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INFECTIOUS DISEASES

A population-based prevalence study of hepatitis A, B and C virus using oral fluid in Flanders, Belgium

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Abstract. Ten years after the first seroprevalence study performed in Flanders, the aim of this cross sectional study was to follow the evolution of hepatitis A, B and C prevalence. The prevalence of hepatitis A antibodies, hepatitis B surface antigen and hepatitis C antibodies was measured in oral fluid samples collected by postal survey. Using the National Population Register, an incremental sampling plan was developed to obtain a representative sampling of the general population. A total of 24,000 persons were selected and 6,000 persons among them contacted in a first wave. With 1834 participants a response rate of 30.6% was achieved. The prevalence was weighted for age and was 20.2% (95% CI 19.43–21.08) for hepatitis A, 0.66% (95% CI 0.51–0.84) for hepatitis B surface antigen and 0.12% (95% CI 0.09–0.39) for hepatitis C. The prevalence of hepatitis A and C in the Flemish population is lower in 2003 compared with the results of the study performed in 1993. The difference may be due to a real decrease of the diseases but also to differences in the methodology. The prevalence of hepatitis B surface antigen remains stable. Considering the 30% response rate and the high quality of the self-collected samples as reflect of a good participation of the general population, saliva test for prevalence study is a good epidemiological monitoring tool.

Key words: Hepatitis A, Hepatitis B, Hepatitis C, Oral fluid, Postal survey, Prevalence

Abbreviations: IPH = Scientific Institute of Public Health; PSU = Primary Sampling Units

Introduction

Population-based prevalence studies are a valuable tool for surveillance, which provides important data on the susceptible groups and the potential for future outbreaks. In order to monitor the prevalence over time and to evaluate changes in the epidemiological trends, estimates of the prevalence need to be obtained at regular interval. Such a surveillance system is essential for public health applications, allowing policy makers to adapt or to adopt preventive measures.

Depending on the type of hepatitis virus involved, the burden of the disease differs. Viral hepatitis is a primary infection of the liver caused by one of at least six identified types of viral hepatitis. Hepatitis A, hepatitis B and hepatitis C are the most common types. The public health importance of these infections depends on the incidence, transmission route and subsequent morbidity and mortality.

In order to monitor population immunity for hepatitis A, hepatitis B and hepatitis C infections, the Public Health Administration of the Flemish Community decided to repeat a seroprevalence study in the Flemish population at a 10-year interval. The study performed in 1993–1994 used a serum test on a hospital-based population representative of the general population with respect to age and gender [1]. As a hospital based population remains a selected population, in 2003, the study population was selected from the general public and a saliva test was used because it is not invasive, not painful, less expensive and does not require trained people to collect the sample. Prior to the prevalence study, tests for detection of hepatitis antibody or antigen in oral fluid were validated [2–4]. An additional study objective was to evaluate the feasibility of using saliva tests on samples gathered by regular postal services for

prevalence study in order to develop a surveillance tool.

This paper reports on the first population-based postal survey in Belgium using oral fluid technology to collect information on three hepatitis viruses.

Methods

Sampling design

The estimated prevalence of hepatitis in the Flemish population in early 1990 was used in the sample size calculation [1]. A minimum of 1,500 persons had to be involved in the study.

The main elements to consider when designing the sample were to keep a close control over the number of respondents and to obtain a final sample representative for the general population in the respect of a time constraint.

According to the experience of Statistics Belgium in household survey, a 12% response rate was taken into account.

To realize the sampling, Statistics Belgium developed an incremental 2-stage sampling plan. After stratifying the population by province, the first sampling stage consisted in the selection of primary sampling units (PSUs). First, the Flemish population was divided in 15,653 PSUs of different sizes according to individual characteristics of age, sex, and statistical sectors. Then, 3,000 PSUs (including replacements) were selected by probability proportional to size among all PSUs. Finally, fixed number individuals ($G = 8$), called a ''cluster'', were drawn by PSU.

Close control over the number of respondents was foreseen at this sampling stage by applying waves ($W = 4$) for contacting the selected individuals. In a first wave, two persons per cluster were invited to participate in the investigation. As soon as one person responded, the cluster was considered as 'completed'. If no answer came, another person of the cluster was invited in a second wave. The procedure was repeated until the specified number of respondents was reached. With an initial sample $n = 24{,}000$ individuals in $m = 3{,}000$ clusters of size $G = 8$ each, planning $W = 4$ waves and inviting $C = 2$ individuals per wave and cluster, simulations showed that the expected number of respondents would be about 2,151 after the 4 waves [5].

Selection of the study group

Containing all necessary variables to select a representative sample, the Belgian National Population Register (NPR) of 2002 was used by Statistics Belgium.

Procedure

Statistics Belgium attributed an identification number to the 24,000 selected persons and entrusted the SPSS file to the Scientific Institute of Public Health (IPH). The IPH received the database with the coded demographic information for the 24,000 selected persons.

The first two individuals of each selected 'cluster' were activated in the first wave and these 6,000 persons were invited by letter to participate in the study. The first mailing package was sent by Statistics Belgium in order to respect the privacy. The package contained an invitation to participate. This letter explained the objectives of the study. The package also enclosed an informative leaflet about hepatitis and a reply form to send back to the Scientific Institute of Public Health.

IPH and Statistics Belgium completed the database following codes for the different type of responses (no response, positive response, negative response, mail back with e.g. unknown address, moved ...). Three weeks after the mailing, the IPH and Statistics Belgium exchanged the files in order to determine the need to activate the following wave.

IPH received personal data from each person who consented to participate. The IPH sent them a second mailing package with a letter to remind them of the aim of the study, an informed consent, a questionnaire, two swabs and operating instructions. Swab is used as a toothbrush, rubbed against the gum for 1 minute and put back in a tube, before to be sent in prepaid envelope with other documents (e.g. questionnaire, informed consent ...).

All participants were invited to complete a questionnaire including some general information about their immune status: previous hepatitis or vaccine. As risk factor evaluation by questionnaire could induce a drop out so the focus of the study was on the estimation of the prevalence of hepatitis A, B and C, and not to collect information about risk factors.

Although subjects had the right to receive directly their own results, the consent form invited them to give the address of their general practitioner in order to ensure that the communication of a positive result of hepatitis B or C could be done by a health care professional.

Tests

The contents of the swabs (Oracol collection devices) were eluted in 1 ml of transport medium composed by a phosphate buffer saline pH 7.2 with 10% foetal calf serum, 0.2% Tween20, 0.5% gentamicin and 0.2% fungizone [6]. The vials were centrifuged at 2000 rpm for 5 minutes and 1 ml oral fluid was extracted. The oral fluid samples were stored at –20°C until testing. An 'in-house' IgG quantification assay was used to determine the validity of samples [7].

Commercially available ELISA tests were modified to detect hepatitis A, B and C in oral fluid and validated in the laboratory of National Centre of Viral Hepatitis, Institute of Public Health in Brussels [2–4].

The detection of hepatitis A antibodies was performed with an anti-HAV combined IgG/IgM test (ETI-AB-HAVK-PLUS, DiaSorin). During the validation, in order to obtain the best sensitivity and to distinguish vaccine-induced immunity from natural infection, only oral fluid samples with titres higher than 9,000 mIU/ml were considered for natural infection [8, 9]. The sensitivity and specificity were 84.7% (95% CI 72.5–92.4) and 100% (95% CI 93.8–100), respectively [4].

While it was not possible to perform all the tests to define the exact hepatitis B status of the study participants, the choice was made to detect the hepatitis B surface antigen (HBsAg) in order to estimate the spread of the virus in the general population.

The test was modified for the detection of HBsAg was ETI-MAK-4 ELISA, DiaSorin. The sensitivity and specificity were 90.7% (95% CI 76.9–97.0) and 100% (95% CI 93.8–100), respectively [3].

The detection of hepatitis C antibodies was performed with an anti-HCV combined IgG/IgM test (Ortho HCV 3.0 ELISA, Ortho Diagnostics). The sensitivity and specificity were 89% (95% CI 79.0–94.8) and 100% (95% CI 93.8–100) respectively [2].

The number of paired samples ($n = 73$) used to develop these tests was enough, even if quite limited, according to the formula described by Greiner and Gardner [2]. Since the prevalence of hepatitis B and C is low, this is of importance because a slightly lower specificity would result in drastically decreased positive predictive values for HBsAg and anti-HCV antibodies.

Statistics

In order to extrapolate the results of the study to the general population, the age-gender distribution of the realised sample was compared to that of the Flemish population at provincial level. A Chi-square test was used to compare proportions. The inverse of the selection probability of each individual in the realised sample was used as sampling weight. Weighted prevalence was estimated with a 95% confidence interval. Data were processed using SPSS 11.5 and Epi-Info 6.04d software.

Results

Population-based sample

A total of 2,036 persons from the 6,000 individuals contacted in the first wave answered positively for participating to the study. Within the first week after the mailing, the sample size reached 63% and 91% after three weeks. A response rate of 30.6% was achieved with 1,834 returned samples, representing a 3/10,000 tested persons among the population living in Flanders. Of those, four swabs were excluded leaving a total of 1,830 suitable swabs for testing.

The repartition of the participants was representative for the five Flemish provinces. The age and sex profile of the study population closely matched the profile of the Flemish population except for two age groups 0–14 years and ≥65 years (Table 1). The age group 0–14 is slightly over-represented ($\chi^2 = 8.82$; $p < 0.003$) and the age group ≥65 years underrepresented but only for women in two provinces ($\chi^2 = 35.31$; $p < 0.0000$). The second wave was not activated, but a correction for age applied. The proportion of foreign nationality in the Flemish provinces is 4.7%. The proportion of non-Belgians in the study (2.5%) is significantly lower than in the general population ($\chi^2 = 19.53$; $p < 0.00001$).

Given the small number of non-Belgians (19.7%, $n = 26$) Europeans and 0.54% non-Europeans ($n = 10$, coming from 7 different countries) among participants, no distinction between Belgians and non-Belgians was made in the analysis.

Among participants, only 13% did not give a general practitioner's address, informing they preferred to receive directly their own results or that they had no general practitioner at that time. In Belgium, organisation of health care services does not oblige people to elect a general practitioner.

About 99% of participants completed the questionnaire with information about their immune status.

Comparing the results of the present study with the hospital based study performed in 1993, the prevelance of hepatitis A and C is lower while the prevalence of hepatitis B surface antigen seems to be stable (Table 2).

Table 1. Comparison of the study group and the Flemish population by age, gender and province

Variable	Study group (N)	Study group (%)	Flemish population (%)
Age group (years)			
0–14	353	19.3	16.7
15–24	209	11.4	12.0
25–34	193	10.5	12.9
35–44	308	16.8	15.9
45–54	323	17.7	14.3
55–64	231	12.6	11.3
65+	213	11.7	16.9
Gender			
Male	885	48.4	49.6
Female	945	51.6	50.4
Province			
Antwerpen	527	28.8	27.8
West-Vlaanderen	334	18.3	18.8
Oost-Vlaanderen	403	22.0	22.8
Limburg	283	15.5	13.5
Vlaams-Brabant	283	15.5	17.1

Table 2. Results of the prevalence study performed in 1993 (serum, hospital-based population) and 2003 (saliva, population-based), in Flanders, Belgium

	1993	2003
Prevalence (%)		
HAV	55,1(95% CI 53.5–56.7)	20,2 (95% CI 19.43–21.08)
HBsAg	0,7 (95% CI 0.5–1.0)	0,66 (95% CI 0.51–0.84)
HCV	0,87 (95% CI 0.5–1.1)	0,12 (95% CI 0.09–0.39)

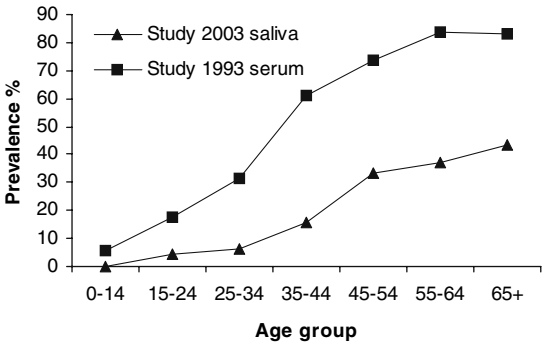

Figure 1. Comparison of HAV prevalence by age group in 1993 and 2003.

Anti-HAV prevalence

Testing of 1,830 oral fluid samples gave a weighted anti-HAV prevalence of 20.2% (95% CI 19.4–21.1).

The age-specific HAV prevalence is shown in Fig. 1. The prevalence rose steadily from 0.3% in children 0–14 years of age to 6.2% in persons aged 25–34. The prevalence doubled to 15.6% and 33.4% in the age group 35–44 and 45–54, respectively. Then the increase levelled off to 43.7% in the oldest age group. From the 357 participants with a positive HAV saliva test, only 6 were non-Belgians.

The gender ratio (M/F) among HAV positive participants was 0.9/1 without significant difference ($p = 0.665$) in prevalence between man (19.4%) and woman (21%).

The number of cases was equally spread in all provinces of the Flemish region.

In the present study, 9.7% of participants declared to be vaccinated against the hepatitis A virus. The number of vaccinated people varies according to age group. The highest proportion of vaccinated people was in the age group 25–34-years old (16.6%) and the smallest in the older than 65 (1.9%). Among them, 20% were positive for HAV infection. Recall bias or were these persons vaccinated without prior control of their natural immunity?

Among participants only 4% mentioned, in the questionnaire, to know their immune status. Among those who mentioned to be negative, 20% were actually positive while among those who mentioned to be positive, 24% were in fact negative.

In Belgium, adults have no personal medical record and as hepatitis A infection can be asymptomatic, health information collected through a self-administrated questionnaire has to be used with caution and possibly checked by tests. In this study, use of tests providing qualitative results did not permit to distinct between negative samples and vaccinated participants.

Anti-HCV prevalence

Testing of 1,830 oral fluid samples gave a weighted anti-HCV prevalence of 0.12% (95% CI 0.09–0.39), with 2 positives HCV samples in a total of 1,830. The patients were a man and a woman, 31- and 38-years old respectively. Both are Belgians but living in different provinces. Both participants ignored their positivity.

HBsAg prevalence

Testing of 1,830 oral fluid samples gave a weighted HBsAg prevalence of 0.66% (95% CI 0.51–0.84) in the general population living in Flanders.

The age-specific prevalence of HBsAg is shown in Fig. 2. The age group 34–44 and 15–24-years old were the most affected with a specific prevalence of 1.3% and 0.9% respectively. Except for one patient coming from another European country all positive cases were Belgian. Despite a majority of positive HBsAg among women, no statistically significant difference in gender was observed.

The positive HBsAg cases ($n = 13$) were spread equally in all Flemish provinces.

About 25% of participants mentioned in the questionnaire to have been vaccinated against hepatitis B virus. The percentage of vaccinated participants decreased sharply from the youngest (53.3%) to the older (0.94%) age group (Fig. 3). This figure is concordant with the vaccine policy, introducing a systematic vaccination in 1999 for two cohorts: infants and 12-year old children. Among participants who mentioned to have been vaccinated, 0.9% was positive for HBsAg.

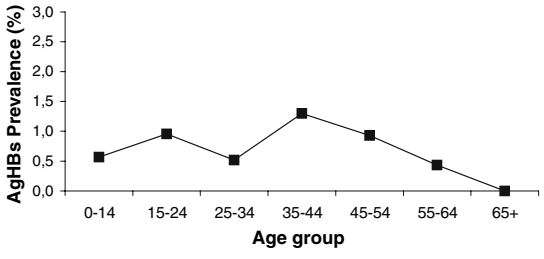

Figure 2. HBsAg prevalence by age group.

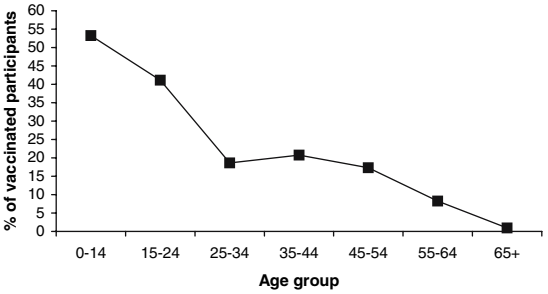

Figure 3. Percentage of HBV vaccinated participants by age group.

Discussion

This study is the first postal population-based survey using non-invasive oral fluid sampling to collect prevalence data on hepatitis A, B and C in Belgium. Besides estimating the prevalence, another purpose was to evaluate the methodology as a monitoring instrument.

A 34% participation rate to this investigation after the first wave can be considered as a good result. The only incentive for people to participate in the study was the possibility of knowing their immune status or their will to contribute to scientific research. According to the sample design, this response rate is obtained after only one contact with selected persons. Other similar prevalence studies gained a higher response rate while using reminders. A 60.4% response rate was achieved in the study of O'Connell et al. [10] after non-respondents received two reminders letters and a phone call. In this study, a press release was also circulating to the national and local press. Morris-Cunnington et al. [11] obtained a 40% response rate using two postal reminders. Reminders proved their effectiveness to increase the response rate [12, 13] but require more follow up of non respondents without having certitude to obtain a final representative sample.

Of the 34% positive answers (n = 2,036), about 90% (n = 1,834) effectively returned the swabs. By reducing the delay between the positive reply and the sending out of the swabs and by choosing carefully the study period, this loss could be minimized. As the first reply was higher than expected a stock shortage for swabs was not anticipated. Secondly, during Christmastime, the swabs dispatch was suspended, as the mail processing is longer during this period. Delay between home sampling and test in the laboratory should not exceed 7 days.

Taking into account the cost of five mails (from the invitation to participate to the communication of the results), laboratory tests and the extra material (e.g. swabs), the cost by participant is about 25 euros. Laboratory tests account for 60% of the sum. Time and human resources to realise such a study do not to be neglected. With 1.5 FTE affected during 18 months, resources needed for this prevalence study were underestimated (e.g. delay to obtain agreement from the Privacy Commission or time to deal with massive answers during two weeks after the first mailing).

Regarding the high response rate, the good quality of the swabs (only four swabs not suitable for testing), the survey is well accepted by the population and the samples correctly taken by participants. Use of swabs to collect saliva through the usual postal services is an appropriate tool for monitoring prevalence in the general population.

Disadvantages of this methodology are illustrated by the main purpose of the study which was the follow up of viral hepatitis prevalence in the population living in Flanders comparing the results of the present study with the hospital based study performed in 1993: sensitivity of the tests and selection bias. The methodologies of both studies are actually too different to permit comparisons of the results.

Compared with the 95% sensitivity of the serum test, the saliva tests have lower sensitivity which can contribute to a slight underestimation of the prevalence.

Selection bias in both studies can explain a probable overestimation of the prevalence in 1993 and an underestimation in 2003. In 1993, the participants were recruited in some departments of eleven hospitals located in urban areas. Some departments were excluded in order to prevent possible selection bias (e.g. gastroenterology). In the present study (2003), people from the general population were invited to participate in the study by mail, having to understand how they had to participate and how to collect the samples themselves. While the recruitment was done in a population more exposed to risk factors for hepatitis B and C in the previous study, the recruiting of participants by mail in the general population probably missed people from some risk groups: people with a lower socioeconomic status, people who already know their immune or vaccination status or subpopulations at higher risk (e.g. drugs users, institutionalised patients, prison population, ...). The disproportion of participants with a foreign nationality in both studies reflects the recruitment bias. The number of participants with a foreign nationality (7.2%) in the previous study was significantly higher (χ^2 = 47.96, $p < 0.00001$) than in the general Flemish population (4.7%). In the current study non-Belgians are underrepresented (2.5%: χ^2 = 19.53, $p < 0.00001$).

This recruitment bias should be taken into account in subsequent study using this methodology to monitor hepatitis prevalence. In order to evaluate whether certain risk groups are missed, more questions about risks should be included in the questionnaire. Moreover additional data about hepatitis among some specific groups should be collected by other surveillance tools as a register of new HCV

cases (e.g. stage at diagnosis, transmission way ...), specific prevalence study among drug users, ...

Hepatitis A is the most frequent form of viral hepatitis but also the less serious one considering the absence of chronic evolution. However, mortality rate can be as high as 0.6% among adults reaching 1.8% in patients older than 50 years [14, 15]. Despite the more hygienic living and housing conditions and the raised living standard of the last decades of the 20th century, the seroprevalence in Flanders was still high in 1993–1994 with 55.1% HAV positives [1]. In the current study, the HAV prevalence was about 20.2%. While the prevalence reached 50% in persons aged 35–44 in 1993 it was only 15.6% in the current study of 2003. In both time periods, age was an important factor with a constant increase from the younger to the older age groups (Fig. 1).

Though underestimated, the results indicate a decreasing trend, resulting in a low prevalence of infection in Belgium, leaving a larger susceptible population. The same trends were observed by the sentinel laboratory network in Belgium [16] but in other European countries also [11, 17–19].

As the potential for future outbreaks rises [18], the severity of infection increasing with age, these changes are important to describe over time. There is no universal hepatitis A vaccination programme recommended in Belgium. It is recommended for travellers to an endemic area and for some additional risk groups [20]. Before vaccination it is recommended to check immunity of people older than 40 years, people who have spent more than one year in tropical areas and people with a previous history of jaundice [20]. Considering the lack of information to interpret prevalence changes over time, surveillance should be reinforced making available arguments to modify these recommendations taking into account the consequences for the overall burden of morbidity of hepatitis A in the present European socio-economical context.

Hepatitis C is the least common of the viral hepatitis studied but can be a serious disease with severe liver complications. Hepatitis C is also the least known of these three types of viral hepatitis, with a probable change in the major transmission ways. The HCV prevalence in 1993 was estimated at 0.87% [1], which is quite different from the 0.12% prevalence in 2003 ($\chi^2 = 11.47$; $p < 0.0007$). The real HCV prevalence in the general population living in Flanders is probably contained between 0.87 and 0.12% but a decrease of the prevalence since the previous study cannot be excluded. Hepatitis C remains a disease with a low prevalence in the Flemish population. Our findings are concordant with the prevalence observed in other European countries, values ranging from 0.1% to 1.5% [21, 22].

Since 1990 all blood donations in Belgium are tested for HCV and the donors selected, giving a blood safety guarantee. Preventive measures have also reduced the transmission risk among some other risk groups, such as patients having haemodialysis [23, 24] or health care workers [21, 25–27].

In Belgium, the HCV prevalence among IV drug users is high, the results vary from a study 78.3% [28] to another 66.2–84.4% [29]. If intravenous drug use remains a major risk factor, it would not be justified to pretend that the hepatitis C problem is nowadays limited to this risk group. Even though the other risk factors are defined as minor (sexual, mother-to-infant transmission, household contact, nosocomial contamination), the results of the study invite more research about the actual transmission routes.

Overall hepatitis B is less frequent than hepatitis A, but 6–10% of HBV infected adults will become carriers, and a substantial proportion of these will develop chronic complications [30].

In 1993–1994, in Flanders 0,7% [1] appeared to be HBsAg positive and no change was observed in the HBsAg prevalence in 2003. The exposure to the hepatitis B virus remains constant in the general population although vaccination is recommended for some risk groups such as health care workers, patients having haemodialysis, [31] ... and a systematic vaccination started in 1999 for two cohorts: infants (three doses at 2-, 3-, 4-months old and a booster at 15-moths old) and 12-year-old children (three doses). Considering the results obtained in countries [32, 33] where the universal vaccination goes on for a long period, more than 10 years, it is probably too early to observe a decreasing trend in Flanders. To reduce the circulation of the hepatitis B virus will be more difficult as the prevalence is low.

In this study no analysis of antibodies nor analysis of HBeAg were performed making difficult to comment more extensively on the evolution of hepatitis B prevalence since HBsAg is a marker of infection but a marker of carrier ship as well.

The long interval between the two prevalence studies has also to be considered while interpreting the observed decline in prevalence. A shorter interval between studies, using the same methodology, would allow more arguments to comment on epidemiological trends and should therefore not exceed 5 years in order to use effectively prevalence study as a surveillance tool.

Conclusion

Even if partly underestimated, the prevalence of hepatitis C infections remains low while the 10-year evolution corresponds to a decrease of HCV in the general population. This finding does not change the requirement to consider hepatitis C as a public health problem but invites consideration of other transmission routes since the major one, blood transfusion, can be considered safe. If the prevalence study in the general population needs to be repeated, it seems

important actually to use an appropriate screening strategy to also identify persons at higher risk.

The low HAV prevalence leaves a large proportion of adults living in Flanders susceptible for the disease, underlying the necessity to repeat prevalence study allowing policy makers to evaluate the vaccine policy.

Hepatitis B surface antigen prevalence remains stable but in order to complete the picture of the hepatitis B prevalence and interpret its evolution, other tests as antiHBc and antiHBs are required.

The evidence that oral fluid collection by postal survey is a good tool for epidemiological surveillance purposes supports the necessity to repeat the study using the same methodology and in a maximum 5-years interval. A well-described epidemiology will help to face the major challenges in the future and firstly, the elimination of the transmission of viral hepatitis thanks to a nationwide prevention program (e.g. vaccine policy, infection control policies, ...).

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