

# CHANGES IN THE EPIDEMIOLOGY OF HEPATITIS B VIRUS INFECTION FOLLOWING THE IMPLEMENTATION OF IMMUNISATION PROGRAMMES IN NORTHEASTERN GREECE

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The objective of this study was to investigate changes in the epidemiology of hepatitis B virus infection in the general population and selected groups of immigrants in the region of northeastern Greece over the last decade in relation to the introduction of hepatitis B vaccination programmes. Two population-based seroprevalence surveys were carried out during the years 1992-1994 and 1998-2006. In total, 25,105 individuals were tested for the presence of hepatitis B virus markers: HBsAg, anti-HBs and anti-HBc. Childhood/adolescence immunisation programmes began early in 1994 in selected groups of immigrants and were complemented by the national vaccination programme in 1998. Between 1992-1994 and 1998-2006, the HBsAg carrier rate declined from 5.4% [95% CI: 4.5-5.9] in adults (20-60 years old) and 1.9% [95% CI: 1.6-2.4] in children/adolescents (5-19 years old) of indigenous residents to 3.4% [95% CI: 2.9-3.8] and 0.6% [95% CI: 0.2-1.4] respectively ( $p < 0.05$ ). In spite of a decrease compared with 1992-1994, the percentage of HBsAg carriers was still relatively high in 1998-2006 among the Muslim religious minority group (8.2% [95% CI: 8.0-8.7] in adults and 2% [95% CI: 1.7-2.4] in children/adolescents) and in immigrants from the former Soviet Union (4.3% [95% CI: 3.6-4.7] in adults and 1.1% [95% CI: 0.8-2.4] in children/adolescents) ( $p < 0.05$  for both selected groups versus general population). The decline of the prevalence of HBsAg in the general population and selected groups of immigrants in northeastern Greece over the last decade supports the effectiveness of the ongoing immunisation programme although the information on the actual number of cases of acute HBV infection is not available.

### Introduction

Although hepatitis B virus (HBV) infection is a major public health problem throughout the world, the geographic variation in the epidemiology of this infection is considerable.

The prevalence of hepatitis B surface antigen (HBsAg) in the general population varies widely between European countries with high to intermediate HBsAg carrier rates in Turkey (8%), Bulgaria (4%), and Greece (2%) [1]. Furthermore, the prevalence of HBV infection in the Russian Federation and Ukraine is classified as intermediate with prevalence of HBsAg ranging from 2% to 7% [2].

In Greece, several recent studies investigated the prevalence of hepatitis B virus markers in certain risk groups, such as blood donors, healthcare workers, injecting drug users, alcoholics, pregnant women and small size populations [3-8]. In addition, a few prevalence studies in the general population are available which show a geographic variation of HBsAg seropositivity from 1.9% to 5% [9-11].

In 1994, a comprehensive hepatitis B immunisation programme was implemented only in the region of Thrace in northeastern Greece which covers three prefectures: Xanthi, Rodopi and Evros. The programme included vaccination of first generation immigrants from former Soviet Union and of the Muslim religious minority group of all ages. In 1998, a national vaccination programme for hepatitis B was started, including vaccination of pregnant women, infants (0-2 years old), children 5-6 years old and adults at high-risk of infection such as hospital workers, sexually active heterosexuals (more than one partner in the past six months), men who have sex with men, individuals diagnosed with a sexually transmitted infection (STI), illicit drug users (injecting, inhaling, snorting, pill popping), sex contacts or close household members of an infected person, children adopted from countries where hepatitis B is common (in Asia, Eastern Europe, and the Middle East), families of children adopted from the countries listed above, immigrants from countries where hepatitis B is common (listed above), individuals born to parents who have emigrated from countries where hepatitis B is common (listed above), recipients of a blood transfusion before 1992, renal dialysis patients and those in early renal failure [12]. Both vaccination programmes (regional and national) are free of charge.

The aim of this study was to evaluate the effectiveness of the HBV vaccination strategies in Thrace by analysing the data from two large population sero-surveys that were conducted in this region, one before and one after the vaccination programmes. Another objective was to identify variables that were independently associated with HBV infection, such as age and origin of residents.

## Material and methods

### Study population

Two community-based sero-surveys were conducted during the years 1992-1994 and 1998-2006 in Thrace. The estimated population of this region is 368,993 inhabitants [13]. A total of 25,105 individuals were investigated by physicians of the Unit of Preventive Medicine of Social Security Institute in Alexandroupolis: 14,483 during the first survey of 1992-1994 and 10,622 in the second survey of 1998-2006. People counted twice in the same serosurvey, foreigners or Greek visitors and adults older than 60 years were excluded from the sample population. The mobile survey unit visited almost all areas of the three prefectures of Thrace: Xanthi, Rodopi and Evros, including urban and rural areas, hard to reach mountains and plains. The adult population consisted of interested volunteers who were informed about the study by the local media. Young people 5-19 years old were included through screening organised stepwise by visiting all schools in the region and stratified according to geographic region (stratified random sampling).

The population sample was divided into the following groups: 1) immigrants born in countries of the former Soviet Union ('immigrant group'), 2) indigenous residents of Greek origin ('majority population group' or 'indigenous residents') and, 3) Muslim Thracians of Turkish origin ('Muslim religious minority group'). The above groups were further divided according to age into two groups: 5-19 and 20-60 years old. Children younger than 5 years of age were not included because the screening for children was organised in schools, while adults older than 60 years did not show interest to participate in the screening program.

### Ethical approval

The study protocol was approved by the Research and Ethics Committee of the Unit of Preventive Medicine of the Social Security Institute of Greece and the Research Committee of the Ministry of Health and Welfare. The purpose and the protocol of the study were clearly explained. Informed consent was requested before a blood specimen was collected from each participant. For children and young participants <18 years old, informed consent was obtained from the parents or guardians. Only consenting volunteers, or children and young participants of consenting parents/guardians were included in the study.

The study was carried out in accordance with guidelines of the Declaration of Helsinki and was approved by the Hellenic Center for Infectious Diseases Control. All participants remained anonymous throughout the survey.

### Serological testing and interpretation

All serum samples were stored at -20°C until testing for HBV markers. The number of samples collected from the study population at first and second survey period is shown in Tables 1, 2 and 3. All blood samples in both studies were tested for the presence of hepatitis B surface antigen (HBsAg). Testing for antibodies to HBsAg (anti-HBs) and antibodies to hepatitis B core antigen (anti-HBc) was performed on all samples in the second serosurvey and on samples from the age group 5-19 only in the first serosurvey. Adults in the first serosurvey were tested for the HBsAg marker only. The tools used were enzyme immunoassay (EIA, Abott Diagnostics, Germany) during the first survey period and a fully automated microparticle enzyme immunoassay (Abbott AxSYM System version 3.0, Abbott Diagnostics, Germany) during the second period.

Individuals with anti-HBs antibodies alone [HBsAg(-)/anti-HBc(-)/anti-HBs(+)] were considered to have evidence of post vaccination immunity whereas those with positive anti-HBc and anti-HBs antibodies [HBsAg(-)/anti-HBc(+)/anti-HBs(+)] were considered to have evidence of past infection. In case of individuals who tested only anti-HBc-positive it was assumed that HBsAg had disappeared in long-term virus-carriers and the titer of anti-HBs was very low (undetectable) or HBsAg seroconversion to anti-HBs would occur later. Finally, individuals with HBsAg (+) only were considered to have evidence of acute HBV infection.

### Statistical analysis

The sample was considered representative of the general population of Thrace between 5-60 years of age, as compared with age and other parameters. Statistical comparisons were performed using chi-squared (X<sup>2</sup>) test with SPSS software (SPSS Inc.). The level of significance was set at 5%.

The method of log-linear models, a type of stepwise logistic regression for discrete variables, was used to identify variables that were independently associated with HBV infection, such as age and origin of residents. The risk of infection for each variable was investigated by measuring both the unadjusted and the adjusted relative risk (RR).

## Results

### First epidemiological survey (1992-1994): Prevalence of HBV markers in selected groups compared to general population

The results of the first survey are shown in Tables 1, 3, and 4. Among the indigenous residents, 205 of the 3,789 adults (5.4%) [95% CI: 4.5-5.9] and 152 of the 7,864 children/adolescents (1.9%) [95% CI: 1.6-2.4] were HBsAg (+). Seroprotection rate varied with age from 1.7% (58/3408) [95% CI: 1.4-2.2] in adolescents to 10% (45/4456) [95% CI: 8.5-12.6] in children (Table 4). Interestingly, the prevalence of HBsAg among adolescents was higher than among children - 2.9% (101/3408) [95% CI: 1.9-2.8] compared with 0.92% (41/4456) [95% CI: 0.7-1.4] (Table 4).

Among the Muslim religious minority group, the prevalence of HBsAg was 9.9% (116/1165) [95% CI: 8.5-9.9] in adults and 5.1% [95% CI: 4.1-5.9] (33/643) in children/adolescents. In the group of immigrants from the former Soviet Union the rates were 5.3% (32/610) in adults [95% CI: 4.7-5.8] and 1.7% (7/412) in children/adolescents [95% CI: 1.1-2.4] (Table 1). The HBsAg prevalence was higher in adults and children/adolescents of

TABLE 1

**Prevalence of hepatitis B surface antigen (HBsAg) before and after the introduction of vaccination programme: results of two seroprevalence studies in Thrace, northern Greece (period A: 1992-1994, period B: 1998-2006), according to ethnic origin and age (n=25,105)**

Age group	HBsAg seroprevalence (%)			
	5-19 years old		20-60 years old	
Period	A	B	A	B
Indigenous residents	1.9 (152/7,864)	0.6 (211/3,538)	5.4 (205/3,789)	3.4 (113/3,338)
Immigrants from the former Soviet Union	1.7 (7/412)	1.1 (4/363)	5.3 (32/610)	4.3 (20/463)
Muslim religious minority	5.1 (33/643)	2 (33/1,632)	9.9 (116/1,165)	8.2 (106/1,288)

Muslim religious minority group than in immigrants and indigenous residents ( $p < 0.001$ ) but the differences between the prevalence rates in immigrants and indigenous residents were not significant.

Seroprotection rate was low among children/adolescents of Muslim religious minority 7.7% (50/643) [95% CI: 6.0-8.4] and 4.6% (19/412) [95% CI: 4.1-5.4] among immigrants of the same age group (Table 3).

Second epidemiological survey (1998-2006): Prevalence of HBV markers in selected groups compared to general population

The findings of HBV markers of people screened in the second epidemiological survey are shown in Tables 1-4.

The prevalence of HBsAg was significantly higher in immigrants from the former Soviet Union both in adults and children/adolescents ( $p = 0.03$  in 5-19 and  $p = 0.001$  in 20-60 years age group) and in the Muslim religious minority group ( $p = 0.0001$  for adults and  $p = 0.0002$  children/adolescents) compared to indigenous residents. In details, prevalence of HBsAg was 8.2% [95% CI: 8.0-8.7] in adults and 2% [95% CI: 1.7-2.4] in children of Muslim religious minority group, 4.3% [95% CI: 3.6-4.7] and 1.1% [95% CI: 0.8-2.4] of immigrants from the former Soviet Union and, 3.4% [95% CI: 2.9-3.8] and 0.6% [95% CI: 0.2-1.4] of indigenous residents, respectively (Table 1). The prevalence of HBsAg was higher in adults and children/adolescents of Muslim

religious minority group than those of indigenous residents and of immigrants from the former Soviet Union ( $p = 0.0001$  and  $p = 0.0007$  for adults and  $p = 0.0002$  and  $p = 0.006$  for children/adolescents, respectively).

The highest proportion of individuals with anti-HBs only positivity was found in the group of 5-19-year-old members of the Muslim religious minority group (73%) [95% CI: 69.5-78.3], followed by children of the indigenous residents (45%) [95% CI: 42.9-48.8] and of the immigrants from the former Soviet Union (38%) [95% CI: 34.9-41.2], ( $p = 0.001$ ) (Table 3). No significant differences were observed among immigrants and indigenous residents ( $p = 0.26$ ).

#### Risk factors for HBV infection

Multivariate analysis showed that older age and the origin of residents (immigrants from the former Soviet Union and residents from Muslim religious minority group) were independent risk factors for HBV infection (HBsAg positivity) (Table 5).

#### Effect of vaccination on pattern of HBV infection

In the indigenous residents the prevalence of HBsAg dropped significantly after the vaccination period, as shown from the two prevalence studies, from 5.4% (205/3789) [95% CI: 4.5-5.9] to 3.4% (113/3338) [95% CI: 2.9-3.8] in the adult group and from 1.9% (152/7864) [95% CI: 1.6-2.4] to 0.6% (211/3538) [95% CI: 0.2-1.4] in the 5-19 years age group (Table 1).

TABLE 2

Prevalence of hepatitis B virus (HBV) markers among adults aged 20-60 years, divided by ethnic origin, in the second seroprevalence study in Thrace, northern Greece, 1998-2006 (n=5,089)

Groups	HBV markers							
	HBsAg (+)		anti-HBc(+) only		anti-HBc (+) and anti-HBs (+)		anti-HBs (+)	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Immigrants from the former Soviet Union (n=463)	20	4.3	31	6.7	77	16.6	15	3.2
Muslim religious minority (n=1,288)	106	8.2	80	6.2	234	18.2	190	14.8
Indigenous residents (n=3,338)	113	3.4	200	6	551	16.5	213	6.4

TABLE 3

Prevalence of hepatitis B virus (HBV) markers in children and adolescents aged 5-19 years, divided by ethnic origin, in the first (period A: 1992-1994) and second (period B: 1998-2006) seroprevalence study in Thrace, northern Greece (n=14,452)

Study population		HBV markers							
		HBsAg (+)		anti-HBc+ only		anti-HBc (+) and anti-HBs (+)		anti-HBs (+)	
Groups	Period	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Immigrants from the former Soviet Union	A (n=412)	7	1.7	7	1.7	14	3.4	19	4.6
	B (n=363)	4	1.1	4	1.2	9	2.6	14	3.8
Muslim religious minority	A (n=643)	33	5.1	15	2.3	44	6.8	50	7.7
	B (n=1,632)	33	2.0	13	0.8	49	3	1,197	73.3
Indigenous residents	A (n=7,864)	152	1.9	102	1.3	197	2.5	103	1.3
	B (n=3,538)	21	0.6	30	0.85	68	1.9	1,581	44.7

Furthermore, despite the relatively low percentages of immunised immigrants of 5-19 years old the prevalence of HBsAg decreased from 1.7% (7/412) [95% CI: 1.1-2.4] to 1.1% (4/363) [95% CI: 0.8-2.4] with a concurrent increase in immunised children/adolescents (5-19 years old) from 4.6% (19/412) [95% CI: 4.1-5.4] up to 38% (14/363)[95% CI: 34.9-41.2] (Table 3).

Moreover, the percentage of immunised individuals in the 5-19 years old group of Muslim religious minority group has markedly increased from 7.7% (50/643) [95% CI: 6.4-8.4] to 73% (1197/1632) [95% CI: 69.5-78.3] while the prevalence of

HBsAg decreased from 5.1% (33/643) [95% CI: 4.1-5.9] to 2% (33/1632) [95% CI: 1.7-2.4] (Table 3).

### Discussion

This study describes the prevalence of HBV markers in the general population of northeastern Greece (Thrace) and in selected immigrant groups and investigates the impact of vaccination programmes – a regional one started in 1994 in northeastern Greece and a national one implemented in 1998. Two large population-based surveys involving in total 25,105 individuals were carried out, the first one in the period 1992-1994 preceding the

TABLE 4

Prevalence of hepatitis B virus (HBV) markers in different age groups of indigenous residents in the first (period A: 1992-1994) and second (period B: 1998-2006) seroprevalence study in Thrace, northern Greece (n=18,529)

Age groups (in years)	Period	(n)	HBV markers							
			HBsAg (+)		anti-HBc (+)		anti-HBc (+) and anti-HBs(+)		anti-HBs (+)	
			(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
5-12	A	4,456	41	0.92	43	0.9	48	1.1	45	10
	B	1,714	5	0.23	14	0.08	14	0.8	1,063	62
13-19	A	3,408	101	2.9	59	1.7	148	4.3	58	1.7
	B	1,824	16	0.9	16	0.8	54	3	518	28
20-30	A	930	17	1.8	*	*	*	*	*	*
	B	800	7	0.9	7	1.3	64	8	41	5.1
31-40	A	1,240	40	3.2	*	*	*	*	*	*
	B	1,010	15	1.4	60	5.9	158	15.6	77	7.6
41-50	A	912	85	9.3	*	*	*	*	*	*
	B	930	57	6.1	67	7.2	207	22.3	70	7.5
51-60	A	707	63	8.9	*	*	*	*	*	*
	B	598	34	5.5	66	11	122	20.4	25	4.1

\*In the first seroprevalence study (period A: 1992-1994) the adult population was not tested for anti-HBc and anti-HBs

TABLE 5

Independent risk factors for HBsAg carrier state. Multivariate analysis of data from the second seroprevalence study in Thrace, northern Greece, 1998-2006

Risk factors	Sample	HBsAg(+)	RR [95% CI] Adjusted	p
<b>Age groups</b>				
5-19 years	3538	21 (0.6%)	1*	
20-30 years	800	7 (0.9%)	2.97 [0.19-13]	>0.05
31-40 years	1010	15 (1.4%)	5.19 [0.19-19.2]	<0.01
41-60 years	1528	91 (5.8%)	16.72 [2.34-57.3]	<0.001
<i>Age group 20- 60 years by ethnic origin</i>				
Indigenous residents	3338	113 (3.4%)	1*	
Muslim religious minority	1288	106 (8.2%)	10.82 [1.78-89.2]	<0.0001
Immigrants from the former Soviet Union	463	20 (4.3%)	1.98 [0.34-19.3]	>0.05
<i>Age group 5-19 years by ethnic origin</i>				
Indigenous residents	3538	21 (0.6%)	1*	
Muslim religious minority	1632	33 (2%)	23 [4.9-136.2]	<0.0001
Immigrants from the former Soviet Union	363	4 (1.1%)	17.9 [2.1-98]	<0.001

\*Reference group

vaccination programmes and the second one in the period 1998-2006 following the implementation of vaccination programmes.

We have observed an impact of the immunisation programmes on the prevalence of HBsAg in all the study groups. Indeed, the HBsAg prevalence declined significantly in children and adolescents of all groups: indigenous residents (from 1.9% in 1992-1994 to 0.6% in 1998-2006), immigrants from the former Soviet Union (from 1.7% to 1.1%) and Muslim religious minority (from 5.1% to 2%). In the adult population, the HBsAg prevalence of indigenous residents also declined from 5.4% to 3.4%, of immigrants from 5.3% to 4.3% and of Muslim religious minority from 9.9% to 8.2%. However, this decline may also be related to factors other than vaccination, such as improvement of quality of life, use of disposable medical equipments and screening of blood donors and pregnant women.

The 3.4% prevalence of HBsAg in the adult indigenous residents (majority population group) obtained in the second survey was within the range reported by other studies (1.9%-5%) in Greece [9-11].

However, compared to other European countries, the burden of hepatitis B in northeastern Greece is higher. The prevalence of HBsAg in the general population varies widely between European countries with high to intermediate HBsAg carrier rates: in Turkey (8%), Romania (6%), Bulgaria (4%), Latvia (2%) and Greece (2%) [1]. In the Slovak Republic, Poland, Czech Republic, Belgium, Lithuania, Italy and Germany the HBsAg prevalence is 0.5%-1.5% and in the Netherlands, Estonia, Hungary, Slovenia and Norway below 0.5% [1,14]. Various studies in the past decade and recent years make comparisons difficult [1,2,14-19]. In France, the prevalence of anti-HBc and HBsAg in persons of French origin was 2.2% and 0.2% [16], in Belgium 6.9% and 0.7% [17], in Spain 10.2% and 0.9% [18], in Germany 8.71% and 0.62% [19], respectively. This might reflect differences in the epidemiology such as lower infection rates in newborns and infants corresponding to lower rates of chronicity.

The incidence of reported HBV cases in the European Union (EU) and European Economic Area / European Free Trade Association (EEA/EFTA) countries has declined over the past ten years from 6.7 cases per 100,000 population in 1995 to 1.5 cases per 100,000 population in 2006 [20]. However, although a notification system exists in Greece for acute and chronic HBV infection, there is significant underreporting because of the lack of compliance of doctors and unclear case definition and therefore changes in epidemiology of hepatitis B cannot rely on the reported incidence of acute hepatitis as in other countries.

In indigenous residents the HBsAg prevalence in age groups 5-19 and 20-60 years old (0.6% and 3.4% respectively) was somewhat higher than usually assumed (0.33-2.3%) in Greece [1]. However, a statistically higher prevalence of HBsAg was observed in age group 13-19 years (0.9%) compared with age group 5-12 years (0.23%), reflecting differences in the proportion of immunised children of these groups (28% and 62%, respectively). These findings support the fact that horizontal transmission from child to child and from mother to child has been eliminated due to vaccination and medical checks. Universal prenatal screening and infant immunisation will contribute to a further decline of HBV infection.

In many European countries immigrants from highly endemic regions are from 5 to 90 times more frequently affected by HBV

than the general population [21-23]. Indeed, in our study, the prevalence of HBsAg among both the adult (4.3%) and the 5-19 years old (1.1%) groups of immigrants from the former Soviet Union was higher compared to the rates in the general population (3.4% and 0.6%, respectively). However, the prevalence of HBsAg among the immigrants of age group 5-19 (1.1%) was lower than that among children of the Muslim religious minority group (2%). Two Greek studies reported prevalence of HBsAg of 2.8% and 2.7% in groups of immigrants aged 12-18 years [22,24]. Higher rates of HBsAg positivity have been reported in other studies such as from the United States and Israel which reported prevalence of HBsAg of 4% in immigrants aged <20 years and 9% in 20-70 years age group [25,26]. Moreover, the children of first generation immigrants continue to have high prevalence of HBV infection as those of Muslim religious minority group. The overcrowded families may facilitate child to child transmission of HBV in the family.

We have also found a high rate of HBsAg prevalence (8.2%) in adults of Muslim religious minority. This high rate may be explained by higher risk of exposure such as poor adherence to standard control measures such as absence of screening pregnant women, childbirth at home, early weddings at the age of 12 years.

With respect to the strength of our study we should clarify that the sample adult population consisted of all interested adults whereas for the younger participants aged 5-19 years old samples stratified by age and geographic area were collected without selection bias from the participants at schools. We consider this study group to be representative of the general population of Thrace as compared with age and other parameters. Finally, this standardised methodology has been widely used and allows future comparative analyses to be performed [27].

Our study has several limitations. The results relied on serological data collected in the two surveys. The registration reporting system from the results recorded did not distinguish acute from chronic HBV infection for adults in the first survey since they were only screened for the marker HBsAg. Therefore, also some adult patients with a resolved or past infection could not be in the register. Incidence data and data regarding complications of chronic HBV infection are lacking. However other studies from this area provide such information [28,29].

In conclusion this study indicates the decline of the prevalence of HBsAg in the general population and selected groups of northeastern Greece over the last decade reflecting the effectiveness of HBV vaccination. Despite that, HBsAg prevalence remains high in certain communities such as immigrants and Muslim religious minority. Prevention programmes based on education and specific precautions for transmission along with vaccination are important.

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