Epidemiol. Infect. (2016), **144**, 3365–3375. © Cambridge University Press 2016
doi:10.1017/S0950268816001849

Impact and long-term protection of hepatitis B vaccination: 17 years after universal hepatitis B vaccination in Tunisia

H. CHAOUCH[1,2,3]*, W. HACHFI[1,2], I. FODHA[4], O. KALLALA[4], S. SAADI[4], A. BOUSAADIA[2], F. LAZRAG[2], I. BOUGMIZA[5], M. AOUNI[3], A. TRABELSI[4], E. BEN JAZIA[2,6] AND A. LETAIEF[1,2]

[1] *Department of Infectious Diseases,University Hospital Farhat Hached, Sousse, Tunisia*
[2] *Viral Hepatitis Research Unit (UR12SP35), University Hospital Farhat Hached, Sousse, Tunisia*
[3] *Laboratory of Infectious Diseases and Biological Agents, Faculty of Pharmacy, University of Monastir, Monastir, Tunisia*
[4] *LR14SP02;Laboratory of Microbiology, University Hospital Sahloul, Sousse, Tunisia*
[5] *Department of Community Medicine, University of Sousse, Sousse, Tunisia*
[6] *Department of Gastro-enterology, Viral Hepatitis Research Unit (UR12SP35), University Hospital Farhat Hached, Sousse, Tunisia*

Received 29 February 2016; Final revision 11 June 2016; Accepted 24 July 2016; first published online 18 August 2016

SUMMARY

Hepatitis B virus (HBV) vaccination has been part of the Expanded Programme of Immunization (EPI) in Tunisia since 1995. The aim of this study was to evaluate, for the first time, the impact of mass vaccination in Tunisia 17 years after this programme was implemented, and in parallel, assess the long-term persistence of anti-HBs antibody in the vaccinated Tunisian population. A total of 1422 students were recruited (703 vaccinated, 719 non-vaccinated). HBV seromarkers were checked. None of the students from either group had positive HBsAg. The overall prevalence of anti-HBc was 0·8%. A Significantly higher prevalence of anti-HBc was noted in unvaccinated students than in vaccinated (1·4% *vs.* 0·3%, $P = 0·02$). The overall seroprotection rate (anti-HBs titre $\geqslant$10 mIU/ml) was 68·9% in vaccinated subjects. Seroprotection rates and geometric mean titres decreased significantly with increasing age, reflecting waning anti-HBs titre over time. No significant difference was detected between seroprotection rates and gender or students' area of origin. Incomplete vaccination was the only factor associated with an anti-HBs titre <10 mIU/ml. This study demonstrates the excellent efficacy of the HBV vaccination programme in Tunisia 17 years after its launch. However, a significant decline of anti-HBs seroprotection has been observed in $\geqslant$15-year-old adolescents which places them at risk of infection. Additional studies are needed in hyperendemic regions in Tunisia.

Key words: Anti-HBs antibody, hepatitis B vaccine, protective immunity, Tunisia.

INTRODUCTION

Hepatitis B virus (HBV) infection and its complications remain a major worldwide health problem with more than 350 million chronically infected people and nearly one million deaths every year from acute or chronic sequelae of HBV infection, such as fulminant hepatitis, liver cirrhosis and hepatocellular carcinoma [1, 2]. Therefore, HBV infection is an important public health candidate for prevention, early diagnosis, and treatment [3]. Universal HBV vaccination is considered to be the best way to prevent HBV infection

* Author for correspondence: Miss H. Chaouch, Department of Infectious Diseases, University Hospital Farhat Hached, 4000 Sousse, Tunisia.
(Email: chawech_houda@yahoo.fr)

in areas with high and intermediate endemicity where the infection is predominantly transmitted at younger ages [4]. In 1991, the World Health Organization (WHO) recommended universal infant immunization against HBV, with a target for worldwide implementation by 1997 [5, 6]. As of 2008, 177 countries had implemented HBV vaccine into their national immunization programme as a routine vaccine given to all infants, which led to substantial reduction in the global burden and transmission of HBV [7].

In Tunisia, a country with an intermediate HBV endemicity, the prevalence of hepatitis B surface antigen (HBsAg) and HBV infection range from 5·3% to 7·8% and 28·5% to 48·5%, respectively [8, 9], with predominance of genotype D [10, 11]. The predominant mode of HBV transmission was the horizontal route, and HBV infection had already occurred before age 20 years especially in hyperendemic areas [9]. HBV vaccination has been part of the Expanded Programme of Immunization (EPI) in Tunisia since 1995. During the first 10 years, the vaccination schedule consisted of three paediatric doses (10 μg recombinant HB vaccine) given at ages 3, 4 and 9 months intramuscularly in the deltoid or thigh area. Since 2006, the first administration was advanced to birth following a three-dose schedule (0, 2, 6 months). In addition, all pregnant women are screened for HBsAg and administration of post-exposure prophylaxis is indicated for infants born to HBsAg-positive women within 24 h of birth. As a result of this programme, and considering the high vaccination coverage in Tunisia (94%; Ministry of Health, Immunization coverage, April 2015) [12], almost all Tunisians citizens born after 1995 have been vaccinated. However, to date, there is no available local data on the epidemiological survey of HBV markers after implementation of the universal vaccination programme.

Previous worldwide data have disclosed a significant decrease in newly acquired HBV infection, carrier rate, and hepatitis B-related mortality in countries where universal vaccination has been implemented [13, 14]. Results of various studies revealed that 90–99% of vaccinated healthy neonates and adults develop protective levels of antibody against hepatitis B surface antigen (anti-HBs) after primary vaccination [15, 16]. However, persistence of this protective antibody was unknown and is still debated. Hence, it is important to assess if primary vaccination can confer protection until adolescence and early adulthood, when increased risky behaviour can increase the risk of contracting HBV infection [17, 18]. Data of immunity duration in vaccinated infants are scarce, mainly in countries at intermediate and low endemicity, and requires further corroboration [19].

The objectives of the present study were to describe the change in the HBV endemicity in Tunisia for the first time 17 years after implementation of the mass hepatitis B vaccination programme, and to evaluate the efficacy and the long-term persistence of immune protection in the vaccinated Tunisian population.

MATERIALS AND METHODS

Study design and sampling

A cross-sectional, seroepidemiological survey was conducted in 2012 in the region of Sousse, located in the Eastern Centre of Tunisia. During the study period the population of Sousse was 622 100 inhabitants, of whom 171 000 (27·5%) were aged 10–25 years [20].

The targeted population included students aged 12–21 years in public colleges in the governorate of Sousse during the school year of 2012–2013. This study population constituted a representative sample of college students. Two groups were targeted: (1) vaccinated students, aged 12–17 years, born between 1995 and 2000, i.e. up to 5 years after the introduction of universal vaccination, and (2) non-vaccinated students, aged 18–21 years, born near the start of the universal HBV vaccination programme (birth years 1991–1994).

A stratified, two-stage, random cluster sampling was performed to select colleges. First, we sampled colleges, and then we sampled classes from selected colleges. Distribution of colleges in urban, suburban and rural regions was taken into account during sampling. Sample size calculation was estimated as a total of 1344 students with an expected HBV seroprevalence of 3% in the vaccinated group and 7% in the unvaccinated group. An allocation ratio between the two groups was equal to 1 (±5% level of accuracy), with a power of 90% and a confidence level of 95%.

Data collection

We assessed students' vaccination history by examining school medical records or their personal health booklet. This booklet, which is used to record a person's vaccination history, is provided to the parents of all newborns by the hospital. According to data recorded in the health booklet, students born before

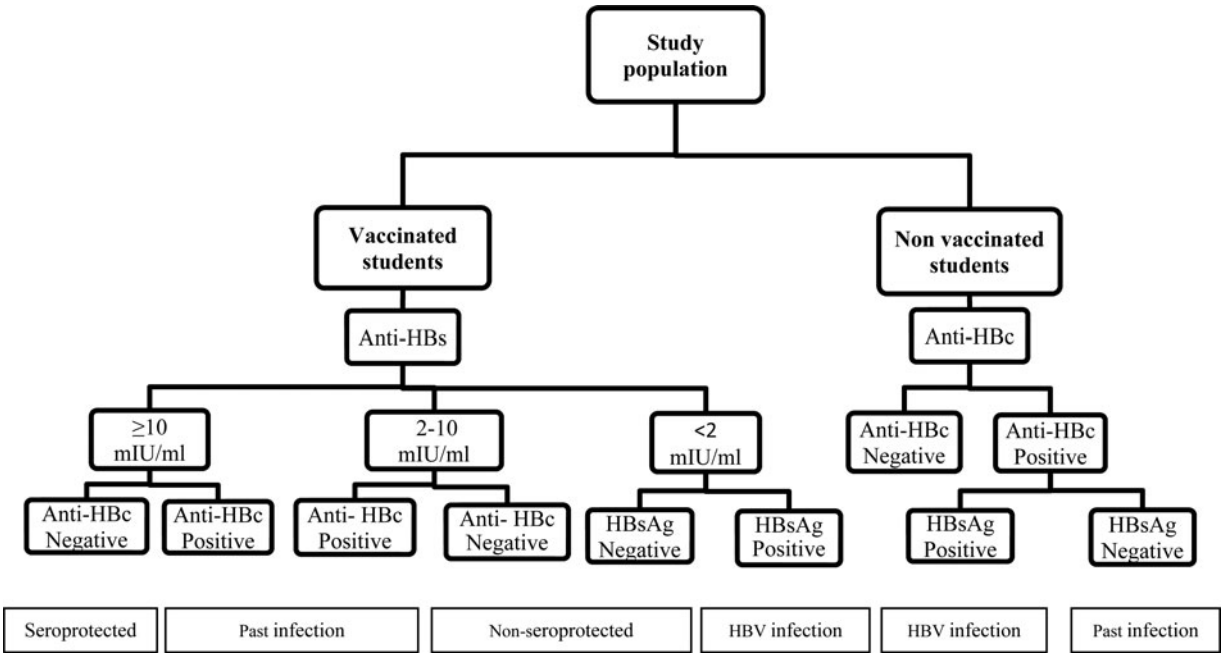

Fig. 1. Study flowchart.

1995, who were vaccinated against HBV, were systematically excluded from the study.

In addition to collecting serum samples, vaccination dates, gender, date of birth and educational level were recorded using a structured questionnaire. Five physicians were involved with completion of questionnaires, and blood samples were obtained by four nurses.

All participants were voluntary and were informed of the study's purpose. Written informed consent was obtained from all participants or their parents for those aged <18 years.

Definitions and serological study design

HBV serum markers, including HBsAg, anti-HBs, and anti-HBc were checked in two groups according to the study's flowchart summarized in Figure 1. Anti-HBc and HBsAg were checked by microparticle enzyme immunoassay (Murex; Abbott, USA), anti-HBs was checked by Cobas e411 Elecsys analyser (Roche, USA) using original Roche kits for anti-HBs.

Complete vaccination was defined as a subject receiving three doses of vaccine. Incomplete vaccination was considered when the student received only one or two doses of vaccine.

Throughout the study, we took HBsAg to be the marker of HBV infection, anti-HBs to be the marker of immunity, either from vaccination or infection, and anti-HBc to be the marker of past infection. Seronegativity was defined as being negative for all three hepatitis B markers HBsAg, anti-HBs and anti-HBc. Anti-HBs concentration $\geqslant$10 mIU/ml was defined as protective; whereas, individuals with anti-HBs titre <10 mIU/ml were considered as non-seroprotected. The cut-off and upper detection limits for anti-HBs was 2 mIU/ml and 1000 mIU/ml, respectively. When calculating the geometric mean titre (GMT) values, a concentration of 1000 mIU/ml was used for values >1000 IU/ml.

Statistical analyses

Sample size was calculated using Epi-Info v. 6.04 (CDC, USA). Data were entered and analysed, using SPSS software v. 11.0 (SPSS Inc., USA). The results on seroprevalence and immune response to HBV vaccination are presented with point estimates and two-sided 95% confidence intervals (CIs). The outcomes for anti-HBs are expressed as seroprotective rates and GMT. χ^2 or Fisher's exact tests were used for analysing categorical data when relevant. Student's *t* test was used for analysing continuous data when relevant. A descriptive analysis was followed by univariate analysis using a χ^2 test or Fisher's exact test to identify factors associated with the level of long-term immunogenicity. A two-sided *P* value <0·05 was considered statistically significant.

Table 1. *Characteristics and seroprevalence of hepatitis B markers of the study population.*

	Non-vaccinated students ($n = 719$)	Vaccinated students ($n = 703$)
Characteristics		
Age at enrolment (mean ± S.D.)	19·03 (±1·01)	14·25 (±1·13)
Gender		
Female	535 (74·4)	454 (64·6)
Male	184 (25·6)	249 (35·4)
Origin		
Urban	452 (62·9)	380 (54·1)
Suburban	122 (17)	323 (45·9)
Rural	145 (20·2)	–
Vaccination history*		
Complete	–	573 (96·8)
Incomplete	–	19 (3·2)
HBV markers		
HBsAg positive	0	0
Anti-HBc(+)	10 (1·4)	2 (0·3)
Anti-HBs(+)	9 (1·2)	579 (82·4)
Anti-HBs(+), Anti-HBc(-)	–	577 (82·3)
Anti-HBs(+), Anti-HBc(+)	9 (1·2)	2 (0·3)
Anti-HBs(-), Anti-HBc(-)	–	124 (17·6)
Anti-HBs response		
<10 IU/ml	2 (0·27)	218 (31·1)
⩾10 IU/ml	7 (0·97)	485 (68·9)

Data given are number (%).
* Vaccine dose date was recorded exactly in 592 of vaccinees.

Ethical standards

This study was conducted in accordance with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

RESULTS

Demography

A total of 1422 apparently healthy students aged from 12 to 21 years, from 12 colleges, were included in this study. The first group consisted of 703 vaccinated students (mean age 14·2 ± 1·1 years, male:female sex ratio 0·54). In the second group, 719 unvaccinated students were enrolled (mean age 19 ± 1 years, sex ratio 0·34) (Table 1).

Effect of vaccination on pattern of HBV infection markers

None of the students from the two groups had positive HBsAg. The overall prevalence of anti-HBc was 0·8%. A Significantly higher prevalence of anti-HBc was showed in unvaccinated students than in those vaccinated [1·4% *vs.* 0·3%, odds ratio (OR) 4·944, 95% CI 1·079–22·643, $P = 0·02$]. The frequency distribution of the HBV markers in the groups is given in Table 1. No association was found between students' origin and HBV markers, including anti-HBc and anti-HBs levels.

HBV vaccination history

In the vaccinated group, vaccine doses were recorded exactly in 592 (84·2%) cases, 573 (96·8%) of which had completed three doses of vaccine (Table 1). Incomplete vaccination rate was significantly higher in students from suburban areas compared to urban areas (5% *vs.* 1·8%, OR 2·153, 95% CI 1·185–5·651, $P = 0·02$).

Long-term response to HBV vaccine

Distribution of anti-HBs in vaccinated children

The overall rate of anti-HBs seroprotection in vaccinated subjects, based on a titre of anti-HBs ⩾10 mIU/ml, was 68·9% (485/703). Of these, 42% (204/485) had anti-HBs titres ⩾100 mIU/ml. An anti-HBs level of <10 mIU/ml was recorded in 31% of vaccinees (218/703) (Table 1).

Anti-HBs in different age groups

Significant difference was detected in age groups and in protective anti-HBs rates. The seroprotection rate and anti-HBs mean titre acquired through vaccination decreased significantly with age. The prevalence of protective anti-HBs titre in the 12–14 years age group (birth year 1998–2000) was 75·1% with a GMT of 154·5 mIU/ml. It decreased significantly to 60·8% with GMT values of 115·8 mIU/ml for the 15–17 years age group (birth year 1995–1997) (OR 2·231, 95% CI 1·410–2·694, $P < 0·0001$) (Table 2). In order to assess any trends for vaccination seroprotection in the study participants, the rate of protective anti-HBs and GMT values were assessed and stratified according to age (Fig. 2). It became evident that anti-HBs protective titre and GMT values decreased, respectively, from 78% and 130 mIU/ml for birth year 2000 (age 12 years after vaccination), to 52·4% and 38·1 mIU/ml for birth year 1995 (age 17 years after vaccination). In addition, the prevalence of students with a value <10 mIU/ml increased from 22% for birth year 2000 to 47·6% for birth year 1995. The prevalence of students featuring a serum

Table 2. *Distribution of anti-HBs seroprotection rate and geometric mean titre (GMT) by age group, gender, vaccination history and origin*

	Year of vaccination	Mean age (years)	No. tested	Anti-HBs response <10 mIU/ml *n* (%)	Anti-HBs response ⩾10 mIU/ml *n* (%)	GMT (mIU/ml) mean (±S.D.)
Age groups (years)						
12–14	1998–2000	13·4	399	99 (24·8)	300 (75·1)*	154·5 (255)**
15–17	1995–1997	15·3	304	119 (39·1)	185 (60·8)	115·8 (229)
Subtotal	1995–2000	14·2	703	218 (31)	485 (68·9)	137·7 (244·7)
Gender						
Male	1995–2000	14·1	249	76 (30·5)	173 (69·4)	140·8 (249·8)
Female	1995–2000	14·3	454	142 (31·2)	312 (68·7)	132·7 (234·3)
Subtotal	1995–2000	14·2	703	218 (31)	485 (68·9)	137·7 (244·7)
Vaccination history						
Complete	1995–2000	14·2	573	172 (30)	401 (70)***	139·4 (246·2)****
Incomplete	1995–2000	14·1	19	10 (52·6)	9 (47·5)	52 (91·6)
Not recorded	1995–2000	14·1	111	36 (32·4)	75 (67·5)	102 (105·3)
Subtotal	1995–2000	14·2	592	218 (31)	485 (68·9)	137·7 (244·7)
Origin						
Urban	1995–2000	14	380	112 (29·5)	268 (70·5)	141·9 (250·2)
Suburban	1995–2000	14·4	323	106 (32·8)	217 (67·2)	132·8 (238·2)
Subtotal	1995–2000	14·2	703	218 (31)	485 (68·9)	137·7 (244·7)

* $P < 0{\cdot}0001$, ** $P = 0{\cdot}03$, *** $P = 0{\cdot}03$, **** $P = 0{\cdot}001$ for participants with complete *vs.* incomplete vaccination history.

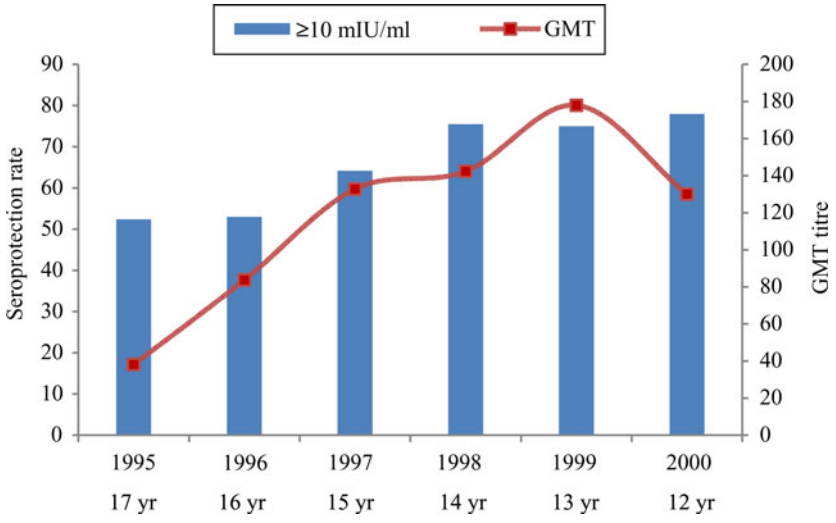

Fig. 2. Distribution of anti-HBs seroprotection rate and geometric mean titre (GMT) 12–17 years after vaccination in the vaccine study population.

anti-HBs level >100 mIU/ml declined from 35% for birth year 2000 to 10·4% for birth year 1995 (Fig. 3).

Comparative analysis of anti-HBs between males and females

No significant difference was observed in the effectiveness and protective anti-HBs levels of the HBV vaccine between males and females (Table 2).

Comparative analysis of anti-HBs between different students' area of origin

No association was found between protective anti-HBs levels and students' area of origin (Table 2).

Correlation with the vaccine history

The effectiveness and protective anti-HBs acquired through vaccination were significantly associated

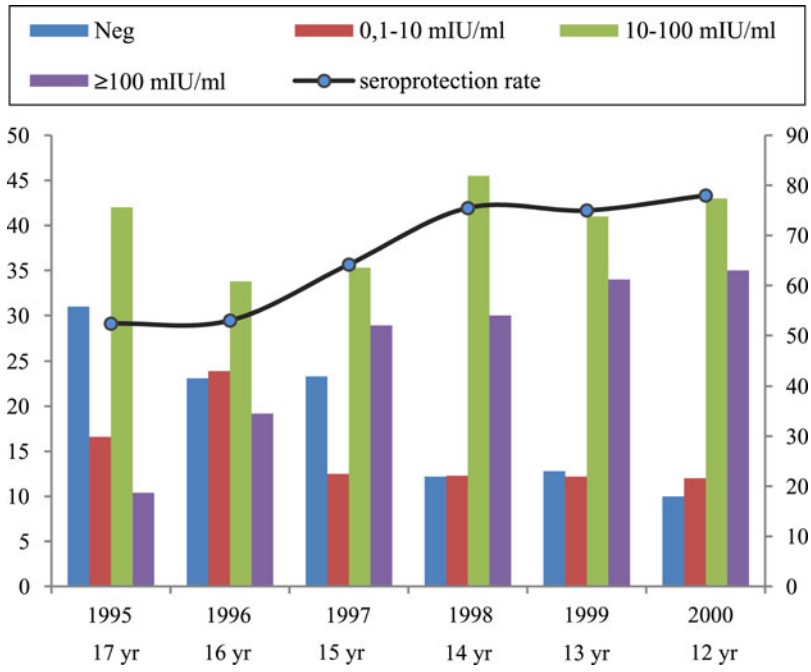

Fig. 3. Anti-HBs levels in students born during the period from birth years 1995–2000 (12–17 years of vaccination).

with history of vaccination. In fact, the seroprotection rate decreased significantly between subjects who received complete vaccination and those who received an incomplete course of HBV vaccine (OR 2·463, 95% CI 1·043–5·817, $P = 0·03$). This was supported by a decline in GMT values, from 139·4 mIU/ml in children who received complete vaccination, to 52 mIU/ml in those who received an incomplete course ($P = 0·001$) (Table 2).

DISCUSSION

This is the first seroepidemiological survey of HBV markers conducted in Tunisia after implementation of the universal vaccination programme in 1995. Results of this study contribute to evaluation of the impact of vaccination, providing epidemiological data of HBV infection and seroprotection over a lengthy vaccination programme (17 years).

The main results delineated a significant decrease of anti-HBc antibody, as markers of HBV infection, in students born after the implementation of the vaccination programme. In fact, comparison of results between unvaccinated and vaccinated students showed that anti-HBc-positive rates decreased from 1·4% to 0·3%. No positivity for HBsAg was detected in either of the groups.

In comparing our results to the seroepidemiological survey of HBV markers conducted in Tunisia before implementation of the universal vaccination programme, we observed a marked decline in HBV infection, attested by HBsAg and anti-HBc prevalences. In fact, in the 1995 survey, the mean prevalence rates of HBsAg and anti-HBc in the population aged between 10 and 20 years were 8% and 33% in meso-endemic areas, and 2% and 11% in hypoendemic areas, respectively [9]. In the present survey, HBsAg was negative in all subjects aged 12–21 years, and the overall anti-HBc prevalence declined to 0·8%.

The significant decrease of anti-HBc in vaccinated students compared to those unvaccinated, and the marked decline in HBV markers in the entire group's age compared to the seroepidemiological survey conducted in Tunisia before implementation of the vaccine programme, suggest the important role of universal vaccination in the decrease of HBV endemicity.

Previous worldwide studies have also revealed the significant decrease in HBsAg carriers and anti-HBc seropositivity in children and adolescents after the mass hepatitis B vaccination programme [21–28]. In Italy, the first Mediterranean country where mass HBV vaccination was introduced, no HBV infection was observed in vaccinated persons 17 years after vaccination [29]. In addition, a marked decline of acute HBV infection has been seen in Italy since the introduction of this mass vaccination programme [30–32].

According to the last survey conducted in Tunisia before implementation of the universal vaccination

programme, a significant decrease of HBV markers was observed in all the group's ages, even in those who were not vaccinated during infancy. In fact, in the 1995 survey, the mean prevalence rates of HBsAg and anti-HBc in the population aged between 15 and 25 years were 2% and 17% in hypoendemic areas, respectively [9]. In the present survey, HBsAg was negative in the 17–22 years age group (unvaccinated group), and anti-HBc declined to 1·4%. In light of these results, we believe that the decrease found in unvaccinated persons is connected to the decrease in HBV carriers in the area since introduction of universal vaccination, which therefore reduces the risk of contagion chain for those unvaccinated by reducing the spread of HBV in the area.

Absence of infection in this study could be attributed to the high vaccination rate (96·8%) of the three-dose HB vaccine in our study population. In 2014, the WHO strongly recommended focus on coverage of the birth dose and the three-dose vaccination regimen to control HBV infection in populations with intermediate or high HBV endemicity [7].

Other beneficial factors could contribute towards the elimination of HBV carriage. It was shown previously that urbanization plays an important role in the natural control of HBV [33, 34]. It is therefore possible that improvement in socioeconomic status of most people in Sousse may have a positive impact on the control of HBV infection and explain the absence of positive HBsAg in our study population.

The present study showed an overall anti-HBs seroprotection rate of 68·9%, 17 years after the implementation of the universal vaccination programme. Efficacy of HBV vaccination for different age groups from several countries shows heterogeneous results. While some studies reported that 75–90% of vaccinees had protective titres of anti-HBs from 1 to 24 years after primary vaccination [23, 35, 36], in others, only 7·3–40% found this response [37–39]. These different data are reported in Table 3.

Protective anti-HBs acquired through vaccination were significantly associated with history of vaccination. Indeed, the seroprotection rate is significantly lower in persons who received an incomplete course of HBV vaccine, suggesting the role of the second and third doses as boosters. The immune response rates were not associated with gender, as noted in the majority of studies [35, 36, 40], while some authors suggest a significantly better immune response for women [41–43].

Another interesting finding of the present study is the linear decline of seroprotection rates with increasing age, from 79·3% to 52·4%, respectively, 12 and 17 years after primary vaccination. This remarkable decline in seroprotection rates was further confirmed by the significant decline in the GMT values over the past 17 years. These results were consistent with a series of studies [36, 44–47] reported from several countries (Table 3). A meta-analysis of 46 studies showed that the proportion of children vaccinated who retained anti-HBs concentrations >10 mIU/ml fell from 75% at age 5 years to 20% at age 20 years [19]. However, how long the effect of the immunological memory will protect vaccinated persons from future infection remains unknown.

Previous serological studies have shown that a third to half of vaccinees may have low (<10 mIU/ml) or undetectable levels of anti-HBs by ages 10–15 years [36, 13]. In the present study, 31% had lost protective levels of antibody 12–17 years after primary vaccination. Several studies reported that individuals with waning (<10 mIU/ml) or absent concentrations of anti-HBs, long after primary vaccination, can mount a rapid and vigorous anti-HBs anamnestic response to a challenge dose of hepatitis B vaccine [28, 39, 45, 46, 48–50] (Table 3). These observations indicate that neonate immunization offers a lasting protection without the need for a booster dose, even when protective antibody was undetectable. Nevertheless, some studies have revealed the absence of an anamnestic response in about 20–30% of persons 20 years after primary vaccination [22, 51]. Moreover, cases of chronic HBV infection were reported after vaccine-induced protecting antibodies had disappeared [52]. However, in their latest guidelines, the WHO and the European Consensus Group on Hepatitis B Immunity do not recommend a booster dose to sustain long-term immunity in immunocompetent persons after primary immunization [7, 53]. In present study, a significant decline of anti-HBs seroprotection has been observed in $\geqslant$15-year-old adolescents with puberty-associated risky behaviours, suggesting the need of additional follow-up and testing of the anamnestic response to establish whether a primary course of vaccination in infancy may confer lifelong protection or whether boosters may be needed at this age.

On the other hand, Jack *et al.* suggested that anti-HBs concentration of 10 mIU/ml measured 1–3 months after administration of the last dose of the primary vaccination course is considered a reliable marker of longer protection against infection [54]. Subsequently, they recommended determination of anti-HBs levels in children after primary vaccination to provide long-term immunity against HBV infection.

The variability of results about seroprotective rates, long-term persistence of anti-HBs and the anamnestic

Table 3. *Studies in different regions with different endemicity showing seroprotection after infant vaccination programme, the breakthrough infections (HBsAg, anti-HBc) and anamnestic response to a booster dose*

Duration of follow-up, years	No. of subjects	Country	No. of doses and schedule (months)	Seroprotection* (%)	HBsAg(+)	Anti-HBc(+)	Anamnestic response†	Ref., year of publication
10	606 vaccinated	Germany	3 doses: 0, 1, 6	78%	NT	NT	97·2%. After 1 booster dose at 10 years if anti-Hbs <10 mIU/ml	[45], 2012
10	1212 vaccinated	Italy	3 doses: 3, 5, 11	64%	0	1%	97%. After 1 booster dose at 10 years if anti-Hbs <10 mIU/ml	[28], 2005
10	663 vaccinated	Canada	3 doses: birth, 1, 6	86·4%	NT	NT	100%. After 1 booster dose at 10 years if anti-Hbs <10 mIU/ml	[46], 2013
10	146 vaccinated	Iran	3 doses: birth, 2, 6	47·9%	0	7·5%	95%. After1 booster dose at 10 years if anti-Hbs <50 mIU/ml	[48], 2006
6–11	242 vaccinated	Egypt	3 doses: 3, 4, 6	39·3%	NT	NT	NT	[44], 2009
15	663 vaccinated	Canada	3 doses: birth, 1, 6	76·7%	NT	NT	100%. After 1 booster dose at 15 years if anti-Hbs <10 mIU/ml	[46], 2013
15	105 vaccinated	USA (Micronesia)	3 doses: birth, 2, 6	7·3%	0	7·6%		[37], 2008
17	1704 vaccinated	Italy	3 doses: 3, 5, 11	84·2%	0	0	NT	[35], 2015
16–18	1355 vaccinated	Saudi Arabia	3 doses: birth, 1, 5	38%	0	0	NT	[38], 2008
15–17	5981 vaccinated	Taiwan	4 doses: birth, 1, 2, 12	37%	1·6	4·1	69·7%. After 1 booster dose anti-Hbs <10 mIU/ml	[45], 2008
18	840 vaccinated	Iran	3 doses: birth, 2, 6	48·9%	NT	NT	NT	[36], 2014
20	1204 non-vaccinated 6388 vaccinated	Taiwan	4 doses: birth, 1, 2, 12	74%	7·4% non-vaccinated 2·2% vaccinated	23·5% non-vaccinated 6·7% vaccinated	NT	[23], 2007
20	300 vaccinated	Iran	3 doses: birth, 2, 6	37%	0	0	97%. After 1 booster dose at 20 years if anti-Hbs <10 mIU/ml	[49], 2014

Table 3 (*cont.*)

Duration of follow-up, years	No. of subjects	Country	No. of doses and schedule (months)	Seroprotection* (%)	HBsAg(+)	Anti-HBc(+)	Anamnestic response†	Ref., year of publication
20	843 vaccinated	Taiwan	4 doses: birth, 1, 2, 12	33·6%	1·4%	2·7%	82·2%. After 1 booster dose at 20 years if anti-Hbs <10 mIU/ml	[22], 2007
24	402 vaccinated	China	3 doses: birth, 1, 6	30·2%	1%	6·7%	84·5%. After1 booster dose at 24 years if anti-Hbs <10 mIU/ml	[39], 2011
12–17	719 non-vaccinated 703 vaccinated	Tunisia	3 doses: 3,4, 6	68·9%	0	1·4% non-vaccinated 0·3% vaccinated	NT	This study

NT, Not tested.
* Anti-HBs titre ⩾10 mIU/ml.
† An anamnestic response has defined as a rise within 10–28 days in anti-HBs concentrations to ⩾10 mIU/ml in subjects seronegative for anti-HBs antibodies (or with levels <10 mIU/ml) before challenge dose and as an increase in anti-HBs titres in subjects with seroprotective levels of anti-HBs before challenge.

response may be attributed to differences in genetic and environmental factors, type and dose of the vaccine (plasma-derived or recombinant), age of initial vaccination, schedule of immunization, body mass index and time intervals between vaccine administrations, but they may also be caused by methodological and statistical variations [22, 36, 19].

In conclusion, universal hepatitis B vaccination in Tunisia has resulted in progress towards the prevention and control of hepatitis B infection. Seventeen years after the implementation of universal vaccination, inhabitants of our region aged <21 years are protected against HBV infection. These findings should be demonstrated in other more endemic Tunisian regions. The HBV vaccination programme not only reduced the perinatal and horizontal transmission of HBV in vaccinated persons but also reduced horizontal transmission of HBV to unvaccinated persons born up to 5 years before the start of the programme. By decreasing the carrier pool, continuation of HBV immunization should prevent HBV infection in the children of Tunisia, and, subsequently, adults as well. Our data provide evidence that a strong immunological memory persists for >15 years after immunization of healthy adolescents with a primary course of hepatitis B vaccination. However, a significant decline of anti-HBs seroprotection has been observed in ⩾15-year-old adolescents with puberty-associated risky behaviours. Therefore, additional follow-up is warranted to establish whether a primary course of vaccination in infancy may confer lifelong protection or whether boosters may be needed at this age.

ACKNOWLEDGEMENTS

The authors thank the following people for their help in recruiting students and drawing blood: the team of the Department of Infectious Diseases and the team of Regional Center of Blood Transfusion, University Hospital Farhat Hached, Sousse, Tunisia. This study received no financial support.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Lee WM.** Hepatitis B virus infection. *New England Journal of Medicine* 1997; **337**: 1733–1745.
2. **Specialist Panel on Chronic Hepatitis B in the Middle East.** A review of chronic hepatitis B epidemiology

and management issues in selected countries in the Middle East. *Journal of Viral Hepatology* 2012; **19**: 9–22.
3. **Chen DS.** Hepatitis B vaccination: the key towards elimination and eradication of hepatitis B. *Journal of Hepatology* 2009; **50**: 805–816.
4. **Ott JJ, *et al.*** Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 2012; **30**: 2212–2219.
5. **Alavian SM, Fallahian F, Lankarani KB.** Implementing strategies for hepatitis B vaccination. *Saudi Journal of Kidney Diseases and Transplantation* 2010; **21**: 10–22.
6. **Goldstein ST, *et al.*** A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *International Journal of Epidemiology* 2005; **34**: 1329–1339.
7. **World Health Organization Publication.** Hepatitis B vaccines: WHO position paper. *Weekly Epidemiological Record* 2009; **84**: 405–420.
8. **Triki H, *et al.*** Seroepidemiology of hepatitis B, C and delta viruses in Tunisia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1997; **91**: 11–14.
9. **Ben-Alaya-Bouafif N, *et al.*** Heterogeneity of hepatitis B transmission in Tunisia: risk factors for infection and chronic carriage before the introduction of a universal vaccine program. *Vaccine* 2010; **28**: 3301–3307.
10. **Hannachi N, *et al.*** Molecular analysis of HBV genotypes and subgenotypes in the Central-East region of Tunisia. *Virology Journal* 2010; **7**: 302.
11. **Meldal BH, *et al.*** A novel hepatitis B virus subgenotype, D7, in Tunisian blood donors. *Journal of General Virology* 2009; **90**: 1622–1628.
12. **Ben Farhat E, *et al.*** Bulletin of the National Vaccination Programme, April 2015 [in French]. Tunisian Ministry of Health, 2015.
13. **FitzSimons D, *et al.*** Hepatitis B vaccination: a completed schedule enough to control HBV lifelong? Milan, Italy, 17–18 November 2011. *Vaccine* 2013; **31**: 584–590.
14. **Shen LP, *et al.*** Epidemiological changes in hepatitis B prevalence in an entire population after 20 years of the universal HBV vaccination programme. *Epidemiology and Infection* 2011; **139**: 1159–1165.
15. **Shokri F, Jafarzadeh A.** High seroprotection rate induced by low doses of a recombinant hepatitis B vaccine in healthy Iranian neonates. *Vaccine* 2001; **19**: 4544–4548.
16. **Qawasmi M, *et al.*** Age-dependent decrease of anti-HBs titers and effect of booster doses using 2 different vaccines in Palestinian children vaccinated in early childhood. *Human Vaccines & Immunotherapeutics* 2015; **11**: 1717–1724.
17. **Zhang H, *et al.*** Seroprevalence and risk factors for hepatitis B infection in an adult population in Northeast China. *International Journal of Medical Sciences* 2011; **8**: 321–331.
18. **Floreani A, *et al.*** Long-term persistence of anti-HBs after vaccination against HBV: an 18 year experience in health care workers. *Vaccine* 2004; **22**: 607–610.
19. **Schonberger K, *et al.*** Determinants of long-term protection after hepatitis B vaccination in infancy: a meta-analysis. *Pediatric Infectious Disease Journal* 2013; **32**: 307–313.
20. **National Institute of Statistics.** (http://www.ins.nat.tn/indexfr.php). Accessed 8 February 2012.
21. **Ang LW, *et al.*** Seroepidemiology of hepatitis B virus infection among adults in Singapore: a 12-year review. *Vaccine* 2013; **32**: 103–110.
22. **Su FH, *et al.*** Hepatitis B seroprevalence and anamnestic response amongst Taiwanese young adults with full vaccination in infancy, 20 years subsequent to national hepatitis B vaccination. *Vaccine* 2007; **25**: 8085–8090.
23. **Chang HC, *et al.*** Seroprevalence of hepatitis B viral markers among freshmen – 20 years after mass hepatitis B vaccination program in Taiwan. *Journal of the Formosan Medical Association* 2007; **106**: 513–519.
24. **Ni YH, *et al.*** Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. *Annals of Internal Medicine* 2001; **135**: 796–800.
25. **Whittle HC, *et al.*** Long-term efficacy of continuing hepatitis B vaccination in infancy in two Gambian villages. *Lancet* 1995; **345**: 1089–1092.
26. **Da Villa G, *et al.*** Long-term epidemiological survey of hepatitis B virus infection in a hyperendemic area (Afragola, southern Italy): results of a pilot vaccination project. *Research in Virology* 1998; **149**: 263–270.
27. **Jeong SH, *et al.*** Changes in the intrafamilial transmission of hepatitis B virus after introduction of a hepatitis B vaccination programme in Korea. *Epidemiology and Infection* 2010; **138**: 1090–1095.
28. **Salleras L, *et al.*** Dramatic decline in acute hepatitis B infection and disease incidence rates among adolescents and young people after 12 years of a mass hepatitis B vaccination programme of pre-adolescents in the schools of Catalonia (Spain). *Vaccine* 2005; **23**: 2181–2184.
29. **Zanetti AR, *et al.*** Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. *Lancet* 2005; **366**: 1379–1384.
30. **Stroffolini T, *et al.*** The impact of the hepatitis B mass immunisation campaign on the incidence and risk factors of acute hepatitis B in Italy. *Journal of Hepatology* 2000; **33**: 980–985.
31. **Mele A, *et al.*** National Surveillance System for Acute Viral Hepatitis Collaborating Group. Acute hepatitis B 14 years after the implementation of universal vaccination in Italy: areas of improvement and emerging challenges. *Clinical Infectious Diseases* 2008; **46**: 868–875.
32. **Banatvala J, Van Damme P, Oehen S.** Lifelong protection against hepatitis B: the role of vaccine immunogenicity in immune memory. *Vaccine* 2000; **19**: 877–885.
33. **Kew MC.** Progress towards the comprehensive control of hepatitis B in Africa: a view from South Africa. *Gut* 1996; **38** (Suppl. 2): S31–36.
34. **Tsebe KV, *et al.*** The first five years of universal hepatitis B vaccination in South Africa: evidence for elimination of HBsAg carriage in under 5-year-olds. *Vaccine* 2001; **19**: 3919–3926.

35. **Coppola N, *et al.*** The long-term immunogenicity of recombinant hepatitis B virus (HBV) vaccine: contribution of universal HBV vaccination in Italy. *BMC Infectious Diseases* 2015; **15**: 149.
36. **Norouzirad R, *et al.*** Serum levels of anti-hepatitis B surface antibody among vaccinated population aged 1 to 18 years in ahvaz city southwest of iran. *Hepatitis Monthly* 2014; **14**: e13625.
37. **Bialek SR, *et al.*** Persistence of protection against hepatitis B virus infection among adolescents vaccinated with recombinant hepatitis B vaccine beginning at birth: a 15-year follow-up study. *Pediatric Infectious Disease Journal* 2008; **27**: 881–885.
38. **Alfaleh F, *et al.*** Long-term protection of hepatitis B vaccine 18 years after vaccination. *Journal of Infection* 2008; **57**: 404–409.
39. **Zhu CL, *et al.*** Presence of immune memory and immunity to hepatitis B virus in adults after neonatal hepatitis B vaccination. *Vaccine* 2011; **29**: 7835–7841.
40. **Chen CC, *et al.*** Epidemiology of hepatitis B virus infection among young adults in Taiwan, China after public vaccination program. *Chinese Medical Journal* 2007; **120**: 1155–1158.
41. **Lim WL, Wong DA, Cheng KC.** Immune response to hepatitis B vaccine in health care workers in Hong Kong. *Hong Kong Medical Journal* 1996; **2**: 138–140.
42. **Baghianimoghadam MH, Shadkam MN, Hadinedoushan H.** Immunity to hepatitis B vaccine among health care workers. *Vaccine* 2011; **29**: 2727–2729.
43. **Morris CA, *et al.*** Intradermal hepatitis B immunization with yeast-derived vaccine: serological response by sex and age. *Epidemiology and Infection* 1989; **103**: 387–394.
44. **Afifi SS, *et al.*** Serum level of anti-hepatitis B surface antigen among newborns and fully vaccinated infants and children aged 6 to 11 Years. *Australian Journal of Basic and Applied Sciences* 2009; **3**: 3239–3245.
45. **Behre U, *et al.*** Long-term anti-HBs antibody persistence and immune memory in children and adolescents who received routine childhood hepatitis B vaccination. *Human Vaccines & Immunotherapeutics* 2012; **8**: 813–818.
46. **Gilca V, *et al.*** Antibody persistence and the effect of a booster dose given 5, 10 or 15 years after vaccinating preadolescents with a recombinant hepatitis B vaccine. *Vaccine* 2013; **31**: 448–451.
47. **Theeten H, *et al.*** Universal hepatitis B vaccination in Belgium: impact on serological markers 3 and 7 years after implementation. *Epidemiology and Infection* 2014; **142**: 251–261.
48. **Jafarzadeh A, Montazerifar SJ.** Persistence of anti-HBs antibody and immunological memory in children vaccinated with hepatitis B vaccine at birth. *Journal of Ayub Medical College* 2006; **18**: 4–9.
49. **Bagheri-Jamebozorgi M, *et al.*** The persistence of anti-HBs antibody and anamnestic response 20 years after primary vaccination with recombinant hepatitis B vaccine at infancy. *Human Vaccines & Immunotherapeutics* 2014; **10**: 3731–3736.
50. **Poovorawan Y, *et al.*** Persistence and immune memory to hepatitis B vaccine 20 years after primary vaccination of Thai infants, born to HBsAg and HBeAg positive mothers. *Human Vaccines & Immunotherapeutics* 2012; **8**: 896–904.
51. **Lu CY, *et al.*** Humoral and cellular immune responses to a hepatitis B vaccine booster 15–18 years after neonatal immunization. *Journal of Infectious Diseases* 2008; **197**: 1419–1426.
52. **Lu CY, *et al.*** Waning immunity to plasma-derived hepatitis B vaccine and the need for boosters 15 years after neonatal vaccination. *Hepatology* 2004; **40**: 1415–1420.
53. **Banatvala J, *et al.*** Are booster immunisations needed for lifelong hepatitis B immunity? *Lancet* 2000; **355**: 561–565.
54. **Jack AD, *et al.*** What level of hepatitis B antibody is protective? *Journal of Infectious Diseases* 1999; **179**: 489–492.