ARTICLE IN PRESS

ELSEVIER

Vaccine 3528 (2002) 1–7

Vaccine

www.elsevier.com/locate/vaccine

Impact of universal vaccination programmes on the epidemiology of hepatitis B: 10 years of experience in Italy

Paolo Bonanni [a,*], Giovanna Pesavento [a], Angela Bechini [a], Emilia Tiscione [a], Francesco Mannelli [b], Cristiana Benucci [b], Antonella Lo Nostro [a]

[a] *Public Health Department, University of Florence, Viale G.B. Morgagni 48, 50134 Florence, Italy*
[b] *Pediatric Hospital A. Meyer, Florence, Italy*

Abstract

Ten years have elapsed since routine vaccination of infants and of 12-year-old adolescent was implemented in Italy.

In this period, evidence has accumulated on the epidemiological impact of universal immunisation.

Coverage is on average >90% and is ≥95% in many areas of the country. Incidence of acute hepatitis B, that was already declining before 1991, was further decreased by routine vaccination programmes.

This is particularly evident in adolescents and young adults (cohorts involved by mandatory vaccination), while incidence shows little changes in older subjects according to data of the last years. Prevalence of hepatitis B virus (HBV) markers detected by sero-epidemiological studies on anonymous sera confirms both the very high coverage with hepatitis B vaccination and the virtual absence of chronic HBsAg carriers in cohorts involved by routine vaccination programmes. The system of passive surveillance on adverse events following hepatitis B vaccination supports the excellent safety record of hepatitis B vaccines.

In a hyperendemic area of Southern Italy, where a pilot programme was firstly implemented, it was also possible to document the decline of the involvement of hepatitis B in chronic liver pathologies (from 48% in 1982 to 18% in 1997).

If coverage rates are maintained at the present levels, elimination of HBV transmission in Italy may be envisaged in few decades.

Keywords: Hepatitis B; Vaccination programmes; Epidemiology

1. Introduction

Hepatitis B is one of the major threats to public health in the world. More than 350 million people are chronic carriers and 1 million die each year for diseases caused by hepatitis B virus (HBV) infection. Among them, liver cirrhosis and hepatocellular carcinoma are the most important chronic consequences. For this reason, hepatitis B vaccine is also rightly considered as the first largely used vaccine against cancer.

In 1992, the World Health Assembly endorsed the recommendations of the Global Advisory Group of the Expanded Programme on Immunisation that hepatitis B vaccine be integrated into national immunisation systems of all countries by 1997.

Italy was one of the first industrialised countries to introduce a programme of routine vaccination against hepatitis B [1]. After a careful evaluation of both epidemiological and economic data [2,3], a double cohort policy of mandatory immunisation (infant and 12-year-old children) was chosen, together with active offer of free-of-charge vaccination to high-risk groups. The aim of the programme was to reduce, and, in the long term, to eliminate the transmission of the infection by rapidly creating 24 generations of immune subjects within the first 12 years of implementation. Law n.165 introducing compulsory vaccination was issued on 27 May 1991, but it was implemented in the whole country as of the beginning of 1992. Since 10 years have now elapsed, and the end of the adolescent programme is foreseen for the year 2003 (when the first children immunised as infants will reach the year 12 of life), it seems useful to verify the impact of routine immunisation on the epidemiology of the infection by analysing the trend and the change of several indicators.

2. Steps for the control of hepatitis B through routine vaccination

When the decision to introduce a universal programme of hepatitis B vaccination is taken, the first step is to implement it and to monitor coverage. This may be accomplished

* Corresponding author. Tel.: +39-55-3262437; fax: +39-55-3262436.
E-mail address: bonanni@dsp.igiene.unifi.it (P. Bonanni).

0264-410X/02/$ – see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0264-410X(02)00580-7

by comparing the vaccination register (number of subjects receiving the basic immunisation course) with the number of subjects of the same age group supplied by the birth register of each municipality where the study is performed. If a significant number of irregular immigrant is present, a precise estimate of their number and of their immunisation status should be obtained in order to get the real coverage in that area. A possible verification of coverage data may be obtained by evaluating the number of subjects within a certain age group (whose vaccination status is unknown) who test positive for anti-HBs shortly after the presumed time of completion of the immunisation course.

A further short-middle term consequence of the implementation of routine vaccination is the decline of the incidence of hepatitis B in the age cohorts covered by immunisation. As to this aspect, it is particularly important to investigate the risk factors and reasons of possible cases occurring in age groups subject to mandatory or recommended vaccination. When a case occurs in a vaccinated subject, it should be investigated whether this is due to a wild type or to a mutant virus, in order to monitor the long term efficacy and effectiveness of the currently used vaccine and foresee possible need of changes in vaccine composition.

Safety issues are becoming increasingly important in the perception of the public with regard to acceptance of routine immunisation. Since frequent and minor side effects are usually reported during clinical trials preceding vaccine registration, but rare and important adverse events are seen only after a large use of the new product, it is necessary to perform a continuous post-marketing surveillance to verify the safety in the field.

In a later phase, it is important to check the changing prevalence of HBV markers in different age groups as a consequence of the implementation of immunisation programmes.

The last step consists in the verification of a decline in the incidence and prevalence of chronic liver diseases (particularly cirrhosis and primary liver cancer) consequent to the wide use of routine immunisation in different cohorts of population.

3. Impact of hepatitis B routine vaccination in Italy

3.1. Schedule

In Italy, all pregnant women are subject to mandatory screening for HBsAg during the third trimester of pregnancy. The Italian schedule of vaccination consists of three doses given at 3, 5 and 11–12 months of life in infants born to HBsAg negative mothers. If born to a HBsAg positive mother, the newborn is administered a dose of specific immune globulin immediately after birth together with the first dose of vaccine at a different site. The vaccination course is completed by other three doses at 1, 2 and 11–12 months of life. Twelve-year-old adolescents are given three doses of vaccine according to a 0, 1, 6 months schedule, either at school or at vaccination services in health districts [1].

3.2. Monitoring of coverage

Data on coverage with hepatitis B vaccine should theoretically be available through systematic collection of the number of subjects immunised reported to the Department of Prevention of each Local Health Agency, which, on turn, should report data to each Regional Health Authority.

In reality, collection of data is almost always not complete, is performed differently in the various regions of the country, and in some cases only the number of doses administered is collected, with lacking information about number of completed and incomplete vaccination courses. For these reasons, the administrative method is not a reliable system (except for some particular situations) for monitoring coverage with mandatory and recommended vaccinations in Italy.

'Ad hoc' surveys based on the cluster sampling method as defined by the World Health Organisation were therefore performed in 1993 and in 1998 on cohorts of children born in different Italian regions in 1991 and 1996, respectively [4,5]. In the last survey, data relative to compulsory vaccinations showed a very good coverage throughout the country. In particular, coverage with three doses of hepatitis B vaccines ranged from 89.1% in Molise to 100% in Val d'Aosta (Table 1).

A study was also performed in 1998 in Tuscany (a region with 3.5 million inhabitants) on more than 22,000 children and adolescent living in different municipalities representative of the entire region. Birth certificates of newborns and of 12-year olds registered in such municipalities were used to calculate the yearly number of subjects eligible for the

Table 1
Coverage with three doses of hepatitis B vaccine (95%confidence intervals) at 24 months of age in 20 Italian Regions, 1998 (source: 5)

	Mean (95% CI)
Abruzzo	94.8% (91.4–98.2)
Basilicata	99.1% (97.8–100)
Bolzano	85.6% (80.6–90.7)
Calabria	94.8% (91.7–97.9)
Campania	97.6% (81.3–93.9)
Emilia R.	97.6% (95.7–99.6)
Friuli V.G	97.6% (95.7–99.6)
Liguria	97.6% (95.3–100)
Lombardia	97.6% (95.7–99.6)
Marche	94.8% (90.4–99.1)
Molise	89.1% (82.0–96.3)
Piemonte	98.6% (95.8–100)
Puglia	93.0% (89.1–96.9)
Sardegna	95.2% (92.4–98.0)
Sicilia	91.1% (86.1–96.1)
Toscana	95.2% (92.4–98.0)
Trento	98.1% (96.3–99.9)
Umbria	98.6% (97.0–100)
Val d'Aosta	100
Veneto	97.6% (95.7–99.6)

study. Coverage was defined as the proportion of eligible subjects who completed the three-dose vaccination regimen within 6 months after the scheduled time of last vaccine administration according to a vaccination certificate registered at local health districts. Overall, 95.0% of children and 95.4% of adolescents belonging to cohorts subject to mandatory immunisation were vaccinated between 1992 and 1997 [6]. Since that time, coverage has remained virtually unchanged. This is confirmed by the last estimates of the Italian Ministry of Health (February 2002) that indicate for Tuscany a coverage of 94.7% with three doses of HB vaccine in children <24 months of age in the year 2000 (data not shown). However, there are still some areas where coverage is sub-optimal; in particular, in some disadvantaged regions of the South, some adolescents escape school attendance and are not reached by immunisation services. Moreover, these subjects belong to social classes where the risk of HBV infection is higher [7].

As an indirect way to confirm the high immune coverage against hepatitis B infection of the children, adolescent and young adults cohorts in our region, we analysed a sub-set of data in a recently performed sero-epidemiological survey to investigate the prevalence of HBV markers in Tuscany. In the year 2000, 681 sera from subjects aged 1–50 years were collected in Florence. Samples from subjects of paediatric age were obtained from the emergency service of a paediatric hospital after exclusion of subjects with immune deficiency or applying for acute infectious diseases. Sera from older subjects were collected in two labs performing routine chemical chemistry analysis. Immune deficient subjects were excluded.

All samples were made anonymous by laboratory personnel, the only available data remaining age and sex. No information was available on vaccination status. The results of anti-HBs tests on samples in the age range 1–23 years, expressed as percentage of seroprotected subjects (titre ≥ 10 mIU/ml), are shown in Fig. 1. It is possible to note that between 1 and 4 years of age, seroprotection ranged between 97 and 100%. The increasing interval between immunisation and time of blood drawing explains the progressive decrease in the percentage of anti-HBs positive subjects between 5 and 8 years that, however, remains at values of 79–93%. The clear drop of positive subjects between 9 and 11 years of age is due to the fact that such samples belong to cohorts not subject to mandatory immunisation (some of them may have been immunised under request of their parents). The increase from 12 to 14 years reflects the progressive completion of the vaccination course in adolescent cohorts. The proportion of seropositive subjects remains high between 15 and 20 years (range 79–100%), while it is notably lower in individuals aged 21–23 years, who belong to cohorts not subject to compulsory vaccination. Therefore, also an analysis of anonymous sera confirms the excellent level of immunity to HBV acquired by age cohorts included in the programme of mandatory immunisation.

3.3. Decrease in the incidence of acute hepatitis B

Two surveillance systems for acute hepatitis are available in Italy. The first consists in the collection of official notifications for all infectious diseases at the Ministry of Health. According to the data from this surveillance system (Table 2) notified cases in Italy passed from 4124 in 1988 to 1575 in 1999 (cases reported in 1987 are not completely reliable because that was the first year when differentiation among types of viral hepatitis was introduced).

The other system of surveillance, called Integrated Epidemiological System of Acute Viral Hepatitis (SEIEVA) was started in 1985 with voluntary participation of local health districts throughout the country. They covered a large proportion of the Italian population and supplied information on type of hepatitis, on demographic characteristics of cases and on presumed sources of infection. Because the population covered was not the entire Italian population, data are expressed as rates of cases per 100,000 individuals (Fig. 2). It should be underlined that the trend of acute hepatitis B

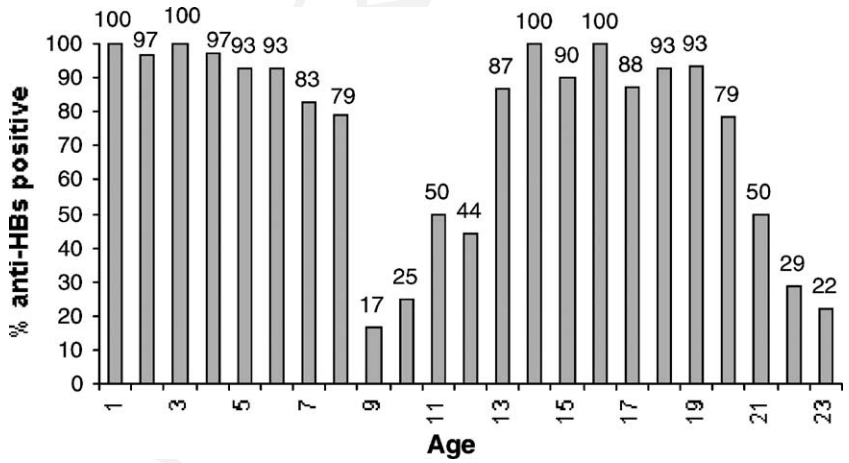

Fig. 1. Prevalence of anti-HBs reactivity (≥10 mIU/ml) in anonymous sera collected from subjects aged 1–23 years living in Florence (year 2000).

Table 2
Cases of viral hepatitis notified in Italy, 1982-1999 (source: Ministry of Health)

	Type				Total
	A	B	NANB	Non-specified	
1982	–	–	–	–	26.509
1983	–	–	–	–	28.251
1984	–	–	–	–	33.815
1985	–	–	–	–	18.386
1986	–	–	–	–	16.085
1987	2.007	2.189	662	10.062	14.920
1988	1.567	4.124	1.549	2.652	9.892
1989	1.295	3.915	1.510	1.510	8.230
1990	2.572	3.640	1.532	1.983	9.727
1991	2.764	3.260	1.560	1.363	8.947
1992	6.046	3.423	1.902	1.724	13.095
1993	3.308	3.344	1.788	982	9.422
1994	3.531	2.733	1.569	551	8.384
1995	1.434	2.600	1.455	390	5.879
1996	8.651	2.248	1.149	394	12.442
1997	9.952	1.996	932	303	13.183
1998	2.962	1796	845	122	5.725
1999	1.693	1575	788	101	4.157

notifications was already declining before the compulsory vaccination programme was introduced. The decrease was particularly evident in the age class 15–24 years, which traditionally showed the highest incidence rates. The reason of such decrease have been analysed elsewhere [8]. However, a further acceleration occurred shortly after the implementation of the routine immunisation programme.

In Tuscany, we recently collected data on notifications of acute hepatitis B and information on disease cases thanks to the collaboration of regional health authorities. Vaccination status and number and time of possible doses administered were collected for each notified case of acute disease.

Modifications of incidence between 1994 and 2001 were calculated by 5-year age groups in the age range 0–29 years, 10-year age groups between 30 and 49 years, and globally over 50 years of age. The results of the study are reported in Fig. 3. It is possible to note that, while incidence rates remained virtually unchanged in the age cohorts over 25 years, a clear decline was registered particularly in the age groups 15–19 years (from 7.3 to 1.3/100,000) and 20–24 years (from 14.3 to 3.7/100,000). The decline in the latter age group was particularly evident in 2001, when subjects aged 20 and 21 years belonged to cohorts subject to mandatory vaccination.

We further investigated the vaccination status of acute hepatitis B cases in the same time interval (1994–2001) and found that only five subjects had received a full immunisation course (three doses). The two cases occurring in 1994 regarded a 11-month-old infant and a 36-year-old woman. The former was a baby affected by Down's syndrome who had received the second dose of vaccine later than scheduled due to a cardiac surgery intervention. It is likely that infection was acquired during hospitalisation after a single dose. The woman had received the last vaccine dose in September 1993, while acute disease occurred in March 1994. However, an anti-HIV reactivity had been occasionally detected during hospitalisation for acute hepatitis B. One case occurred in the year 2000 in a 2-year-old girl of Chinese ethnicity. She was born to a HBsAg positive mother, and resulted to have received three doses of vaccine at birth and at 1 and 3 months of age, respectively. No documentation existed on administration of specific immune globulin immediately after birth and on receipt of the fourth dose at 1 year of age, as required by the Italian law. The two cases reported in 2001 regarded two men of 50 and 36 years of age, respectively. The reason for vaccination was unknown for the former, and no post-vaccination testing had been performed. The other man was an intravenous drug abuser

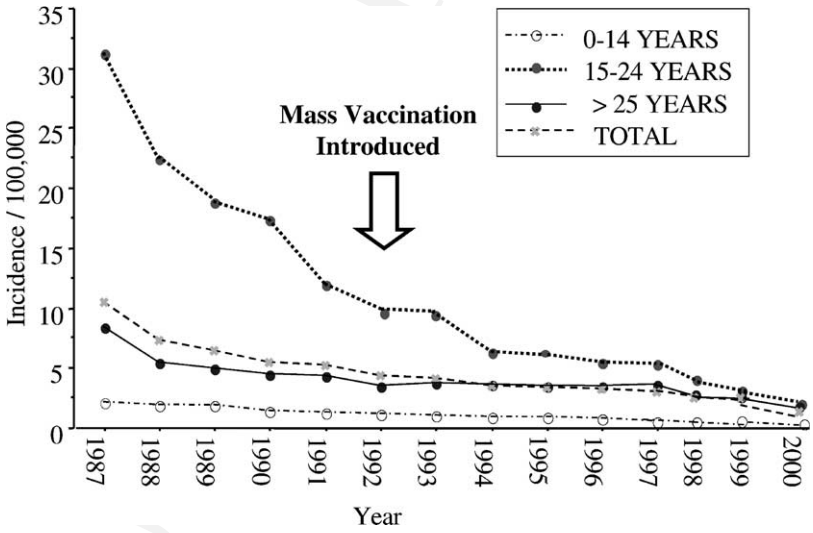

Fig. 2. Incidence of acute viral hepatitis B in Italy by age groups (Data from SEIEVA—Integrated Epidemiological System of Acute Viral Hepatitis).

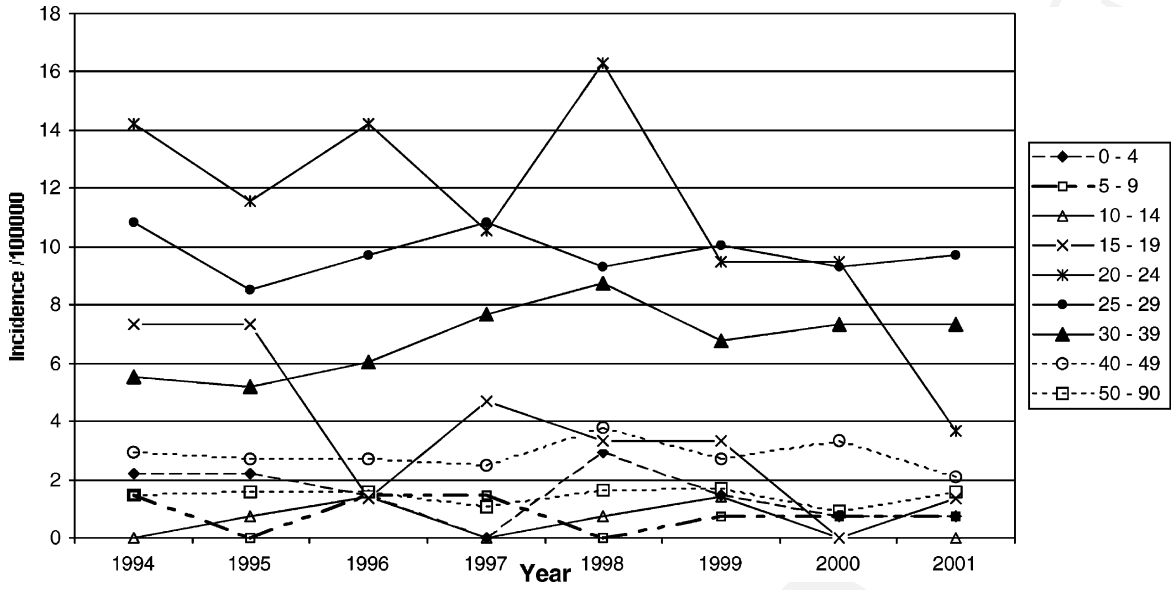

Fig. 3. Incidence of acute hepatitis B by age in Tuscany, Central Italy (1994–2001).

without post-vaccination testing. Samples from these two subjects were sent to the National Institute of Health (Istituto Superiore di Sanità) for molecular sequencing (results not yet available). Overall, it is possible to conclude that no evidence exists of a secondary failure of vaccination in Tuscany in the last 8 years. Primary non-response seems the most likely reason for cases occurring in adult age.

3.4. Importance of mutant viruses

Viral mutations reflect attempts by the virus to evade the mechanisms that potentially endanger its existence and to survive in a hostile environment. HBV mutants may be naturally occurring or originate under the pressure of the host's immune system or as a reaction against introduced antibodies [9]. Several immune escape mutants have been described, the most frequent of which involves the second loop of the *a* determinant (substitution glycine–arginine at amino acid 145: G145R mutant). G145R was described as responsible of breakthrough infections in passively/actively immunised infants born to HBsAg/HBeAg positive mothers [10] and during passive immune prophylaxis after transplantation [11]. From a public health point of view, the problem is to verify whether these mutants tend to spread and to become the predominant population of circulating viruses. In that case, a change in the composition of currently used vaccines should be envisaged. A recently published study performed in Italy was intended to address such problem [12]. The research involved 522 children born to HBsAg positive mothers from 1985 to 1994 in three public hospital in the Campania region. All babies were immunised with passive plus active prophylaxis immediately after birth. Among mothers whose complete serological status at delivery was traceable, 8.3% were also HBeAg positive.

In 1998/99, 17/522 children (3.3%) had acquired an asymptomatic infection as shown by the presence of anti-HBc alone ($n = 14$; none of them was HBV-DNA positive) or with simultaneous presence of HBsAg ($n = 3$). Two of the three carrier children had a wild type HBV (subtype ayw) similar to that of the respective mother. The third child had a double mutation within the '*a*' determinant and high level of anti-HBs. His mother had a wild type HBV (subtype ayw).

Five to 14 years after immunisation, 79% of children still had protective levels of anti-HBs. The authors of this large study on subjects belonging to a risk group where vaccine-induced escape mutants were firstly identified conclude that HBV mutants does not raise concern at present about the efficacy of universal vaccination programmes in Italy. Nevertheless, it is important to maintain a careful surveillance on the possible expansion of already known variants and emergence of new mutants, particularly as immune populations grow in number.

3.5. Surveillance on adverse events

In clinical trials preceding registration, all commercially available hepatitis B vaccines proved to be safe, and to cause minor side effects in proportion not superior to those registered in subjects receiving placebo, except for local pain, that was reported in up to 30% of vaccinees [13–15].

As for all vaccines, only frequent adverse events can be identified during phase 2 and phase 3 clinical trials, while rare effects (<1/1000 doses) can only be evaluated during post-marketing surveillance [16].

The occurrence of anaphylaxis after hepatitis B vaccination is proven, although it is a rare event (1/600,000 doses distributed). However, no fatal case is reported.

A number of other adverse events have been reported as temporarily associated with hepatitis B vaccination. Most of them are represented by chronic syndromes with unknown or partially known aetiology. Media attention is frequently attracted by these allegations, causing a fall in the confidence of the public in hepatitis B immunisation. However, it must be distinguished between hypothesis generation and hypothesis testing. It should also be considered that when a possible association between vaccination and adverse event is suggested by surveillance data, case reports and case series, we only see one side of the overall picture. As a matter of fact, we do not know whether the same event is occurring also in non-immunised subjects, and how many vaccinees are not experiencing the adverse event.

The recent allegations on the possible relationship between hepatitis B vaccination and demyelinating diseases like multiple sclerosis (MS) that had a large echo in France (where they caused a dramatic drop of coverage) had only a very limited impact in Italy.

The US Institute of Medicine recently reviewed the data of nine epidemiological studies and concluded that evidence favoured rejection of a causal relationship between hepatitis B vaccine administered to adults and incident multiple sclerosis or multiple sclerosis relapse.

Data of passive surveillance on adverse events following hepatitis B vaccination collected by the Italian Ministry of Health for the years 1991–1996 show that seven cases of paraesthesia, three cases of brachial plexus neuropathy, two cases of Guillain-Barré and one case of convulsions were reported as temporarily associated with immunisation. If we consider these figures vis-a-vis the millions of doses administered each year in the country, we can understand that the record of safety of hepatitis B vaccines is impressive. For these reasons, in September 1999 the Ministry of Health stated that, in view of a merely hypothetical and unproven damage, hepatitis B vaccination showed to be highly effective, and, therefore, the recommendation to immunise all infants and adolescents remained unchanged.

3.6. Changing prevalence of HBV markers and first impact on chronic liver disease

The previously cited sero-epidemiological study performed on sera collected from 681 subjects in Florence in the year 2000 was mainly intended to verify the trend of HBV markers after implementation of routine vaccination programmes.

Anti-HBc prevalence analysed by 10-year age group between 1 and 50 years showed that infection with HBV is extremely rare up to 20 years of age (no reactivity (0/239) between 1 and 10 years, and 0.9% (1/206) reactive samples in the 11–20-year age group). On the other hand, in the age groups 21–40 years, 9.3% of subjects (15/161) were anti-HBc positive, and the prevalence rose to 17.3% (13/75) in the 41–50 age group. The difference between age cohorts subject to mandatory vaccination (1–20 years) and not involved in routine immunisation (21–50 years) is statistically highly significant ($P = 0.0001$).

With regard to HBsAg prevalence, only 6/644 (0.9%) tested samples resulted reactive, and all of them were individuals not belonging to cohorts subject to compulsory immunisation.

A clear decline of HBV markers after implementation of routine hepatitis B vaccination was demonstrated in Afragola, an hyperendemic area in the Campania region, where a pilot project of universal infant hepatitis B vaccination was introduced in 1983. Incidence of acute hepatitis B before vaccination was 63/100,000; anti-HBc and HBsAg prevalence rates were 66.9 and 13.4%, respectively.

In 1997, 15 years after the implementation of the programme, the incidence had dropped to 3/1000,000 population. Anti-HBc prevalence declined to 34.2%; HBsAg prevalence was only 3.7%, and the change was particularly evident in young children and adolescents (from 6.8% in 1982 to 0.7% in 1997) [17].

Since chronic consequences of HBV carriage develop over a long time interval, it is too early to document the impact of routine vaccination on chronic active hepatitis, cirrhosis and hepatocellular carcinoma.

However, in the previously cited study in Afragola, it was possible to demonstrate that HBV was involved in 48% of chronic liver pathologies in 1982, but only in 18% in 1997 [17].

4. Conclusions

Since Italy was the first industrialised country to introduce universal hepatitis B vaccination in a double cohort of population (infant + adolescent), the surveillance on the changing epidemiology of HBV infection and related diseases may be of interest also for other countries. In the 10 years of implementation, a set of data has become available to document the impact of immunisation programmes.

A high coverage with three doses of vaccine in both cohorts was documented since the first years after introduction of routine vaccination [5,6]. Coverage with hepatitis B in infants and adolescents is on average >90%, and exceeds 95% in many areas of Italy. Sero-epidemiological data on anonymous sera collected in Tuscany confirm the high level of protection in the cohorts subject to mandatory vaccination.

Surveillance on acute hepatitis B cases consistently shows a decline of notifications, especially in the 15–24 years age group. Data from Tuscany and from the rest of Italy show the virtual absence of acute HB cases in subjects belonging to compulsorily vaccinated cohorts who completed the immunisation course.

In Tuscany, no case of reactivity for HBsAg was detected in age groups covered by universal immunisation, and only one sample was anti-HBc positive in a series of samples collected in the year 2000. The difference between immu-

nised and non-immunised cohorts was statistically highly significant. Long-term surveillance on children born to HBsAg positive mothers shows the occurrence of a very limited number of asymptomatic and almost always self-limiting infections in vaccinees. At present, mutant viruses do not pose a threat to universal vaccination programmes in the country.

However, the steady incidence in older age groups, and the demonstration of the role of sexual and iatrogenic exposures [18] stress the importance to complement routine immunisation with non-immunological preventive measures.

In conclusion, the data presented in our paper consistently demonstrate the deep epidemiological impact 'on the field' of 10 years of implementation of the universal vaccination programme against hepatitis B in Italy and confirm that elimination of HBV transmission in the country in the middle-long term is a feasible goal.

References

[1] Bonanni P. Implementation in Italy of a universal vaccination programme against hepatitis B. Vaccine 1995;13(Suppl 1):S68–71.
[2] Crovari P, Cuneo Crovari P, Coppola RC, Icardi GC, Bonanni P. Efficacia e sicurezza dei vaccini contro l'epatite B. In: Fondazione SmithKline, editor. La vaccinazione contro l'epatite B: una scelta prioritaria di politica sanitaria ed economica. Milano, Franco Angeli Editore; 1990. p. 59–84.
[3] Dellamano R. Vaccinare contro l'epatite B: un costo senza ritorno o un buon investimento? In: Ghetti V, editor. La Vaccinazione di Massa contro l'Epatite B in Italia. Basi Fisio-patologiche, Epidemiologiche e Socio-economiche. Milano, Franco Angeli Editore; 1991. p. 31–76.
[4] The Italian Vaccine Coverage Survey Working Group. Childhood vaccination coverage in Italy: results of a seven-region survey, Bull WHO 1994;72:885–95.
[5] Salmaso S, Rota MC, Ciofi degli Atti ML, Tozzi AE, Kreidl P. and the ICONA Study Group: infant immunisation coverage in Italy: estimates by simultaneous EPI cluster surveys of regions. Bull WHO 1999;77:843–51.
[6] Bonanni P, Colombai R, Gasparini R, et al. Impact of routine infant and adolescent hepatitis B vaccination in Tuscany, Central Italy. Pediatr Infect Dis J 1999;18:677–82.
[7] Stroffolini T, Cialdea L, Tosti ME, Grandolfo M, Mele A. Vaccination campaign against hepatitis B for 12-year-old subjects in Italy. Vaccine 1987;15:583–5.
[8] Bonanni P, Crovari P. Success stories in the implementation of universal hepatitis B vaccination: an update on Italy. Vaccine 1998; 16:S38–42.
[9] Francois G, Kew M, Van Damme P et al. Mutant hepatitis B viruses: a matter of academic interest only or a problem with far-reaching implications? Vaccine 2001;3799–3815.
[10] Carman WF, Zanetti AR, Karayiannis P, et al. Vaccine-induced escape mutant of hepatitis B virus. Lancet 1990;336:325–9.
[11] Hawkins AE, Gilson RJ, Gilbert N, et al. Hepatitis B virus surface mutations associated with infection after liver transplantation. J Hepatol 1996;24:8–14.
[12] Mele A, Tancredi F, Romanò L, et al. Effectiveness of hepatitis B vaccination in babies born to hepatitis B surface antigen-positive mothers in Italy. J Infect Dis 2001;184:905–8.
[13] Szmuness W, Stevens CE, Harley EJ, et al. Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. N Engl J Med 1980;303:833–41.
[14] Francis DP, Hadler SC, Thompson SE, et al. The prevention of hepatitis B with vaccine: report of the centers for disease control multi-center efficacy trial among homosexual men. Ann Intern Med 1982;97:362–6.
[15] Lewis E, Shinefield HR, Woodruff BA, et al. Safety of neonatal hepatitis B vaccine administration. Pediatr Infect Dis J 2001;20:1049–54.
[16] Anthony BF. FDA perspective on regulatory issues in vaccine development. In: Williams JC, Goldenthal KL, Burns DL, Lewis Jr BP, editors. Combined vaccines and simultaneous administration: current issues and perspectives, vol. 754. New York: Annals of the New York Academy of Sciences; 1995. p. 10–16.
[17] Da Villa G, Piccinino F, Scolastico C, et al. Long-term epidemiological survey of hepatitis B virus infection in a hyperendemic area (Afragola, southern Italy): results of a pilot vaccination project. Res Virol 1998;149:263–70.
[18] Stroffolini T, Mele A, Tosti ME, et al. The impact of the hepatitis B mass immunisation campaign on the incidence and risk factors of acute hepatitis B in Italy. J Hepatol 2000;33:980–5.