The prevalence of chronic hepatitis B virus (HBV) infection in Denmark is not clear. The primary aim of this study was to estimate the prevalence of chronic HBV infection in Denmark. The capture–recapture method was used to estimate the total population diagnosed with chronic HBV infection in Denmark using four nationwide registers. The population with undiagnosed chronic HBV infection was estimated by incorporating data from a two-year nationwide HBsAg screening programme in pregnant women. We identified 4,466 individuals with chronic HBV infection in the four registers until the end of 2007, and the capture–recapture estimate of the total population diagnosed with chronic hepatitis B was 7,112 (95% confidence interval (CI): 6,953–10,747). Only 17% of the identified patients attended recommended clinical care according to national guidelines. Including undiagnosed patients, the current population alive with HBV infection was 10,668 (95% CI: 10,224–16,164), corresponding to a prevalence of 0.24% (95% CI: 0.23–0.37%) in the Danish population older than 15 years. The estimated prevalence of chronic HBV infection among adults in Denmark was lower than reported from other northern European countries. Only half of the infected population had been diagnosed, and a minority attended specialised clinical care.

Background

Hepatitis B virus (HBV) infection is a major global health problem. More than 350 million people are chronically infected with HBV, causing 500,000 to 1,200,000 deaths annually [1,2]. The reported incidence of acute hepatitis B in Denmark has been declining for the last two decades to 0.4 per 100,000 per year; in contrast, the incidence of diagnosed chronic hepatitis B has increased to 5.2 per 100,000 per year [3]. Universal childhood vaccination is not a part of the Danish hepatitis B prevention programme, as this is targeted at high prevalence groups. However, since November 2005, all pregnant women have been screened for HBV. People who inject drugs (PWID) are offered a HBV test and vaccination free of charge, and programmes for this have been implemented in prisons and drug treatment centres. Other high prevalence groups (migrants from HBV-endemic countries, men who have sex with men (MSM) etc.) are recommended for HBV testing and vaccination, but the coverage of this intervention is unknown [4,5].

The exact number of persons with chronic infection in Denmark is unknown as a national seroprevalence survey has never been performed. Notification of chronic HBV infection to the national register of notifiable diseases has been mandatory since 2000, but reporting rates are low [6]. The National Board of Health estimated the prevalence of chronic hepatitis B in Denmark at 0.28% (15,000 patients) in 2002 [7]. Based on the national screening of pregnant women for hepatitis B from 2005 to 2007, the prevalence of hepatitis B was estimated at 0.2 to 0.3% (13,500 persons) in 2007 [8].

The primary aim of this study was to estimate the prevalence of chronic hepatitis B (both diagnosed and undiagnosed) in Denmark. A secondary aim was to estimate the coverage of diagnosed patients by national registers.

Methods

All persons with permanent residence in Denmark are assigned a 10-digit personal identification number.
This number was used to identify patients with a diagnosis of chronic HBV infection in the four different nationwide registers. Persons without a valid personal identification number were excluded from the analysis (mean: 1% in source registers).

**Registers**

**Laboratory register (DANVIR)**
This is a research database that includes patients tested for hepatitis B and C in 14 of the 17 Danish laboratories performing HBV and hepatitis C virus (HCV) testing [9]. This database included data on 280,643 persons tested for hepatitis B as of 31 December 2007. Samples reactive for HBsAg were retested with a confirmatory HBsAg assay, and only confirmed positives were included in this analysis. All tests were commercial kits approved by the health authorities, but each laboratory chose its own supplier and thus the test kit used varied between laboratories and over time in the individual laboratories.

**National patient register**
This national register was established in 1977 and records all discharge diagnoses from hospitals in Denmark according to ICD-8 and, from 1994, ICD-10 codes. Since 1995, the register has also included diagnoses from hospital outpatient visits. From this register we extracted all individuals registered with chronic HBV infection with or without delta agent (ICD-10 diagnosis codes DB18.0 and DB18.1).

**The Danish database for chronic hepatitis B and C (DANHEP)**
This nationwide clinical database was established in 2002. It includes patients older than 15 years with chronic viral hepatitis attending medical care in one of the 14 specialised medical units treating patients with viral hepatitis in Denmark. From this database we included all patients registered with chronic HBV infection.

**Communicable diseases register**
This national register of notifiable diseases has been recording acute hepatitis B since 1980 and chronic hepatitis B since 2000. Although reporting is mandatory for any clinician diagnosing a patient with hepatitis B infection, reporting rates are low [6,10]. We included all patients reported with chronic HBV infection. The register has been estimated to cover 35–40% of all diagnosed individuals [6,10].

**The civil register**
This register was established in 1968 and stores information on vital status and residency as well as immigration and emigration for all Danish residents. From this register we extracted vital status and residency data.

**Definition of chronic HBV infection**
Classically, chronic hepatitis B infection is defined as two positive HBsAg tests measured at least six months apart. Anti-HBc IgM is positive in the early state of infection and becomes negative after months of infection [11]. Thus patients who are HBsAg-positive and anti-HBc IgM negative are likely to be chronically infected.

For DANHEP, the national patient register, and the register for communicable diseases, the case definition was two positive HBsAg tests six months apart, as specified by the National Board of Health [7]. This definition could not be used for the laboratory register, as many patients had not been tested twice. In addition, many HBsAg-positive patients with only one test had not been tested for anti-HBc IgM. As the vast majority of patients reported with acute hepatitis B are native Danes, we included place of origin as a criterion in the case definitions used for the laboratory register:

1. **Definite chronic hepatitis B**: two samples at least six months apart positive for HBsAg;
2. **Definite or likely chronic hepatitis B**: As in 1., or one sample positive for HBsAg and negative for anti-HBc IgM;
3. **Definite or possible chronic hepatitis B**: As in 1. or 2., or one sample positive for HBsAg, anti-HBc IgM not done, and the patient born in a country of high endemicity.

Estimates were calculated with all three case definitions, but detailed results are only presented for the estimates based on definition 3.

**Study population**
We included all cases identified with chronic HBV infection diagnosed before 31 December 2007 in any of the registers described above. As the laboratory register had three definitions, three different study populations were extracted. We linked these populations with data from the civil register, extracting vital status, residency, immigration and emigration information. We excluded patients who were younger than 16 years, had no assigned address, or were reported dead, missing or emigrated by 31 December 2007.

**Statistical analysis**
We estimated the prevalence of chronic HBV infection in the Danish population in the following two steps:

Firstly, the population with diagnosed chronic hepatitis B was calculated by capture–recapture analysis of an overlap table of the four source registers stratified by age (three groups), sex, geographical region (five) and calendar time (first diagnosis before versus after year 2000) [12,13]. The calculation was based on log-linear modelling using the statistical programme GLIM 4 [14,15]. The model contained 60 strata, and in total 113 different models, including all possible two-way and three-way interactions, were fitted to the overlap data for each individual stratum. The Akaike
information criterion was used to find the best fitting model, but we also calculated a weighted estimate for each overlap pattern. This weighted estimate was averaged across all fitted models using the Schwartz criterion as a weight. Some of the 113 models produced unrealistically high estimates; therefore, if an estimate was more than five times the observed number, then a weight of zero was attached to that estimate in order to decrease the influence of what we considered to be unrealistic estimates. If the best model suggested by the Akaike information criterion resulted in an estimate that differed markedly from the weighted estimate, we used the Schwartz criterion to obtain the best fitting model. When the choice between the Akaike and Schwartz criterion was not clear, we chose the model that gave an estimate closest to the ratio of known to estimated number of infected patients found in other strata. The 95% confidence intervals (CI) for the total estimate were calculated by bootstrap analysis of 1,000 samples [13,15].

Secondly, the proportion of patients with chronic hepatitis B who had not been diagnosed (never tested) was calculated from a complete two-year national screening programme for HBsAg among pregnant women performed from 2005 to 2007 [8]. We identified the overlap between the four source registers and women identified in the screening programme. The proportion of all HBsAg-positive women identified in the screening programme who were present in one or more of the source registers, gave a direct estimate of the diagnosed fraction of the population with hepatitis B (as well as a direct estimate of the prevalence of hepatitis B among pregnant women). We used the national
estimate of the ‘hidden proportion’ (e.g. diagnosed with hepatitis B but not present in any of the four source registers) to calculate the total number of pregnant women diagnosed with hepatitis B. Assuming the same proportion of diagnosed HBV infection outside the national screening programme (e.g. among drug users, MSM etc.) we calculated the total prevalence of HBV in Denmark (Figure). A further bootstrap analysis, which also included a binomial distribution to account for the coverage rate, was used to obtain a 95% CI of the total prevalence.

Statistical analysis was performed using SPSS version 19 and GLIM 4. The study was approved by the Danish Data Protection Agency (J. 2008-41-2402).

Results
The four source registers identified 5,547 patients with chronic hepatitis B of whom 1,081 (19.5%) were excluded due to death, emigration, unknown address or age under 16 years. A total of 4,466 patients were included in the study. The 4,466 were identified as cases of possible chronic HBV infection in one or more of the four registers using definition 3 in the laboratory database (“definite or possible HBV infection”) (Table 1).

Of the identified population, 72% (n=3,192) were registered in DANVIR, whereas the other registers identified 28–41% (1,242–1,821) (Table 1). Women accounted for around 50% of the population in all four registers. Three quarters (n=3,345) of those who were registered with HBV infection were younger than 40 years when first registered in any of the registers, and 69% (n=3,095) of cases were registered after the year 2000.

The estimated total population diagnosed with chronic HBV infection was 7,112 (95% CI: 6,953–10,747), corresponding to a prevalence of 0.16% (95% CI: 0.16%–0.24%) among Danish adults [16] (Table 2). This included a ‘hidden’ population of 2,646 (37%) individuals diagnosed with chronic HBV infection but not registered in any of the registers. The prevalence was a little higher among men than women and in most of the regions the prevalence was lowest in the group aged 16 to 25 years. Copenhagen represented 42% (n=2,996) of all diagnosed cases, and only 4% (n=313) came from the North Denmark region, corresponding to, respectively, 0.23% and 0.07% of the adult population. This is consistent with the regional distribution of immigrants in Denmark: in 2008, 48% of immigrants lived in the Copenhagen region and 6% in the North

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Persons identified with chronic hepatitis B according to four nationwide registers on 31 December 2007 (n=4,466)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>3,192</td>
</tr>
<tr>
<td>% of total</td>
<td>71.5</td>
</tr>
<tr>
<td>Male (%)</td>
<td>48.3</td>
</tr>
<tr>
<td>Age (%)</td>
<td>&lt;25 years: 36.8, 25–40 years: 40.8, ≥40 years: 22.4</td>
</tr>
<tr>
<td>Region (%)</td>
<td>North: 5.7, Central: 20.8, South: 23.2, Zealand: 6.5, Copenhagen: 43.7</td>
</tr>
<tr>
<td>Year of inclusion (%)</td>
<td>2000 or earlier: 30.4, After 2000: 69.6</td>
</tr>
</tbody>
</table>
Among the 7,112 individuals with chronic HBV infection, 50% were registered in the laboratory register, 26% in the National patient register, 22% in the register of communicable diseases and 17% in the clinical register (DANHEP).

By the stricter case definitions we identified 3,675 patients diagnosed with definite chronic HBV infection (two HBsAg tests positive six months apart), and 4,003 patients diagnosed with definite or likely chronic HBV infection (one sample HBsAg positive and anti-HBc IgM negative). The corresponding estimates were respectively 6,121, and 6,815 diagnosed patients.

Estimation of the undiagnosed population with chronic hepatitis B infection

Of 140,376 pregnant women tested for HBsAg in Denmark in the years 2005 to 2007, 381 were identified with chronic hepatitis B, and of these, 185 were registered in one or more of the registers described above. Adjusting for 37% diagnosed but not present in the source registers, this corresponded to 66.6% (254/381) (95% CI: 62–71%) [12]. Assuming the same diagnostic coverage among patients with chronic HBV infection in the general population as in this group of pregnant women, the total national estimate was 10,668 (95% CI: 10,224–16,164), corresponding to a prevalence of 0.24% (95% CI: 0.23–0.37%) in the Danish population older than 15 years.

Table 2

<table>
<thead>
<tr>
<th>North</th>
<th>Central</th>
<th>South</th>
<th>Zealand</th>
<th>Copenhagen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of estimate for Denmark (%)</td>
<td>4</td>
<td>18</td>
<td>24</td>
<td>11</td>
<td>42</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>0.07</td>
<td>0.13</td>
<td>0.18</td>
<td>0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>Population of Denmark 15 years (x1,000)</td>
<td>465</td>
<td>993</td>
<td>959</td>
<td>658</td>
<td>1,121</td>
</tr>
<tr>
<td>Age</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>&lt;25 years</td>
<td>88 (28)</td>
<td>386 (29)</td>
<td>495 (29)</td>
<td>206 (27)</td>
<td>841 (28)</td>
</tr>
<tr>
<td>25–40 years</td>
<td>147 (47)</td>
<td>440 (34)</td>
<td>658 (38)</td>
<td>304 (40)</td>
<td>1,230 (41)</td>
</tr>
<tr>
<td>≥40 years</td>
<td>78 (25)</td>
<td>486 (37)</td>
<td>581 (33)</td>
<td>247 (33)</td>
<td>925 (31)</td>
</tr>
<tr>
<td>Sex</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Male</td>
<td>154 (49)</td>
<td>781 (60)</td>
<td>978 (56)</td>
<td>373 (49)</td>
<td>1,626 (54)</td>
</tr>
<tr>
<td>Female</td>
<td>159 (51)</td>
<td>531 (40)</td>
<td>756 (44)</td>
<td>384 (51)</td>
<td>1,370 (46)</td>
</tr>
<tr>
<td>Entrance in register</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>2000 or earlier</td>
<td>93 (30)</td>
<td>330 (25)</td>
<td>656 (38)</td>
<td>134 (18)</td>
<td>877 (29)</td>
</tr>
<tr>
<td>After 2000</td>
<td>220 (70)</td>
<td>982 (75)</td>
<td>1,078 (62)</td>
<td>623 (82)</td>
<td>2,119 (71)</td>
</tr>
<tr>
<td>Total</td>
<td>313 (100)</td>
<td>1,312 (100)</td>
<td>1,734 (100)</td>
<td>757 (100)</td>
<td>2,996 (100)</td>
</tr>
</tbody>
</table>

NA: not applicable.

Discussion

In this large register-based study we estimated the adult population alive and diagnosed with chronic hepatitis B infection to be 0.16% in Denmark. Including undiagnosed cases, the estimated prevalence of chronic hepatitis B infection for persons aged 16 years or older on 31 December 2007 was 0.24%.

The majority of patients were diagnosed after the year 2000, and unexpectedly, we did not find an increasing prevalence with age. This probably reflects an increase in immigration, and an increased focus on the disease, as indicated by the fact that reporting of chronic HBV infection to the register of communicable diseases became mandatory in 2000, and antenatal screening was implemented in 2005. Most patients were younger than 40 years when first diagnosed with HBV infection. Studies performed 10 to 30 years ago have found that the population with chronic hepatitis B in Denmark primarily consists of immigrants from high endemic countries infected by vertical transmission, and immigrants are younger than the general Danish population [6,18]. The population of foreign origin has increased from 3% to 10% of the total Danish population during the last 30 years [17]. This also explains why the overall prevalence of HBV infection among pregnant women overall has increased from 0.11% in 1971 to 0.26% in 2007, while in the same period, the prevalence among pregnant native Danes fell from 0.11% to 0.01% [8,18]. In accordance with this, a Norwegian study found that chronic HBV infection was more likely to be diagnosed...
among immigrants than native Norwegians, and more likely among 20 to 29 year-olds than among 50 to 59 year-olds [19].

It was disappointing to us that only 28% of the observed population (corresponding to 17% of the estimated population with the diagnosis) had attended an outpatient clinic that specialised in viral hepatitis (registered in the clinical register, DANHEP). In accordance with this, only one third of the observed cases were reported to the public health register, as previously reported [6,10]. This suggest that increased efforts will be necessary to assure that chronic hepatitis B patients receive the appropriate clinical care - as specified in current Danish guidelines [5]. This is important, as the treatment possibilities for hepatitis B have greatly improved in the last decade [20].

Our study has several limitations: capture–recapture analysis requires a closed population and the same case definition in each register used in the analysis [21]. Ideally there should be independency between the different registers, although any dependencies to some degree can be explained and accounted for using log-linear modelling. The case definition was assumed to be the same in the three clinical registers but expanded in the laboratory register, as 18% of HBSAg positives did not have a follow-up test registered to fulfil the classical definition of chronic hepatitis B. Patients who tested positive for anti-HBc IgM were excluded to avoid inclusion of patients with acute hepatitis B of whom more than 90% eventually cleared the infection [11]. However, it is well known that patients with chronic hepatitis B may be intermittently anti-HBc IgM-positive [11]. In contrast, using the geographical definition (patients with only one HBSAg-positive sample and not tested for anti-HbC IgM) we would include patients of foreign origin with acute hepatitis B. This would underestimate the true number of infected individuals. In a sensitivity analysis among patients with definite chronic hepatitis B (two HBSAg six months apart), we found that 92% (1,225/1,337) of those tested were anti-HBc IgM-negative and 90% (1,874/2,083) were immigrants (for 1,592 patients no country of origin was registered). Among the pregnant women identified in the national screening programme 96% (341/355) were immigrants (for 26 of 381, country of origin was not available). By excluding patients with chronic HBV infection who were anti-Hbc IgM-positive and chronically infected Danes with only one HBSAg positive sample, our capture–recapture estimate would become too large.

The issue of independency between the registers may only have been partially resolved. A patient from the clinical registers was more likely to be represented also in the laboratory register because being tested for hepatitis B is a prerequisite for entering any of the other three registers. Those registered in the clinical database (DANHEP) attended a specialised clinical unit that should report to the register for communicable diseases (mandatory since 2000), and those patients should also have a diagnosis of chronic hepatitis B in the national patient register. It is obvious in Table 1 that this was not always the case. We did adjust for interactions between the registers in the statistical model, but as appearance in one register increased the likelihood of appearing in another register, the estimate of the hidden population might still be too small. In addition, as our model did not produce stable estimates for several of the cells in the final table, we truncated these cell estimates at five times the observed number. The truncation due to instability of the model may have underestimated the true number of infected patients.

From the capture–recapture model, we were only able to estimate the diagnosed number of patients with chronic HBV infection, thereby missing the number of patients in the population who had never been tested. Therefore we estimated the test coverage in a national cohort of pregnant women and used this to estimate the prevalence in the adult population. Apart from immigrants, major populations with chronic hepatitis B in Denmark are PWID and MSM. It is very likely that these populations have lower test rates than pregnant women. For PWID, we have recently estimated the HCV test coverage rate to 54%, and we believe this to be comparable to their HBV test rate as these tests are usually performed simultaneously among drug users [9]. For MSM, we have not been able to find any data on test rate, but MSM have been targeted for HBV screening and vaccination for decades. However when compared to the estimated absolute size of these populations in Denmark and their estimated prevalence of HBSAg (497,000 immigrants: 2.7% HBSAg; 50,000, MSM: 1–3% HBSAg; 13,000 PWID: 2–4% HBSAg), a 10% change in test rate for MSM and PWID will add only 150 and 16 cases to the total estimate, indicating that our estimate is rather robust to variation in test rates in these groups. Calculating the total Danish population with chronic hepatitis B based on subpopulation sizes and prevalence of chronic hepatitis B resulted in a total of 16,000 persons (0.36% of the adult population), suggesting that our estimate of the diagnosed population (67%) may be too high. Private practitioners participating in the study on pregnant women reported that 58% of cases had been known before the survey, and using this proportion, our national estimate would rise to 12,626 [8]. Correspondingly, an estimate from the United States (US) reported only 35% diagnosed HBV patients [22].

We found that HBV was evenly distributed among men and women and prevalence of infection decreased with age. The majority of patients in our study were diagnosed before the age of 40 years. The cohort with the pregnant women was almost completely younger than 40 years; therefore our estimate of test coverage may not be valid in the older population. Our estimates corresponded to a prevalence of 0.53% for persons under 25 years-old, 0.39% for persons 25 to 39 years-old and 0.13% for persons 40 years and older. In particular,
we expect the test rate among male immigrants to be lower, as this group has less contact to the health care system than pregnant women. Our total estimate is sensitive to this because a 10% change in test rates represents 1,000–1,300 cases. This may also explain why our register-based estimate was 20% smaller than the estimate based solely on the screening programme of pregnant women (13,500 cases in Denmark) [8].

The even sex distribution and falling prevalence with age in our study results are different from most published studies where up to two thirds were men and prevalence increased with age. In Sweden, a national serosurvey performed in 1991 found only 0.06% infected, but increasing exposure with age (anti-HBc positives), 60% females, and no significant geographical variation [23]. In Germany, the prevalence of chronic hepatitis B was 0.6% in 1998, with a three times higher prevalence among males and exposure (anti-HBc positives) increasing with age [24]. In France (metropolitan areas), HBsAg prevalence was 0.65%, and males were five times more likely to be infected [25]. Finally, in the US from 1999 to 2008, the National Health and Nutrition Examination Survey (NHANES) survey reported an adult prevalence of 0.38%, with an odds ratio for males of 2.3 and a maximum at 50 to 59 years of age [24]. Thus our estimated prevalence was higher than the 1991 Swedish study but lower than the French and German screening programmes.

Conclusion
We estimated the prevalence of chronic hepatitis B in the adult population in Denmark to be 0.24%, confirming the low prevalence previously estimated. However, one third of the infected were undiagnosed, and the national registers showed low coverage, with only 17% of identified hepatitis B patient attending specialised clinical care. These data suggest that screening for hepatitis B should be improved and that Denmark is far from fulfilling the intention that all identified patients with chronic hepatitis B should attend appropriate specialist care. Our study is six years old, but unfortunately ongoing research suggests that this has not improved significantly since.

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