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SPECIAL REPORT

Second national serum bank for population-based seroprevalence studies in the Netherlands

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ABSTRACT

In 2006/2007 a large serum bank was established by means of a cross-sectional population-based study. This serum bank will be used to evaluate the Dutch national immunisation programme (NIP) by serosurveillance and additional immunological and epidemiological research. In this paper we describe the design of this population-based cross-sectional serosurvey and report the participation rates as well as general characteristics of the study population. A similar serum bank was collected in 1995/1996.

Dutch inhabitants (aged 0-79 years, men and women) were invited from 40 municipalities throughout the country and also from eight additional municipalities known with low vaccination coverage (LVC). An oversampling of the migrant population was performed.

Blood samples were obtained from all participants accompanied with extensive information on demographic and epidemiological data, such as vaccination history, risk factors and travelling.

In addition, sociodemographic data are available from individuals who declined to participate (non-response survey). Overall 33% of all invitees were included in this study. The serum bank comprises 6386 sera in the nationwide sample including the extra sample of immigrants (n=646) and 1518 sera from the LVC municipalities. The sera will be analysed for antibodies against all NIP antigens but will also be used for other infectious diseases research. Results of this second serosurveillance study will contribute to the discussion whether it is needed to reconsider the schedule and/or the vaccine components of the current National Immunisation Programme.

KEYWORDS

Immunisation, serosurveillance, serum bank, study design, the Netherlands, vaccines

INTRODUCTION

Vaccination of children through national immunisation programmes (NIP) has effectively reduced many of the target diseases and their complications. Despite lower disease incidence and smaller numbers of cases evaluation of the NIP is still essential. In this situation the role of serosurveillance, i.e. the assessment of antibody levels in serum in the evaluation of the NIP, becomes more evident. Groups with low immunity, e.g. as a result of waning of natural or vaccine-induced antibodies, could be identified. When necessary, vaccination strategies could be changed to prevent future epidemics of disease.

Although over the last decades the essential role of cellular immunity has become evident; for many pathogens antibody levels can be used as a surrogate for protection at the population level. Furthermore, even when correlates of protection are unclear (e.g. pertussis), insight into (the combination of) antibody levels, antibody affinity and response kinetics give an important indication about the protection and occurrence of infection.

In 2006/2007 a large study was conducted in the Netherlands to set up a population-based serum bank in which three additional substudies were incorporated (PIENTER 2). This is the second national Dutch serum bank, as a similar study was performed in 1995/1996 (PIENTER 1).[1] Results of this first study have been published.[2-6] The participation in the European Seroepidemiology Network (ESEN and ESEN2) enabled us

to compare our results internationally.[7-11] By repeating such studies we get insight into the changes of the population's immunity over time and in changes in infection pressure. Since the collection of the first serum bank in 1995, the NIP has changed (*table 1*). Schedules and vaccines used in the current National Immunisation Programme in the Netherlands can be found at http://www.rivm.nl/rvp/rijks_vp/vac_schema. An overview of the changes in recent years can be found in the annual report.[12]

In this paper we describe the design of this population-based cross-sectional serosurvey and report the participation rates as well as general characteristics of the study population. We focus on the improvements and additions implemented in the design and methods compared with the first PIENTER study.

Table 1. *Major changes in Dutch immunisation programme from 1997 onwards*

Year	Vaccination	Change in programme
1997	DTP-IPV-Hib	Schedule from 3,4,5 to 2,3,4 months
2001	aP	Introduction of booster at age of 4 years simultaneously administered with DT-IPV booster
2002	MenC	Introduction of conjugate MenC at age of 14 months simultaneously administered with MMR vaccine, catch-up for children up to 19 years
2005	aP	Change from whole cell to acellular vaccine in the first year of life
2006	Pneumo	Introduction of pneumococcal vaccination at 2,3,4, 11 months, simultaneously administered with DTPa-IPV-Hib (-HepB)
2009	HPV	Introduction for girls at age of 12 years with catch-up for girls up to 17 years

METHODS

The main objective of the PIENTER 2 study is to estimate age-specific seroprevalence to diseases included in the NIP for the following groups: general population, orthodox reformed individuals and non-Western immigrants. The participants were asked to donate blood and to complete a questionnaire.

Design of the study

General population

To assess the overall and age-specific seroprevalence for vaccine preventable diseases a nationwide sample was drawn in the Netherlands, using a two-stage cluster sampling technique. The study design was kept similar to that of the first serum bank in 1995/1996.[1] The Netherlands were first divided into five geographical regions of approximately equal population size. Within each of the five geographic regions, eight municipalities (e.g. clusters) were randomly drawn with a probability proportional to their size. Initially, in each of these 40 municipalities an age-stratified sample of 380 individuals was drawn from the population register. The age strata were 0 years, 1-4 years and thereafter intervals of five years 5-9, ..., 75-79 years. With exception of the first two age strata, 20 individuals per age stratum were sampled. In the first two age strata in each municipality, 40 individuals were sampled because of an expected lower response rate.[1] Due to the low response in certain age strata (0 and 20-39 years), the number of invited individuals was increased in these age strata during the study to 80 (infants) and 40 respectively. In total 17,223 individuals were invited in the nationwide sample.

Oversampling: communities with low vaccination coverage

Special attention was given to the group of orthodox reformed individuals who refuse vaccination for religious reasons and are socio-geographically clustered. The potential for epidemics of NIP diseases is high in this group because susceptibility levels increase as a result of absence of vaccine-induced antibodies, as was shown in the PIENTER-1 study.[1] Indeed, several outbreaks have occurred in these communities, namely polio type 3 in 1992/1993, measles in 1999/2000, rubella in 2004 and mumps in 2007/2008.[13-17] To assess overall and age-specific seroprevalence estimates in the low vaccination communities (LVCs) and in individuals who refuse vaccination based on religious grounds, an extra sample was taken in eight additional municipalities with low immunisation coverage. The vaccine coverage in these municipalities for three DTP-IPV immunisations in 2007 (birth cohort 2005) varied between 71 and 83%, which is considerably lower than the nationwide coverage (95%). Individuals were divided into three age strata: 0-9, 10-49 and 50-79 years. These age strata were chosen for the following reasons:

- Vaccinations in the NIP were given to children up to 9 years of age so within this group the effectiveness of the current NIP can be assessed.
- Individuals between 10 and 49 years could have participated in the NIP so that the effectiveness of the NIP in the longer term could be assessed.
- Individuals of 50 years and older did not participate in the NIP as no such programme was available at that time.

Antibodies measured in the group of participants of 50 years and older will mainly be derived upon natural infection. Similar to the nationwide sample an age-stratified sample of initially 380 individuals per municipality was

drawn. Also here the number of invited individuals was increased in certain age strata during the study. In total 4366 individuals were invited in the low immunisation coverage sample.

Additional aims in PIENTER 2 compared with the PIENTER 1 study

Oversampling: migrant population

Assessing the seroprevalence estimates in non-Western immigrants was added to the aims of the PIENTER 2 study. In the PIENTER 1 study it was shown that the number of non-Western immigrants was too low to reliably assess the seroprevalence in this group. This group has become a relatively large group in the Netherlands (11% of the total population in 2007, while 8% in 1996) and little is known about the immunity against vaccine preventable diseases. Van der Wal *et al.*[18] showed that in 2003 the vaccination coverage for DTP-IPV for the first-generation immigrants of 5-12 years of age born in Surinam, Morocco or Turkey and living in Amsterdam varied between 82 and 86%, which was lower than the average vaccination rate of 93%. Further, Pauw-Plomp *et al.*[19] showed that in 1984 the vaccination coverage for DTP-IPV for second generation immigrants of 1 to 14 years of age, whose mothers were born in Turkey or Morocco, was 41 and 43%, respectively, whereas 19 years later (in 2003) the vaccination rates were similar for 5-12 year old children with indigenous parents and children of immigrants.[18] It is unknown whether the seroprevalence in non-Western immigrants is currently different from the seroprevalence in the indigenous Dutch. For example, certain infectious diseases are still endemic in these non-Western countries, different immunisation schemes are used and frequent travelling to these countries takes place.

To assess the seroprevalence in non-Western immigrants extra individuals were invited from 12 municipalities of the nationwide sample. The number of migrant inhabitants, the distribution of country of births and the urbanisation degree were decisive factors in selecting the municipalities. The sampling of the individuals from the population register within each municipality was random, similar to the nationwide sample.

Twelve immigrant groups were distinguished by age group, country of birth and first or second generation (*table 2*). In total 2558 individuals were invited in this extra sample.

Table 2. *Immigrant groups were distinguished according to country of birth, age group and first and second generation*

Country of Birth	Age group	Generation
Morocco and Turkey	0-9 years	First generation
	0-9 years	Second generation
	10-49 years	First and second generation
	50-79 years	First and second generation
Suriname, Netherlands Antilles and Aruba	0-9 years	First generation
	0-9 years	Second generation
	10-49 years	First and second generation
	50-79 years	First and second generation
Other non-Western countries	0-9 years	First generation
	0-9 years	Second generation
	10-49 years	First and second generation
	50-79 years	First and second generation

Sampling additional objectives

Three additional studies were incorporated in this population-based serum collection, in contrast to the previous PIENTER project. The first additional study, which is part of the European modelling project Polymod, will provide insight into the spread of air-borne infections by estimating the number of social contacts between individuals by means of a diary.[20] About 1000 participants in the nationwide sample were randomly asked to complete the diary. The diary contained detailed questions on characteristics of social contacts with different individuals during one weekday including age, sex, location, duration, frequency and occurrence of physical contact.

The second additional study will provide insight into genetic differences between vaccine responders. For this purpose an extra blood sample or buccal swab for children less than five years old was taken for DNA isolation. The material will be used for research on genetic factors involved in cellular and humoral responses to infections, e.g. CD40, inducible co-stimulatory molecule (ICOS) or polymorphisms in Toll-like receptors.[21,22]

The third additional study aims to estimate the seroprevalence of food allergies by measuring IgE and assessing the suggested association of vaccination with (reported) allergies.[23] A special question on having disorders (e.g. COPD/asthma, eczema, hay fever, allergies and more specific certain food allergies) and whether these disorders were diagnosed by the general practitioner was included in the questionnaire.

Data collection

Data were collected from February 2006 to June 2007 in collaboration with the Public Health Service, an organisation well known to the local inhabitants. Each invited individual received a letter of invitation, along with a brochure containing information on the study, a questionnaire, an informed consent form, and a prescheduled appointment for blood donation. All invited individuals were asked to complete the questionnaire at home and to visit a clinic for the blood collection.

The questionnaire contained questions on demographic characteristics, vaccination history, health perception and diseases, activities possibly related to infectious diseases (e.g. travelling, profession, gardening), information related to sexually transmittable diseases for 15 to 79 year olds and opinion on vaccination-related topics for 0 to 14 year olds. The vaccination history of the participants was either checked by copying the vaccination certificates brought by the individuals to the clinic or a copy was retrieved from the regional vaccine administration offices archives.

Residents born in a foreign non-Western country received a letter of invitation in their own language (Turkish) or a partly translated letter in English, French and Arabian along with a Dutch version. To increase the response all invited individuals were approached by phone or mail one week before the consultation appointment. Individuals who refused to participate were asked to complete the questionnaire or as a second possibility to answer some questions for the non-response survey (by telephone or mail). Invitees who did not show up at one of the clinics were sent a reminder along with a non-response questionnaire. This questionnaire contained a limited number of questions on demographic characteristics, vaccination history and health perception. From all invited persons information on age, gender, residence, country of birth and country of birth of both parents was available from the population register. Participants were offered a gift voucher.

The study proposal was approved by the Medical Ethics Testing Committee of the foundation of therapeutic evaluation of medicines (METC-STEG) in Almere (clinical trial number: ISRCTN 20164309).

Serum processing and storage

A blood sample was taken at the clinic and transported to the laboratory the same day, where the blood samples were stored in a cold room (4°C) overnight. From adults a maximum of 22 ml blood was taken and depending on their age and the degree of discomfort, less blood (0.1 to 8 ml) was taken from children. The next day the blood was centrifuged (10 min at 2500 rpm) and divided into portions of 5 ml serum. One tube of serum per participant was thawed and aliquoted with a robot (Tecan 150) into 10 separate micronic blocks with different volumes and stored at -80°C until analysis. The other tubes with serum were stored at -80°C in different freezers. The blood samples and buccal swabs reserved for DNA isolation were stored at – 20°C.

Serology

With an NIP expanding over the past years and up till now consisting of 23 vaccine components involving 11 pathogens, it is crucial to have antibody determination techniques at one's disposal which are less serum consuming and take less analysis time than the already established methods such as ELISA. Multiplexing techniques (eg. Multiplex Immuno Assay, MIA with Luminex) are now state of the art in measuring large, population-based serum banks. Diphtheria, tetanus and pertussis (Pertussis toxin, Filamentous haemaglutin, Pertactin, Fimbriae 2/3) can be measured simultaneously with a small volume of serum.[24] Also the MIA determination of antibodies against *Neisseria* menigitidis type C (MenC), A, Y, W135 and *H. influenzae* type b (Hib) has been described.[25,26] This MIA technique has been described for other (candidate) NIP components such as human papilloma virus (HPV)[27,28] or is commercially available as a kit, e.g. mumps-measles-rubella-varicella or pneumococci. In the former population-based study this MIA technique was not yet available and most antibody levels were determined using the ELISA technique. Information on the functionality of the antibodies will be obtained using a neutralisation assay (NT) or serum bactericidal assay (SBA) similar to the former PIENTER study. Compared with the MIA, ELISA and especially the NT are labour intense techniques.

RESULTS

Serum bank

In total we have collected 6386 (32%) sera in the nationwide sample including the extra sample of immigrants (n=646, 25%) and 1518 (35%) sera in the low immunisation coverage sample. *Table 3* shows the participation and the obtained materials in the nationwide and in the low immunisation

Table 3. *Number of participants in the nationwide and low immunisation coverage sample, PIENTER 2 project 2006-2007, the Netherlands*

	Nationwide sample	Low immunisation coverage sample
	n (%)	n (%)
Total invited	19,781	4366
Materials obtained at clinic:		
• Blood and questionnaire	6351 (32.1%)	1517 (34.7%)
• Blood, no info questionnaire	35 (0.2%)	1 (0.02%)
• DNA	6207 (31.4%)	1469 (33.6%)
• Only questionnaire (visited consult)	135 (0.7%)	43 (1.0%)
• Only information population register	7 (0.04%)	
• Vaccination booklet	4583	932
• Diary	824	
Materials obtained otherwise:		
• Questionnaire (no consult visit)	1200 (6.1%)	354 (8.1%)
• Short questionnaire	1652 (8.4%)	450 (10.3%)
• Only information population register	10,402 (52.6%)	2001 (45.8%)

coverage sample. The number of persons who visited the clinic for blood collection (i.e. participants) was 8089. Almost all participants (95%) gave consent for DNA isolation. The vaccination history was confirmed for 68% (5515 of the 8089) of the total number of participants and for 4843 of the 6042 (80%) who were eligible for the vaccination programme. In total 824 of the 1162 (71%) diaries were completed by participants in the nationwide sample for the study on social contacts between individuals.

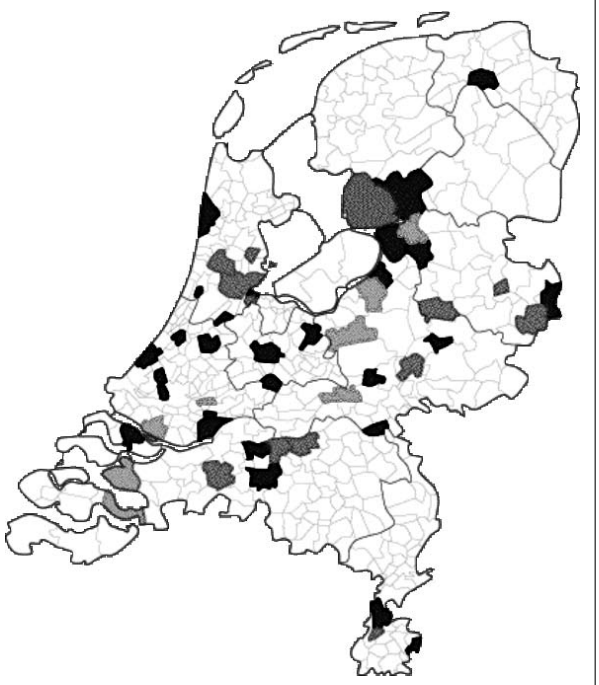
From the participants with a blood sample and a questionnaire, the highest response in the nationwide sample was seen in women aged 10-49 and 50-79 years and in the low immunisation coverage sample in women aged 10-49 years (*table 4*). Based on ethnicity the highest response was seen in indigenous Dutch and the lowest in individuals born in Morocco or Turkey. Overall, the response rate was higher in second-generation immigrants compared with first-generation immigrants.

In all age strata in the nationwide sample more than 260 participants are included (*figure 2*).

The number of participants in the age groups 1-4 and 5-9 years was highest (509 and 610 participants respectively) but note that the number of invited persons in the age group 0 and 1-4 years was twice as high as in the older age groups. The number of participants per age strata in the low vaccination coverage sample varied between 69 (35-39 years and 50-54 years) and 196 (1-4 years).

The median age in the nationwide sample was somewhat lower than in the low immunisation coverage sample and both were lower than the median age in the Dutch population, which is 38 (*table 5*; IQR: 19-54) (data, 1 January 2007, from the Central Bureau of Statistics (CBS). Further, 19% of the Dutch population considers themselves Protestant Christian, 28% Roman Catholic, 10% had another

Figure 1. *Selected municipalities in this study*



Black and dark grey municipalities are included in the nationwide sample, in the dark grey municipalities oversampling of immigrants also took place and the light grey municipalities are included in the low immunisation coverage sample.

Figure 2. *Number of participants per age stratum in the nationwide sample, stratified by gender*

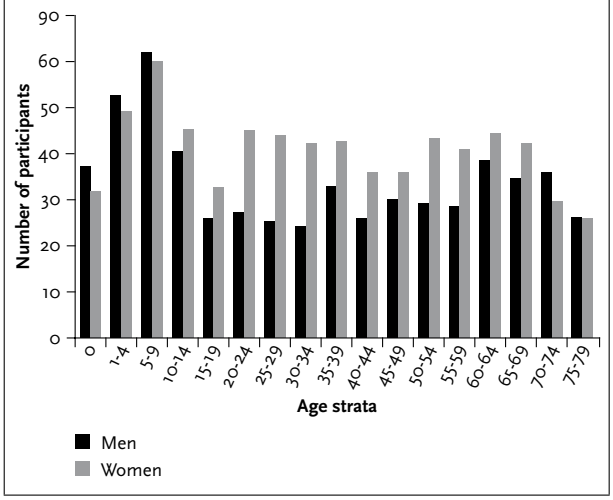


Table 4. *The response rate of participants with a blood sample and a questionnaire aged 0-79 years for age, gender, ethnicity and first and second generation in the nationwide sample and in the low immunisation coverage sample*

	Nationwide sample n (response rate %)	Low immunisation coverage sample n (response rate %)
Age and gender:		
• 0-9 years male/female	759 (27%)/705 (26%)	224 (32%)/188 (28%)
• 10-49 years male/female	1166 (26%)/1619 (38%)	290 (33%)/353 (44%)
• 50-79 years male/female	967 (36%)/1135 (38%)	220 (35%)/242 (35%)
Ethnicity:		
• Indigenous Dutch	4864 (36%)	1453 (35%)
• Morocco and Turkey	334 (21%)	3 (30%)
• Suriname and the Netherlands	352 (25 %)	3 (50%)
• Antilles and Aruba		
• Other non-Western countries	358 (23%)	11 (31%)
• Other Western countries	443 (29%)	47 (31%)
Generation:		
• First	799 (23%)	21 (26%)
• Second	688 (26%)	43 (36%)

Table 5. *Age and religion characteristics of participants with blood and a questionnaire aged 0-79 years in the nationwide sample and in the low immunisation coverage sample*

	Nationwide sample	Low immunisation coverage sample
	n (%)	n (%)
Median age (interquartile range)	34 (11-56) years	29 (9-55) years
Religion:		
• Protestant	1470 (23%)	1143 (76%)
• Roman Catholic	1806 (29%)	53 (4%)
• Other religion	812 (13%)	40 (3%)
• No religion	2215 (35%)	273 (18%)
Specific Protestant Christian religion:		
• Orthodox reformed	71 (5%)	326 (30%)
• Reformed bond	159 (12%)	294 (27%)
• Other specific Protestant Christian religion	1133 (83%)	450 (42%)

religion and 43% had no religion according to CBS, which is similar for the nationwide sample in our study. As expected, the percentage of individuals who consider themselves Protestant, orthodox reformed, and reformed bonders is much higher in the low immunisation coverage sample.

Seroprevalence estimates for all vaccine-preventable diseases in the NIP are part of this study: *Bordetella Pertussis* (Pertussis toxin, Filamentous haemaglutinin, Pertactin and Fimbriae2/3), diphtheria, tetanus, *Neisseria menigitidis* type C, A, Y, W135 and *H. influenzae* type b, *Streptococcus pneumoniae* (pneumo), mumps, measles, rubella, polio, hepatitis B, human papilloma and varicella viruses. Measuring antibody levels as a correlate of protection is generally accepted for several diseases and cut-off values are described. However, for other diseases (pertussis in particular) the correlate of protection and the antibody cut-off values are not established and research is ongoing.

The seroprevalence against other infectious diseases such as gastrointestinal infections (*Salmonella*, *Campylobacter*, *Noro* and hepatitis A), zoonotic infections (Q fever, *Toxoplasma*, *Toxocara*, *Echinococcus*, hepatitis E), vector borne infections (lyme, West Nile, dengue) or infections related to sexually transmitted diseases (herpes simplex virus, hepatitis C) can also be investigated.

DISCUSSION

Around ten years after the first serum bank, a second serum bank of the general Dutch population has been established with the aim to evaluate the Dutch national immunisation programme. The availability of a questionnaire and individual vaccination data is an important additional value for these population-based studies in contrast to a collection of residual sera.

Furthermore, oversampling of specific groups, such as orthodox reformed individuals who refuse vaccination on religious grounds (both studies) and immigrant groups (present study), enables us to study whether the protection against the target diseases of the NIP in these groups needs additional attention. In addition to the current target diseases, insight into the seroprevalence in the population against diseases for which a vaccine will be available in the near future, or already is available, i.e. varicella zoster and HPV, can be obtained.

In contrast to the first study, information on contact patterns of individuals in the population was collected in this study, which offers the unique opportunity to directly link the infection frequency according to age with age-specific contact patterns. Furthermore, additional studies on the incidence of allergies and genetic factors involved in vaccine responsiveness were incorporated in the study design.

The design is extensive and costly but efficient since a broad spectrum of study questions including both NIP and broader infectious diseases issues could be addressed. Also in relative terms, the costs of the study were only a very small proportion of the budget spent on the NIP in total.

Large serological studies have been done in other countries. The NHANES study in the United States is an important source of data for a variety of nutritional and health parameters[29,30] and the European Sero-Epidemiology Network (ESEN) aims at coordinating and harmonising serological surveillance in Europe. Some countries in the ESEN network obtained their serum bank through population-based random sampling whereas other countries could only collect residual sera. Although residual sera provide valuable information on immunity against infectious diseases[31] the sets of residual samples might not be representative for the general population and might lead to bias towards a more hospitalised or medical subpopulation.

In the first Dutch population study performed in 1995/1996 (PIENTER 1) the response rate was higher than in the present study (50 *vs* 33%). The designs were identical between the two studies to ensure maximal comparison of seroprevalence estimates and input for modelling analysis. There are several reasons which might explain the lower response rate in the current study. Most importantly, the study design included municipalities from which the participants were invited. Compared with 11-12 years ago the municipality boundaries in the Netherlands have become much larger, which means that invitees were expected to travel much further. In addition, most non-participating invitees stated that they were too busy and had no time to visit the clinic or did

not want to donate blood. Other recent large studies in the Netherlands reported a response rate of 26%[32] or 44%.[33] The slightly higher financial compensation and the individual limited health checks included in the Amsterdam study might have contributed to the higher response rate in Amsterdam.

The materials obtained in the PIENTER 2 study are well characterised and extensive information is available on the participants. However, although the design of the study ensures random selection of the participants, the response rate of 33% makes a non-response bias plausible. A thorough non-response analysis will therefore be performed by analysing the data from the non-response questionnaires and the data from the registers of the municipalities. Data from all Dutch inhabitants from the Central Bureau of Statistics will be used to interpolate the PIENTER 2 study to the general population. Part of the non-response bias in characteristics, which is most likely to be associated with immune status for vaccine-preventable disease (e.g. age, gender, country of birth), can be compensated by weighting the frequencies of seropositives to the Dutch population.

Antibody measurements and the corresponding analysis of the seroprevalences for all vaccine-preventable diseases in the NIP are in progress. Antibody levels above cut-off values are indicative for protection against disease. However, for some vaccines, vaccine-induced antibody levels will decrease within a few years after vaccination and eventually reach a pre-vaccination level.[34] Although antibody levels are low, protection based on memory immunity might be present. This indicates that serosurveillance based on the analysis of seroprevalence of antibodies alone will not provide a complete overview of or insight into immunity against infectious diseases. Further laboratory tests using functional assays (Opsonophagocytosis assay, NT and SBA) and better insight into more profound immunological processes such as memory immunity are essential to evaluate the present national immunisation programme completely.

CONCLUSION

We have established a new population-based serum bank together with additional information from questionnaire, vaccination booklet and diary. This material will primarily be used to evaluate the Dutch immunisation programme. Results of this second serosurveillance study might contribute to the discussion whether it is necessary to reconsider the schedule and/or the vaccine components of the current NIP. Certainly it will strengthen our insight into the Dutch immunisation programme and it will contribute to the confidence in the NIP and to the continuation of its success.

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