
Seroepidemiology of hepatitis A and hepatitis B virus in Luxembourg

J. MOSSONG^{1,2*}, L. PUTZ^{1,2}, S. PATINY¹ AND F. SCHNEIDER¹

¹ *Laboratoire National de Santé, Luxembourg*

² *CRP-Santé, Luxembourg*

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SUMMARY

A prospective seroepidemiological survey was carried out in Luxembourg in 2000–2001 to determine the antibody status of the Luxembourg population against hepatitis A virus (HAV) and hepatitis B virus (HBV). One of the objectives of this survey was to assess the impact of the hepatitis B vaccination programme, which started in May 1996 and included a catch-up campaign for all adolescents aged 12–15 years. Venous blood from 2679 individuals was screened for the presence of antibodies to HAV antigen and antibodies to hepatitis B surface antigen (anti-HBs) using an enzyme immunoassay. Samples positive for anti-HBs were tested for antibody to hepatitis B core antigen (anti-HBc) using a chemiluminescent microparticle immunoassay to distinguish between individuals with past exposure to vaccine or natural infection. The estimated age-standardized anti-HAV seroprevalence was 42·0% [95% confidence interval (CI) 39·8–44·1] in the population >4 years of age. Seroprevalence was age-dependent and highest in adult immigrants from Portugal and the former Yugoslavia. The age-standardized prevalence of anti-HBs and anti-HBc was estimated at 19·7% (95% CI 18·1–21·3) and 3·16% (95% CI 2·2–4·1) respectively. Anti-HBs seroprevalence exceeding 50% was found in the cohorts targeted by the routine hepatitis B vaccination programme, which started in 1996. Our study illustrates that most young people in Luxembourg are susceptible to HAV infection and that the hepatitis B vaccination programme is having a substantial impact on population immunity in children and teenagers.

INTRODUCTION

Infections with hepatitis A virus (HAV) or hepatitis B virus (HBV) are important public health problems, which can both, in principle, be prevented by vaccination. The natural history and epidemiology for these two viruses are very different. HAV infection causes acute self-limited diseases and usually follows oral ingestion of virus and is spread by faecal shedding

[1]. The prevalence of HAV infection is influenced by general hygiene conditions, like toilet facilities, water supplies and food preparation [2]. HBV infection is acquired through sexual intercourse, mother-to-infant transmission, or direct parenteral exposure, but horizontal transmission is also thought to occur among young children [3]. HBV can cause persistent infection (carrier state), chronic hepatitis and hepatocellular carcinoma [3].

The hepatitis A vaccine is not currently included in the routine vaccination schedule. In November 2001 the national body advising the Minister of Health on vaccination policy issued guidelines for preventing

* Author for correspondence: Dr J. Mossong, Laboratoire National de Santé, PO Box 1102, L-1011 Luxembourg.
(Email: joel.mossong@lns.etat.lu)

transmission of hepatitis A; vaccination was recommended for the following risk groups: child-care workers and workers in collective institutions, personnel in the food sector, personnel in water-treatment plants and persons planning to travel to endemic countries. The vaccine is not reimbursed by the national sickness funds (Union des Caisses de Maladie). The hepatitis B vaccine was officially included in the vaccination schedule in May 1996 including a catch-up campaign for all adolescents aged 12–15 years following an agreement of the Luxembourg Directorate of Health and the National Sickness Funds. The vaccination schedule adopted at the time was for babies to get the first dose at 2–3 months, the second dose at 3–5 months and the third dose at 10–12 months; for unvaccinated adolescents, the first dose at 12–18 years, followed by further doses at 1-month and 6-month intervals. Adolescents aged 12–15 years were administered a paediatric dose, whereas adolescents aged 16–18 years were administered the adult dose.

In 2000–2001, the National Laboratory of Health and the Public Research Centre of Health conducted a seroprevalence survey within the framework of the European Sero-Epidemiological Network (ESEN) 2. One of the aims of this serosurvey was to assess the level of immunity in the Luxembourg population against vaccine-preventable hepatitis, which forms the basis of this study. The results of the serosurvey for the other six vaccine-preventable infections have been (or are in the process of being) published elsewhere [4–6].

METHODS

Survey design

A prospective sample design with different recruitment methods for children/adolescents and adults was followed to allow achievement of sample sizes according to the specifications of the ESEN 2 project [7], i.e. 100 samples in each age year band in those aged 0–19 years and 200 samples for each of the age groups 20–24, 25–29, 30–34, 35–39, 40–49, 50–59 and ≥ 60 years.

Children and adolescent samples were collected in randomly selected primary schools (seven schools out of 109 in total) and secondary schools (three schools out of 23 in total) from different geographical regions in Luxembourg. All pupils and their respective parents were given a leaflet explaining the aims of the study including a short description of the diseases involved.

Written consent of study participants or their parents if participants were <18 years old was obtained. Participation rates in primary and secondary schools were 47.2%, but depended on age with highest rates among 12- to 15-year-olds (>60%) and lowest for the youngest and oldest school members. Given that in Luxembourg children start kindergarten at 4 years of age, no serum samples were collected from children aged ≤ 3 years.

Serum samples of adults were obtained from adult volunteer blood donors at the national Red Cross Centre, adults undergoing compulsory premarital testing (prior to marriage, all couples need to undergo the following compulsory laboratory tests: syphilis, TB skin test and blood group for males and toxoplasmosis, rubella, syphilis, TB skin test and blood group for females; HIV testing is offered on a voluntary basis), and non-hospitalized adult volunteers coming for routine blood tests at the National Health Laboratory.

All study participants were offered test results via a doctor of their choice who could give advice on additional vaccinations if deemed necessary.

Serology

Serum samples were tested with an automated BEP[®] 2000 analyzer (Dade Behring, Marburg, Germany) using a commercial Enzygnost[®] Anti-HAV antigen kit (Dade Behring) with reported sensitivity and specificity of 98.5 and 96.6% respectively, according to the manufacturer.

Serum samples were tested with an automated BEP[®] 2000 analyzer (Dade Behring) for the presence of antibodies to hepatitis B surface antigen (anti-HBs) using the Enzygnost[®] Anti-HBs II kit (Dade Behring) with reported sensitivity and specificity of 100 and 99.6%. Seropositivity was defined according to the manufacturer's instructions: samples with absorbance higher than (or equal to) the mean absorbance of the negative controls plus 0.08 were defined as positive.

To distinguish vaccinated from naturally infected persons, all anti-HBs-positive sera were tested for the presence of antibody to hepatitis B core antigen (anti-HBc) using Architect[™] anti-HBc assay (Abbott Laboratories, Abbott Park, IL, USA). Serum samples with sample-to-cutoff relative light units of ≤ 0.9 were considered as negative, and samples with sample-to-cutoff relative light units of ≥ 6 were considered as positive. Serum samples with values between 0.9 and 6 sample-to-cutoff relative light units were retested

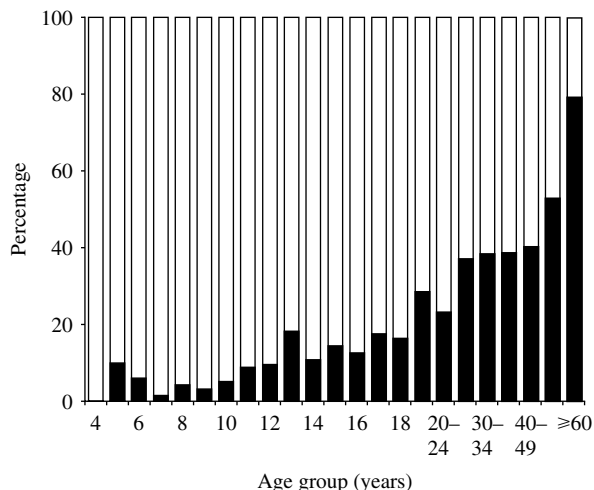


Fig. 1. Seroprevalence of anti-hepatitis A antibodies in Luxembourg in 2000–2001 by age group. Seroprevalence is increasing with age. ■, Anti-HAV positive; □, anti-HAV negative.

using Abbott AxSYM anti-Hbc assay (Abbott Diagnostics Division, Wiesbaden, Germany). Thirty-eight out of the 48 sera positive with the Architect assay in this range were negative with the AxSYM assay. As a result of the poor agreement, the results of the more established AxSYM system were used for determining antibody status of this subset of sera.

Statistical analysis

Seroprevalence of hepatitis A and hepatitis B antibodies was calculated for both gender and age groups following standardization according to the population of Luxembourg in 2000 [8]. Associations between serological status and independent variables – site of sample collection, age (categorized as shown in Fig. 1), sex and nationality [Luxembourg, Portuguese, other European Union (EU) and non-EU] – were tested using the χ^2 statistic. Risk factors for being antibody positive were determined using multivariate logistic regression. All calculations were done using STATA 8.0 (StataCorp, College Station, TX, USA).

RESULTS

Hepatitis A epidemiology

A total of 717 (26.8%) of the 2679 obtained serum samples were found to be positive for anti-HAV antibodies, corresponding to an overall age-standardized prevalence of 42.0% [95% confidence interval (CI) 39.8–44.1] in the Luxembourg population >4 years

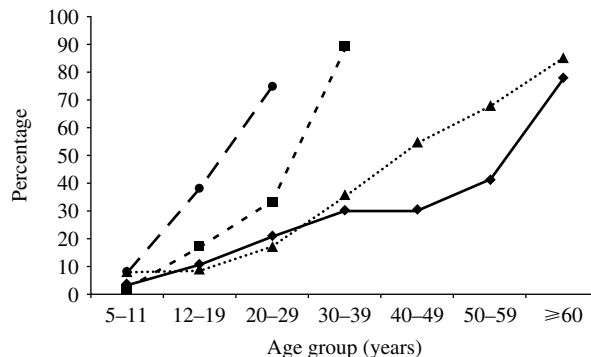


Fig. 2. Observed seroprevalence of anti-hepatitis A antibodies by age group and nationality. Only data-points based on more than 20 serum samples are shown. —◆—, Luxembourgish; --■--, Portuguese;▲....., other EU; -.-●-.-, non-EU.

of age. Seroprevalence was highly age-dependent as can be seen in Figure 1 with seroprevalence rising from <5% in primary schoolchildren to nearly 80% in persons aged ≥ 60 years old. HAV seroprevalence was found to differ according to nationality. Figure 2 shows age-specific seroprofiles by nationality in the survey sample. In adolescents and adults aged <30 years, HAV seroprevalence was substantially higher in non-EU study participants compared to other nationalities ($P < 0.001$). In the 30–39 years age group, seroprevalence was three times higher in Portuguese compared to Luxembourgish and other EU study participants ($P < 0.001$). In the 40–59 years age group, seroprevalence was also found to be higher in other EU nationalities compared to Luxembourgers ($P < 0.001$). No significant association was found between seroprevalence and gender ($P = 0.97$). Seroprevalence was found to be homogenous within the six primary schools and the three secondary schools after controlling for nationality.

Hepatitis B epidemiology

In total, 765 (28.6%) of the study participants were found to be positive for anti-HBs, corresponding to an overall age-standardized anti-HBs prevalence of 19.7% (95% CI 18.1–21.3) in Luxembourg. Of the 765 anti-HBs-positive study participants, 49 (6.4%) were also anti-HBc positive, i.e. their HBV antibody status was indicative of having acquired natural infection, and the remaining 716 study participants were anti-HBc negative, indicating that their antibody status was most probably due to having been vaccinated. The age-standardized overall seroprevalence of anti-HBc antibodies in all study participants was

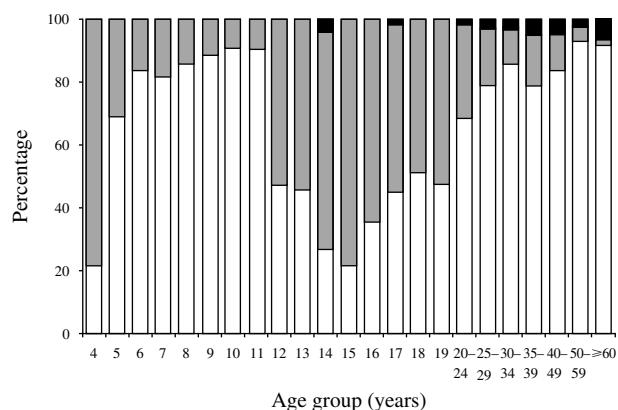


Fig. 3. Seroprevalence of hepatitis B markers in Luxembourg population by age group in 2000–2001. Only sera that tested positive for anti-HBs antibodies were also tested for anti-HBc antibodies. ■, Anti-HBs positive and anti-HBc positive, natural infection; ▒, anti-HBs positive and anti-HBc negative, vaccinated; □, anti-HBs negative, susceptible.

3.16% (95% CI 2.2–4.1). This figure must however be considered to be an underestimate as we did not test for anti-HBc in anti-HBs negative individuals.

The distribution of HBV antibody status by age in Figure 3 reveals strikingly the impact of the mass vaccination programme started in 1994, with high anti-HBs seroprevalence in the adolescent age groups targeted by the vaccination programme and in the 4-year-olds, which were the first cohort to have received the vaccine.

In children and pupils going to primary and secondary schools, there was a statistically significant difference of anti-HBs seroprevalence by nationality ($P=0.004$). Whereas 42 and 37% of Luxembourg and Portuguese schoolchildren respectively, were anti-HBs positive, this rate was lower at 30 and 29% for pupils of other EU and non-EU nationalities respectively, even after controlling for age. In adults aged ≥ 20 years, anti-HBs seroprevalence was similar by nationality ($P=0.55$) at an average of 16.6%. No difference by sex could be observed ($P=0.18$) over the whole age range.

Among the 765 anti-HBs-positive study participants, older age [odds ratio (OR) 1.11, 95% CI 1.09–1.14] and non-EU nationality (OR 17.2, 95% CI 4.5–65.5) were statistically significant risk factors for being anti-HBc positive.

DISCUSSION

To our knowledge, it is the first time that such a prospective population-based serosurvey on vaccine-

preventable viral hepatitis has been conducted in Luxembourg. A notification system of transmissible diseases exists, but published data are aggregated for infectious or viral hepatitis only and not hepatitis A, B.

The collected serum bank is large (2673 serum samples correspond to 0.6% of the total resident population), but a large sample size is no guarantee for representativity. Selective participation probably had an impact, as a substantial fraction of adults were recruited from routine blood donors and heterosexual participants (premarital test), which means that known risk groups – in particular men who have sex with men and intravenous drug users – with increased exposure to hepatitis A and B are underrepresented in the study sample. However, as reported previously, we could not detect any significant difference in participation between nationalities ($P=0.075$) in school children [4].

Our study shows clearly that both for HAV and HBV seroprevalence, age and nationality play an important role but gender does not. With hindsight it would have been appropriate to include participants below the age of 4 years to be able to assess the impact of the HBV vaccination programme in these cohorts, but a ‘random’ sample collection in this age group is difficult to implement.

For hepatitis A, our results indicate that in recent decades, incidence of natural infection in Luxembourg is low, and that the large majority of children, school children and young adults are susceptible to acquire this infection if exposed, e.g. either during large outbreaks in low-endemic countries (e.g. [9]) or on travel in intermediate/high endemic countries. The majority of the older section of the population (at least >50 years), on the other hand, has evidence of immunity from past infection. Our data are thus very similar to seroprevalence surveys in other developed countries [10–13] indicating declining incidence in the past. To what extent the low HAV rate of seropositives in children, adolescents and young adults is due to natural infection or vaccination is impossible to assess with currently available assays. A recent serosurvey among persons aged <45 years in the United Kingdom found that vaccination proved to be the most important determinant of seropositivity [14].

Similar to results in the Belgian serosurvey, we found that participants originating from non-EU countries (in our case mainly immigrants from countries of the former Yugoslavia) had a higher

seroprevalence indicating that these regions were, at least until recently, intermediate endemic and that travellers would benefit from vaccination as recommended by the National Board advising the Minister, the HPA in Britain and CDC in the United States [1]. Interestingly we also found a high rate of HAV seropositives in adult Portuguese immigrants, which form the largest foreign community in Luxembourg (13.4% of the total population in 2001). As a large part of this community regularly travel back to their country of origin on holidays for a substantial duration, it is impossible to know whether they acquired HAV infection prior to emigrating to Luxembourg or whether HAV infection might still be endemic in some regions of Portugal. A recent serosurvey in Northern Portugal in 2001 found similarly high seroprevalence rates (75%) in the 30–39 years age group, although the authors concluded that the lower prevalence of anti-HAV antibody in younger age groups was indicative of improving sanitary conditions [15].

For hepatitis B, our study indicates clearly that the mass vaccination programme initiated in 1994 is bearing fruit and that the large majority of the cohorts targeted by the vaccination programme will possess protective antibody titres. It is interesting to note that seroprevalence in 4- and 13-year-olds does not exceed 80%. A recent vaccine coverage survey in Luxembourg in January–February 2002 estimated that 94.5% of infants had received three doses of hepatitis [16], but no figures are available of the cohort of 4-year-olds in our study, who were born in 1996. Thus, it remains unclear to what extent the 20% who were seronegative in our study failed to receive three doses of vaccine or failed to respond adequately to vaccine. Community-based studies have suggested that approximately 80–90% of vaccinated children have protective responses to hepatitis B vaccine [17, 18], although this figure tends to be higher (>95%) in clinical studies [19]. The difference in anti-HBs prevalence between 4-year-olds and teenagers suggests that vaccine uptake is probably higher in young children than in adolescents because the hepatitis B vaccine is integrated in the routine schedule, whereas adolescents probably have to visit their GP specifically for this vaccine.

Our study indicates that seroprevalence of anti-HBc was strongly age-dependent with highest rates in the oldest age groups. Foreign nationality was again an independent risk factor indicating that a proportion of HBV infection could have been acquired

prior to immigrating to Luxembourg. Our estimate of 3.16% anti-HBc prevalence in our study population is most probably an underestimate as we did not test for anti-HBc in anti-HBs-negative individuals. A recent study in a general population in Germany has shown that ~20% of anti-HBc-positive individuals do not have HBsAg antibodies [20]. In another study in Flanders, this figure was ~10% [21].

It is important to note that our study was initially designed to investigate seroprevalences of eight vaccine-preventable infections and thus sample sizes were set much higher for the youngest age groups. As such, the sample design was not optimal for estimating seroprevalence of anti-HBc in the oldest age groups. Moreover, because a substantial fraction of serum samples in the older age groups were obtained from blood donors, it is clear that estimation of the prevalence of chronic carriers would not be possible.

Our study illustrates that most young people in Luxembourg are susceptible to HAV infection, whereas older individuals and immigrant adults have higher seroprevalences. The hepatitis B vaccination programme which started in 1996 is having a substantial impact on population immunity in children and teenagers and will help to reduce hepatitis B transmission.

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DECLARATION OF INTEREST

None.

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