)	2009	National	-	116	46.6	37.2 56.1
Czech rep. (32)	2009	National	-	353	22.4	18.1 27.1
Estonia (32)	2002	Subnational	Ida-Viru, Tallinn	100	90.0	82.4 95.1
Finland (32)	2009	National	-	682	60.5	56.8 64.3
France (32)	2006	Subnational	Five cities	362	41.7	36.6 47.0
Germany (33)	2001-2003	Subnational	Warstein	1512	75·0	72.7 77.2
Greece (32)	2009	National		1751	55.0	52.6 57.4
Hungary (32)	2009	National	-	667	24.4	21.2 27.9
Ireland (32)	2003	Subnational	Dublin	65	72.3	60.0 82.7
Italy (32)	2000	National	-	628	72.9	69.3 76.4
Latvia (32)	2007	Subnational	Riga	406	74.4	69.9 78.6
Lithuania (32)	2000	National	-	693	79.0	75.7 81.9
Luxembourg (32)	2005	National	-	268	81.3	76.2 85.8
Malta (32)	2009	National	-	121	30.6	22.5 39.6
Netherlands (32)	2008	Subnational	Rotterdam	65	86.2	75.3 93.5
Norway (32)	2009	National	-	3972	72.9	71.5 74.3
Poland (32)	2009	Subnational	Eight cities	950	59.2	56.0 62.3
Portugal (32)	2009	National	-	895	83.1	80.5 85.5
Romania (32)	2009	Subnational	Bucharest	449	82.9	79.0 86.2
Slovakia (32)	2009	Subnational	Bratislava	98	50.0	39.7 60.3
Slovenia (32)	2009	National	-	401	23.4	19.4 27.9
Spain (32)	2001-2003	Subnational	Barcelona, Madrid, Seville	912	77.2	74.3 80.0
Sweden (32)	2008, 2009	Subnational	Stockholm county, Sotckholm, Gothenburg	683	75.5	72.2 78.7
Turkey (32)	2008	Subnational	Gaziantep	168	5.3	2.5 9.9
UK (32)	2008, 2009	Subnational	England & Wales, Northern Ireland, Scotland	3112	45.3	43.6 47.1

* CI Confidence interval
No estimate of anti-HCV prevalence for IDUs was available for Denmark, the FYR Macedonia, Iceland, Liechtenstein and Switzerland.
For EMCDDA data (32) original sources are available online: http://www.emcdda.europa.eu/stats11/inftab0

Table S3.4a Migrants: HBsAg prevalence (%), Europe, 2000-2009.

Country (Reference)	Study period	Country of birth / ethnicity	Status	N	Prevalence (%)	Remark
Greece (147)	not reported	Albania	Refugees	130	15.4	
Italy (148)	1997	Albania	Refugees	670	13.6	
Italy (149)	2005-2006	South America	Refugees	130	10.7	
Italy (150)	2005	Africa, Asia	Refugees	556	10.7	
Italy (151)	2005	Sub-Sahara Africa	Undocumented migrants	182	9.3	
Italy (152)	2003-2004	Several countries	Refugees	890	9.3	
Spain (153)	2001-2004	Several countries	Residents	1,905	7.7	
Italy* (154)	2000	Turkey (Kurds)	Refugees	368	6.8	
United Kingdom (155)	2000	Somalia	Residents	448	5.7	
Greece (156)	2002	Roma	Residents	118	4.2	Children only
Italy (157)	1999	Kosovo	Refugees	526	2.9	
Italy* (154)	2000	Iraq (Kurds)	Refugees	637	2.2	
Netherlands (158)	2004	Several countries	Residents	205	1.0	

* One publication on 2 migrant groups

Table S3.4b Migrants: anti-HCV-Ab prevalence (%), Europe, 2000-2009.

Country	Study period	Country of birth / ethnicity	Status	N	Prevalence (%)	Remark
Hungary (159)	2004	Roma	Residents	64	23.4	
Spain (153)	2001-2004	Several countries	Residents	1,848	3.1	
Greece (147)	not reported	Albania	Refugees	130	2.3	
Italy (151)	2004-2005	Sub-Saharan Africa	Undocumented migrants	182	2.2	
Netherlands (158)	2004	Several countries	Residents	205	1.5	
Italy (160)	2002-2006	Several countries	Residents	120	0.8	Children only
Italy (157)	1999	Kosovo	Refugees	526	0.7	
Italy (148)	1997	Albania	Refugees	670	0.3	
Italy* (154)	2000	Turkey (Kurds)	Refugees	368	0.1	
Greece (156)	2002	Roma	Residents	216	0.0	Children only
Italy* (154)	2000	Iraq (Kurds)	Refugees	637	0.0	

Supporting information 5: PRISMA checklist [116]

TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	✓
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	✓
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	✓
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	✓

METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	✓
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	✓
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	✓
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	✓
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	✓
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	✓
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	✓
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	✓
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	✓
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	✓
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	✓
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	n.a.
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	✓
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	✓
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	✓
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	✓
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	✓
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	✓
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	n.a.
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	✓
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	✓
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	✓
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	✓

Chapter 5

Discussion

Discussion

This Thesis presents results of a decade of studying the epidemiology of HBV in the Netherlands, England & Wales and at a European level, starting in 2003. These studies mainly aimed to increase the understanding of the local epidemiology of HBV and to assess the effectiveness of HBV vaccination programmes in order to inform public health policy for prevention and control of HBV. In this final Chapter, I discuss what these studies add to what was already known. Subsequently, I outline the implications of this Thesis for HBV surveillance, control and research.

1. EPIDEMIOLOGY OF HBV IN THE NETHERLANDS

Prevalence in the general population

What was known

The corrected HBsAg prevalence estimate from a 1996 HBV prevalence study in the general Dutch population (the Pienter-1 study) was 0.1%. The anti-HBc prevalence was 2.1%. Non-Dutch ethnicity and travel abroad for 3 months or longer were the only risk factors for infection identified (1-3).

What this Thesis adds

Our 2007 national seroprevalence study (the Pienter-2 study) showed that 10 years after the 1996 study the prevalence of HBV infection did not increase (Chapter 2.1). The Netherlands therefore continues to be among countries with the lowest HBV prevalence in the world (Chapter 4.1, 4,5). We estimated that about 40,000 people are living with chronic HBV infection in the Netherlands. In 2008, Marschall *et al.* published an estimate of the HBsAg prevalence (HBsAg 0.3-0.5%) that was considerably higher than the prevalence found in 1996 and 2007 (6). Marschall's estimate was based on the 1996 seroprevalence study with a correction for under-representation of migrants, assuming the prevalence in migrants reflects their country of origin. Later evidence suggests this assumption may not be sufficiently valid (7). Since in our 2007 seroprevalence study migrants were over sampled, we believe the estimated prevalence is representative of the general population including migrants. However, since illegal migrants and some other, less numerous high-risk groups such as injecting drug users are less well represented, we may have still slightly underestimated the HBV prevalence.

The infection prevalence decreased in indigenous Dutch, likely due to the implementation of various prevention strategies in the past decades such as adequate screening of blood products, improved vaccination of health care workers and travelers (Chapter 2.1). Increases in migration to the Netherlands had no discernable impact on the prevalence in the indigenous Dutch. The same conclusion was drawn for HIV (8). Similarly, increases in migration to the UK did not have an impact on patterns of HBV incidence and transmission (Chapter 3.1).

Prevalence among first and second generation migrants

What was known

The 1996 Pienter-1 seroprevalence study showed that non-Dutch participants had a higher prevalence of infection than native Dutch, particularly those of Turkish descent (1-3). However, due to the low number of participating migrants, a sufficiently precise estimate of the HBV prevalence among FGM in the Netherlands and insight into risk factors for infection were not available. An increased risk of HBV among second generation migrants (SGM) was deemed plausible but never demonstrated. This assumption was the basis for the selective national infant vaccination programme for SGM (9,10).

What this Thesis adds

FGM had a 10-times higher prevalence of chronic infection than indigenous Dutch[1], with those born in Asia at increased risk[2]. These findings match numerous studies of HBV prevalence in migrants in developed countries (11-13). Contrasting with previous findings, SGM were also at increased risk of

1 Chapter 2.1, table 1
2 Chapter 2.1, table 5

infection: They were 5-times as likely to be anti-HBc positive compared to indigenous Dutch[3], for SGM children this prevalence ratio was even 12[4]. These findings from our seroepidemiological study were corroborated by our case-control study, showing that SGM children have a 7-times higher risk of acquisition of HBV infection whilst resident in the Netherlands than the indigenous population[5]. A recent study from Amsterdam confirmed the increased HBV infection risk of SGM (14). These findings are consistent with our studies in England & Wales, in which we demonstrated an increased risk of acute HBV infection in residents of South Asian descent [6].

Risk factors for acquisition of HBV infection among Dutch residents

What was known
Previous assessments of risk factors for HBV infection among the general population in the Netherlands were mostly based on case-only descriptions of notified acute HBV infections (15-17). This study design uses a priori assumptions regarding the hierarchy of risk factors for transmission, is unable to identify risk factors among high risk groups, and does not allow quantification of risks associated with certain exposures. These studies estimated that about 60% of HBV infections in the Netherlands was acquired by sexual contact (15-17) and suggested that men who have sex with men (MSM) and heterosexuals with a partner from an endemic country are important high-risk groups.

What this Thesis adds
We confirmed that among Dutch residents male homosexual contact is the most important risk for acquisition of HBV infection, with a 146 times higher odds of infection than heterosexual females[7]. MSM who had 3 or more partners in the past 6 months were at higher risk than those with 0 or 1 partner[8]. Male heterosexuals had approximately double the risk of HBV infection of heterosexual females[9]. Among heterosexuals having had 2 sexual partners in the past 6 months and having a foreign born partner were independent risk factors[10]. Our seroepidemiological study (Chapter 2.1) confirmed the independently increased risks of HBV infection when being a SGM and having a sexual partner from an endemic country. In adult SGM, about 30% of the infections were attributable to having a foreign-born sexual partner[11]. A considerable part of the HBV infections in SGM children are acquired during travel to endemic regions[12], frequently through parenteral exposures[13]. A newly identified risk factor was having had a blood transfusion in the Netherlands in the past[14].
Injecting drug use (IDU) continues to be an important route of acquisition of HBV infection in Europe (18-20). Previous studies suggested this is no longer the case in the Netherlands (21). Our analyses confirmed this[15].

2. EFFECTIVENESS OF PUBLICLY FUNDED HBV VACCINATION PROGRAMMES IN THE NETHERLANDS

What was known
HBV vaccine coverage among infants of mothers with chronic infection, children of migrants, and of the universal infant vaccination programme is high compared to national targets and coverage figures reported from elsewhere (22-25). Since HBV vaccines are generally efficacious, this suggests the Dutch programmes are effective in protecting the target population. However, for several reasons the effectiveness of the two HBV vaccination programmes targeting groups at highest risk of HBV infection remained uncertain. First, the coverage achieved by programme for population groups at risk of HBV due to high-risk behaviour is difficult to assess, since the size of the target populations (including MSM, injecting drug users (IDUs) and commercial sex workers (CSWs)) are not well known. Furthermore, drawing conclusions based on the number of case reports is problematic as effects of changes in risk-behaviour are difficult to disentangle from effects of the vaccination programme. A previous assessment suggested the programme had been insufficiently effective to control HBV infection in the targeted groups (26).
Second, regarding the programme for infants born to mothers with chronic HBV infection, the uncertainty was related to the national vaccine schedule used, which did not contain a birth dose of vaccine be-

3 Chapter 2.1, table 2
4 Chapter 2.1, table 3
5 Chapter 2.2, table 4b
6 Chapter 3.1, paragraph 3.4, and Chapter 3.2, Figure 1
7 Chapter 2.2, Table 1
8 Chapter 2.2, Table 3
9 Chapter 2.2, Table 1
10 Chapter 2.2, Table 2b: OR 4.1 and 8.9, respectively.
11 Chapter 2.1
12 Chapter 2.1, Table 3
13 Chapter 2.2, table 4b
14 Chapter 2.1, Tables 2, 3 and 4
15 Chapters 2.1, 2.2 and 2.3

tween 1989 and 2006. This contrasted with WHO and Dutch Health Council recommendations (27,28). The evidence base for the Dutch schedule without a birth dose was derived from locally performed trials in the 1980s (29). However, these had been insufficiently powered to detect important differences in effectiveness. Other uncertainties that remained were whether the selective programme for children of migrants was acceptable to the population, and whether the hexavalent vaccine used in this programme may elicit insufficient immune responses (30,31).

What this Thesis adds

The programme for behavioural high-risk groups has effectively reduced the incidence of HBV in the Netherlands by preventing HBV infection among men who have sex with men (MSM) (Chapter 2.3). This is an important finding to guide policy on HBV prevention in MSM in the Netherlands and elsewhere. The discrepancy with an earlier evaluation can be explained by newer methods used (coalescence analyses) and the longer observation period since the start of the vaccination programme (26). Mathematical modeling had indeed projected that impact of the programme would need several years to be discernable (32). The relative absence of homophobia, marginalisation and stigmatisation of MSM in the Netherlands compared to most other countries in the world is likely to have had a beneficial effect on improving access to prevention services (33). It is therefore uncertain whether the success of the MSM vaccination programme can be replicated elsewhere. It is also uncertain whether a similar programme would be able to curb transmission in other high-risk groups such as IDUs.

Molecular epidemiological analyses furthermore identified the emergence of a clonal A2 HBV strain among heterosexuals in the Netherlands (Chapter 2.3). This may be explained by factors including a relative advantage of this strain in causing chronic infection or a higher viral load (34-37). This could explain the spread of genotype A2 infections among adult risk groups worldwide (38). It is also consistent with findings from a recent study from Amsterdam, showing that an unexpectedly high proportion of MSM (23%) developed chronic infection of acquisition of HBV (39).

We confirmed that in the Netherlands the HBIg and vaccine coverage among infants born to mothers with chronic HBV infection is very high (98% in 2007) (Chapter 2.4). The proportion of infants acquiring HBV infection despite vaccination was 0.8% (8 children of 1044) for the schedule without a birth-dose, and 0.7% (5 children of 738) for the schedules with a birth dose[16]. All children in this analysis had received HBIg at birth. The difference between the effectiveness of the schedules was not statistically significant. However, also in our study the sample size was insufficient to detect small differences. Interestingly, the analyses of anti-HBc levels in children of HBsAg positive mothers suggested there may have been several breakthrough infections that were cleared by the age of 1,5 years (Chapter 2.5).

The question regarding the effectiveness of a schedule without a birth dose of HBV vaccine is no longer relevant since in 2006 a birth dose was added to the programme for children of mothers with chronic HBV infection in the Netherlands. The question was never relevant for countries where HBIg is not given at birth, including most developing countries, since there is sufficient evidence that in the absence of HBIg a birth dose of vaccine is essential (40). Another rational is logistical in nature: higher vaccine coverage is more likely when vaccines are given at birth rather than later. An important finding in Chapter 2.3 was that Chinese women with chronic HBV infection are more likely to transmit the virus to their infants than other chronically HBV infected women[17], probably related to the specific genotypes and higher viral load in this group (38,41). Some of these infections may be preventable by antiviral (and HBIg) treatment during pregnancy (42-44). Antiviral treatment is recommended in the Netherlands for pregnant women with a high viral load (45). The current guideline for prenatal care for HBsAg positive pregnant women recommends referral for an assessment of the indication for antiviral treatment for HBeAg positive women (46). Implementation of guideline, especially among women of Chinese descent, should be monitored and where needed improved. In our study, 2 of the 12 mothers where breakthrough infection occurred were HBeAg negative. More information on the prevalence of a high viral load among HBeAg negative women is needed.

After HBV vaccination of infants of migrants was implemented in 2003, the incidence of acute HBV notifications in young children decreased significantly (Chapter 2.6). However, mainly due to changes in surveillance case definitions we were unable to conclude that this reduction was a result of the selective vaccination programme. However, the question whether the infant vaccination programme targeted

16 Chapter 2.4, Table 3
17 Chapter 2.4, Table 3

at certain ethnic groups is effective is no longer relevant for the Netherlands since universal vaccination was introduced in August 2011. However, countries without universal infant programmes such as Denmark, Hungary, Iceland, Norway, Sweden, Switzerland and the UK (47) should consider introducing HBV vaccination for infants of migrants from endemic countries, since there is evidence both of increased risk of HBV infection in this group[18] and of acceptability of the programme to parents of children in the target population (Chapter 2.7).
In Chapter 2.8 we studied the immunogenicity of Infanrix hexa, a vaccine that is used in the Dutch national immunisation programme concomitantly with Prevenar (conjugated 7-valent pneumococcal vaccine) since 2006. We found that over 99% of 164 children studied developed an adequate immune response against HBV (anti-HBs ≥ 10IU/L). The immune response to one pertussis component was lower in Infanrix hexa vaccinated children than in those vaccinated with Infanrix IPV/Hib. Furthermore, the geometric mean concentration of anti-HBs antibodies was lower than expected. The clinical relevance of these findings remains uncertain but is probably limited.

3. EPIDEMIOLOGY OF HBV INFECTION IN ENGLAND & WALES

What was known
In the late-1980s to early 1990s, the incidence of acute HBV notifications in England & Wales decreased from 2.7 to 1.4 per 100,000 per year, with IDU the most frequent route of transmission (48,49). As in other developed countries, HBV was known to be more prevalent among UK ethnic minorities than among its indigenous populations (11,50).

What this Thesis adds
The incidence of HBV in England & Wales in the late 1990s up to 2000 remained at the level reported for the early 1990s. IDU remained the most frequent route of transmission[19]. Over half of new infections could have been prevented had sufficient coverage been achieved of the UK's current selective vaccination programme[20]. Between 2002 and 2008, IDU still accounted for over 20% of cases of acute HBV in England & Wales (51). Strengthening of selective vaccination, particularly of IDUs, is therefore needed.
We were the first ever to demonstrate an increased incidence of infection among the South Asian ethnic minority in England & Wales[21], later corroborated by findings in Chapter 3.2. Therefore, the option of targeting UK ethnic minority children for HBV vaccine should be explored, especially since we demonstrated such a programme in the Netherlands was feasible, acceptable and probably effective[22].

4. PREVALENCE OF HBV AND HCV INFECTION IN THE EUROPEAN NEIGHBOURHOOD AND COST-EFFECTIVENESS OF SCREENING

What was known
A HBV prevalence review published in 2004 reported estimates for 11 European countries (5). For HCV, two European prevalence reviews were published. Esteban reviewed the HCV prevalence in Europe in 2008 (52), but studies on blood donors were included limiting the general population representativeness. A study from 2009 used expert opinion as a main source of data, which makes the validity of conclusions difficult to ascertain (53).
Regarding the cost-effectiveness of screening for HCV, an earlier review included studies published up to March 2007 and was partly sponsored by the pharmaceutical industry (54). It concluded that HCV screening of former or current IDUs was cost-effective. Systematic reviews of cost-effectiveness of screening for HBV infections have not been published.

What this Thesis adds
We provide updated HBsAg and anti-HCV-Ab prevalence estimates for 13 countries in the European neighbourhood showing that countries in the South and West of the EU, and Turkey, have much higher prevalences than countries in the North and West of the EU (Chapter 4.1). Now that treatment options for chronic HBV and HCV infection have improved, policies for secondary prevention, including screening and access to treatment, are a priority. Our review of evidence for cost-effectiveness concludes that HCV screening of IDUs and HBsAg screening of pregnant women are cost-effective interventions. We found some evidence for cost-effectiveness of HBsAg screening of migrants.

18 Chapter 2.1, 2.2, 3.1 and 3.2
19 Chapter 3.1, Table 2
20 Chapter 3.1 Table 2
21 Chapter 3.1 paragraph 3.4
22 Chapters 2.6 and 2.7

5. IMPLICATIONS OF THIS THESIS FOR HBV SURVEILLANCE

- The assessment of HBV infection prevalence in a population requires carrying out a seroepidemiological study, since acute and chronic HBV infections are often asymptomatic. To obtain representative estimates, migrant populations need to be oversampled. Assuming the prevalence among migrants equals that in the country of origin is not sufficiently valid.

- HBV incidence and trends in the most frequent routes of transmission among adults can be fairly reliably assessed using case based information only, provided cases are interviewed by trained public health nurses and reporting is adequate. Among children, where HBV infection is usually asymptomatic, seroprevalence studies are the only way to assess HBV infections.

- For about a third to a quarter of acute HBV infections, the route of transmission remains unknown. Trends in the number of cases over time with an unknown route of transmission are remarkably similar to those in the main transmission group (IDUs in the UK and MSM in the Netherlands). This suggests a considerable proportion of cases with an unknown route of transmission were acquired by IDU or were in MSM. This is corroborated by molecular typing of acute HBV cases in the Netherlands.

- Routine monitoring of the HBV vaccination coverage in target populations is adequate to monitor the effectiveness of HBV vaccination programmes, provided sufficiently precise estimates for the size of the target populations are available. For behavioural high risk groups, where this is not the case, additional surveillance methods including molecular epidemiology, phylodynamics and behavioural surveillance are required to assess the programme's effectiveness.

- Molecular epidemiology combines information on the genetic variability of pathogens with demographic and behavioural characteristics of its hosts to obtain insight into transmission patterns (55). The resolution of genetic information needed is determined by the epidemiological questions. In Chapter 2.4 we applied phylogenetic methods to HBV-DNA sequence data to study clustering of cases of acute HBV infection. For about a third to a quarter of acute HBV cases the route of acquisition of infection remains unclear, and molecular typing can help to obtain insight into which risk group these cases are likely to belong to. This analysis suggested that a considerable proportion of male cases with an unknown route of transmission were likely acquired by undisclosed male homosexual contact. This is important since it implies that vaccination of MSM may also be effective to lower the number of cases without a reported route of transmission. Usefulness of data can be further enhanced by applying phylodynamic methods. In contrast, routine typing of *chronic* HBV cases is of little use to study transmission patterns, since in the Netherlands people with chronic HBV infection are predominantly migrants who acquired the infection at birth prior to migration. Strains in this group therefore reflect global diversity of HBV rather than transmission patterns in the Netherlands.

- We enhanced the information obtained from molecular epidemiology by applying coalescence analysis, a phylodynamic method (Chapter 2.4). This considers molecular epidemiological data in the dimension of time, by taking into account the date of sampling of the virus and HBV's evolutionary rate. This results in insight into changes in genetic diversity over time, which can reflect intensity of transmission. The results of the coalescence analyses together with those following the traditional methods of analyses suggested the selective vaccination programme effectively reduced the incidence of HBV among MSM in the Netherlands. Coalescence based methods are likely to be of even more value in the context of infections or countries where surveillance is less optimal than is the case for HBV in the Netherlands.

- HBV genetic variants that can cause the virus to escape vaccine induced immunity, detection by regular diagnostics and antiviral treatment have been described (56). The prevalence of these variants is still low in the general population but needs careful monitoring.

- Now that treatment options for HBV are improving, HBV surveillance should include monitoring of delays in testing, treatment and care, to inform interventions to improve access to care.

- At a European level, standardisation of methods

for surveillance of acute HBV infection, including common case definitions, is a priority. The added value of molecular surveillance of acute HBV infection, as was demonstrated in the Netherlands, needs to be explored.

6. Implications of this Thesis for further research

Epidemiology, immunology, molecular epidemiology and phylodynamics

- The evidence that the Netherlands are a very low prevalence country for HBV is robust, repeated assessments of this will only be necessary every 10 years or so. The increased prevalence in Dutch residents born in midor highly endemic countries is also evident, and this information should feed into screening policy to allow early treatment to prevent liver disease. Less, but still convincing information is available regarding the increased risk of FGM and SGM for acquisition of HBV whilst resident in the Netherlands. However, routes of transmission and cost-effectiveness of catch-up vaccination for FGM and SGM need exploration.

- Regarding the prevention of perinatal transmission by antiviral treatment during pregnancy, current guidelines recommend an assessment of the indication for this only for HBeAg positive women. Information regarding the adherence to this guideline is necessary to allow potential improvements. Among the cases of perinatal transmission we documented, 2 out of 12 mothers were HBeAg negative. This may suggest that the current guideline of only referring HBeAg positive women for antiviral treatment is inadequate. Therefore, further evidence on the prevalence of a high viral load among HBeAg negative women is needed.

- We found an indication that the anti-HBs titer resulting from concomitant HBV and pneumococcal vaccination was lower than expected. Explanations and the clinical relevance of this finding need further documentation.

- In this Thesis we used both epidemiological and coalescence based methods to obtain insight into the intensity of HBV transmission in the Netherlands and England & Wales ([23]). These methods provide relative estimates of transmission intensity. Absolute estimates of the incidence would allow a comparison with results of standard surveillance, and could hence provide insight into important parameters such as the true (age-specific) rate of underreporting and asymptomatic infection. However, mathematical theory and tools to translate genetic diversity into incidence are lacking and need development.

- A key question to understand transmission of HBV and impact of e.g. early treatment on prevention is which proportion of new cases is caused by chronic versus acute infections. Methods used in this Thesis are inadequate to answer this. Phylodynamic methods that are likely to be able to contribute to answering this question are being developed (57).

- Coalescence based analyses were proven to be of large added value to understand infectious disease transmission patterns in the recent and distant past (58,59). Most relevant for public health policy, however, is the application of these methods to evaluate interventions. Apart from the work presented in Chapter 2.4 of this Thesis, based on methods developed earlier (60), this is rarely done. It is likely that there is much added value in resource poor settings where standard surveillance is problematic. Further research of assessing the methods' validity and robustness when applied to interventions for various infections and in different contexts is therefore needed

Methods and impact of screening, testing and early treatment

- In Chapter 2.1 we estimated that about 40,000 people in the Netherlands are living with chronic HBV infection, a relatively small number compared to the millions infected in e.g. Romania and Turkey[24]. Interventions to prevent severe liver disease and death in these people are available, particularly now that improved antiviral drugs are being developed. However, between 40%-80% of people with chronic hepatitis are unaware of their infection and can hence not access these (61,62). Research priorities to build the evidence for screening policies include:
  - HBV disease progression rates in natural history of infection and under treatment,
  - long-term effectiveness of HBV treatment to prevent progression,
  - methods to optimize participation in screening and referral,
  - methods to optimize the detection of HBV (and

23 Chapter 2.3 and Chapter 3.1

24 Chapter 4.1, Table 1a

HCV) in clinical samples to allow high throughput at low costs, e.g. nucleic acid detection in dried blood spots,
- cost-effectiveness and feasibility of combined screening for HBV, HCV and other infections/ diseases in high-risk populations,
- needs assessment of general population screening for HBV (and HCV) in countries with medium to high prevalence such as Romania and Turkey,
- the effect of testing and treatment on lowering the infectiousness of HBV carriers and reducing HBV transmission, and the development of dynamic HBV transmission models that can take such an effect into account.

Viral characteristics and co-infections

- The emergence of a clonal genotype A2 strain among cases of HBV acquired among adults in the Netherlands, UK, USA and Japan raises the question whether this is merely the consequence of seeding this strain in high-risk populations, or whether it reflects intrinsic advantages of this virus for transmission. The latter may include an increased capability to evade the immune system and cause chronic infection or a high infectiousness e.g. due to a relatively high viral load. Interestingly, co-infection with genotype G seems restricted to individuals infected with genotype A2 (63). If truly so, this also likely impacts on the natural history of infection and transmission. Cohort studies of high-risk populations such as the Amsterdam cohort studies coupled with studies following up acute HBV cases in low risk populations are needed to answer some of these questions.

- Co-infection of HBV with HCV, HAV and HIV is known to adversely affect the outcome of treatment and infection (64,65). However, prevalence of co-infections are rarely imported and need further documentation.

- Recent studies suggest quantitative HBsAg titer testing may be a valid surrogate to assess the viral load (66,67). The role of quantitative HBsAg assays to assess the indication for antiviral treatment, to monitor treatment effect, and to identify pregnant women at high risk of perinatal transmission needs further assessment.

- There are indications that the natural history of HBV infection and viral load are genotype dependent (41,68). In contrast, a recent Danish study suggests this is not related to differences in viral load by genotype (69). Further work is needed to understand the virological and epidemiological differences between HBV genotypes and genosubtypes.

7. Implications of this Thesis for the prevention and control of HBV infection in the Netherlands, England & Wales and the European neighbourhood

- We demonstrated the effectiveness of the selective HBV vaccination programme to control HBV infection among MSM in the Netherlands. Since universal HBV vaccination was implemented only for infants in 2011, the programme coupled with sensitive surveillance, will need to continue for the foreseeable future.

- Outreach vaccination and strong programmatic coordination are likely explanations for the success of the Dutch HBV vaccination programme for behavioural high-risk groups. Other countries who fail to curb HBV transmission in high-risk populations, such as the UK, USA and Japan, are recommended to consider adopting a similar approach.

- The effectiveness of the Dutch programme to prevent perinatal HBV transmission may be enhanced when pregnant women with high viral loads are treated with antivirals, as is currently recommended by Dutch guidelines. It may be of particular importance for women of Chinese origin, given the increased risk in this group for perinatal transmission. Monitoring of the implementation of the guidelines, the safety and effectiveness is urgently needed.

- Now that treatment options for HBV are improving it is a public health priority to avoid delays in testing, treatment and care, and to ensure equity in access to these.

- There is robust evidence of an increased risk of HBV infection among children of migrants in low-endemic countries for HBV. Targeting these children for HBV vaccination is feasible and acceptable. Countries in the European neighbourhood that have not implemented universal infant HBV vaccination programmes[25] should implement selective HBV vaccination programmes for children of migrants from endemic countries.

25 Including Denmark, Hungary, Iceland, Norway, Sweden, Switzerland and the UK

- There is clear evidence that antenatal HBsAg screening is cost-effective. Countries in Europe that have selective antenatal HBV screening programmes should implement universal antenatal screening. A birth-dose of vaccine in the national vaccination programme is not an argument against this, since the coverage of national programmes is usually too low for optimal prevention of perinatal transmission, and it does not include HBIg. The evidence that antiviral treatment during prevalence can reduce the risk of perinatal transmission further supports the need for universal antenatal HBV screening.

- Screening migrants from endemic countries for chronic HBV infection is likely to be cost-effective. Policies and models of best practice need to be developed for this.

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Summary

This Thesis resulted from a decade of studying the epidemiology of hepatitis B virus (HBV) infection in the Netherlands, England & Wales and at a European level, starting in 2003. The studies mainly aimed to improve the knowledge of the local epidemiology of HBV and to assess the effectiveness of HBV vaccination programmes, in order to inform public health policy for prevention and control of HBV and its associated burden of disease and death in the Netherlands and elsewhere.

Chapter 1.1 includes a general overview of the main virological, clinical and epidemiological characteristics of HBV and the methods for its primary and secondary prevention. HBV is a DNA virus that causes hepatitis and can be transmitted between humans. HBV infection can resolve or result in chronic infection. People developing chronic HBV infection are at high risk of cirrhosis and hepatocellular carcinoma (HCC), which is often fatal. There are up to 200-fold differences in the risk of HBV infection in the general population between countries and regions. HBV infection can be prevented by hygienic precautions and safe sexual practices, active immunisation with HBV vaccine and in some situations by passive immunisation using hepatitis B immunoglobulin (HBIg). Treatment of chronic HBV infection can reduce the risk of cirrhosis, HCC and death. Secondary prevention is possible by early detection and treatment of chronic infection. This may contribute to primary prevention by reducing the infectiousness of infected individuals. HBV vaccination programmes in the Netherlands have up to 2011 only been targeted at selected groups in the populations at increased risk, such as infants with one or two parents born in an endemic country, infants of mothers with chronic HBV infection and behavioural high-risk groups including e.g. men who have sex with men (MSM). In addition, from August 2011 onwards, all infants are offered HBV vaccine within the Dutch National Immunisation Programme. Chapter 1 finishes by outlining that surveillance is the main tool to design and evaluate public health programmes for HBV prevention and control.

Chapter 2 focuses on the Netherlands, starting with a study of the prevalence in the general Dutch population in 1996 and 2007, and risk factors for infection in 2007 (Chapter 2.1). This study made use of serum and questionnaire data collected in two national seroprevalence studies (the Pienter studies), in which about 7,200 and 6,200 randomly selected Dutch residents participated. In 2007, the weighted anti-HBc prevalence was 3.5% (95% CI 2.2–5.5) and the HBsAg prevalence was 0.2% (95% CI 0.1–0.4). In indigenous Dutch participants, the anti-HBc prevalence was lower in 2007 than in 1996 (p=0.06). First-generation adult migrants (FGMs) had a 13-fold greater risk of being HBsAgand/or HBV-DNA-positive than indigenous Dutch participants. In indigenous Dutch participants, risk factors for anti-HBc positivity were older age and having received a blood product before 1990. In FGMs, being of Asian origin was a risk factor. In second-generation migrants, having a foreign-born partner and injecting drug use were risk factors. FGMs are the main target group for secondary HBV prevention in the Netherlands.

Chapter 2.2 focuses on routes of transmission of HBV in the Netherlands. These were studied using information from 120 notified cases of acute HBV infection reported in 1999-2000 and nearly 4,000 population sampled 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.

The following Chapters in this section (2.3-2.8) all focus on assessing the effectiveness of HBV vaccination programmes. *Chapter 2.3* deals with the HBV vaccination programme for behavioural high-risk groups. This commenced in 2002, targeting men

having sex with men (MSM), drug users, commercial sex workers and heterosexuals who change partners frequently. The latter group was excluded from the programme in 2007. We studied all 1687 routinely notified cases of acute HBV infection reported between 2004 and 2010 using molecular epidemiology and phylodynamic methods. The incidence of reported acute HBV infection decreased from 1.8 to 1.2 per 100,000 inhabitants between 2004 and 2010, mostly due to a reduction in the number of cases in MSM. Men were overrepresented among cases with an unknown route of transmission, especially among genotype A2 cases mainly associated with transmission through male homosexual contact. The genetic diversity of non-imported genotype-A strains obtained from MSM decreased from 2006 onwards, suggesting the HBV incidence among MSM decreased. We concluded that the selective HBV-vaccination programme for behavioural high-risk groups reduced the incidence of acute HBV infection in the Netherlands, predominantly by preventing HBV infections in MSM. Effectiveness of such a programme on a national level was never demonstrated before. Targeting high-risk MSM was probably essential to the programme's success. A considerable proportion of cases in men who did not report risk behaviour was probably acquired through male homosexual contact. Our findings support continuation of the programme in the Netherlands and adopting a similar approach in other countries where HBV transmission is focused in high-risk adult groups.

Chapter 2.4 includes an assessment of the effectiveness of the vaccination programme for children of mothers with chronic HBV infection. In the Netherlands, different hepatitis B vaccination schedules have been used for these children. All schedules included a birth dose of hepatitis B immunoglobulin (HBIg). However, a birth dose of HBV vaccine was only introduced in the national programme in 2006 while in Amsterdam a birth dose was given since the programme's start. This allowed an assessment of the added value of a birth dose of HBV vaccine. We studied infants born to HBV infected mothers between 1.1.2003 and 30.6.2007, using national databases and a separate database for Amsterdam. Of 2,657 infants registered in the national database, 91% were registered to have received HBIG and at least three hepatitis B vaccinations. In Amsterdam, this coverage among 413 children at risk was higher (96%, $p < 0.01$). Serological test results for 2121 infants (80%) indicated that 13 (0.6%) were HBsAg positive. A mother of Chinese descent was the only risk factor for perinatal HBV infection identified (RR 9.1, 95% CI 3.1–26.8). Among infants who received HBIg at birth, we did not detect an increased risk of perinatal HBV infection when the first dose of hepatitis B vaccine was administered after the first week of life. A shorter period between last vaccination and testing, and having received more doses of hepatitis B vaccine were independently associated with a higher anti-HBs titer. We concluded that infants born to Chinese mothers are at increased risk of perinatal HBV infection. The viral load of all HBsAg positive pregnant women of Chinese origin should therefore be assessed to determine whether there is an indication for anti-viral treatment during pregnancy.

In *Chapter 2.5* we studied the same group of children born to HBsAg positive mothers, using national data that did not include Amsterdam. In addition to the children with break-through infection, three older children were found with high levels of anti-HBc, anti-HBs and anti-HBe whilst being HBsAg and HBV DNA negative, indicating resolved HBV infection. In the group of older children (1.5–5 years of age, n = 728), about half of the HBV-infected children (3 of 7) had already cleared their infection at the time of sampling. Two of the 12 HBV-infected children (17%) had a high level of anti-HBs. An evaluation of the efficacy of a new intervention programme to prevent vertical HBV transmission should analyse HBV markers in serum collected when the children are older than 1.5 years. Testing of children born to HBV-infected mothers should include an HBsAg test, since a protective level of anti-HBs does not exclude chronic infection.

The last Chapters in this section (2.6-2.8) focus on the HBV vaccination programme for children of migrants, which was in operation between 2003 and 2011. In *Chapter 2.6* we studied the effectiveness and impact of this programme by assessing the HBV vaccine coverage and HBV notifications in the target population. The HBV incidence was determined in children aged 0-4 years and born after (2003-7) and before (1990-2002) the introduction of the HBV vaccination programme. The HBV vaccine coverage in the target population was 89.6% (96,186/107,338) in the period 2003-5. There were 37 notified acute infections in the pre-vaccination birth cohort 1990-2002 (incidence $2.9/10^6$ person-years), compared with one in the post-vaccination birth cohort 2003-7 (incidence $0.3/10^6$ person-years). The incidence rate ratio for the 2003-7 birth cohort compared with the 1990-2002 birth cohort was 0.12 (95% CI 0.02

to 0.87; p=0.04). We concluded that the incidence of HBV notifications in children born after the introduction of targeted childhood HBV vaccinations was lower than the incidence in children born before the start of this vaccination programme. Although this is consistent with the adequate HBV vaccine coverage found, the interpretation is hampered by a change in case definition for notification in 1999. The results are of importance to policy makers in both The Netherlands and other countries that have a targeted HBV vaccination programme for infants of migrants from endemic countries.

Subsequently, we assessed in *Chapter 2.7* the acceptability of this programme by conducting a questionnaire survey among parents of children in the HBV target population (130 participants) and children not targeted for HBV vaccination (69 participants). Only 13 of 83 parents (16%) within the HBV-eligible group whose child was registered as 'incompletely vaccinated' for HBV reported that they refused a vaccine for their child. Risk factors for HBV refusal were a low risk perception of HBV, a high socioeconomic status and one parent born in The Netherlands. Within the non-eligible group, we found that 9% (95% CI: 3–22%) of the parents whose child was fully vaccinated with DPT-IPV-Hib had a negative attitude towards universal HBV vaccination.

Finally, in *Chapter 2.8*, we assessed the immunogenicity of the hexavalent vaccine, including HBV vaccine, used in the programme for children of migrants between 2006-2011 and currently offered to all Dutch neonates. This vaccine is administered, concomitantly with pneumococcal vaccine (Prevenar) in a 4-dose schedule at 2, 3, 4 and 11 months of age. We assessed the immunogenicity of the HBV component of Infanrix hexa co-administered with Prevenar, and compared pertussis and Hib components in Infanrix hexa with the standard Infanrix-IPV + Hib vaccine. Target thresholds for immune responses were achieved for all antigens studied. Over 99% (163/164) of children vaccinated with Infanrix hexa achieved an adequate immune response (≥10 mIU/ml) to the HBV component and peak anti-HBs geometric mean concentration (GMC) was 2,264 mIU/ml (95% CI: 1,850–2,771 mIU/ml). This anti-HBs GMC was about 3 times lower than has been reported following Infanrix hexa vaccination without concomitant other vaccines. The clinical relevance of this remains uncertain but is probably limited. The GMC of a pertussis component, filamentous hemagglutinin (FHA), of Infanrix-hexa was significantly lower in children vaccinated with Infanrix hexa and Prevenar than in children vaccinated with Infanrix-IPV + Hib. Despite very high rates of seroconversion for the HBV component of Infanrix hexa, its long-term immunogenicity and effectiveness should be monitored after concomitant vaccination.

Chapter 3 includes studies carried out in England & Wales in the first years of the new millennium 2000. In *Chapter 3.1* we analysed routine laboratory surveillance data of acute HBV infection in England & Wales between 1995–2000 with data on migration and global HBsAg prevalence. The estimated annual incidence of HBV infection in England & Wales was 7.4 per 100,000 (adjusted for underreporting and asymptomatic infections). Injecting drug use was the most frequently reported route of transmission. The number of cases attributed to heterosexual contact was fairly stable, whereas the number of cases in men having sex with men decreased. These observations continue trends reported for the early 1990s. Transmission during childhood was rarely reported, but was more frequent among South Asians (UK residents descendent from countries including India, Pakistan and Bangladesh). The incidence in South Asians was relatively high, and their main risk factors were medical treatment overseas and heterosexual contact. For about a third of cases of acute HBV infection no route of transmission is reported, but analysis of secular trends and age distribution suggest that many of these may be related to injecting drug use. Endemic transmission gives rise to only a small proportion of all new chronic infections, with the vast majority arising from immigration of established HBV carriers. We concluded that the incidence of acute HBV infection in England and Wales remained low, with a similar pattern of reported routes of transmission compared to the early 1990s. The UK prevalence of HBV infection is dependant on global rather than national immunisation policy. We recommended improving immunisation coverage among injecting drug users, which is expected to also reduce the number of cases without a risk factor reported. In addition, immunisation strategies that better suit the needs of ethnic minorities need to be explored.

In *Chapter 3.2* we focused on the South Asian subpopulation in England & Wales by using acute HBV infections and associated risk factors reported by laboratories in England and Wales between 1988 and 2000. We used "Nam Pehchan" software to assign South Asian ethnicity using names of cases that were held temporarily on the laboratory surveillance database. We found that the incidence of acute HBV infection is higher among South Asian than among

non-South Asian UK residents, and infections in South Asians occur more often during childhood. We recommended that the UK's immunisation policy should better protect ethnic minority children against HBV infection. To date, this recommendation has unfortunately not been followed.

In *Chapter 4* we report the findings of a systematic literature review into the prevalence of HBV and hepatitis C virus infection in 34 countries in the European neighbourhood and five population subgroups, and the cost-effectiveness of screening for chronic HBV and HCV infections, in order to inform screening policies. Of 1759 and 384 papers found in the prevalence and cost-effectiveness search respectively, we included 124 and 22 papers after checking the paper's quality. We used decision rules to calculate weighted prevalence estimates by country. The HBsAg and anti-HCV-Ab prevalence in the general population ranged from 0.1%-5.6% and 0.4%-5.2% respectively, by country. For IDUs, men who have sex with men and migrants, the prevalence of HBsAg and anti-HCV-Ab was higher than the prevalence in the general population in all but 3 countries. There is evidence that HCV screening of IDUs and HBsAg screening of pregnant women is cost-effective. HBsAg screening of migrants is likely to be cost-effective. We concluded that the prevalence of chronic HBV and HCV infection varies widely between European countries. Anti-HCV-Ab screening of IDUs and HBsAg screening of pregnant women are European public health priorities. HBsAg screening of migrants is likely to be cost-effective. Cost-effectiveness analyses may need to take effect of antiviral treatment on preventing HBV and HCV transmission into account.

In *Chapter 5*, the general discussion, I discuss what this Thesis adds to what was already known (summarised in Table 1 below), its implications for HBV surveillance and research (Table 2), and recommendations for prevention and control of HBV (Table 3).

Table 1: What this Thesis adds to what was already known about HBV

HBV epidemiology in the Netherlands

- About 40,000 people are living with chronic HBV infection in the Netherlands.
- The prevalence of HBV infection in the general Dutch population did not increase between 1996 and 2007; The Netherlands continue to be among countries with the lowest HBV prevalence in the world.
- Among indigenous Dutch, the prevalence of HBV infection decreased between 1996 and 2007. Having had a blood transfusion in the past was a risk factor for infection.
- Increases in migration to the Netherlands have had no impact on the HBV prevalence in the indigenous Dutch.
- First generation migrants (FGM) had a 10-times higher prevalence of chronic infection than indigenous Dutch. The vast majority of these infections were most likely acquired prior to migration.
- Second generation migrants (SGM) were also at a somewhat increased risk of HBV infection compared to indigenous Dutch. Among adult SGM, this is mainly caused by having a foreign born sexual partner. Among SGM children, travel to endemic regions explains part of the increased risk.
- Among Dutch residents male homosexual contact is the most important risk for acquisition of HBV infection, with those having had 3 or more partners in the past 6 months at increased risk. Among heterosexuals having had 2 sexual partners in the past 6 months and having a foreign born partner were independent risk factors.
- Injecting drug use is no longer an important route of transmission for HBV in the Netherlands, in contrast to other countries including England & Wales.
- Chinese women with chronic HBV infection are more likely to perinatally transmit the virus to their infants than other chronically HBV infected women.

Vaccination programmes

- The programme for behavioural high-risk groups has effectively reduced the incidence of HBV in the Netherlands by preventing HBV infection among men who have sex with men (MSM).

- The HBIg and vaccine coverage among infants born to mothers with chronic HBV infection is high (>95%). Among infants who received HBIg at birth, the proportion of infants acquiring HBV infection despite vaccination did not differ between those having received the schedule without and with a birth dose (0.8% and 0.7%, respectively).
- Among HBsAg positive pregnant women the risk of perinatal transmission to their infant was higher for women of Chinese descent compared to other women. Antiviral treatment during pregnancy can reduce this risk, and may also be indicated for some HBeAg negative women.
- After the HBV vaccination programme for infants of migrants was implemented in 2003, the incidence of acute HBV notifications in young children decreased significantly. Due to changes in surveillance case definitions we were unable to conclude that this reduction was a result of the vaccination programme. The programme was acceptable to parents of children in the target population.
- Over 99% of children vaccinated with Infanrix hexa concomitantly with Prevenar developed an adequate immune response against HBV (anti-HBs ≥ 10IU/L). However, the geometric mean concentration of anti-HBs antibodies was somewhat lower than expected.

HBV epidemiology in England & Wales

- The incidence of HBV in England & Wales in the late 1990s up to 2000 remained at the level reported for the early 1990s. IDU remained the most frequent route of transmission.
- Over half of new infections could have been prevented had the UK's current selective vaccination programme been implemented properly.
- The South Asian ethnic minority in England & Wales is at increased risk of acquisition of HBV infection.

HBV epidemiology in Europe

- Countries in the South and West of the EU, and Turkey, have much higher HBV and HCV prevalences than countries in the North and West.
- Now that treatment options for chronic HBV and HCV infection have improved, policies for secondary prevention, including screening and access to treatment, are a priority.
- There is robust evidence that HBsAg screening of pregnant women is cost-effective. HBV screening of migrants is probably cost-effective, depending on factors including their HBsAg prevalence.

Table 2. Implications for HBV surveillance and research

HBV surveillance

- Assuming the HBV prevalence among migrants equals that in their country of origin is not sufficiently valid.
- HBV incidence and trends in the most frequent routes of transmission among adults can be assessed using case-based information only. Among children, seroprevalence studies are needed.
- Routine monitoring of HBV vaccination coverage needs to be complemented by additional surveillance methods for those target groups where the population size is uncertain.
- Molecular typing of acute HBV is of added value to provide insight in routes of transmission and incidence. Information obtained can be enhanced by applying phylodynamic methods.
- Monitoring of HBV genetic variants that escape vaccine induced immunity, detection by regular diagnostics and antiviral treatment is necessary to timely detect increases in the occurrence of these.
- HBV surveillance should include monitoring of delays in testing, treatment and care, to inform interventions to improve access to care.
- At a European level, standardisation of methods for surveillance of acute HBV infection is a priority. The added value of molecular surveillance of acute HBV infection needs exploration.

Epidemiology, immunology, molecular epidemiology and phylodynamics

- Regarding HBV infection in FGM and SGM, further research is needed into the routes of transmission and cost-effectiveness of catch-up vaccination.
- Regarding prevention of perinatal transmission, it is a priority to study whether antiviral treatment is given when needed. To assess whether current referral guidelines are adequate, the prevalence of a high viral load among HBeAg negative women needs documentation.
- The anti-HBs titer after concomitant HBV and pneumococcal vaccination was lower than expected. Explanations and the potential clinical relevance of this need further documentation.
- Mathematical and phylodynamic theory and tools to translate genetic diversity into incidence, and to attribute new infection to either acute or chronic infections, need development.
- Further research of assessing the validity and robustness of coalescence based methods when applied to interventions regarding various infections and contexts is needed.

Methods and impact of screening, testing and early treatment

- The evidence for screening of chronic HBV needs to be strengthened. Priorities include assessing progression rates with and without treatment, methods to optimise participation to screening, efficient testing, referral and treatment, cost-effectiveness of combining screening for HBV with screening for other infections such as HCV and HIV, needs assessment for general population HBV screening in mid-and high prevalence countries in Europe such as Romania, and the effect of testing and treatment on preventing new infections.

Viral characteristics and co-infections

- Intrinsic characteristics regarding natural history of disease and transmission parameters associated with HBV genotypes and clones need clarification.
- HBV genetic variants and co-infection of HBV, HCV and HIV need further monitoring.

Viral diagnostics

- The role of quantitative HBsAg testing needs further exploration.

Table 3. Recommendations for prevention and control of HBV infection in the Netherlands and beyond

- The selective HBV vaccination programme for behavioural high risk groups needs to be continued in the Netherlands for the coming decades.
- Other countries which fail to curb HBV transmission in high-risk populations, such as the UK, USA and Japan, are recommended to consider adapting a similar approach.
- The effectiveness of the Dutch programme to prevent perinatal HBV transmission may be enhanced when pregnant women with high viral loads receive antiviral treatment. Monitoring of the implementation of this, especially among women of Chinese descent, is urgently needed.
- Now that treatment options for chronic HBV infection are improving it is a public health priority to ensure optimal access to care by e.g. avoiding delays in testing, referral and treatment.
- European countries that have not implemented universal infant HBV vaccination should consider targeted HBV vaccination for infants of migrants.
- There is robust evidence that antenatal HBsAg screening is cost-effective. All European countries should therefore implement this using best practice models.
- Screening of migrants from endemic countries for chronic HBV infection is likely to be cost-effective. Policies and models of best practice need to be developed for this.

SAMENVATTING

Onderhavig proefschrift bevat de resultaten van epidemiologisch onderzoek naar hepatitis B-virusinfectie (HBV-infectie) in Nederland, Engeland en Wales en op Europees niveau dat werd uitgevoerd tussen 2003 en 2012. Dit onderzoek was gericht op het vergroten van de kennis van de lokale epidemiologie van HBV-infectie en op het beoordelen van de effectiviteit van HBV-vaccinatie programma's. Deze informatie is van belang voor het volksgezondheidsbeleid ten aanzien van HBV-infectie, hetgeen is gericht op het verminderen van de ziektelast en sterfte door HBV-infectie in Nederland en elders.

In *Hoofdstuk 1* worden ter introductie algemene virologische, klinische en epidemiologische kenmerken van het HBV en de methoden voor de primaire en secundaire preventie beschreven. Het HBV is een DNA-virus dat hepatitis veroorzaakt en overdraagbaar is van mens tot mens. Een HBV-infectie kan genezen, maar ook leiden tot een chronische infectie. Een chronische HBV-infectie kan leiden tot ernstige leverziekten, zoals cirrose en hepatocellulair carcinoom (HCC). Deze aandoeningen zijn vaak fataal.
Het deel van de algemene bevolking dat ooit met het HBV is geïnfecteerd verschilt enorm, tot wel 200 maal, tussen landen en regio's in de wereld. HBV-infectie kan worden voorkómen door hygiënische voorzorgsmaatregelen wanneer mensen in aanraking komen met bloed en lichaamsvloeistoffen, en door veilig seksueel gedrag. Daarnaast is preventie van infectie mogelijk door actieve immunisatie met een HBV-vaccin en door passieve immunisatie met hepatitis-B-immunoglobuline (HBIg). Behandeling van chronische HBV-infectie vermindert het risico op cirrose, HCC en overlijden. Secundaire preventie is daarom mogelijk door vroege opsporing en behandeling van chronische HBV-infectie. Dit draagt hoogstwaarschijnlijk ook bij aan primaire preventie van HBV-infectie door vermindering van de besmettelijkheid van geïnfecteerde individuen die behandeld worden.
HBV-vaccinatie-programma's in Nederland waren tot 2011 alleen gericht op bepaalde groepen in de bevolking met een verhoogd risico op infectie, zoals kinderen van wie één of beide ouders geboren zijn in een HBV-endemisch land, zuigelingen van moeders met een chronische HBV-infectie en personen die door hun gedrag hoger risico lopen op HBV-infectie, waaronder bijvoorbeeld mannen die seks hebben met mannen. Sinds augustus 2011 wordt aan alle zuigelingen HBV-vaccin aangeboden binnen het Nederlandse Rijksvaccinatieprogramma. Hoofdstuk 1 besluit met een onderbouwing van het belang van surveillance voor het ontwerpen en evalueren van volksgezondheidprogramma's voor HBV-preventie en -bestrijding.

Hoofdstuk 2 richt zich op Nederland, te beginnen met een studie van de prevalentie in de algemene Nederlandse bevolking in 1996 en 2007, en risicofactoren voor infectie in 2007 (*Hoofdstuk 2.1*). Deze studie maakte gebruik van serum en vragenlijsten die verzameld werden in twee nationale seroprevalentiestudies (de Pienter studies) onder ruim 6000 willekeurig geselecteerde Nederlanders. In 2007 was de gewogen anti-HBc prevalentie 3.5% (95% betrouwbaarheidsinterval (BI) 2.2-5.5); de HBsAg prevalentie was 0.2% (95% BI 0.1-0.4). Onder autochtone Nederlanders was de anti-HBc prevalentie in 2007 lager dan in 1996 ($p = 0.06$). Eerste generatie volwassen migranten (EGM) hadden een 13-maal hoger risico op HBsAg- en/of HBV-DNA-positiviteit dan autochtone Nederlanders. Onder autochtone Nederlanders waren hogere leeftijd en ontvangst van een bloedproduct voor 1990 risicofactoren voor anti-HBc positiviteit. Onder EGM was een Aziatische afkomst een risicofactor. Onder tweede generatie migranten waren het hebben van een in het buitenland geboren partner en injecterend drugsgebruik risicofactoren. EGM zijn de belangrijkste doelgroep voor de secundaire preventie van HBV in Nederland.
Hoofdstuk 2.2 richt zich op de transmissieroutes van het HBV in Nederland. Deze zijn onderzocht met behulp van gegevens van 120 gemelde gevallen van acute HBV-infectie gerapporteerd in 1999-2000. Deze werden vergeleken met gegevens van bijna

4000 personen uit de algemene bevolking (controles). Het risico op acute HBV-infectie was verhoogd bij mannen die seks hebben met mannen (MSM), waarbij diegenen met drie of meer partners in de afgelopen 6 maanden een hoger risico hadden dan MSM met minder partners. Bij kinderen, volwassen vrouwen en heteroseksuele mannen was het hebben van ouders geboren in een hepatitis B-endemisch land een significante risicofactor. Voor volwassen vrouwen en heteroseksuele mannen werd dit risico grotendeels verklaard doordat deze groep vaker een buitenlandse partner heeft. Voor kinderen werd het risico deels verklaard door parenterale blootstelling in het buitenland.

De volgende *Hoofdstukken 2.3 tot en met 2.8* beschrijven studies naar de effectiviteit van HBV-vaccinatie-programma's in Nederland. *Hoofdstuk 2.3* gaat over het HBV-vaccinatie programma voor gedragsgebonden hoog-risico groepen. Dit programma werd landelijk geïmplementeerd in 2002, en is gericht op MSM, druggebruikers, prostituees en heteroseksuelen met wisselende partners. Deze laatste groep werd uitgesloten van het programma in 2007. We bestudeerden alle 1687 gevallen van acute HBV-infectie die gemeld werden in het kader van de infectieziektewet tussen 2004 en 2010, met moleculair epidemiologische en fylodynamische methoden. De incidentie van acute HBV-infectie daalde van 1.8 naar 1.2 per 100,000 inwoners tussen 2004 en 2010, voornamelijk door een daling in het aantal gevallen bij MSM. Mannen waren oververtegenwoordigd bij de groep gevallen met een onbekende route van overdracht, vooral wanneer dit genotype A2 gevallen betrof. Dit genotype is geassocieerd met transmissie via mannelijke homoseksuele contacten. De genetische diversiteit van niet-geïmporteerde genotype-A-stammen vant MSM daalde vanaf 2006, hetgeen suggereert dat de HBV-incidentie onder MSM is afgenomen. We concludeerden dat het selectieve HBV-vaccinatie programma voor gedragsgebonden risicogroepen de incidentie van acute HBV-infectie in Nederland verminderde, voornamelijk door de preventie van HBV-infecties bij MSM. Effectiviteit van een dergelijk programma op nationaal niveau was nooit eerder aangetoond. Het vaccineren van met name hoog risico MSM was waarschijnlijk essentieel voor het succes van het programma. Een aanzienlijk deel van de gevallen bij mannen die geen risicogedrag rapporteerden werd waarschijnlijk opgelopen door mannelijk homoseksueel contact. Onze bevindingen ondersteunen de voortzetting van het HBV-vaccinatieprogramma voor gedragsgebonden risicogroepen in Nederland, en pleiten voor een soortgelijke aanpak in andere landen waar HBV transmissie voornamelijk plaatsvindt onder volwassenen met risicogedrag.

Hoofdstuk 2.4 bevat een beoordeling van de effectiviteit van de vaccinatie voor kinderen van moeders met een chronische HBV-infectie. In Nederland zijn verschillende HBV-vaccinatie schema's gebruikt voor deze kinderen. In alle schema's werd een dosis HBV-immunoglobuline (HBIg) direct na de geboorte aangeraden. Ten aanzien van HBV-vaccinatie werd pas in 2006 landelijk een dosis vaccin aangeraden direct na de geboorte; in Amsterdam werd deze geboorte dosis vaccin gegeven sinds het begin van het programma. Deze situatie gaf de mogelijkheid de effectiviteit van een geboorte dosis HBV-vaccin te onderzoeken. We bestudeerden zuigelingen van HBV-geïnfecteerde moeders tussen januari 2003 en juli 2007 met behulp van nationale databases en een aparte database voor Amsterdam. Van 2657 kinderen in de onderzoekspopulatie kreeg 91% HBIG en ten minste drie HBV-vaccinaties. In Amsterdam was deze vaccinatiegraad hoger (96%, p <0.01). Serologische resultaten voor 2121 kinderen (80%) gaf aan dat 13 kinderen (0.6%) een perinatale HBV–infectie hadden opgelopen (HBsAg positiviteit). Een moeder van Chinese afkomst was de enige risicofactor voor perinatale HBV-infectie die werd vastgesteld (RR 9.1, 95% BI 3.1-26.8). Onder zuigelingen die HBIg kregen werd geen verhoogd risico op perinatale HBV-infectie gevonden wanneer de eerste dosis HBV-vaccin werd toegediend na de eerste week van het leven. Een kortere periode tussen de laatste vaccinatie en de serologische test en het hebben ontvangen van meer doses van het HBV-vaccin waren onafhankelijk geassocieerd met een betere bescherming (hogere anti-HBs titer). We concludeerden dat zuigelingen van HBV-geïnfecteerde Chinese moeders een hoger risico op perinatale HBV-infectie lopen dan zuigelingen van andere HBsAg positieve zwangeren. Alle zwangeren van Chinese afkomst zouden beoordeeld moeten worden ten aanzien van de indicatie voor anti-virale behandeling tijdens de zwangerschap.

In *Hoofdstuk 2.5* werd dezelfde groep van kinderen van HBsAg-positieve moeders bestudeerd op basis van landelijke gegevens met uitzondering van Amsterdam. Naast de kinderen met doorbraak infectie werden 3 oudere kinderen gevonden met hoge anti-HBc, anti-HBs en anti-HBe titers terwijl ze HBsAg en HBV DNA negatief waren, hetgeen wijst op een genezen HBV-infectie. In de groep van ou-

dere kinderen (1.5-5 jaar, n=728) hadden 3 van de 7 HBV-geïnfecteerde kinderen hun infectie op het moment van de bemonstering geklaard. Van de 12 HBV-geïnfecteerde kinderen hadden 2 (17%) een hoge anti-HBs titer. Evaluaties van de effectiviteit van interventies om perinatale HBV-transmissie te voorkomen zouden serologische gegevens moeten gebruiken van kinderen die ouder zijn dan 1,5 jaar. Om perinatale infectie uit te sluiten is een HBsAg test noodzakelijk; een beschermende anti-HBs titer sluit namenlijk chronische infectie niet uit.

De laatste hoofdstukken in dit deel (2.6-2.8) richten zich op de HBV-vaccinatie voor kinderen van migranten, hetgeen tussen 2003 en 2011 een onderdeel was van het Nederlandse Rijksvaccinatieprogramma. In *Hoofdstuk 2.6* onderzochten we de effectiviteit en impact van dit programma door zowel de HBV-vaccinatiegraad als de meldingen van HBV-infecties in de doelgroep te bestuderen. De HBV-incidentie onder kinderen van 0-4 jaar werd vergeleken tussen de groepen geboren vóór en na invoering van het HBV-vaccinatieprogramma (respectievelijk 1990-2002 en 2003-2007). De HBV-vaccinatiegraad in de doelpopulatie was 89,6% (96.186/107.338) in de periode 2003-2005. Er waren 37 geregistreerde acute infecties in het pre-vaccinatie geboortecohort 1990-2002 (incidentie 2.9/10^6 persoonsjaren), vergeleken met slechts één in het post-vaccinatie geboortecohort 2003-2007 (incidentie 0,3/10^6 persoonsjaren). De incidentie ratio voor het 2003-2007 geboortecohort in vergelijking met het 1990-2002 geboortecohort was 0.12 (95% BI 0.02-0.87; p=0.04). We concludeerden dat de incidentie van HBV-meldingen bij kinderen die geboren zijn na de invoering van het selectieve vaccinatieprogramma lager was dan de incidentie bij kinderen geboren vóór de start van dit vaccinatieprogramma. Dit is in lijn met de hoge HBV-vaccinatiegraad die gevonden werd. Het interpreteren van de resultaten werd echter bemoeilijkt doordat het meldcriterium voor HBV-infectie in 1999 gewijzigd werd. De resultaten zijn van belang voor beleidsmakers in zowel Nederland als andere landen met een selectief HBV-vaccinatieprogramma voor kinderen van migranten uit endemische landen.

Vervolgens hebben we in *Hoofdstuk 2.7* de aanvaardbaarheid van het HBV-vaccinatieprogramma voor kinderen van migranten onderzocht door het uitvoeren van een vragenlijstonderzoek onder ouders van kinderen in de doelpopulatie voor HBV-vaccinatie (130 deelnemers) en andere kinderen (69 deelnemers). Slechts 13 van 83 ouders (16%) binnen de HBV-vaccinatie doelgroep wiens kind werd geregistreerd als 'onvolledig gevaccineerd' voor HBV meldden dat zij een vaccin voor hun kind hadden geweigerd. Risicofactoren voor HBV-weigering waren een lage risicoperceptie van HBV, een hoge sociaal-economische status en één ouder geboren in Nederland. Binnen de groep die niet in aanmerking kwam voor HBV-vaccinatie vonden we dat 9% (95% BI: 3-22%) van de ouders wier kind wel volledig gevaccineerd was met DPT-IPV-Hib een negatieve houding ten opzichte van universele HBV-vaccinatie had.

Tenslotte wordt in *Hoofdstuk 2.8* de immunogeniciteit van het hexavalente vaccin (Infanrix hexa), dat ook HBV-vaccin bevat, beoordeeld. Dit vaccin werd gebruikt in het HBV-vaccinatieprogramma voor kinderen van migranten tussen 2006 en 2011 en wordt momenteel aangeboden aan alle Nederlandse pasgeborenen. Dit vaccin wordt in een 4-dosisschema op de leeftijd van 2, 3, 4 en 11 maanden toegediend, gelijktijdig met pneumokokkenvaccin (Prevenar). Wij hebben de immunogeniciteit van de HBV-component van het hexavalente vaccin, gelijktijdig toegediend met Prevenar, beoordeeld. Verder vergeleken we de immunogeniciteit van de kinkhoest en *Haemofilus Influenzae* b (Hib) componenten tussen het hexavalente vaccin en het pentavalente vaccin (Infanrix-IPV en Hib-vaccin). Voor alle onderzochte onderdelen van het vaccin werd een adequate immuunrespons bereikt. Meer dan 99% (163/164) van de kinderen die gevaccineerd werden met Infanrix hexa behaalde een adequate HBV-immuunrespons (anti-HBs titer ≥ 10 mIU/ml). De anti-HBs geometrisch gemiddelde concentratie (GMC) was 2264 mIU/ml (95% BI : 1850-2771 mIU / ml). Dit is ongeveer drie maal lager dan gerapporteerd is voor Infanrix hexa vaccinatie zonder gelijktijdige toediening van andere vaccins. De klinische relevantie van deze bevinding is onzeker, maar waarschijnlijk beperkt. De GMC van één pertussis component (filamenteuze hemagglutinine (FHA)) was lager bij kinderen gevaccineerd met Infanrix hexa en Prevenar dan bij kinderen gevaccineerd met Infanrix-IPV en Hib. Wij concludeerden dat de immunogeniciteit van de HBV-component van Infanrix hexa adequaat is, maar dat het van belang blijft lange-termijn immunogeniciteit te beoordelen.

In *Hoofdstuk 3* wordt onderzoek beschreven dat in Engeland en Wales uitgevoerd werd in de eerste jaren van het millennium 2000. In *Hoofdstuk 3.1* worden laboratorium surveillance gegevens van

acute HBV-infectie in Engeland en Wales tussen 1995 en 2000 geanalyseerd, samen met gegevens over migratie en schattingen van internationale HBsAg prevalenties. De geschatte jaarlijkse incidentie van HBV-infectie in Engeland en Wales was 7.4 per 100,000 (gecorrigeerd voor onderrapportage en asymptomatische infecties). Injecterend drugsgebruik was de meest frequent gemelde wijze van overdracht. Het aantal gevallen toegeschreven aan heteroseksuele contacten was stabiel, terwijl het aantal gevallen bij mannen die seks hebben met mannen afnam. Deze epidemiologische bevindingen zijn onveranderd ten opzicht van de jaren 1990. Infecties bij kinderen werden zelden gemeld, maar kwamen frequenter voor bij Zuid-Aziaten (inwoners van het Verenigd Koninkrijk afkomstig uit Zuid-Aziatische landen, waaronder India, Pakistan en Bangladesh). De incidentie in Zuid-Aziaten was relatief hoog. Hun belangrijkste risicofactoren voor HBV-infectie waren een medische behandeling in het buitenland en heteroseksueel contact. Voor ongeveer een derde van de gevallen van acute HBV-infectie werd geen waarschijnlijke overdrachtsroute gemeld. De analyse van de seculiere trends en leeftijdsopbouw suggereren dat een aanzienlijk deel van deze infecties gerelateerd was aan injecterend drugsgebruik. Endemische transmissie leidt tot slechts een klein deel van alle nieuwe chronische infecties; de overgrote meerderheid is het gevolg van immigratie van chronisch besmette indivuen. We concludeerden dat de incidentie van acute HBV-infectie in Engeland en Wales stabiel laag bleef, met een vergelijkbaar patroon van de gemelde routes van overdracht ten opzichte van het begin van de jaren 1990. De prevalentie van HBV-infectie in het Verenigd Koninkrijk is meer afhankelijk van mondiale dan nationale vaccinatieprogramma's. De vaccinatiegraad onder injecterende drugsgebruikers zou verbeterd moeten worden. Hierdoor zal naar verwachting ook het aantal gevallen zonder een gemelde risicofactor ook dalen. Daarnaast zou onderzocht moeten worden hoe de bestaande vaccinatiestrategieën beter kunnen aansluiten bij de behoeften van etnische minderheden.
In *Hoofdstuk 3.2* hebben we ons gericht op de Zuid-Aziatische subpopulatie in Engeland en Wales door het bestuderen van acute HBV-infecties en de daarmee samenhangende risicofactoren welke zijn gerapporteerd door laboratoria in Engeland en Wales tussen 1988 en 2000. We gebruikten 'Nam Pehchan' software om op basis van de naam van de ziektegevallen diegene van Zuid-Aziatische afkomst te identificeren. We vonden dat de incidentie van acute HBV-infectie hoger was onder Zuid-Aziatische dan onder niet-Zuid-Aziatische inwoners van het Verenigd Koninkrijk. HBV-infecties bij Zuid-Aziaten komen relatief vaak voor op kinderleeftijd. Het Britse immunisatiebeleid zou allochtone kinderen beter moeten beschermen tegen HBV-infectie. Tot op heden is deze aanbeveling helaas niet opgevolgd.

In *Hoofdstuk 4* rapporteren we de resultaten van een systematische literatuurstudie naar de prevalentie van HBV- en hepatitis C-virus (HCV)-infectie in 34 Europese landen en 5 subgroepen van de bevolking, en naar de kosteneffectiviteit van screening op chronische HBV- en HCV-infecties. Het doel van deze exercitie was het onderbouwen van het beleid ten aanzien van HBV screening. Van 1759 en 384 artikelen welke zijn gevonden in respectievelijk de prevalentie en kosteneffectiviteit zoekopdracht werden 124 en 22 artikelen opgenomen in de analyse. We gebruikten beslisregels om gewogen prevalentie schattingen te berekenen per land. De HBsAg en anti-HCV-Ab prevalentie in de algemene populatie varieerde van 0.1% -5.6% en 0.4% -5.2% respectievelijk per land. In alle onderzochte landen, met uitzondering van 3, was de prevalentie van HBsAg en anti-HCV-Ab onder injecterend drug gebruikers (IDU's), mannen die seks hebben met mannen en migranten hoger dan de prevalentie in de algemene bevolking. HCV screening van IDU's en HBsAg screening van zwangere vrouwen is kosteneffectief. HBsAg screening van migranten is waarschijnlijk kosteneffectief. We concludeerden dat de prevalentie van chronische HBV- en HCV-infectie sterk verschilt tussen de Europese landen. Het invoeren van anti-HCV-Ab screening van IDU's en HBsAg screening van zwangere vrouwen heeft prioriteit voor de Europese volksgezondheid. HBsAg-screening van migranten is waarschijnlijk kosteneffectief. Bij kosteneffectiviteit analyses is het waarschijnlijk van belang rekening te houden met het effect van antivirale behandeling op het voorkómen van HBV- en HCV-overdracht.
In *Hoofdstuk 5,* de algemene discussie, bespreek ik wat dit proefschrift toevoegt aan wat reeds bekend was (samengevat in Tabel 1 hieronder), de implicaties daarvan voor HBV-surveillance en -onderzoek (Tabel 2), en aanbevelingen voor preventie en bestrijding van HBV-infectie (Tabel 3).

Tabel 1: Wat dit proefschrift toevoegt aan wat reeds bekend was t.a.v. HBV-infectie

HBV- epidemiologie in Nederland

- Ongeveer 40,000 mensen in Nederland hebben een chronische HBV-infectie.
- De prevalentie van HBV-infectie in de algemene Nederlandse bevolking is niet toegenomen tussen 1996 en 2007; Nederland blijft behoren tot de landen met de laagste prevalentie van HBV-infectie in de wereld.
- Onder autochtone Nederlanders daalde de prevalentie van HBV-infectie tussen 1996 en 2007. Ontvangst van een bloedtransfusie in het verleden was een risicofactor voor infectie.
- Toename van de migratie naar Nederland had geen invloed op de HBV-prevalentie onder autochtone Nederlanders.
- Eerste generatie migranten hadden een 10-voudig hogere prevalentie van chronische infectie dan autochtone Nederlanders. De overgrote meerderheid van deze infecties waren waarschijnlijk voorafgaand aan de migratie opgelopen.
- Tweede generatie migranten (TGM) hadden een enigszins verhoogd risico op HBV-infectie in vergelijking met autochtone Nederlanders. Onder volwassen TGM werd dit met name veroorzaakt door het hebben van een in het buitenland geboren seksuele partner. Onder TGM kinderen verklaarde reizen naar endemische gebieden een deel van het verhoogde risico.
- Mannelijk homoseksueel contact is de belangrijkste risicofactor voor het oplopen van een HBV-infectie, waarbij diegene met 3 of meer partners in de afgelopen 6 maanden een hoger risico hadden dan MSM met minder partners. Onder heteroseksuelen waren het hebben gehad van 2 seksuele partners in de afgelopen 6 maanden en het hebben van een in het buitenland geboren partner onafhankelijke risicofactoren.
- Intraveneus drugsgebruik is niet langer een belangrijke route van overdracht van HBV in Nederland, in tegenstelling tot veel andere landen, waaronder Engeland en Wales.
- Chinese vrouwen met een chronische HBV-infectie hebben meer kans om het virus perinataal doorgeven aan hun kinderen dan andere chronische HBV-geïnfecteerde vrouwen.

Vaccinatieprogramma's

- Het HBV-vaccinatieprogramma voor gedragsgebonden risicogroepen heeft een daling van de incidentie van HBV in Nederland veroorzaakt, met name door het voorkomen van HBV-infectie bij mannen die seks hebben met mannen.
- De HBIg en vaccinatiegraad onder kinderen van moeders met een chronische HBV-infectie is hoog (> 95%) in Nederland. Onder zuigelingen die HBIg ontvingen, hebben wij geen verschil gevonden tussen het risico op doorbraakinfectie tussen de vacicnatieschema's met en zonder een geboorte dosis vaccin (0.8% en 0.7%, respectievelijk).
- Onder HBsAg-positief zwangere vrouwen is het risico van perinatale transmissie naar hun kind hoger voor vrouwen van Chinese afkomst in vergelijking met andere vrouwen. Antivirale behandeling tijdens de zwangerschap kan dit risico verminderen en is mogelijk ook geïndiceerd voor sommige HBeAg-negatieve vrouwen.
- Nadat het HBV-vaccinatie-programma voor kinderen van migranten werd geïmplementeerd in 2003 nam de incidentie van acute HBV-meldingen bij jonge kinderen aanzienlijk af. Door veranderingen in het meldcriterium in de onderzoeksperiode is het moeilijk te beoordelen of deze daling een gevolg is van het vaccinatieprogramma. Het programma was aanvaardbaar voor ouders van kinderen in de doelgroep.
- Meer dan 99% van de kinderen die gevaccineerd werden met Infanrix hexa en Prevenar ontwikkelde een adequate immuunrespons tegen HBV (anti-HBs ≥ 10 IU/ L). Echter de geometrische gemiddelde concentratie van anti-HBs antilichamen was lager dan verwacht. De klinische relevantie hiervan is onzeker, maar waarschijnlijk beperkt.

HBV- epidemiologie in Engeland en Wales

- De incidentie van HBV in Engeland en Wales in de late jaren 1990 tot 2000 bleef op hetzelfde niveau als gerapporteerd voor het begin van de jaren 1990. Injecterende drug gebruik bleef de meest voorkomende wijze van overdracht.

- Meer dan de helft van de nieuwe infecties had voorkomen kunnen worden wanneer het huidige selectieve Britse HBV-vaccinatieprogramma volledig geïmplementeerd was.
- De Zuid-Aziatische etnische minderheid in Engeland en Wales loopt een verhoogd risico op het oplopen van HBV-infectie.

HBV-epidemiologie in Europa

- Landen in het zuiden en westen van de EU en Turkije hebben een veel hogere HBV- en HCV-prevalentie dan landen in het noorden en westen.
- Nu de behandelingsmogelijkheden voor chronische HBV- en HCV-infectie zijn verbeterd, heeft het prioriteit het beleid voor secundaire preventie, met inbegrip van screening en toegang tot behandeling, te verbeteren.
- Er is overtuigend bewijs dat HBsAg screening van zwangere vrouwen kosteneffectief is. HBV-screening van migranten is waarschijnlijk kosteneffectief, afhankelijk van factoren waaronder hun HBsAg prevalentie.

Tabel 2. Implicaties voor HBV-surveillance en -onderzoek

HBV-surveillance

- De aanname dat de HBV-prevalentie onder migranten gelijk is aan de prevalentie in hun land van herkomst is waarschijnlijk onvoldoende geldig.
- HBV-incidentie en trends in de meest voorkomende routes van overdracht bij volwassenen kunnen goed worden beoordeeld met behulp van individuele gegevens van ziektegevallen. Bij kinderen zijn HBV-infecties vaak asymptomatisch en zijn seroprevalentie studies nodig.
- Routinematige monitoring van de HBV-vaccinatiegraad alleen is onvoldoende om de effectiviteit van de HBV-bestrijding in gedragsgebonden hoog-risicogroepen te onderzoeken, met name omdat de omvang van deze populaties onzeker is.
- Moleculaire typering van acute HBV-infectie is van toegevoegde waarde om inzicht in routes van transmissie en incidentie te verkrijgen. De verkregen informatie kan worden verbeterd door toepassing van fylodynamische methoden.
- Surveillance van HBV-genetische varianten die ontsnappen aan door vaccinatie verkregen immuniteit, diagnostiek en antivirale behandeling is van belang om tijdig eventuele toename in de prevalentie van deze varianten te detecteren.
- HBV-surveillance moet ook toegang tot diagnostiek, behandeling en zorg monitoren zodat deze waar nodig verbeterd kunnen worden.
- Op Europees niveau heeft de standaardisatie van methoden voor surveillance van acute HBV-infectie prioriteit. De toegevoegde waarde van de moleculaire surveillance van acute HBV-infectie zou onderzocht moeten worden.

Epidemiologie, immunologie, moleculaire epidemiologie en fylodynamica

- Ten aanzien van HBV-infectie in eerste en tweede generatie migranten is verder onderzoek nodig naar de routes van transmissie en kosteneffectiviteit van inhaalvaccinatie.
- Met betrekking tot de preventie van perinatale transmissie heeft het onderzoeken of antivirale behandeling wordt verstrekt wanneer daarvoor een indicatie is prioriteit. Om te beoordelen of de huidige richtlijnen voor verwijzing adequaat zijn, zou de prevalentie van een hoge virale concentratie bij HBeAg-negatieve vrouwen onderzocht moeten worden.
- De anti-HBs titer na gelijktijdige toediening van HBV- en pneumokokkenvaccin was lager dan verwacht. Verklaringen hiervoor en de eventuele klinische relevantie vergen nader onderzoek.
- Wiskundige en fylodynamische theorie en methoden moeten ontwikkeld worden om gegevens over genetische diversiteit te vertalen naar incidentieschattingen. Deze zijn dan ook relevant in het onderzoek naar welk deel van de acute infecties veroorzaakt wordt door acute en welk deel door chronische infecties.

- Verder onderzoek is nodig naar de waarde en de robuustheid van coalescentie gebaseerde methoden die de effectiviteit van interventies onderzoeken ten aanzien van verschillende infecties en contexten.

Methoden en impact van screening, testen en vroege behandeling

- De 'evidence base' voor het screenen op chronische HBV-infectie moet worden versterkt. Prioriteit moet gesteld worden aan het vergelijken van progressie van leverziekte bij HBV-geinfecteerden met en zonder behandeling, methoden om de deelname aan de screening te optimaliseren, efficiënt testen, verwijzing en behandeling, de kosteneffectiviteit van het combineren van screening op HBV met screening op andere infecties zoals HCV en HIV, indicatiestelling voor algemene bevolking HBV-screening in midden- en hoge prevalentie landen in Europa zoals Roemenië en het effect van de behandeling op het voorkomen van nieuwe infecties.

Virale kenmerken en co-infecties

- Intrinsieke kenmerken ten aanzien van het natuurlijk beloop van infectie en transmissie parameters, geassocieerd met verschillende HBV-genotypes en klonen behoeven opheldering.
- HBV-genetische varianten en co-infectie van HBV, HCV en HIV vergen nadere surveillance.

Virale diagnostiek

- De rol van kwantitatieve HBsAg test vergt nader onderzoek.

Tabel 3. Aanbevelingen voor preventie en bestrijding van HBV-infectie in Nederland en daarbuiten

- Het selectieve HBV-vaccinatie programma voor gedragsgebonden hoog-risicogroepen moet de komende decennia worden voortgezet in Nederland.
- Andere landen waar HBV-transmissie voornamelijk plaatsvindt in hoog-risicogroepen, zoals het Verenigd Koninkrijk, de Verenigde Staten en Japan, wordt aangeraden een soortgelijk programma te implementeren.
- De effectiviteit van het Nederlandse programma voor de preventie van perinatale HBV-overdracht kan worden versterkt als zwangere vrouwen met een hoge viral load behandeld worden met antivirale behandeling. Monitoring van de uitvoering van behandeling, vooral onder vrouwen van Chinese afkomst, is dringend nodig.
- Nu behandelingsmogelijkheden voor chronische HBV-infectie verbeteren, heeft het prioriteit voor de volksgezondheid om een optimale toegang te garanderen tot zorg, bijvoorbeeld door het vermijden van vertragingen in de diagnostiek, verwijzing en behandeling.
- Europese landen die geen universele HBV-vaccinatie van zuigelingen hebben, zouden gerichte HBV-vaccinatie voor zuigelingen van migranten moeten overwegen.
- Er is overtuigend bewijs dat prenatale screening HBsAg kosteneffectief is. Alle Europese landen zouden dit derhalve moeten implementeren.
- Screening van migranten uit endemische landen voor chronische HBV-infectie is waarschijnlijk kosteneffectief. Beleid en optimale methoden voor implementatie moeten worden ontwikkeld.

Dankwoord

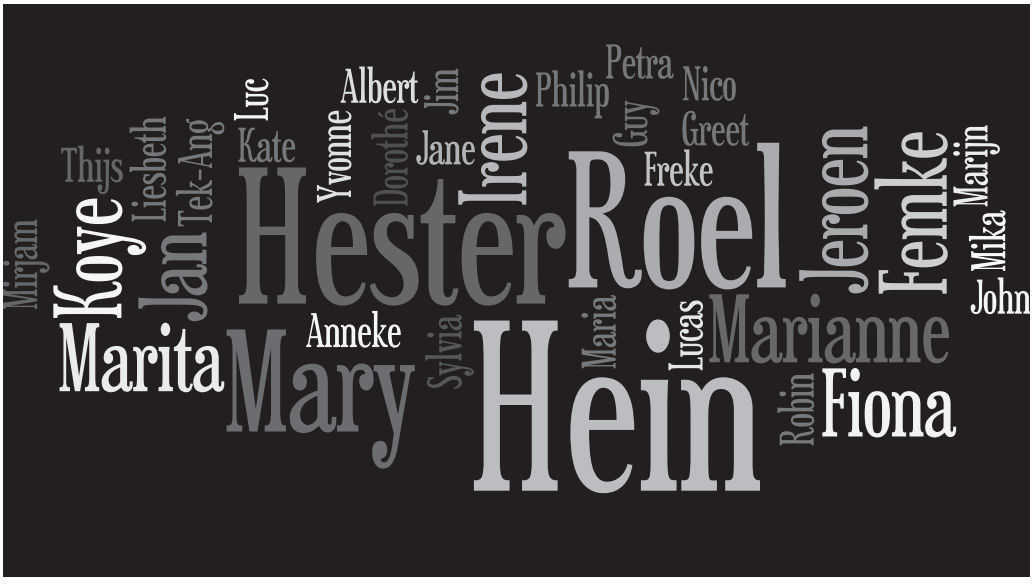
Utrecht, 24 oktober 2012

'Ik dacht dat jij al lang gepromoveerd was' is wat ik het meest hoorde toen ik her en der ging melden dat het proefschrift nu bijna af was. Ik weet niet precies wat ik daar nou van moet vinden. Ik ben in ieder geval blij dat het nu zover gekomen is, met hulp van velen, die ik hier wil bedanken.

Als eerste mijn promotor, Roel Coutinho. Zowel inhoudelijk als in het proces heeft hij enorm geholpen door op het juiste moment de juiste dingen te zeggen, op een prettige manier. En een snellere reactie op stukken dan welke co-auteur dan ook, hoe is dat eigenlijk mogelijk voor een directeur vraag ik me af. Ik denk dat ik deels het antwoord gehoord heb: niet overmatig perfectionistisch zijn, en vroeg opstaan. Tja. Co-promotor Hein Boot heeft me erg geholpen met zijn virologische expertise en voortdurende enthousiasme over het project promoveren. Het proefschrift als opstapje in plaats van het struikelblok zoals ik het soms zag. Hein, ik hoop dat ik nog heel lang met je samen kan werken.

Mijn leidinggevenden, Hester de Melker, Marina Conyn-van Spaendonck en Marianne van der Sande wil ik bedanken voor het bieden van de mogelijkheid te promoveren. Ik realiseer me dat het een luxe is dat ik dat deels onder werktijd kon doen. Ik kan me niet een prettiger direct leidinggevende voorstellen dan Hester, die goed is in epidemiologie en een erg prettige, menselijke werksfeer weet te scheppen. Daarnaast biedt ze goede adviezen op allerlei vlak. Ik ga inderdaad proberen eerst nu eens ervan te genieten dat dit proefschrift nu af is, daarna zien we verder. Alle andere co-auteurs van de artikelen in dit proefschrift wil ik bedanken voor het vertrouwen en de constructieve samenwerking, met name Jane Whelan, Jan Hontelez en Hein Boot, die de 1e auteur zijn van artikelen die in het proefschrift zijn opgenomen. Zonder co-auteurs (Figuur 1) was dit proefschrift er niet gekomen!

Figuur 1. Co-auteurs in dit proefschrift



Ik ben erg blij dat ik door Matty de Wit, Eveline Geubbels en Marc Sprenger de infectieziekte-epidemiologie heb leren kennen, toen in 1999 bleek dat klinisch werk in het ziekenhuis niet de richting was die ik op wilde. Dankzij de enthousiaste verhalen van Marina Conyn-van Spaendonck heb ik vervolgens kennis gemaakt met het Europese Programma voor Interventie Epidemiologie Training (EPIET). Door de toegewijde begeleiding van Roland Salmon, Alain Moren, Tom Grein, Mike Rowland en vele anderen heb ik tijdens het EPIET fellowship bij CDSC Wales in 1999-2001 in de infectieziekte-epidemiologie definitief mijn richting gevonden. Samen met de 'EPIET-familie' en EAN heb ik veel geleerd en lol beleefd op de meest fantastische plekken in Europa, en ben ik een echte Europeaan geworden.

Onder andere door Natasha Crowcroft werd tijdens de EPIET vaccine module in Glasgow in 2001 mijn interesse in vaccinaties gewekt. Dit leidde tot de samenwerking met Mary Ramsay bij het CDSC in Colindale, Londen. Zij introduceerde mij in het onderzoek aan hepatitis B, en staat daarmee aan de wieg van dit proefschrift. Daarnaast heeft ze mijn werk op allerlei manieren geïnspireerd: inhoudelijk, de onderwerpen, methoden en vooral ook de manier van werken. Marita van de Laar introduceerde me, tijdens een etentje in Londen, in het hepatitis B werk in Nederland.

Terug in Nederland bleek de afdeling EPI een leuke werkomgeving met veel lieve collega's binnen en buiten de RVP groep: teveel om op te noemen. De goede sfeer, gezellige praatjes in ons keukentje en bereidwilligheid mee te denken en werken maakt dat ik het een fijne plek vind om te werken. De andere collega's binnen het CIb, met name die van LIS, LCI en BBA, wil ik ook bedanken voor de samenwerking en kritische discussies. Ik heb de oprichting van het CIb van dichtbij mee mogen maken, en ben overtuigd van het belang van het centrum voor de infectieziektebestrijding en kwaliteit van wetenschappelijk onderzoek op dat vlak in Nederland.

Ik hoop dat ik dit dankwoord van mijn proefschrift niet nodig heb om duidelijk te maken dat de vriendschappen die ontstonden tijdens school, universiteit, EPIET, werk en daarbuiten, in Nederland en elders, heel belangrijk voor mij zijn. Mijn vrienden zijn een bron van inspiratie, gezelligheid, ontspanning, steun en liefde.
Paranimfen Bettina Zevenbergen en Richard Pebody bedankt dat jullie de 18e december en de weg ernaar toe naast me willen staan, en Irene Veldhuijzen, fijn dat je het promotiefeest tijdens de langste nacht mee wilde helpen organiseren.

Mijn ouders en familie zijn de basis voor mijn leven en werk. Dank voor het vertrouwen in mij en mijn keuzes, en de vrijheid en mogelijkheden die er altijd waren.

Susan

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Utrecht, 24th October 2012

"I thought you got your PhD ages ago" was the comment made most frequently when I mentioned my PhD thesis was now near completion. I do not know exactly what to think of this. Anyway, I am very glad that the PhD is now nearly finished. Below I'd like to acknowledge and thank everyone who has helped me to get to this stage.

First my supervisor, Roel Coutinho. Both in terms of content and process he greatly helped by saying the right things at the right time, in a pleasant way. He responded faster than anyone to draft papers I sent around. I did wonder how this is actually possible for a director, but I think I heard part of the answer: not being an extreme perfectionist, and getting up early. Well. Co-supervisor Hein Boot has helped tremendously with his virological expertise and constant enthusiasm about the PhD project. The thesis as a stepping stone rather than a stumbling block, as I sometimes tended to see it. Hein, I hope we can work together for a long time.

I would like to thank my line-managers, Hester de Melker, Marina Conyn-van Spaendonck and Marianne van der Sande for having the opportunity to do this PhD. I realise it is a luxury this could be partly done during working hours. I cannot imagine a nicer boss than Hester, who is an excellent epidemiologist and is able to create a very pleasant work atmosphere. In addition, she offers good advice on various levels. I will indeed try to now firstly enjoy completing this thesis. All other co-authors of the articles in this thesis (Figure 1) I would like to thank for the trust and constructive collaboration, particularly Jane Whelan, Jan Hontelez and Hein Boot, first authors of articles that are included in this thesis. Without co-authors this thesis would not have been there!

Figure 1. Co-authors in this thesis

I am very glad that through Matty de Wit, Eveline Geubbels and Marc Sprenger I got to know about infectious disease epidemiology at the National Institute for Public Health and the Environment (RIVM), when in 1999 I decided that clinical work in the hospital was not the direction I wanted to pursue. Thanks to the enthusiastic stories of Marina Conyn-van Spaendonck I decided to apply for a fellowship of the European Programme for Intervention Epidemiology Training (EPIET). Through the dedicated guidance of Roland Salmon, Alain Moren, Tom Grein, Mike Rowland and many others during EPIET fellowship at CDSC Wales in 1999-2001 I found my career in infectious disease epidemiology. Together with the 'EPIET family' and EAN I learned a lot and had great fun in the most fantastic places in Europe, and I became a true European.

My interest in vaccine preventable diseases sparked during the EPIET vaccine module, in Glasgow 2001, where Natasha Crowcroft was one of the facilitators. This was further developed by working with Mary Ramsay at CDSC in Colindale, London, who got me started on hepatitis B and was thus at the inception of the work in this Thesis. She has been inspiring my work in many aspects: topics, methods and ways of working. I am glad that subsequently Marita van de Laar introduced me, at a dinner party in London, to the hepatitis B work in the Netherlands.

Back in the Netherlands, the EPI department proved to be a fun environment with many dear colleagues within and outside the RVP group: too many to mention. The good atmosphere, cozy chats in our kitchen and willingness to think and work together makes it a great place to work. I would also like to thank the other colleagues within the Centre for Infectious Disease Control (CIb), especially those of LIS, LCI and BBA, for the cooperation and critical discussions. I was around at the time the CIb was established and am convinced of the importance of the Centre for infectious disease control and the quality of scientific research in this field in the Netherlands.

I hope that I do not need the Acknowledgement section of my Thesis to make clear that friendships that developed during school, college, EPIET, work and elsewhere, in the Netherlands and beyond, are very important to me. My friends are a source of inspiration, support, fun and love.
I'd like to thank my Para Nymphs Bettina Zevenbergen and Richard Pebody for their support prior to and at the PhD defence on the 18th of December, and Irene Veldhuijzen, for helping to organise the PhD party during the longest night.

My parents and family are the foundation for my life and work. They have always been confident in me and my choices. I am grateful that freedom and opportunities have always been there.

Susan

Curriculum Vitae

Susan Josien Maria Hahné was born on the 30th of June 1970 in Vaassen, The Netherlands. After completing secondary education at the Catholic 'Veluws' College in Apeldoorn in 1988, she studied Health Sciences at the Catholic University in Nijmegen between 1988 and 1993. Upon completion, she studied Medicine at the same university, completing her doctoral degree in 1997 and the physician degree in 1998. She then worked for 2 years in clinical internal medicine (Leiden University Medical Centre and the St Anthonius hospital in Nieuwegein).

Realising prevention is preferable to cure, she moved her career to Public Health by starting a job with the National Institute for Public Health and the Environment (RIVM) in Bilthoven, the Netherlands, in 1999. Whilst working there she was awarded a two-year fellowship with the European Programme for Intervention Epidemiology Training (EPIET), to be based at CDSC Wales in Cardiff, UK, between 1999 and 2001.

Understanding that vaccination, after clean water, is the single most effective intervention to prevent death and disease due to infections, she decided around 2001 to focus her subsequent work on the epidemiology of vaccine preventable diseases. Between 2001 and 2004 she was a Specialist Registrar in Public Health with the then Public Health Laboratory Service (now Health Protection Agency) in Colindale, London, and was granted a Fellowship of the UK Faculty of Public Health in 2008.

In 2005 she returned to the Netherlands to take up a post with the RIVM, Centre for Infectious Disease Control (CIb), department of Epidemiology and Surveillance (EPI) in the team of vaccine preventable diseases, with a focus on measles, mumps, rubella, and since 2006, hepatitis B. In 2007 she started a PhD on the epidemiology and control through vaccination of hepatitis B virus (promoter Prof. Roel Coutinho) and subsequently enrolled as an 'outside' PhD student with the University of Utrecht, the Netherlands. She completed her PhD Thesis in 2012 and continues her current position. In addition, she has been a board member (2001-2004) and President (2005-2007) of the EPIET Alumni Network (EAN), Editor for Epidemiology and Infection (since 2007), and board member of the biodynamic community supported agriculture society the 'Flea Beetle' (now called Amelis' hof) (since 2009).

Curriculum Vitae

Susan Josien Maria Hahné werd geboren op 30 juni 1970 in Vaassen. Na het voltooien van het Atheneum aan het Katholiek Veluws College in Apeldoorn in 1988, studeerde ze Gezondheidswetenschappen aan de Katholieke Universiteit in Nijmegen tussen 1988 en 1993. Na behalen van het doctoraal examen in 1993 studeerde ze Geneeskunde aan dezelfde universiteit (doctoraat in 1997 en arts diploma in 1998). Ze werkte vervolgens twee jaar in de klinische interne geneeskunde (Leids Universitair Medisch Centrum en het St. Anthonius ziekenhuis in Nieuwegein).

Omdat voorkomen beter is dan genezen, koos ze in 1999 voor de Volksgezondheid door te gaan werken bij het Centrum voor Infectieziekte-epidemiologie (CIE) van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) in Bilthoven. Vervolgens werd ze aangenomen voor een tweejarige fellowship bij het Europese programma voor Interventie Epidemiologie Training (EPIET). Hiervoor werkte ze tussen 1999 en 2001 bij het Communicable Disease Surveillance Centre (CDSC) Wales in Cardiff, Verenigd Koninkrijk.

Omdat vaccinatie, na schoon water, de meest doeltreffende maatregel is om ziekte en sterfte door infecties te voorkomen, besloot ze rond 2001 zich te richten op de epidemiologie van door vaccinatie te voorkomen ziekten. Tussen 2001 en 2004 was ze een 'Specialist Registrar' in Volksgezondheid bij de toenmalige Public Health Laboratory Service (nu Health Protection Agency) in Colindale, Londen. Deze specialisatie werd in 2008 bekroond met een lidmaatschap (Fellowship) van de Faculteit van Volksgezondheid in het Verenigd Koninkrijk (het Britse equivalent van de Nederlandse specialisatie Arts Maatschappij en Gezondheid).

In 2005, keerde zij terug naar het RIVM, Centrum Infectieziektebestrijding (CIb) afdeling Epidemiologie en Surveillance (EPI), binnen de projectgroep Rijksvaccinatieprogramma, met een focus op mazelen, bof, rodehond en, sinds 2006, hepatitis B. In 2007 begon ze met de samenstelling van een proefschrift over de epidemiologie en bestrijding door middel van vaccinatie van hepatitis B-virus (promotor prof. dr. Roel Coutinho). Vervolgens werd ze een buiten promovendus bij de Universiteit van Utrecht. Zij voltooide haar proefschrift in 2012 en continueert haar huidige baan. Naast deze werkzaamheden was ze bestuurslid (2001-2004) en voorzitter (2005-2007) van het EPIET Alumni Netwerk (EAN), Editor voor het tijdschrift *Epidemiology and Infection* (sinds 2007), en bestuurslid van de biologisch-dynamische Pergola 'de Aardvlo' (nu 'Amelis 'hof') (sinds 2009).

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