National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

PIENTER 2 study
2006-2007

PIENTER 2 study

A summary of the main findings

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Pienterproject

Preface

The Pienter 2 project enabled the RIVM to monitor the protection of the general population against several infectious diseases primarily those included in the national immunization program (NIP). The design of the Pienter 2 study was kept similar to the former Pienter study (1995/1996) with the exception of minor logistic modifications, assuring maximal comparison between the two studies.
Because the NIP is widely accepted and the vaccine coverage is high, it needs careful monitoring and attention. Demographic changes of our population, implementation of new vaccines and/or vaccine combinations, changes in dynamics of infectious diseases and their pathogens, changes in risk factors for these diseases and the threat of emerging (zoonotic) infections underline the need for regular monitoring.

The Pienter 2 results will contribute to answer the following type of questions:
- What effect have the different changes in the NIP on the protection of the population?
- Can the NIP be improved?
- Who are the vulnerable groups?
- How can the NIP protect the vulnerable groups of the population in a better way?
- Is there a need to change policy on vaccination against infectious diseases e.g., in case of an outbreak?

We have performed the study during 2006 and 2007 by collecting serum samples and questionnaires (N = 8,000) with a dedicated team of experienced members. The contact with the participating municipalities and their public health institutes was very constructive. Especially the contact with the participants during the numerous site visits was inspiring and warm. The contribution of all participants was eminent and is greatly appreciated.

Now most of the serological and epidemiological analyses planned have been performed and are published or will be made public in the near future. The analyses for polio and diphtheria are underway. Because the Pienter 2 project is considered as one of the important products of the RIVM, it seems now appropriate to combine all scientific results, thereby providing an overview of the broad use of all the Pienter materials and data. This overview has resulted in this booklet, which contains quite an impressive list of relevant data.

A representative large serum bank, provided regularly updated, makes it possible to estimate baseline seroprevalance data of diseases from which we don't know the existence at the moment and that might have the potency of becoming a major public health issue in the future. The emergence of some infectious diseases (Q fever, disease caused by specific influenza strains or Smallenberg virus) demonstrates the importance of the availability of such large serum banks. The Pienter 1 and 2 studies can be used for this purpose and part of the material is reserved for this.

The need and the power of the Pienter study are demonstrated with this overview. In the meantime the NIP has already changed a lot since Pienter 2 (introduction of vaccines against pneumococci, human papillomavirus and hepatitis B, and vaccine changes). Because one of the tasks of the RIVM is to carefully monitor the effects of those changes in the program on the protection of the Dutch population against infectious diseases, a Pienter-like follow up study is advised.

We are proud to present here a summary of the main findings of the Pienter 2 study.

Fiona van der Klis, *Project leader Pienter 2*

Liesbeth Mollema, *Researcher epidemiology Pienter 2*

Yolanda van Weert, *Research nurse Pienter 2*

Summary

The Pienter 2 project has been very successful in generating relevant information about the protection against infectious diseases, primarily those vaccine preventable diseases which are included in the national immunization program (NIP). Serological surveillance has become more and more important in view of (much) lower disease incidence due to the success of the NIP. In addition to infectious diseases covered in the NIP, other infectious disease related public health issues were investigated. At the start of the study we could not foresee the spread of the Mexican influenza or the rise in zoonotic infectious diseases like Q fever. With the Pienter study materials we were able to generate important data serving as the population baseline situation for these new emerging infectious diseases.
The possibility to compare the results from both Pienter studies turned out to be of great added value. This was a challenge as laboratory techniques have developed since the first Pienter study. Possible trends and shifts in serological protection over the 10-12 years between the two studies could be detected.

The available results have been made public by many international and national peer-reviewed publications and presentations at (inter) national congresses and meetings.
Use of the available materials and data for public health investigations is encouraged, provided conform the informed consent, and different disciplines have taken this opportunity. The strength of modeling and bio-informatics discipline has become more evident in the last years and the data from the Pienter study are unique and perfect for these analyses.

In this summary the highlights of the Pienter 2 study are listed in the text boxes below.

General population is well protected against **tetanus**. Tetanus vaccination could be reduced by a more restrictive use of post-exposure prophylactic boosting after injury (only to specific risk groups).

(Herd) Immunity against **MenC** is very good due to an unexpected long persistence of protective antibody levels in individuals who have participated in the mass campaign.

The infection frequency of ***B. pertussis*** has more than doubled between the Pienter 1 and 2 studies in the Netherlands. This high circulation of pertussis emphasizes the need for good protection of unvaccinated infants.

If incidence of **MenC** disease increases due to renewed circulation, a MenCC booster immunization at adolescence may be appropriate.

The vaccine induced immunity against **Hib** in 6-11 months children is reduced. Maintaining surveillance of Hib disease is important.

There is no relation between antibody levels and **pneumococcal disease** for unvaccinated older age groups. Can vaccination of the elderly solve this problem?

The prevalence of both **hepatitis B and C** in the general population is low and did not differ between 1995/6 and 2006/7. First generation migrants are the most important risk group for hepatitis B and C.

HPV seroprevalance is associated with age of sexual debut and is highest for the migrant part of the population. Vaccination would also be beneficial for women older than 12 years.

Loss of protection by maternal antibodies against **measles, mumps and rubella** is achieved well before the time of first MMR-vaccination at 14 months.
During a measles epidemic, temporarily decreasing the age of the first MMR-vaccination should be considered.

The Dutch population is generally well-protected against **measles, mumps and rubella**. However, unvaccinated children from religious communities are at high risk. Indeed, the outbreak of mumps among unvaccinated children within these religious groups in 2007 could have been predicted by their very low antibody levels in 2006 during Pienter 2.

Dutch children are infected with **varicella zostervirus** at an earlier age (5 years) compared with other European countries. If varicella vaccination is introduced, a high coverage is needed to prevent a shift of infections to older age as those infections will be more serious.

Vaccination against **hepatitis A** should also target people >40 years of age.

Children younger than two or three years of age will benefit most of **influenza** vaccination as the highest attack rates for influenza A viruses were observed in those children. At seven years of age, all children had developed influenza specific immunity.

An increase in **peanut sensitization**, but not in **cow's milk and egg**, was shown in young children and in adults. For the majority of the peanut allergic people, sensitization occurs during childhood.

Almost half of the general population has serological evidence of prior infection with **CMV** and infection occurs frequently during pregnancy. Infection is more prevalent among non-Western migrants and those with a lower socio-economic status.

Before the **Q fever** outbreaks the seroprevalence in the general population was low, suggesting Q fever is a newly emerging problem since spring 2007.

About 8% of the general population aged 15-40 years of age is seropositive for an infection with ***Chlamydia trachomatis***.

The seroprevalence of antibodies against **hepatitis E virus** seems to be lower compared to other European countries, however exposure in the Netherlands suggests zoonotic potential. Half of the infections are obtained in the Netherlands, including those donating blood. A clear risk profile is needed to enable targeted exclusion of blood donors.

Although the seroprevalence for **hantavirus** in the general population is low, it is estimated that the number of reported cases should be higher. This indicates that many cases are not recognized by Dutch physicians.

Contact patterns provide important data on the future spread of infectious diseases. As children in secondary schools make most contacts, they will contribute most to the spread of infections when infected. Ensuring that this group is highly immunized will contribute to a reduced circulation.

The seroprevalence for ***Toxoplasma gondii*** among women of reproductive age decreased, leaving the majority of pregnant women susceptible to primary infection with *Toxoplasma gondii*. Education about dietary and environmental sources of toxoplasma infection remains essential to prevent toxoplasmosis.

Seroprevalence data are more reliable in estimating the incidence of **salmonella and campylobacter** infections than notifications of clinical cases and demonstrate that infections are more prevalent than expected.

Description PIENTER 2 study

Background

In 2006 and 2007 the RIVM carried out the second PIENTER study by order of the Ministry of Health Welfare Sports (VWS). PIENTER is a Dutch acronym for: Peiling Immunisatie Effect Nederland Ter Evaluatie van het Rijksvaccinatieprogramma. The aim of this study is to gain insight into how well the Dutch population is protected against vaccine-preventable diseases either through vaccination or natural infection. The results will enable further improvements of the immunization program to be made as well as identifying those population groups who are less protected. More than 24,000 people between 0-79 years old were invited to participate. They were asked to complete a questionnaire, to provide a blood sample and to bring their vaccination certificate. In addition to a national sample (NS), we also selected a low vaccination coverage sample (LVC) and invited an extra group of non-Western migrants to participate. Three sub studies were integrated in the PIENTER 2 project: 1. to gain insight into the spread of air-borne infections by estimating the number of social contacts between individuals of various age groups; 2. to gain insight into genetic differences between vaccine responders; and 3. to investigate a possible association of vaccination with allergies.

Results

The response (i.e., those with a blood sample and questionnaire) was 33% in the nationwide sample, 25% in the extra sample of migrants and 35% in the LVC sample (table 1). In total a number of 7904 serum samples were available for many sero-epidemiological studies. Of the participants with a blood sample, 96% also gave a blood sample for DNA isolation. For 73% of the participants with a serum sample, who were eligible for the NIP (< 50 years of age), a DPT-iPV-vaccination was confirmed. In total 814 of the 1,162 (70%) diaries were completed. From all invitees about 50% supplied information via the questionnaires. From the other invitees information from the population registers of the municipalities is available. Figure 1 shows the number of participants per age stratum and gender in the national sample.

Table 1 Materials obtained and response in the PIENTER 2 project

	National sample N (%)	Low vaccination coverage sample N (%)
Total invited	19,781	4,366
Total materials present of persons who visited the clinic:		
Blood and questionnaire	6,348 (32%)	1,517 (35%)
Blood no info questionnaire	38	1
DNA*	6,207	1,469
Questionnaire (visited consult)	135	43
Diary*	824	NA
Only information form population register	7	
Materials obtained otherwise:		
Questionnaire	1,200	354
Short questionnaire	1,652	450
Information population register	10,401	2,001

* These materials should not be included in the total number of invited persons

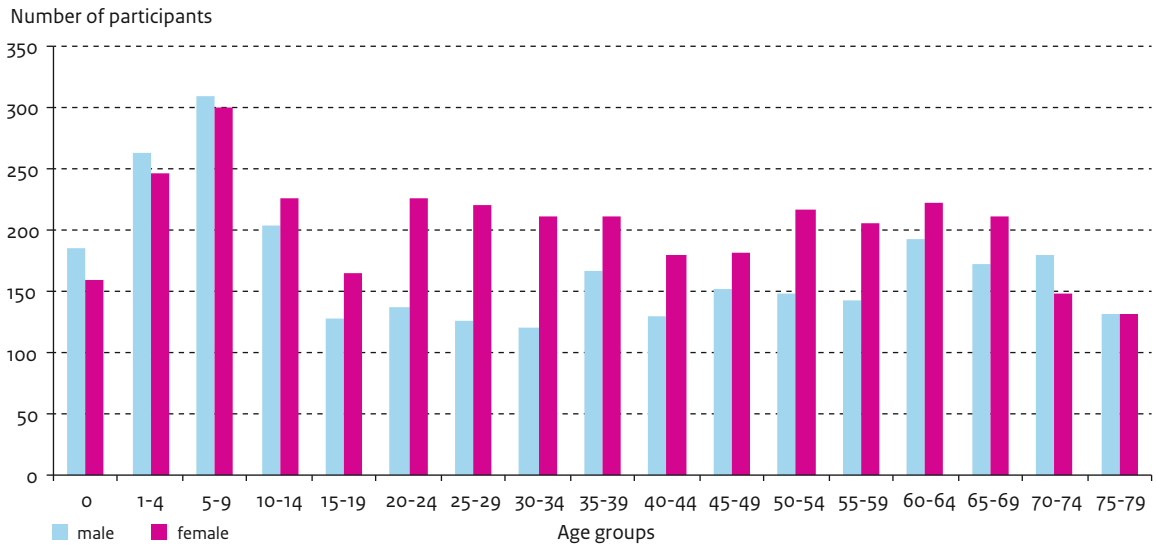

Figure 1 Number of participants (i.e., blood and a questionnaire) per age stratum in the national sample, stratified by gender

Discussion/conclusions

Age-specific antibody levels against the different vaccine preventable diseases in the NIP, but also against other infectious diseases have been determined. The data from the questionnaires have been used for the interpretation of the antibody levels and to obtain information on incidence and risk factors related to infectious diseases. The blood results and questionnaire information have been compared with the results of the first PIENTER study, which was performed ten years ago. These data are reported separately. Furthermore the collected diaries, DNA samples and supplementary questions in the questionnaire, for instance about allergies, have or will be used in additional studies.
The assessment of antibody levels in serum for evaluation of the NIP, by means of large population-

based studies like Pienter, becomes more and more important in view of low disease incidence and smaller number of cases, which is due to the success of the NIP. By repeating such studies within ten years intervals we gain insight into the changes of the immunity of the population over time and in changes in infection pressure to improve the NIP further.

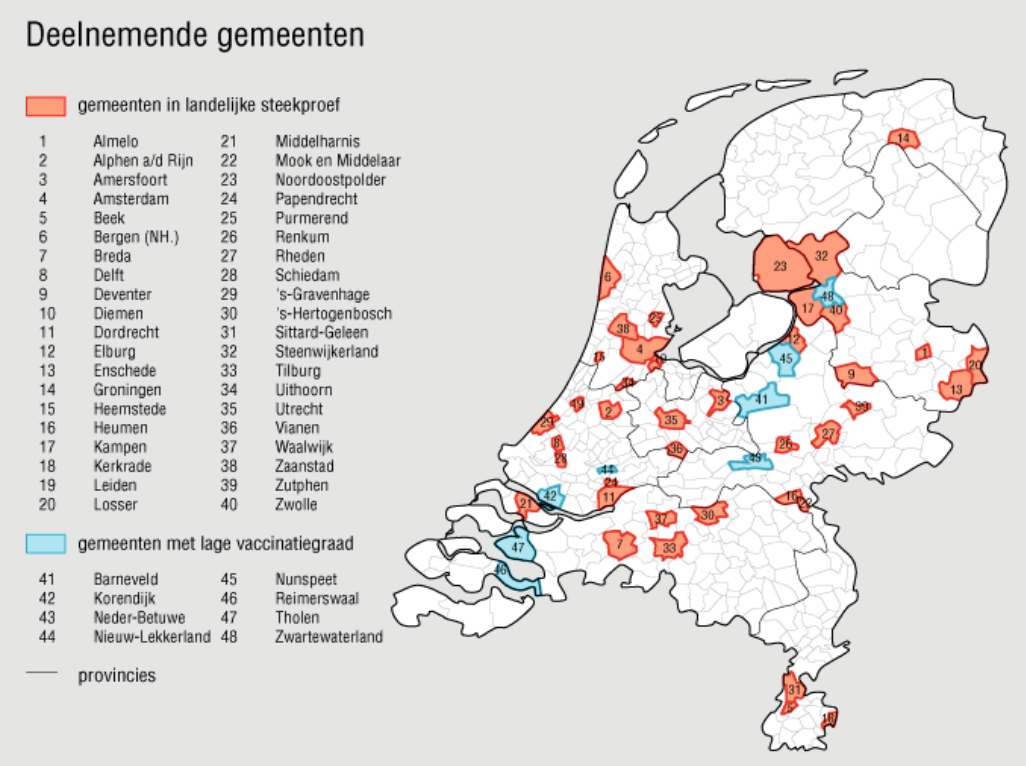

Figure 2 Geographic overview of the participating municipalities in the Pienter 2 study

Publications

Van der Klis FR, Mollema L, Berbers GA, de Melker HE, Coutinho RA. **Second national serum bank for population-based seroprevalence studies in the Netherlands**. *Neth J Med*. 2009 Jul-Aug;67(7):301-8.

Mollema L, de Melker HE, Hahné SJM, van Weert JWM, Berbers GAM, van der Klis FRM. **Pienter 2 project: second research project on the protection against infectious diseases offered by the national immunization programme in the Netherlands**. RIVM report 230421001/2009.

Contact persons: Fiona.van.der.Klis@rivm.nl
Liesbeth.Mollema@rivm.nl

Diseases National Immunization Program (RVP-ziekten)

1. Tetanus

Background

Tetanus is a serious disease and can be lethal without treatment in non-immunized individuals. As a result of successful tetanus vaccination in the national immunization program (NIP), the incidence of tetanus has effectively been reduced in the Netherlands. The average annual number of tetanus cases is two, which occur among unvaccinated persons. The long-term effect of routine vaccination and the current policy of vaccination after injury need to be evaluated to ensure that incidence will remain low or drop even lower. Seroepidemiological studies are useful for such evaluation. Since 1954, routine vaccination against tetanus has been offered to residents of the Netherlands born in 1951 and thereafter. Currently, tetanus vaccination is provided to children at 2, 3, 4, and 11 months of age, with a booster given at 4 and 9 years of age. In addition, *Haemophilus influenzae* type b-vaccination (Hib) conjugated with tetanus toxoid was introduced in 1993 as a single vaccination given at 2, 3, 4, and 11 months; since 2003, it is part of the combination vaccine DTPa-IPV-Hib. There was no catch-up campaign after 1993, but one was conducted in 2002 (1-18 years) after a meningococcal C conjugate (MenC) vaccine with tetanus toxoid as carrier protein was introduced for all children at 14 months of age. In order to identify subgroups susceptible to tetanus and to evaluate whether the policy of tetanus revaccination after injury needs to be revised in view of the recent changes, we aimed to determine the tetanus antitoxin (TT) antibody seroprevalence in the national population and the determinants of TT-antibody concentration. Data from this population-based serum collection (Pienter 2) were compared to data collected about 10 years earlier (Pienter 1).

Results

The overall seroprevalence (cut-off ≥ 0.01 IU/ml) in the nationwide sample was 94% (95% CI 94-95) and the overall TT-GMC was 0.91 IU/ml (95% CI 0.85-0.97). Men had significantly higher seroprevalence and TT-GMC than women (data not shown). For those eligible to participate in routine vaccination the seroprevalence amounted 99% (95% CI 99-100) (Figure 1) and the TT-GMC was 1.5 IU/ml (95% CI 1.4-1.6) (Figure 2). Of our participants with six or more registered tetanus vaccinations, none had a TT-antibody concentration below the minimum protective level. Migrants born in the former Dutch colonies of Suriname, Aruba, and the Netherlands Antilles had distributions of TT-antibody concentration similar to indigenous Dutch individuals. First-generation migrants from other non-Western countries, including Morocco and Turkey, had slightly lower seroprevalences and TT-GMC than the indigenous Dutch. Second-generation migrants from non-Western countries had higher TT-GMC than indigenous Dutch individuals and very high seroprevalences. In the low vaccine coverage sample the seroprevalence of conservative Protestants born in 1951 or thereafter was 36% (95% CI 17-57). Among individuals of other religious groups the seroprevalence was 80% (95% CI 76-85). For non-religious individuals born in 1951 or thereafter the seroprevalence was similar to the general population (96% (95% CI 94-98)).

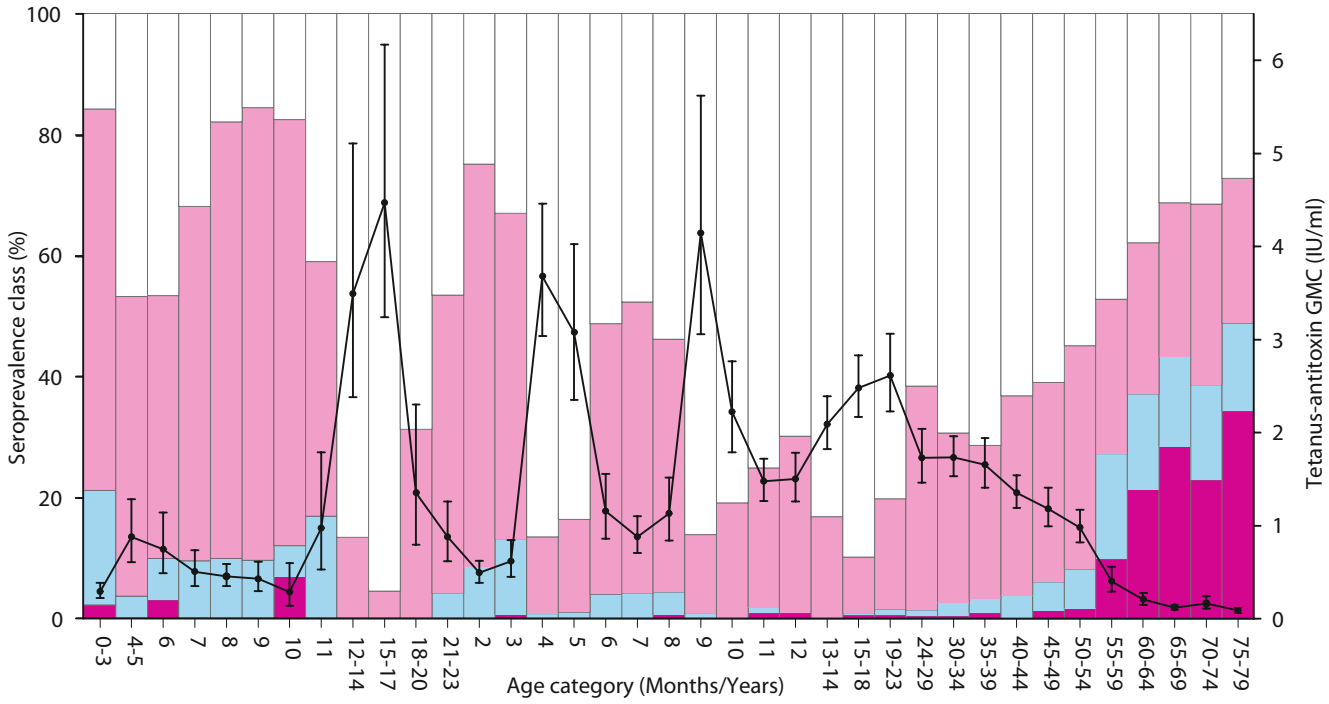

Figure 1 The columns represent the weighted age-group-specific seroprevalence of the general Dutch population (our national sample). The violet column represents a tetanus antitoxin (TT)-antibody concentration of < 0.01 IU/ml; the blue column represents a TT-antibody concentration between ≥ 0.01 IU/ml and < 0.1 IU/ml; the pink column represents a TT-antibody concentration between ≥ 0.1 IU/ml and < 1.0 IU/ml; and the white column represents a TT-antibody concentration of ≥ 1.0 IU/ml. The black line indicates the weighted age-group-specific TT-antibody geometric mean concentration (IU/ml). The error bars show the 95% confidence interval. Note: the first two years of life are presented in months, but thereafter age is presented in years.

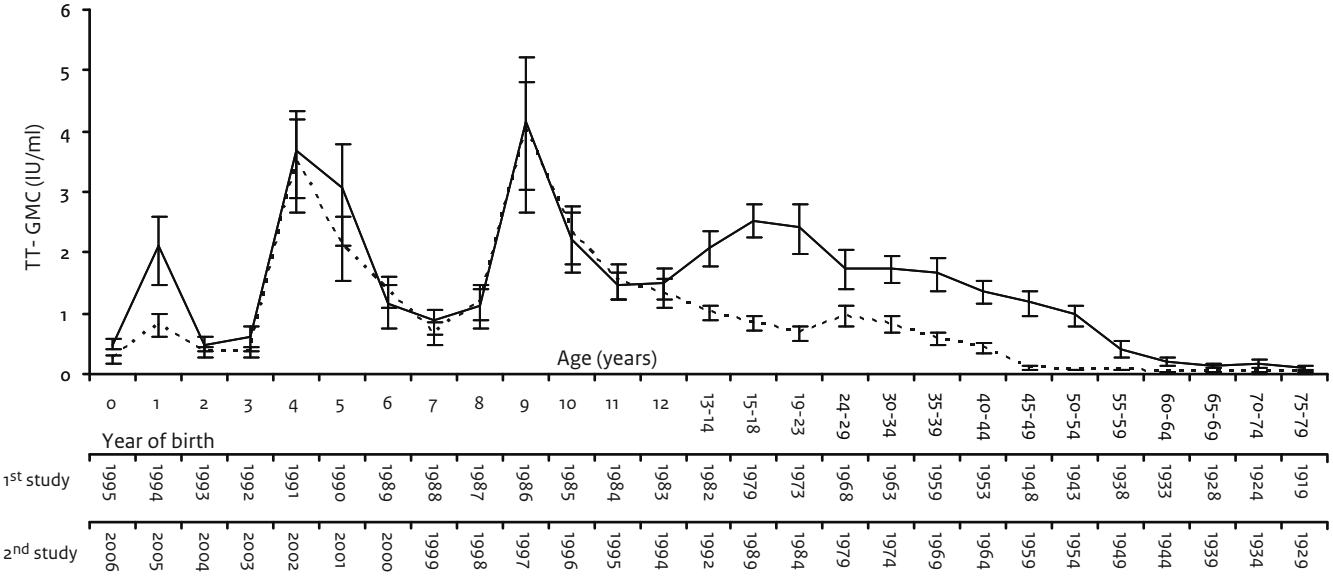

Figure 2 The weighted age-group-specific tetanus antitoxin antibody geometric mean concentration (IU/ml) is plotted by age for Pienter 1 (1995-1996) and Pienter 2 (2006-2007). Separate axes with the median year of birth per age-group are shown for each study. The dotted line represents data of the first serum collection; the continuous line represents data of the second collection. The error bars show the 95% confidence interval.

Discussion/conclusions

Our results show that the Dutch population is very well protected against tetanus due to high participation in the NIP and additional vaccinations because of travel or injury. In addition, the introduction of the MenC vaccine with tetanus toxoid as carrier protein, with its accompanying mass-vaccination campaign, resulted in high TT-antibody concentrations in the targeted age groups. However, a few subgroups have lower seroprevalences: individuals born before 1951 for whom routine vaccination was not yet available, migrants born in or before 1983 in a non-Western country (except Suriname, Aruba, and Netherlands Antilles), and members of some conservative Protestant groups. In conclusion, the Dutch population is very well protected against tetanus. The number of registered vaccinations underestimated the level of protection. To reduce tetanus vaccination, a more restrictive use of post-exposure prophylactic boosting could be explored by offering such vaccination after injury only to specific risk groups, initially guided by a rapid antibody test for all others.

Publication

Steens A, Mollema L, Berbers GA, van Gageldonk PG, van der Klis FR, de Melker HE. **High tetanus antitoxin antibody concentrations in the Netherlands: a seroepidemiological study**. *Vaccine*. 2010 Nov 16;28(49):7803-9.

Contact persons: Hester.de.Melker@rivm.nl
Liesbeth.Mollema@rivm.nl

2. Pertussis (kinkhoest)

Background

In the last decades, an increase of the reported incidence of clinical pertussis cases has been observed in many countries despite a high vaccination coverage. Various explanations have been given for the pertussis re-emergence, including increased awareness, improved diagnostics, waning of vaccine-induced immunity and adaptation of the causative pathogen *Bordetella pertussis* with strains that produce more pertussis toxin. In the Netherlands, despite a consistently high vaccination coverage for decades, increased numbers of pertussis notifications have been observed since 1996 with epidemic peaks every 2–3 years (Figure 1).

Accurate determination of the burden of disease is hampered by reporting artifacts. The infection frequency is more reliably estimated on the basis of the prevalence of high IgG concentrations against pertussis toxin (IgG-PT). The aim was to determine whether the increase in reported pertussis in the last decade is associated with an increase in the number of infections. In Pienter 2 sera, IgG-Ptx concentrations were analyzed using a fluorescent bead-based multiplex immuno assay.

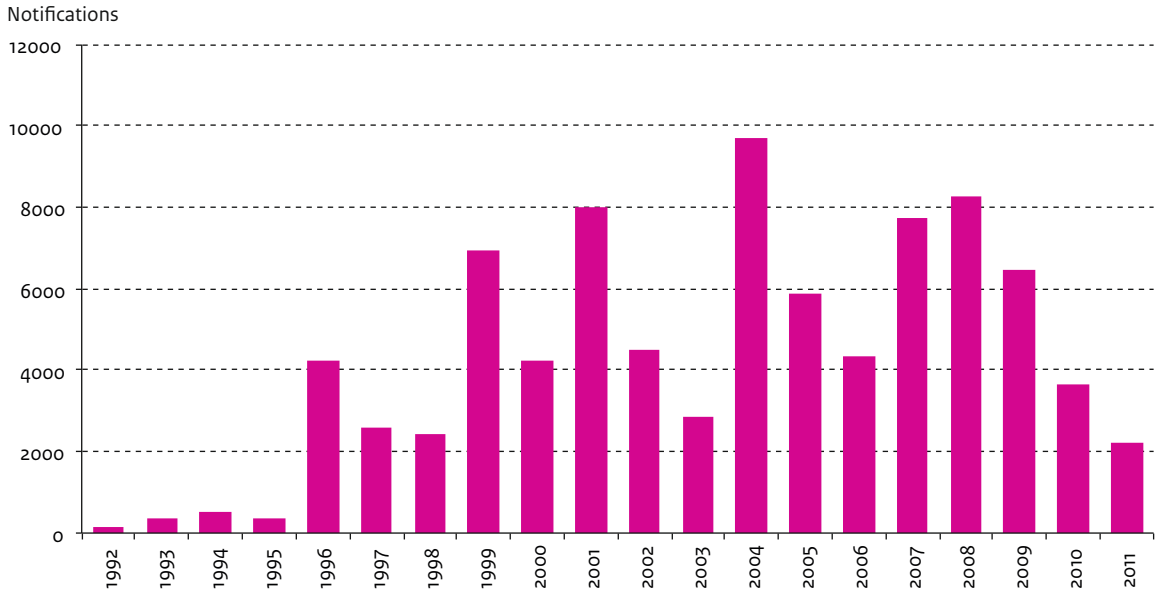

Figure 1 Notifications of pertussis in the Netherlands

Results

In the Pienter 2 study (2006-07), 9.3% (95%CI 8.5-10.1) of the population above 9 years of age had an IgG-PT concentration above 62.5 EU/ml, which is suggestive for pertussis infection in the past year. This means that the infection frequency in adolescents and adults has more than doubled within a decade compared to the Pienter 1 study (1995-96) (4.0%; 95%CI 3.3-4.7) (Figure 2). The reported incidence showed a similar increase as the seroprevalence between both periods.

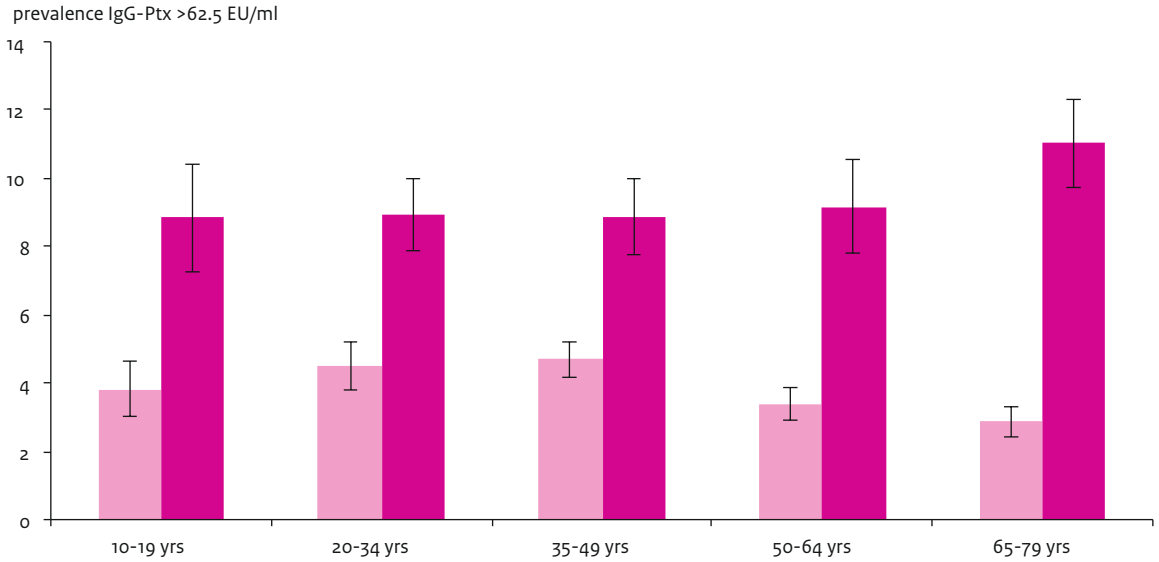

Figure 2: Seroprevalence of specific IgG-PT antibody levels indicative for a recent infection. Pink bars represent Pienter 1 study and violet bars represent Pienter 2 study.

Discussion/conclusions

The Pienter studies have demonstrated that the increase in pertussis incidence in the Netherlands is real. This percentage (>9%) means more than a million infections per year. Only 1% of these cases are reported, probably due to relatively mild infections. Although the vast majority of these older age groups suffer from relatively mild symptoms, they are the main source of infection for the young, not fully vaccinated infants who are at risk for the pertussis associated life-threatening complications (even death).

Although the changes in the vaccination program (implementation of an acellular booster vaccine in 2001 and change from whole cell vaccine to acellular vaccine in first year of life in 2005) have reduced pertussis morbidity in childhood, they have not affected the increased pertussis infection rate in adolescents and adults. Indeed, the high circulation of *B. pertussis* in the latter age-categories may limit the effectiveness of pediatric vaccination.

We believe it would be most efficient to invest in the development of improved vaccines which induce long lasting immunity to reduce the pertussis disease burden. For the short term however, the high circulation emphasizes the need for good protection of unvaccinated infants who are at highest risk for severe pertussis. Vaccinating people in close contact with infants, might be an (cost)effective strategy to prevent transmission of pertussis to infants rather than the introduction of repetitive adolescent/adult booster vaccinations.

Publication

De Greeff SC, de Melker HE, van Gageldonk PGM, Schellekens JFP, van der Klis FRM, Mollema L, Mooi FR, Berbers GAM: **Seroprevalence of pertussis in the Netherlands: evidence for increased circulation of Bordetella pertussis** *Plos One* 2010, 5(12):e14183.

Contact person: Guy.Berbers@rivm.nl

3. Meningococcal C disease

Background

In 2002 a Meningococcal serogroup C (MenC) conjugate vaccine, with tetanus toxoid as carrier protein, was introduced in the Netherlands as a single-dose at 14 months of age. A catch-up campaign was performed targeting all individuals aged 14 months up to 18 years. We determined the MenC-specific immunity before and after introduction of the MenC conjugate vaccine.
The levels of MenC polysaccharide (PS)-specific IgG were analyzed using a fluorescent bead-based multiplex immuno assay (MIA) and serum bactericidal antibody (SBA) titers were measured in the different age cohorts of the Pienter 1 (1995/1996) and Pienter 2 studies (2006/2007). We also measured MenC PS-specific IgM, the IgG1 and IgG2 subclasses and the avidity of the IgG antibodies.

Results

The PS-specific IgG and SBA antibody levels revealed an increasing persistence with age in the catch-up immunized cohorts 4–5 years after their MenCC immunization, gradually increasing from 6 years of age up to 22 years (Figure 1). A comparable pattern was found for antibodies against the carrier protein (tetanus toxoid) in children immunized above 9 years of age. In case of vaccination before the age of 5 years, PS-specific IgG was rapidly lost (Figure 1). Overall, SBA seroprevalence (titers ≥8) increased from 19.7% to 43.0% in the pre- and post-MenC introduction eras, respectively. In non-immunized adults the SBA seroprevalence was not significantly different between the pre- and post-MenC introduction periods, whereas PS-specific IgG was significantly lower in the post-MenC vaccination era compared with the pre-vaccination era.
The age-related persistence of IgG after immunization with the MenCC vaccine seemed to result from an increase of IgG2 levels with age, while IgG1 levels remained stable throughout the different age cohorts. It is noteworthy that the increase in IgG2 correlated with a reduced IgG-avidity with age. These data indicate that the classical characteristics of a T-cell-dependent antibody response as elicited by protein based vaccines might not be completely applicable when conjugate vaccines are administered to older children and adolescents up to 18 years of age. The response elicited by the MenCC vaccine seemed to be more a mixture of both T cell dependent and T cell independent responses in terms of humoral immunological characteristics.

Discussion/conclusions

A single MenCC immunization leads to improved protection compared to naturally elicited immunity. A single MenCC immunization above 5 years of age seems to induce persistent protective antibodies, whereas a single injection at 14 months leads to a rapid waning within a few years of serological protective levels. Since memory responses may not be fast enough to prevent disease, antibody persistence by vaccination seems to be the major way to prevent MenC disease. If the incidence of MenC disease increases due to renewed circulation, a MenCC booster immunization just before entering adolescence may be appropriate. A booster will most likely result in a high level of protective bactericidal antibodies as previously shown in infants and adolescents, and will probably preserve herd-effects, which is requested for those who may be most susceptible to disease, due to a lack of antibodies (cohorts under 14 months of age). But most importantly, a second MenCC immunization will also improve protection in the vaccinated cohorts, as in the years to come a increasing part of children will be less well protected since they received only a primary immunization at 14 months.

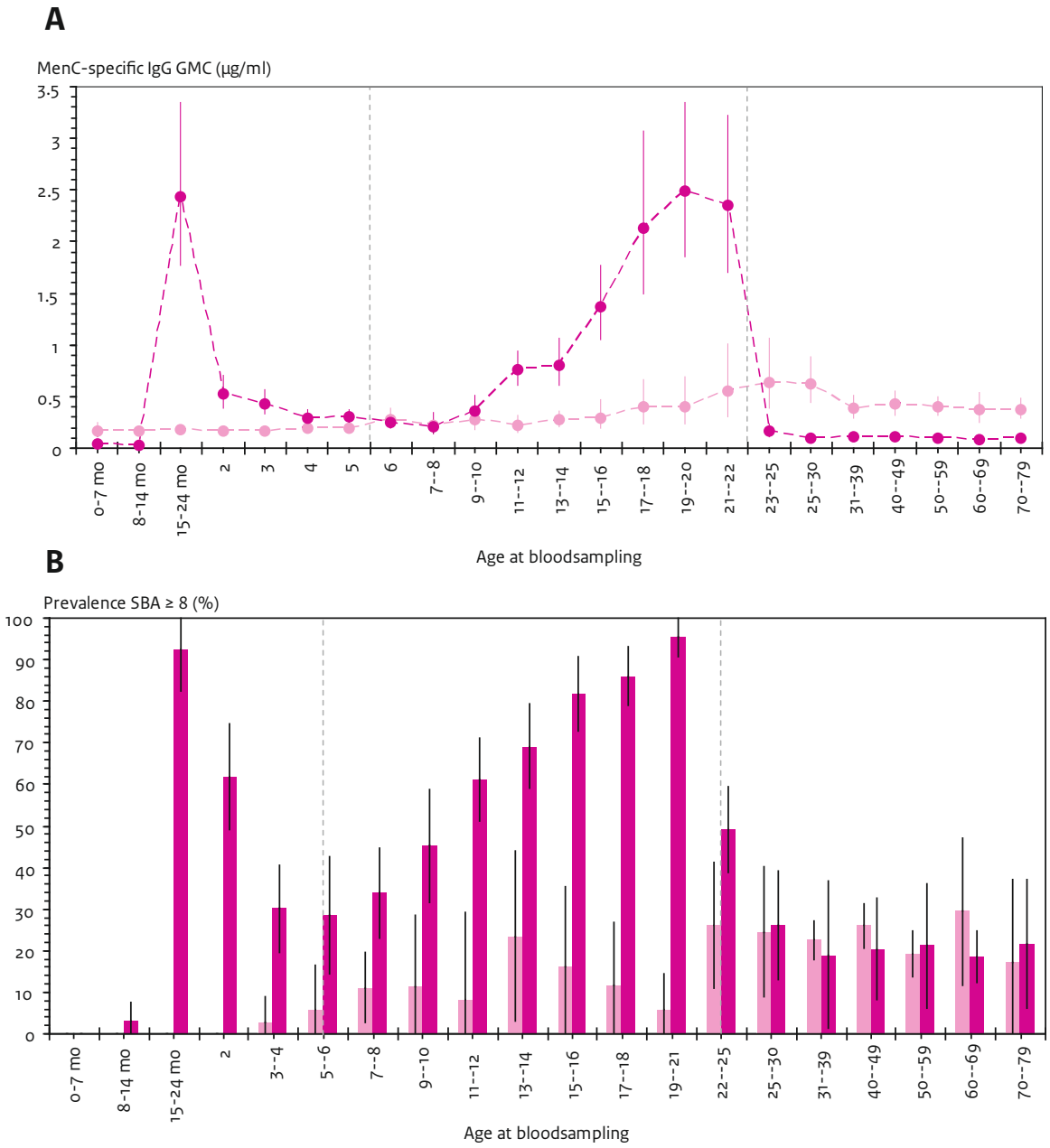

Figure 1 MenC PS-specific IgG (A) and seroprevalence of serum bactericidal antibody levels (SBA, B) within each age-cohort, pre- (pink, Pienter 1) and post-introduction (violet, Pienter 2)of the MenC conjugate vaccine.

Publications

De Voer R. M., Mollema L., Schepp R.M., de Greeff S.C., de Melker H.E., Sanders E.A.M., Berbers G. A.M., van der Klis F.R.M. **Immunity against Neisseria meningitidis Serogroup C in the Dutch Population before and after Introduction of the Meningococcal C Conjugate**. *PLoS ONE* (2010), 5(8), e12144.

De Voer R.M., van der Klis F.R.M., Schepp R.M., Rijkers G.T., Sanders E.A.M & Berbers G.A.M. **Age-related immune responses to Meningococcal serogroup C conjugate vaccination: an Increase in the Persistence of IgG2 Correlating with a Decrease in the Avidity of IgG**. *PLoS ONE* (2011) 6(8): e23497.

Contact person: Fiona.van.der.Klis@rivm.nl

4. Pneumococcal disease

Background

The introduction of the pneumococcal conjugate vaccination in 2006 in the national immunization program will change the immune status against the pneumococcus in the Dutch population. Besides the direct effects such as high antibody concentrations in immunized children against the serotypes included in the vaccine, also changes in the immune status of the non-immunized population due to reduced exposure to pneumococcal vaccine-serotypes may be observed. In this study the IgG antibody concentrations against 13 vaccine serotypes of the pneumococcus were assessed in the Netherlands before the introduction of pneumococcal vaccination. The 13 serotype specific IgG concentrations were assessed simultaneously using a fluorescent bead-based multiplex immuno assay (MIA). The aim of this study was to establish the baseline immune status against the pneumococcus before the introduction of the pneumococcal vaccine and to determine the relationship between IgG concentrations induced by natural exposure to the pneumococci and the occurrence of invasive pneumococcal disease in the Netherlands. The baseline IgG concentrations may be compared with those after the implementation of the pneumococcal vaccination in future seroprevalence studies.

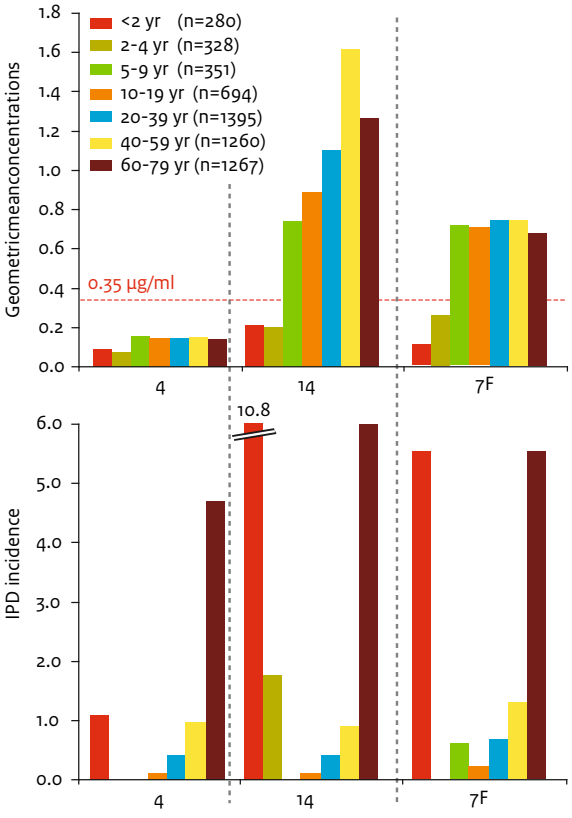

Figure 1. Age stratified geometric mean IgG concentrations and invasive pneumococcal disease incidence.

Results

Overall, the geometric mean IgG concentrations (GMCs) against most of the 13 serotypes in unvaccinated individuals increased with age up to 5-9 years and remained at a plateau thereafter (Figure 1). The highest GMCs were found for antibodies directed against serotype 14 and 19F, whereas antibodies against serotypes 4 and 5 had the lowest GMCs. There was no uniform relationship between the occurrence of serotypes causing invasive pneumococcal disease (IPD) and the GMCs against these serotypes (Figure 1). The data also showed that individuals develop antibodies against an increasing number of different serotypes with increasing age, up to the age of 5-9 years. Increased IPD incidence in the elderly did not seem to be the result of a decline in the GMCs in this age group.

Discussion/conclusions

The seroprevalence before the introduction of the pneumococcal vaccine may serve as a baseline seroprevalence. For most serotypes there was a steady increase of the concentration of anti-pneumococcal antibodies with increasing age and a plateau was reached after the age of 5-9 years. This indicates that carriage of the most prevalent serotypes already occurs before 5 years of age. Comparison of the seroprevalence before and after the implementation of the pneumococcal vaccination may provide insights into the carriage and prevalence of the circulating serotypes before and after the selective pressure of vaccine serotypes.

No clear relationship between IgG concentrations and IPD incidence could be established in this study. But, according to data from a vaccination trial, the IgG concentrations after childhood vaccination are significantly higher, up to a 40-fold, compared to the IgG concentrations after carriage. It is unclear how long the IgG concentrations persist after vaccination. A future Pienter 3 study may provide the answer to this question.

The quantification of IgG concentrations against 13 serotypes in this study anticipated on the introduction of the 13-valent pneumococcal vaccine (Prevenar13, Pfizer, PCV13) as a replacement of the 7-valent vaccine Prevenar. However, in 2011 the 10-valent vaccine (Synflorix, GSK, PCV10) replaced PCV7 in the Dutch immunization program. Although PCV7 has been effective on the IPD incidences of serotypes covered by PCV7, data from 2010 indicate an increase in serotypes that are not covered by PCV7 and PCV10.

Publication

Elberse KE, de Greeff SC, Wattimena N, Chew W, Schot CS, van de Pol JE, van der Heide JG, van der Ende A, van der Klis FR, Schouls LM. **Seroprevalence of IgG antibodies against 13 vaccine *Streptococcus* pneumoniae serotypes in the Netherlands**. *Vaccine*. 2011 Jan 29;29(5):1029-35.

Contact persons: Karin.Elberse@rivm.nl
Leo.Schouls@rivm.nl

5. Hepatitis B and Hepatitis C

Background

Hepatitis B and C virus infect the liver and can cause a broad spectrum of disease outcomes. Chronic infection with these viruses can lead to liver cirrhosis, hepatocellular carcinoma and death. A safe and effective vaccine is available against HBV, but not against HCV. Universal infant HBV vaccination was introduced in August 2011. The aim of testing Pienter sera for evidence of (past) HBV and HCV infection was to obtain a population prevalence estimate, to assess whether this had changed between Pienter 1 and 2, and to identify risk factors for HBV and HCV infection.

Results

Hepatitis B

In 1995-6 (Pienter 1), the weighed population prevalence of anti-HBc and HBsAg was 2.9% and 0.1%, respectively. In 2006-7 (Pienter 2), the anti-HBc population and HBsAg prevalence was 3.5% (95% CI 2.2-5.5%) and 0.2% (0.1-0.4%). The prevalence of HBsAg and anti-HBc did not statistically differ between 1995-6 and 2006-7. However, among indigenous Dutch, the anti-HBc prevalence was lower in 2006-7 than in 1995-6 (p=0.06).

In 2006-7, first generation migrants (FGM) had a 13 times higher risk of being HBsAg and/or HBV-DNA positive than indigenous Dutch. FGM are the main target group for secondary HBV prevention in the Netherlands. Among indigenous Dutch, risk factors for anti-HBc positivity were older age and having received a blood product before 1990. Among FGM, being of Asian origin was a risk factor. Among second generation migrants having a foreign born partner and injecting drug use were risk factors.

Hepatitis C

In Pienter 2, the weighted national HCV seroprevalence was 0.30% (95% CI 0.05 – 0.55%). About 70% of the HCV positive individuals found were born in an HCV endemic country. Numbers were too small to analyse risk factors.

For Pienter 1, Veldhuijzen et al. described a HCV seroprevalence of 0.1% (95% CI: 0.01-0.2%).

Discussion/conclusions

The Pienter surveys are the only source of information on the population prevalence of and population risk factors for hepatitis B and C. This information is important for planning primary and secondary prevention.

For both hepatitis B and C, first generation migrants (FGM) are the most important high prevalence group. Secondary prevention programmes, through early diagnosis and treatment, should be focused on FGM.

Publications

Hepatitis B

S. J. M. Hahné, H. E. de Melker, M. Kretzschmar, L. Mollema, F. R. van der Klis, M. A. B. van der Sande, and H. J. Boot. **Prevalence of hepatitis B virus infection in The Netherlands in 1996 and 2007**. *Epidemiology and Infection*, 2011; nov14; 1-12.

Hepatitis C

Vriend HJ, Op de Coul ELM, van de Laar TJW, Urbanus AT, van der Klis FRM, Boot HJ. **Hepatitis C Virus seroprevalence in the Netherlands**. *Eur. J. Public Health*, 2012; Mar 29 (available on line)

Vriend HJ, Op de Coul ELM, Urbanus AT, Prins M, van Veen MG, Boot HJ. **National and risk group estimates of Hepatitis C Virus prevalence in the Netherlands**. *Submitted*.

Contact persons: hepatitis B: Susan.Hahné@rivm.nl
hepatitis C: Rianne.Vriend@rivm.nl

6. Disease caused by human papilloma virus (HPV)

Background

Human papilloma virus (HPV) is one of the most common sexually transmitted pathogens worldwide. High-risk (hr) HPV, such as types 16 and 18, can cause cervical cancer, other genital cancers, and also oro-pharyngeal cancer. HPV16 and HPV18 are the most common HPV types detected in women worldwide and are responsible for 70% of all cervical cancer cases. When infected with HPV, most genital transient HPV infections regress within two years. Only a small proportion of infected individuals suffer from persistent infections and they are at risk for cervical cancer. Not all HPV infected individuals seroconvert and 20-50% of the women who are carriers of HPV DNA do not have detectable HPV antibodies in their serum. When HPV antibody responses develop after a natural HPV infection, they are relatively stable over time. Therefore, serosurveillance studies will provide information about the HPV seroprevalence in the nationwide population and can be used to estimate lifetime cumulative HPV exposure and past HPV infections. The Pienter 2 study was used to asses the seroprevalence of seven hr-HPV types (16, 18, 31, 33, 45, 52 and 58) and the associated risk-factors before the implementation of the HPV vaccine in the national immunization program (NIP). In the Netherlands, HPV vaccination (Cervarix, including types 16 and 18) was included in the NIP in 2010 for girls 12 years of age with overall vaccination coverage of 50%. In addition, a catch-up vaccination campaign was performed for girls 13-16 years of age. Seroprevalence data from the pre-vaccination era in the Netherlands are scarce, but seroprevalence data can be valuable to provide insight in the HPV incidence in the pre-vaccination era and to evaluate the possible implications of HPV vaccination in the future.

Results

The overall seroprevalence for individuals 0-79 years of age for any of the seven HPV-types amounted to 17.7%; 16.0% for men and 19.4% for women. As expected based on the median age of sexual debut, an increase in seroprevalence was observed in the age cohort of 15-19 years and 20-24 years. This increase in seroprevalence during adolescence is most pronounced for HPV16 in women (4.3% up to 14.1%), but is less clear for the other six HPV serotypes. In the overall seroprevalence a declining trend could be observed from 50 years of age onwards ($p = 0.0125$) (Figure 1).

Focusing on participants older than 14 years of age we found an overall seroprevalence of 22.8%. The seroprevalence was significantly higher among women (25.2%) compared to men (20.3%) ($p = 0.0002$). Seropositivity for two or more HPV types occurred in 10.1% of the individuals older than 14 years of age. The most prevalent HPV serotype among participants older than 14 years of age was HPV16 followed by HPV serotypes 45, 18, 33 and 52. HPV seropositivity for at least one of the tested seven HPV types was significantly associated with ethnicity, marital status, having a casual partner, age of sexual debut and having ever suffered from a sexually transmitted disease. HPV seropositivity for at least one of the seven HPV types tested was significantly higher for migrants from Suriname, Aruba, and the Dutch Antilles. Also marital status, i.e., not married, living together, and divorced were associated with an increased risk for HPV seropositivity compared with married individuals. In contrast, widowed individuals were associated with a decreased risk for HPV seropositivity

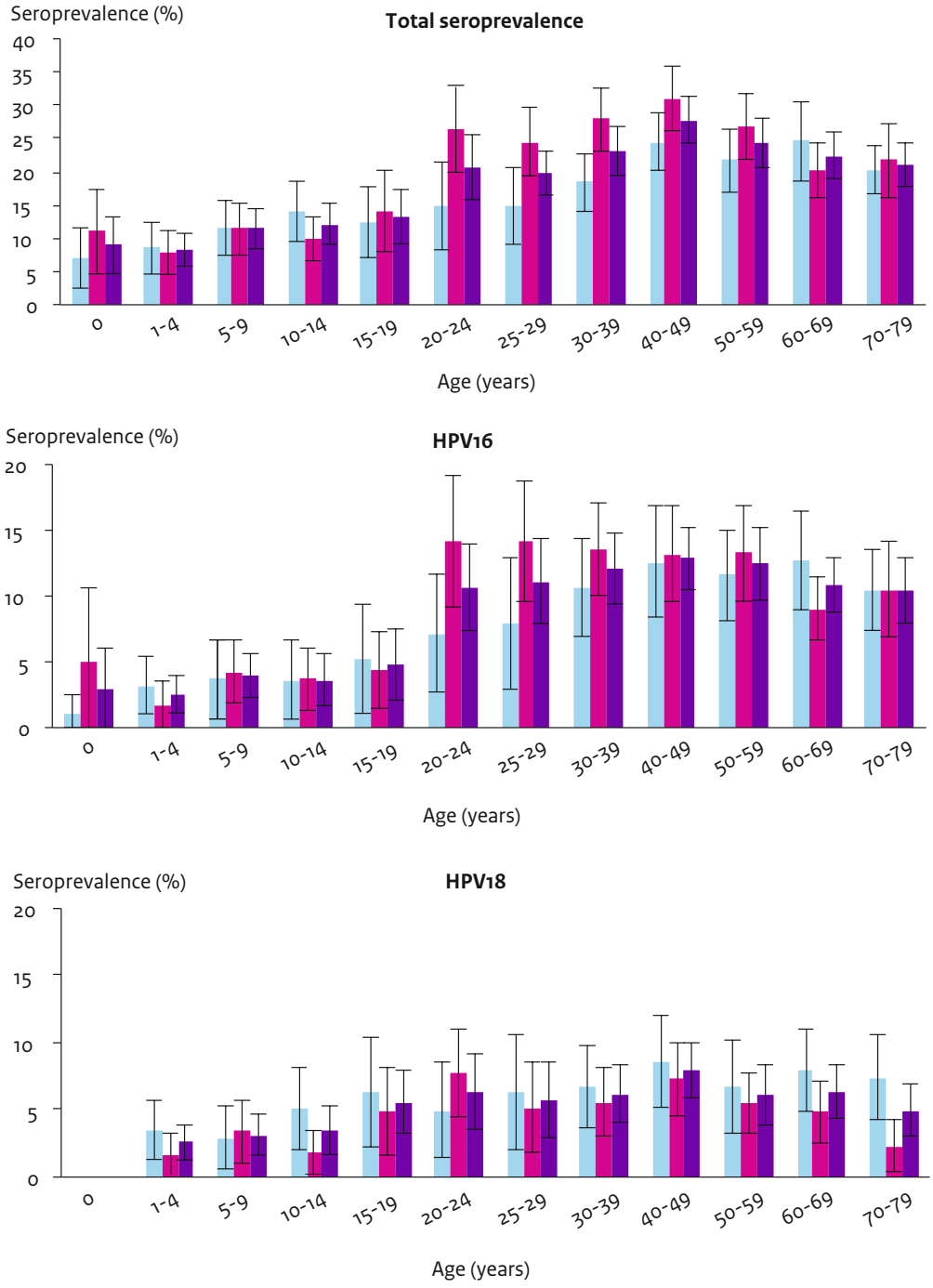

Figure 1 Total HPV seroprevalence and seroprevalence of seven high-risk HPV types in the Netherlands among males (blue bars), females (violet bars), and overall (purple bars). Error bars indicate 95% confidence intervals.

Discussion/conclusions

As nearly all genital HPV infections are sexually transmitted, the sharpest increase in seroprevalence is expected around puberty reflecting the start of sexual activity. This was most pronounced in women. Although it is possible to be infected with multiple HPV types, resulting in the observed multi-seropositivity in 10.1% of the study population, cross-reactivity of HPV specific antibodies should also be taken into account. Studies among men indicate that hr-HPV types are very common among men and HPV infections in men are strongly associated with anal and penile cancers. Because women are currently vaccinated against HPV16 and 18, herd immunity can play a roll in the future in reducing the incidence of HPV infection in men. Moreover, most women, older than 14 years of age, are HPV seronegative and could therefore also benefit from the HPV16/18 vaccination. Our study provides information about the seroepidemiology of seven hr-HPV types in the Netherlands. Seroprevalences in the pre-vaccine era can be used as baseline to evaluate long-term population effects of HPV16/18 vaccination such as effect of vaccination on virus circulation in both women and men. Further studies in measurement of immune responses and interpretation of serological data are needed to make full advantage of this tool used for the monitoring of HPV-vaccination programmes.

Publications

Mirte Scherpenisse, Madelief Mollers, Rutger M Schepp, Hein J Boot, Hester E de Melker, Chris JLM Meijer, Guy AM Berbers, Fiona RM van der Klis. **Seroprevalence of seven high-risk HPV types in the Netherlands.** *Submitted*.

Contact persons: Fiona.van.der.Klis@rivm.nl
Hester.de.Melker@rivm.nl

Preliminary results

7. Disease caused by *Haemophilus influenzae* type b (Hib)

Background

Nationwide vaccination against *Haemophilus influenzae* serotype b (Hib) has been introduced in the Netherlands in April 1993 using a 4-dose vaccination schedule at 3, 4, 5, and 11 months of age. Since then the incidence of invasive Hib disease has declined dramatically from 28.7 per 100,000 children aged 0-4 years in 1992 to 0.80 per 100,000 in 2001. However, despite nearly complete vaccine coverage, a small number of fully vaccinated children in The Netherlands have experienced invasive Hib infection. In 2002 the number of cases invasive Hib disease in Dutch children aged 0 to 4 years started to increase again reaching its peak in 2005 after that the incidence in children aged 0-4 years has dropped again to reach 0.97 per 100,000 in 2009. Genotyping of the Hib isolates obtained from the patients did not reveal the emergence of particular genotypes that were capable of explaining the observed increase. No other factors were found that could explain the increase and as a result the cause of the transient resurgence of Hib disease has remained enigmatic.

The unexpected rise in the incidence of invasive Hib disease emphasizes the importance of post-licensing evaluation of vaccines to assess the efficacy of the vaccination program. Mass vaccination may change the epidemiological dynamics of infectious diseases; e.g., it may cause the elimination of circulation of the pathogen which consequently results in reduced natural exposure that may boost immunity. Serological surveillance may be of particular value for diseases like Hib disease with a small number of cases as a result of mass vaccination with high coverage. In this study we compared the antibody concentrations against Hib polysaccharide from the Pienter 2 study with those obtained in the Pienter 1 study to assess whether any changes in the degree of protection against Hib disease have occurred in the Dutch population 13 years after the introduction of nationwide Hib vaccination.

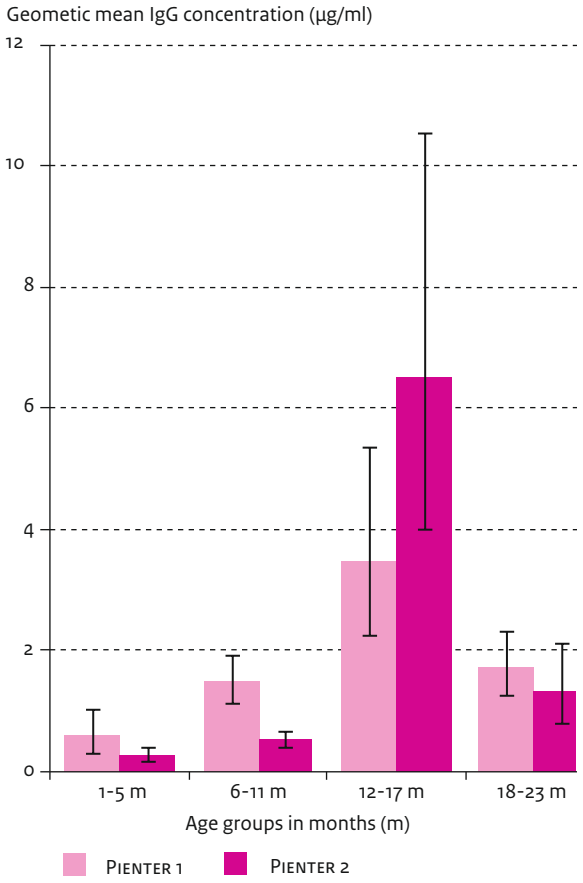

Figure 1. GMCs of IgG against Hib in 0-4 year old children in the national samples for the Pienter 1 and Pienter 2 studies.

Preliminary results

Analysis showed that serum samples of the national sample from children 6-11 months in age (post-primary vaccination) from the Pienter 2 study had approximately 3-fold lower anti-Hib antibody concentrations than samples from children from the same age group from the Pienter 1 study. Although the antibody concentrations were considerably lower in the Pienter 2 samples they still were well above the established protective value of 0.15 µg/ml. No significant differences between the antibody concentrations were found in samples from individuals older than 11 months in age (post-booster or non-vaccinated). In the 6-11 months old children that had received all 3 vaccinations of the primary series the proportion of children with anti-Hib antibody concentrations below the putative protective concentration of ≥ 1 µg/ml was 40%. This was significantly higher than in children with the same age and vaccination status in the Pienter 1 study where this proportion was 60%. Serum bactericidal assays performed on a selection of samples corroborated the finding that children in the Pienter 2 study had lower antibody against Hib than those enrolled in the Pienter 1 study.

Discussion/conclusions

This study revealed that there is a reduction in the vaccine induced immunity after vaccination against Hib in children enrolled in the Pienter 2 study compared to those from the Pienter 1 study. Although the results suggest that a fraction of the vaccinated children may not be sufficiently immunized to protect them against Hib infections during the time period between the last vaccination of the primary series and the booster vaccination, the number of cases of invasive Hib disease in the Netherlands has been very low with an average of 15 cases among children aged 0-47 months annually since 1995 (incidence 0.97 per 100,000 in 2009). It is unlikely that the brief upsurge of cases of invasive Hib disease in 2005 is caused by this reduced immunity.
There may several reasons that can explain the observed decrease in response to the Hib vaccination. Due to the introduction of a nationwide immunization program the major reservoir for Hib has been eliminated leading to considerably reduced circulation of Hib and consequently reduced natural booster effects. This may have contributed to the observed reduced response to Hib vaccination, but it is difficult to measure and its extend remains uncertain.
In the United Kingdom it was deduced that interference by the acellular pertussis vaccine was responsible for a considerable increase in number of cases of Hib vaccine failures in 2002 which occurred mainly in children 1-4 years of age. This has lead to a catch up campaign in 2003 and the introduction of a Hib booster vaccination at the age of 12 months in 2006. A study performed in the UK by Southern et al. showed that children who received a combination vaccine containing acellular pertussis vaccine developed significantly lower anti-Hib antibody titers compared to children who received a combination vaccine containing whole cell pertussis vaccine. However, after receiving a booster dose, there was no longer a statistical difference between the antibody concentrations induced by both combination vaccines. The resemblance between these observations and the results that we obtained is striking. We therefore believe that the most likely explanation for the reduced immunogenicity of Hib vaccine observed in the Pienter 2 study is caused by interference of the acellular pertussis components in the combination vaccine used in the Dutch national immunization program.

Despite the apparent reduced response induced by the Hib vaccine the number of cases of Hib disease in the Netherlands is still very low. Prior to the introduction of Hib vaccination the highest incidence of Hib disease was in 6-11 months old children and currently this is the age group which may be less well protected due to suboptimal response to Hib vaccination, emphasizing the need to remain vigilant and maintain the surveillance for Hib disease. From this study it might be hypothesized that changes in the vaccination schedule and composition of the vaccines may have lead to reduced immune responses against some of the vaccine components. Therefore it is important to perform a Pienter 3 study in a couple of years from now to monitor if further changes in the Dutch national immunization program may have a negative effect on protection against some of the diseases, necessitating modification of the schedule or dosing of the vaccination.

Contact person: Leo.Schouls@rivm.nl

8. Measles (mazelen)

Background

In the Netherlands, children are offered vaccination against measles, mumps and rubella with a combination vaccine (MMR-vaccine) at the age of 14 months and 9 years since 1987, with a three year catch-up programme for pre-school children at its start. This programme was preceded by separate measles vaccination for 14 month-old infants introduced in 1976. In the Netherlands, vaccine coverage for one dose of MMR is 96% and for two doses of MMR 92%. However, in 31 (7%) and 81 (19%) municipalities the vaccine coverage is below 90% (<60%-90%) for one dose and two doses of MMR, respectively.

The most important study questions for the Pienter measles analyses were:

1. Is the Dutch population well-protected against measles, and how does this compare to ten years ago (Pienter 1 study)?
2. What is the risk of a measles outbreak in the religious community?
3. Does waning immunity occur after having received two MMR-vaccinations?

Preliminary results

Figure 1 shows that the measles IgG seroprevalence (level of antibodies ≥ 0.2 IU/ml) in the general population is high for both Pienter 1 and Pienter 2. Figure 2 (measles GMC of IgG by age) shows clearly that IgG concentrations among those vaccinated (until age-group 20-22 years and 29-31 years in Pienter 1 and Pienter 2, respectively) are lower than among cohorts exposed to natural infection by wild type measles virus (after 22 years and 31 years in Pienter 1 and Pienter 2, respectively). For those twice vaccinated and not exposed to natural measles in Pienter 2, the IgG GMC decreased from 1.2 IU/m to 0.6 IU/ml from 10 to 28 years of age. Participants up to age 9 years in Pienter 2 from religious communities (partly) refusing vaccination had a low seroprevalence (63.3% at 5-9 years), thereafter the seroprevalence was above 93% (Figure 3).

Discussion/conclusions

The Dutch population is generally well-protected against measles. In Pienter 2 the level of maternal antibodies in young infants was lower than in Pienter 1, reflecting the lower titers obtained through vaccination than natural infection in women at child-bearing age. This implies that more infants are susceptible. Guidelines for travel and outbreak control may need to take this lower age of susceptibility into account. In vaccinated cohorts a declining antibody level over time was observed. However, antibody levels remained well above the cut-off for protection. Considering the high measles incidence in other European countries and the large susceptible group among children from religious communities refusing vaccination, there is a high risk of a large measles outbreak in the Netherlands.

Contact person: Liesbeth.Mollema@rivm.nl

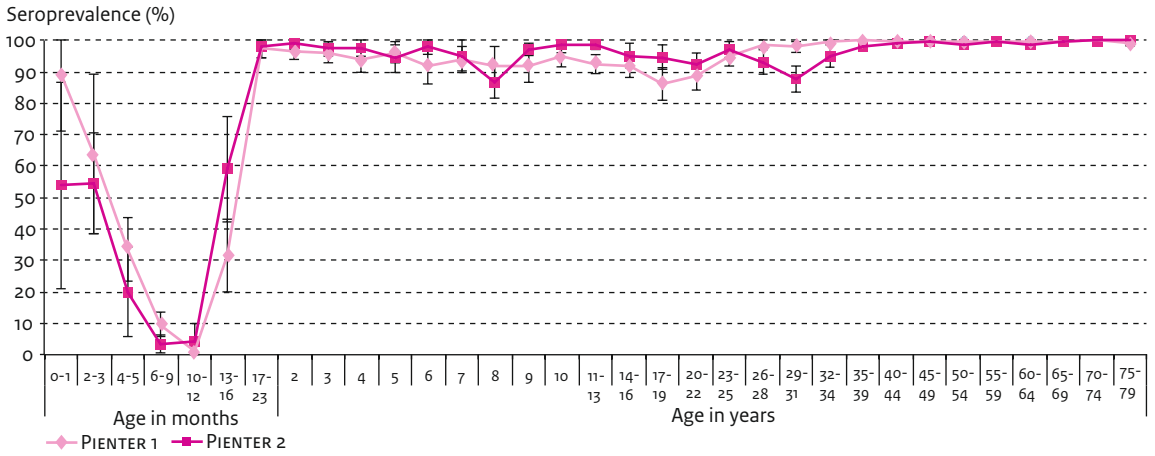

Figure 1. Age-specific seroprevalence of measles IgG antibodies (with 95% CIs) in the general population, Pienter 1 (1995/6) and Pienter 2 (2006/7)

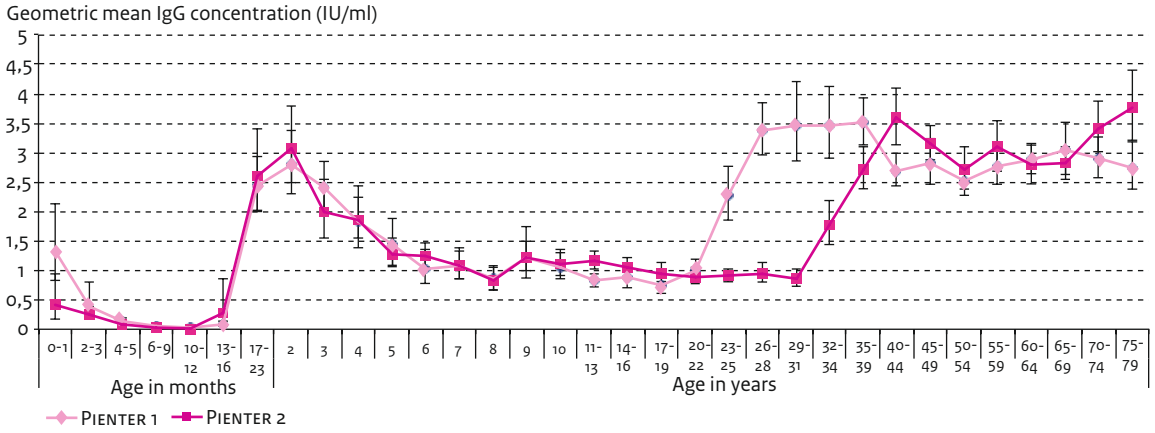

Figure 2. Age-specific geometric mean measles IgG antibody concentrations (with 95% CIs) in the general population, Pienter 1 (1995/6) and Pienter 2 (2006/7)

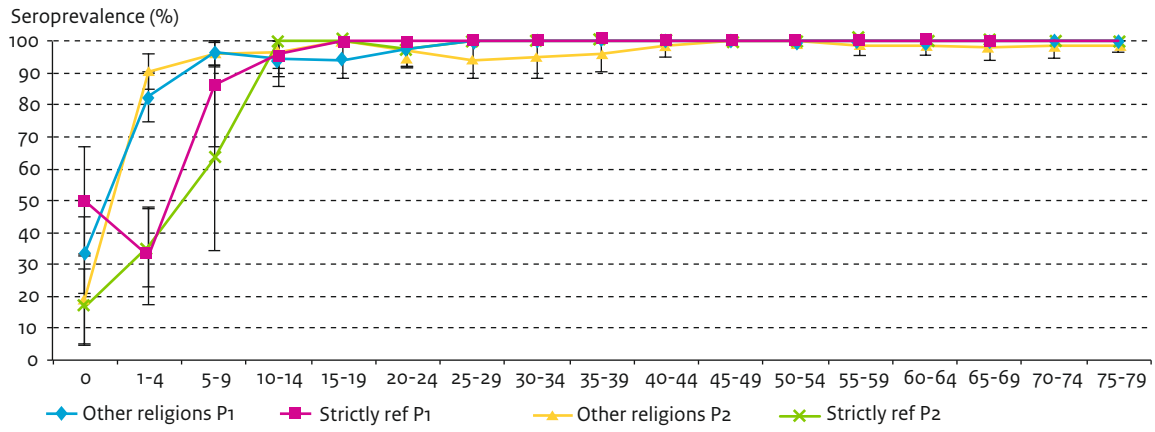

Figure 3. Age specific seroprevalence of measles IgG antibodies (with 95%CIs) in the strictly reformed community refusing vaccination and remaining religions in the low vaccination coverage sample, Pienter 1 (1995/6) and Pienter 2 (2006/7)

9. Mumps (Bof)

Background

Mumps was a common worldwide childhood disease before the introduction of routine vaccination. Normally it causes a relatively benign infection, but it can result in considerable morbidity like orchitis, deafness, symptomatic meningitis and in sporadic cases in mortality.
A combination vaccine of measles, mumps and rubella virus (MMR) has been part of the Dutch National Immunization Program (NIP) since 1987. The MMR vaccine is routinely administered at the age of 14 months with a second dose at the age of 9 years. A high MMRV vaccination coverage of 96% and 92% for respectively the first and second dose has been established since the implementation.

Pienter was designed to obtain insight into the long term protection of the population against vaccine preventable diseases and to assess the effect of any vaccine changes in the NIP. The main research questions concerning the mumps analysis were:

1) How well is the Dutch population protected against mumps and how has this changed compared to ten years ago? (Pienter 1 study).
2) How does immunity wane after one or two doses of MMR vaccination?
3) What are the risks of a mumps outbreak among the religious community?

And in the light of the recent ongoing outbreaks among students, the following question could be added:

4) Are there any signs of waning immunity visible in Pienter 2 among this age group?

Preliminary results

Seroprevalence (the percentage of samples with an antibody concentration ≥ 45 RU/ml) in the nationwide sample is high in as well the Pienter 1 as the Pienter 2 study (Figure 1). However, particular in the age cohorts 3-8 and to a lesser extent in 15-24 years, seroprevalence is lower in Pienter 2 than in Pienter 1. For the cohorts aged 3-8 years the lower seroprevalence appeared to be gender specific, with males having a lower seroprevalence than females. A significant antibody decline with time since last vaccination was observed for those once and twice vaccinated (Figure 2).
The group that refuses vaccination due to religions reasons (LVC) has been divided in a strictly reformed group and a group with other religions. Figure 3 demonstrates that seroprevalence in the younger age groups is lower for the strictly reformed group than for the group with other religions in as well Pienter 1 as Pienter 2. However that difference between both groups is larger in Pienter 2 than in Pienter 1.

Discussion/conclusions

The Dutch population is in general well-protected, there are however some groups that have higher susceptibility, i.e., children aged 6-14 months, 3-8 years and persons aged 15-24 years. In the latter age-groups outbreaks have been reported in 2010 and are still ongoing.
Maternal antibodies decline faster in the Pienter 2 study than in the Pienter 1 study. This indicates that levels of maternal antibodies are lower after vaccination of women of child-bearing age than after natural infection. The faster decline of maternal antibodies in young infants makes them vulnerable for an infection with mumps, since the first vaccination is only given at the age of 14 months. This has little implications as mumps in neonates is not associated with serious disease.
In the strictly reformed group, a large group of children (aged 0-24 years) was susceptible to mumps in 2006/2007; i.e. they are at risk for an outbreak. Note, there was an outbreak among the community with low vaccination coverage in 2007-2008 (i.e., after the Pienter study was performed). The median age in this outbreak was 13 years, corresponding to the above susceptible age group.

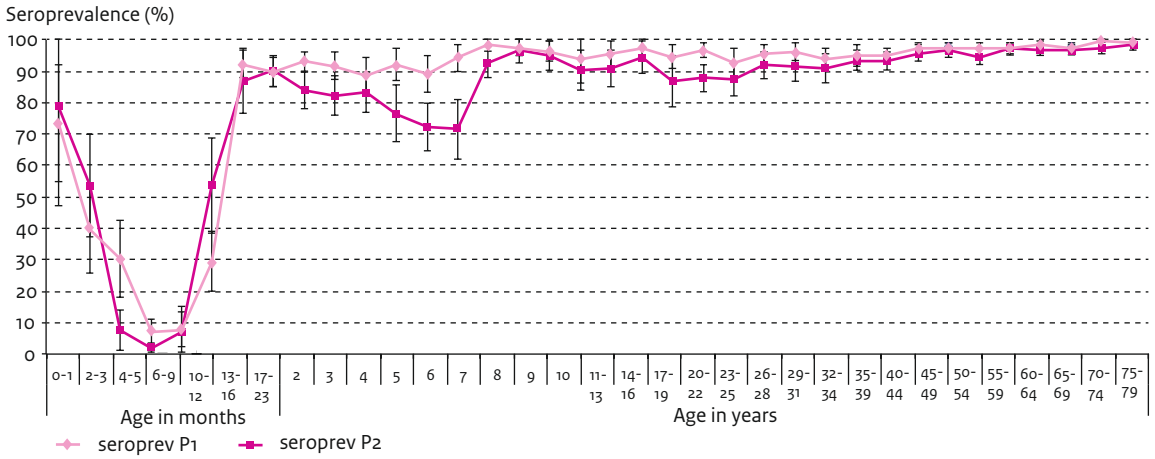

Figure 1. Age-specific seroprevalence of mumps IgG antibodies (with 95% CIs) in the general population, Pienter 1 (1995/6) and Pienter 2 (2006/7)

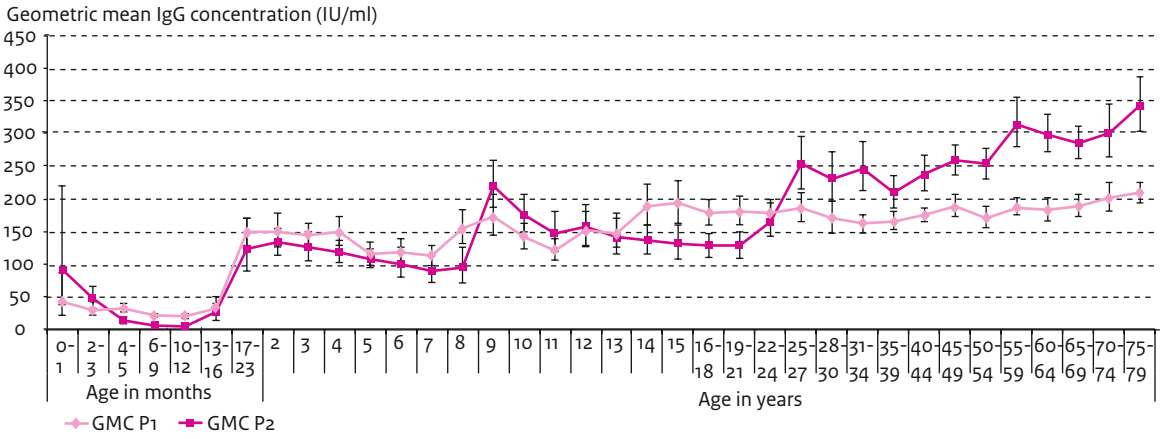

Figure 2. Age-specific geometric mean mumps IgG antibody concentrations (with 95% CIs) in the general population, Pienter 1 (1995/6) and Pienter 2 (2006/7)

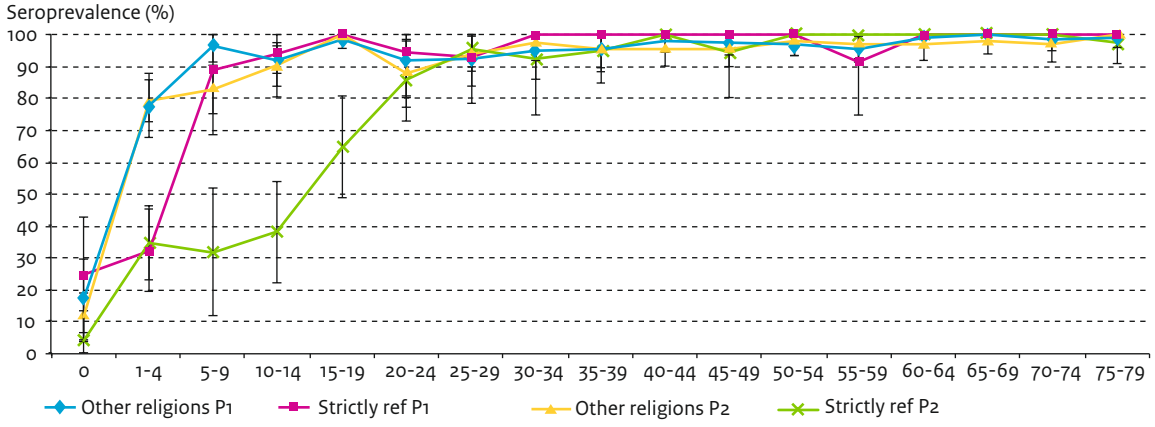

Figure 3. Age specific seroprevalence of mumps IgG antibodies in the strictly reformed community refusing vaccination and remaining religions in the low vaccination coverage sample, Pienter 1 (1995/6) and Pienter 2 (2006/7)

Contact person: Gaby.Smits@rivm.nl

10. Rubella (rode hond)

Background

Rubella, also known as German measles, is a generally mild disease. However, when a woman in early pregnancy becomes infected, it can result in fetal growth retardation, abortion, and congenital rubella syndrome (CRS). Infants born with CRS may have sensi-neural deafness, mental retardation, heart defects or ocular abnormalities.
In the Netherlands, in 1974 an attempt to prevent CRS was made by offering 11 year-old girls a rubella vaccination. The Dutch Health Council however recommended a change to mass vaccination, and in 1987 a combined vaccination of measles, mumps and rubella (MMR) was introduced in the national immunization program (NIP). In the past few years, vaccination coverage has been high, with a coverage around 96% and 92% for respectively infants (age 2 years) and schoolchildren (age 10 years). In case of schoolchildren this is still below the WHO recommended coverage rate of 95%.

Pienter was designed to obtain insight into the long term protection of the population against vaccine preventable diseases and to assess the effect of any vaccine changes in the NIP. The main research questions concerning the rubella analysis were:

1 How well is the Dutch population protected against rubella and how has this changed compared to ten years ago? (Pienter 1 study).
2 What are the risks of a rubella outbreak among the religious community?

Preliminary results

Seroprevalence (the percentage of samples with an antibody concentration ≥ 10 IU/ml) in the nationwide sample is high in the Pienter 1 and the Pienter 2 study (Figure 1). Maternal antibodies decline faster in Pienter 2 than in Pienter 1 and seroprevalence in the 5-8 year age cohort in Pienter 2 is lower than in Pienter 1. Geometric mean concentrations (GMCs) show a clear distinction between antibodies induced by vaccination or by natural infection. This is demonstrated by an increase in antibody concentration in the age cohorts of 8-9 years and 19-20 years for respectively Pienter 1 and Pienter 2 (Figure 2). The group who refuses vaccination due to religions reasons has been divided in a strictly reformed group and a group with other religions. Figure 3 demonstrates that seroprevalence in the younger age groups is lower for the strictly reformed group than for the group with other religions in Pienter 1 and Pienter 2. As result of the rubella outbreak in 2004-2005 the difference between both groups is somewhat larger in Pienter 1 than in Pienter 2.

Discussion/conclusions

The Dutch population is well-protected against rubella. However there are two groups with higher susceptibility: 0-14 month old children, in which maternal antibodies rapidly decline and children belonging to the religious group in the low vaccination coverage areas. Antibody concentrations decrease over time and are lower in vaccinated compared to birth cohorts exposed to wild type virus infection. When there are only vaccinated individuals and there is no natural boosting anymore, antibodies against rubella might continue to decline to a lower level, potentially leading to higher susceptibility. Therefore it is necessary to monitor the antibody levels in the general population.

Contact person: Gaby.Smits@rivm.nl

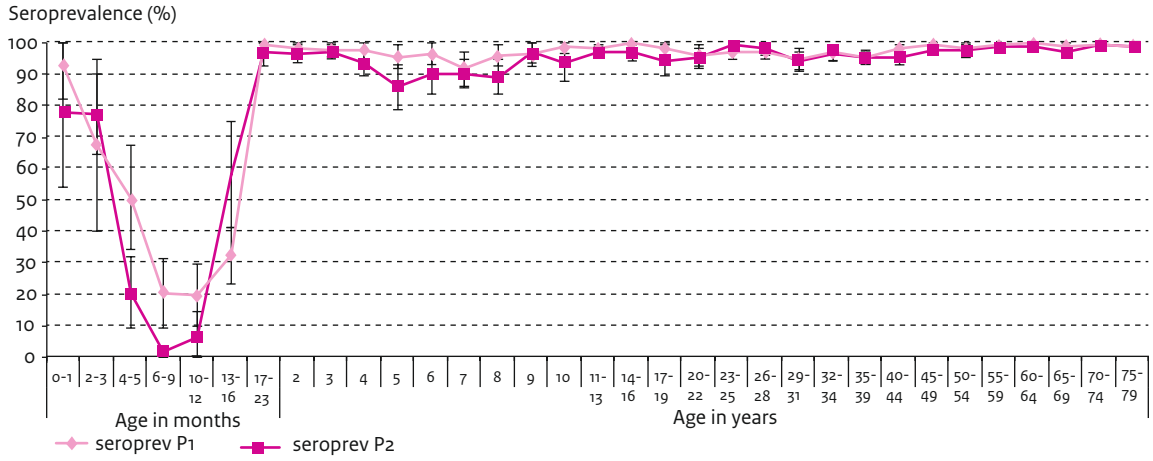

Figure 1. Age-specific seroprevalence of rubella IgG antibodies (with 95% CIs) in the general population, Pienter 1 (1995/6) and Pienter 2 (2006/7)

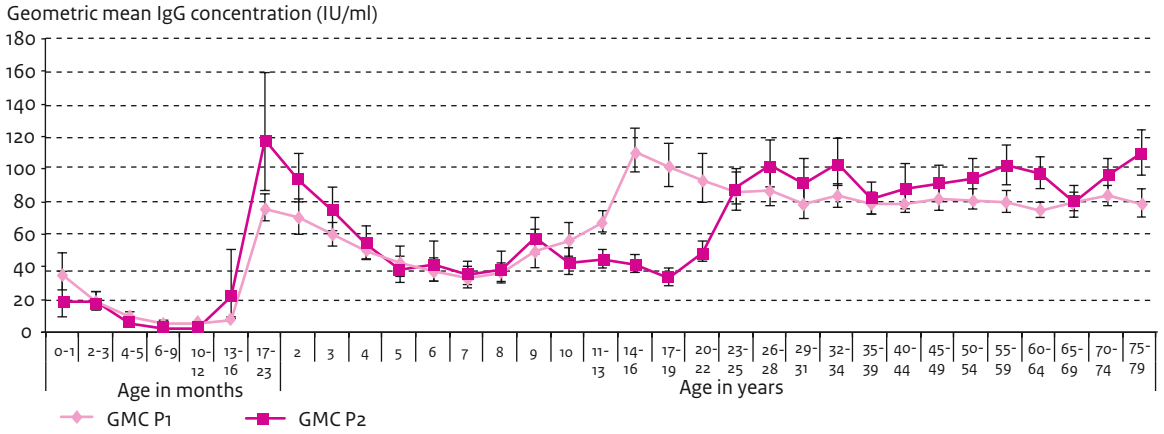

Figure 2. Age-specific geometric mean rubella IgG antibody concentrations (with 95% CIs) in the general population, Pienter 1 (1995/6) and Pienter 2 (2006/7)

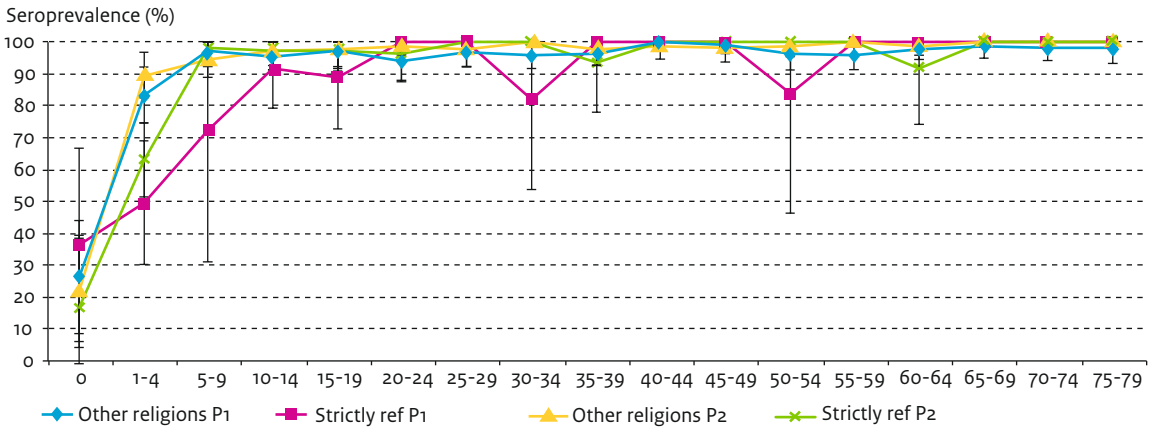

Figure 3. Age specific seroprevalence of rubella IgG antibodies in the strictly reformed community refusing vaccination and remaining religions in the low vaccination coverage sample, Pienter 1 (1995/6) and Pienter 2 (2006/7)

11. Maternal antibodies against measles, mumps, rubella and varicella

Background

Infants born to mothers vaccinated with the trivalent measles-mumps-rubella (MMR) vaccine may receive fewer maternal antibodies and have shorter protected period than infants of women with naturally acquired immunity. As an increasing proportion of mothers has received MMR vaccination, an increasing proportion of infants may be protected for a shorter period by maternal antibodies. It is not known how long infants are protected, and how long they are susceptible to measles, mumps and rubella before the first dose of vaccine is administered. We compared the maternal antibody levels observed in the general population (where mothers are generally compliant with the vaccination program) with these observed infants in the orthodox reformed community (where the vaccine uptake is relatively low and outbreaks of measles, mumps and rubella occur).

Preliminary results

The duration of protection by maternal antibodies of infants in the general population is 3.3 months for measles, 2.7 months for mumps and 3.9 months for rubella. The duration of protection against measles in infants in the general population is 2.0 months shorter than the duration of protection of infants in the orthodox protestant community (Figure 1).

Discussion/conclusions

Loss of protection by maternal antibodies against measles, mumps and rubella is achieved well before the time of first MMR-vaccination at 14 months. The shortened duration of protection against measles for infants of vaccinated mothers offers a strong argument in favour of temporarily decreasing the first age of the MMR-vaccination during a measles epidemic.

The Pienter 2 study was conducted at a time when the oldest mothers have not been targeted by any of the mass vaccination campaigns against measles, mumps and rubella, and when the youngest mothers have been targeted by the regular trivalent measles, mumps, rubella vaccine. Mumps vaccinated women had barely reached the child-bearing age, and due to the late age of administering (at 11 years) the earlier rubella vaccinations, older rubella vaccinated women of childbearing age were possibly exposed to wildtype virus before the vaccination was offered. As a consequence, differences in the maternal antibody level of children whose mothers have different vaccination histories will be masked. Our results show the first signs of the impact of mass vaccination on duration of protection by maternal antibodies. We expect that this impact will increase as the proportion of mothers who is vaccinated with MMR increases, and will also become visible for mumps and rubella in the near future.

Contact person: Sandra.Waaijenborg@rivm.nl

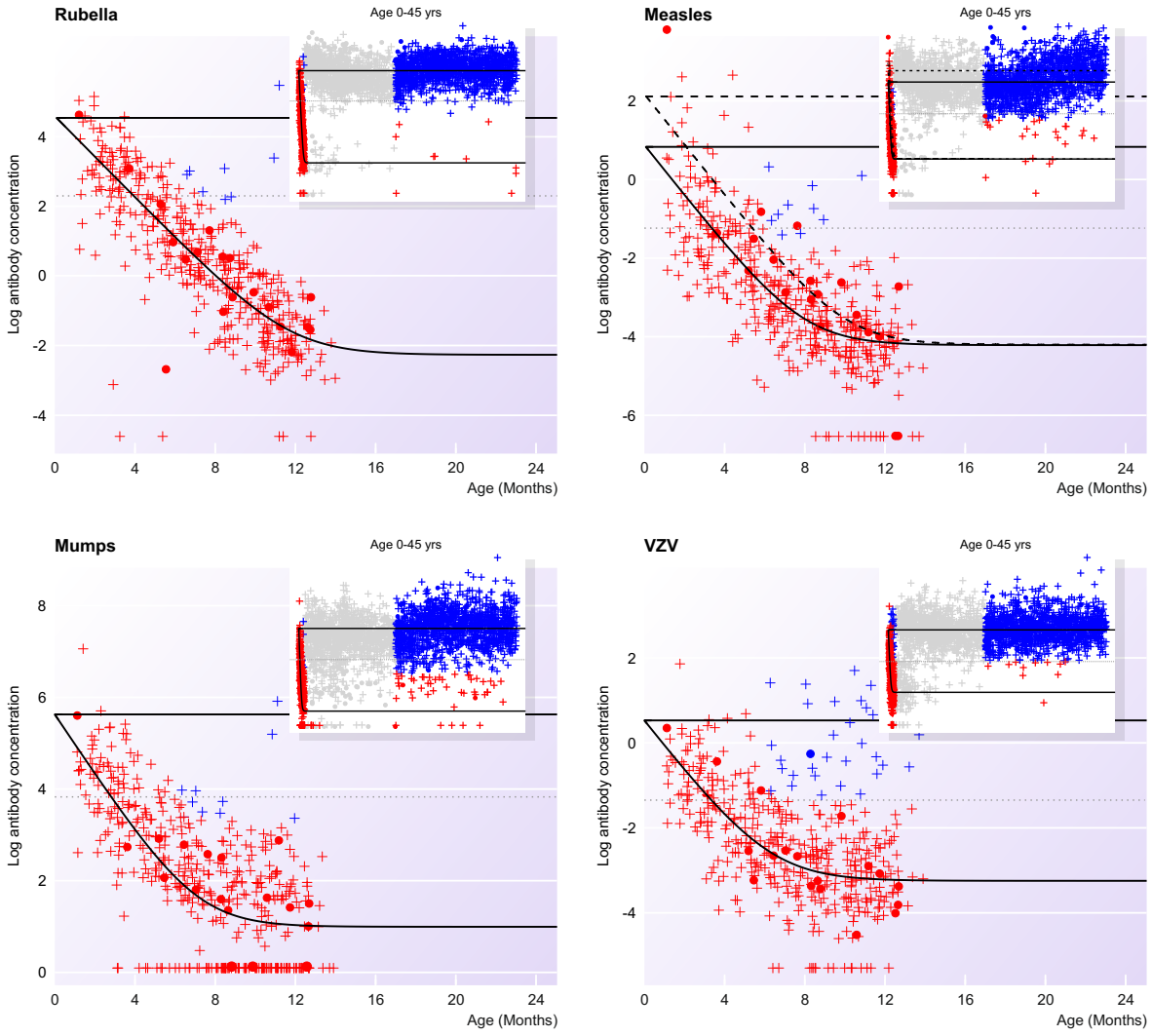

Figure 1. Antibody concentration (log IgG) by age for measles, mumps, rubella and varicella zoster virus (used as control).

The large picture shows all infants up to 15 months and the small picture at the top right-hand corner shows both infants up to 14 months and females aged between 15 months and 45 years. Red markers represent individuals who we assume to have maternal antibodies and/or did not seroconvert; blue markers represent individuals who we assume did seroconvert. Dots are the orthodox protestant individuals and crosses are individuals from the general population. The lines represent the fitted models: the dashed line is age-specific antibody level for orthodox protestant individuals (only observed for measles); the continuous line is the age-specific antibody level for individuals from the general population. The grey horizontal dotted lines are the diagnostic cut-off points. The grey markers in the small picture are females who are not included in the analysis and are only shown for informative reasons.

Other infectious diseases

12. Varicella (waterpokken)

Background

The varicella zoster virus (VZV) causes varicella ("waterpokken") as well as herpes zoster ("gordelroos"). Varicella caused by a primary infection, whereas herpes zoster is caused by reactivation of latent VZV in sensory nerve ganglia. In contrast to varicella, which is mainly a childhood disease, herpes zoster predominantly affects older adults.

Vaccines to prevent varicella and herpes zoster are available. The decision-making process regarding whether or not to introduce varicella vaccination in the Netherlands is ongoing. The Pienter study gives insight in the age at which varicella is contracted and which part of the population gets infected. The age of infection is very relevant when considering VZV vaccination strategies, since the risk on varicella complications rises with age.

Preliminary results

Preliminary results show that the VZV-seroprofiles from the Pienter 1 and Pienter 2 study are comparable (Figure 1). In Pienter 2, the total seroprevalence of VZV antibodies among persons aged 0-79 years was 94.6% (95% CI 93.2-96.0). In Pienter 1 the total seroprevalence was 95.6% (95% CI 94.9-96.3). The seroprevalence increased sharply with age in the Pienter 2 study: 1 year 27.9%, 2 year 52.3%, 3 year 69.8%, 4 year 77.8% and 5 year olds 84.7%, which was comparable to Pienter 1: 18.4%, 48.7%, 59.0%, 75.7% and 93.0%. Furthermore, after the age of 20 years the geometric mean concentration of VZV antibodies was significantly lower for women than for men (Figure 2).

Discussion/conclusions

The Pienter 1 study showed that children in the Netherlands are infected at young age and this is at a younger age compared to other European countries. This notable finding is now confirmed by the Pienter 2 study and could be a plausible explanation for the lower

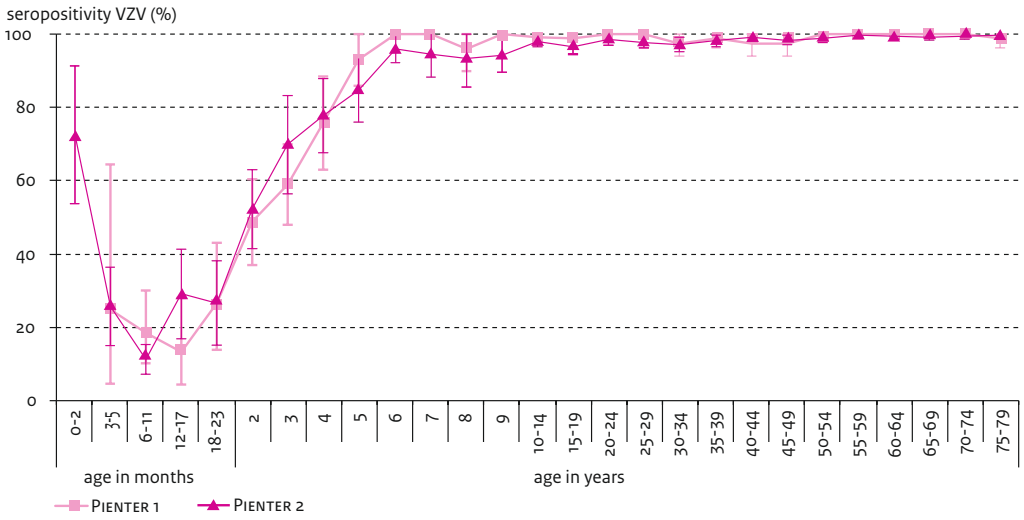

Figure 1. VZV seroprofile per age: Pienter 1 versus Pienter 2 study

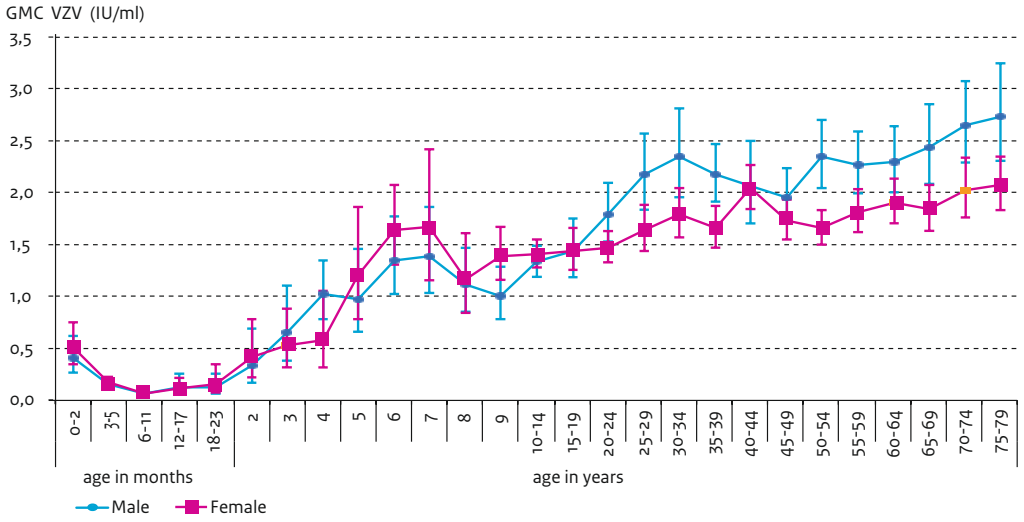

Figure 2. Mean geometric concentrations of VZV antibodies per age and gender in Pienter 2 study

number of varicella related general practitioner consultations, hospital admissions and deaths per 100,000 inhabitants in the Netherlands compared to other countries. The lower geometric mean concentration for women above 20 years of age might be an explanation for the higher incidence of herpes zoster among women.
In the Netherlands, the feasibility of reaching a high vaccination coverage (≥ 85%) is one of the concerns with regard to universal varicella vaccination. Varicella is usually seen as a mild disease and therefore the acceptance of this vaccine by parents might be low. If universal varicella vaccination would be introduced without sufficient vaccination coverage, there is a risk that the mean age of infection (and subsequently varicella related complications) will rise and will result in a situation that is less favorable than the current situation without vaccination in place.

It is important to monitor the VZV seroprofile in the future, especially when universal vaccination would be introduced. At this moment it functions also as a 'control disease' because it is a disease for which vaccination is not included in the National Immunisation Programme and therefore we do not expect large changes in time.

Contact person: Alies.van.Lier@rivm.nl

13. Hepatitis A

Background

Hepatitis A virus (HAV) may cause hepatitis 2-6 weeks after exposure. Infection and vaccination generally results in long-term immunity. In children, HAV infection is often asymptomatic. Symptoms of infection are more severe in the elderly, with a case-fatality rate of 0.8% in people aged >40 years of age. The Pienter 2 study offered the opportunity of investigating the current status of HAV serology in the Netherlands, to get insight in changes of immunity in the population over time and in changes of infection pressure. In addition, we determined current risk factors for hepatitis A infection in order to identify target groups for vaccination strategies in the Netherlands.

Results

The overall seroprevalence increased from 34% in 1995-6 to 39% in 2006-7, mainly due to vaccination of travellers and an increased immigrant population. Figure 1 shows a trend of increasing age of the susceptible population. Risk factors were travelling to, and originating from, endemic regions.

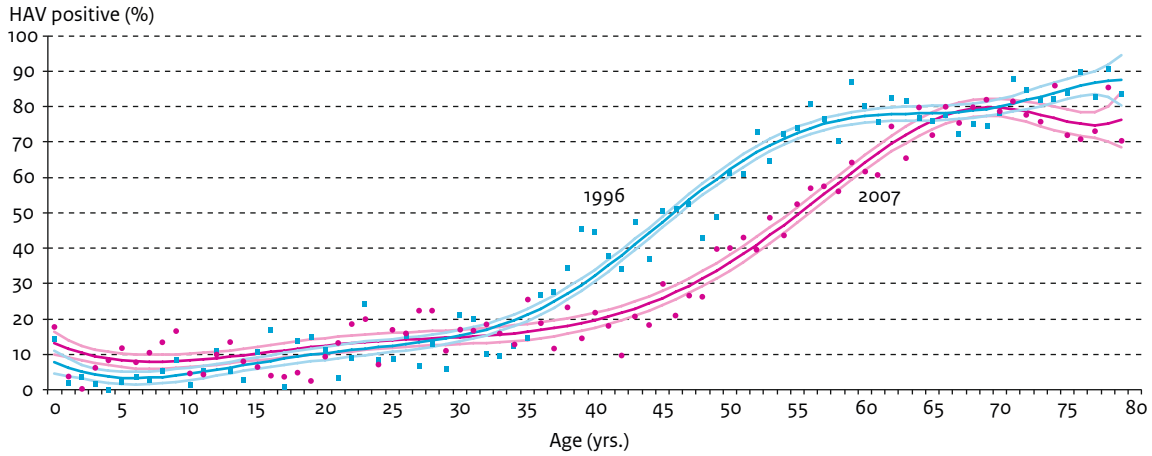

Figure 1. Age-prevalence of hepatitis A antibodies presented per year in age including 90% confidence intervals in non-HAV-vaccinated persons in Pienter 1 (1996-line, blue, n = 7,287, excluding 59 vaccinated participants) and Pienter 2 (n = 5442, 2007-line, violet, excluding 786 vaccinated participants).

Discussion/conclusions

Those aged >40 years and susceptible in the Pienter 2 study would also benefit from HAV vaccination because they are likely to develop clinically serious symptoms after infection, and are increasingly at risk of exposure through imported viruses through foods or travellers. Currently vaccination is targeted at the risk groups identified in this study. The effect of this intervention might be addressed in a next serosurveillance study. The cost-effectiveness of adding elderly people born after World War II as a target group for prophylactic vaccination to reduce morbidity and mortality after HAV infection should be assessed.

Publications

Verhoef L, Boot HJ, Koopmans M, Mollema L, van der Klis F, Reimerink J, van Pelt W. **Changing risk profile of hepatitis A in the Netherlands: a comparison of seroprevalence in 1995-1996 and 2006-2007**. *Epidemiol. Infect*. 2011, 139, 1172-1180.

Contact person: Linda.Verhoef@rivm.nl

14. Influenza A and B

Background

Infection with influenza viruses is an important cause of illness in children. Especially young children with underlying disease are at risk for severe disease after infection with an influenza virus, but also the hospitalization rates attributable to influenza virus infection of young children without underlying disease are similar to those observed among older adults. In addition, the pandemic caused by the influenza A/H1N1(2009) virus has highlighted the importance of influenza viruses as a cause of morbidity and mortality in infants.
To prevent morbidity and mortality of children due to infection with influenza viruses, a number of countries, including the USA, have recommended vaccinating all healthy children 6-59 months of age against influenza. Also in the Netherlands, the implementation of universal influenza vaccination in the national immunization program has been a matter of debate. In various studies, it has been demonstrated that annual vaccination against seasonal influenza is beneficial for children and reduces the transmission of virus. However, the impact of vaccination will be influenced by the immune status of the individuals. Since they will be more at risk to become infected and develop disease, naïve subjects most likely will benefit from vaccination more than children that already have experienced an infection with one or more influenza viruses. In addition, it can be anticipated that with increasing age the chance of having experienced an influenza virus infection also increases. However, since detailed sero-epidemiological studies of this age group are lacking, it was not clear at which age children become infected for the first time and develop influenza virus specific immunity. Therefore, we evaluated the seroprevalence of antibodies against influenza A/H1N1, A/H3N2 and B viruses in children from one month to seven years of age with serum samples collected during the Pienter 2 study.

Results

Serum samples were tested for the presence of antibodies against representative influenza A/H1N1, A/H3N2 and B viruses from multiple influenza seasons using the hemagglutination inhibition (HI) assay at the Department of Virology, Erasmus MC, Rotterdam. Using this assay, we were able to discriminate between antibodies against various antigenically distinct influenza A/H1N1 and influenza A/H3N2 viruses and antibodies to influenza B viruses from B/Victoria/2/87 and B/Yamagata/16/88 lineages.
The seroprevalence of antibodies to influenza was higher in children 1-6 months of age than that of children 7-12 months of age, which likely reflects the presence of maternally derived antibodies. In children >1 year of age, there was a gradual, age-related increase in the seroprevalence of antibodies against all influenza viruses. In all children at seven years of age antibodies against at least one influenza virus were detected (Figure 1). Results were in accordance with epidemiological data from the Netherlands collected between 1999 and 2007.

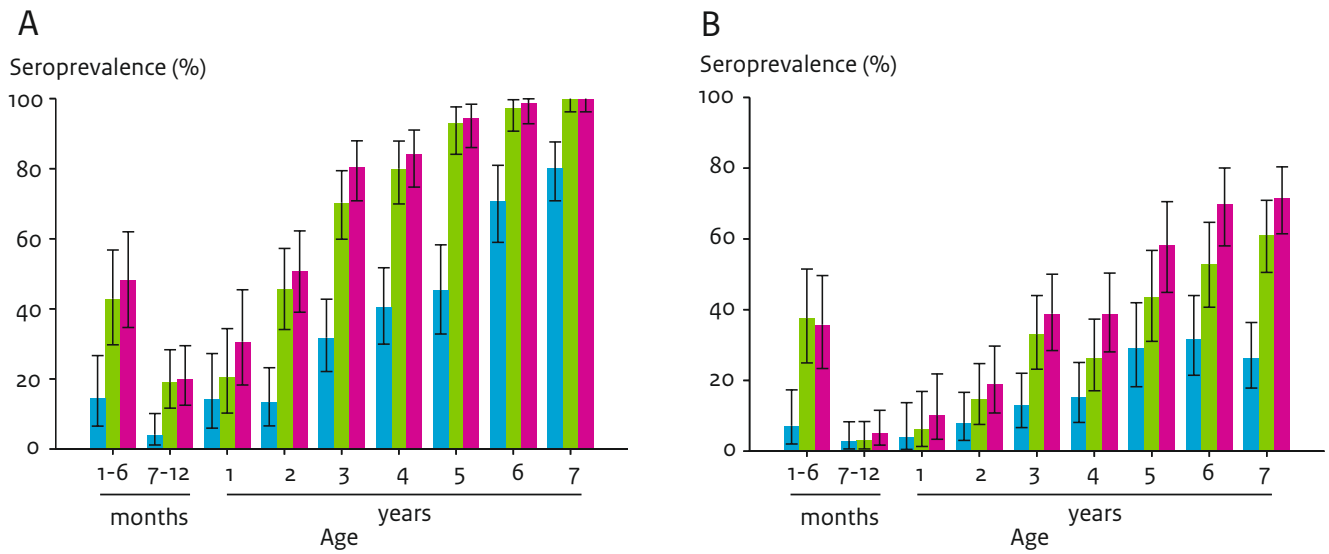

Figure 1. Seroprevalence of antibodies against influenza A and B viruses depends on age. Antibodies against influenza A/H1N1 (blue bars), influenza A/H3N2 (green bars) and all influenza A viruses (red bars) **(A)**. Antibodies against the influenza B viruses from the Victoria-lineage (blue bars), the Yamagata-lineage (green bars) and all influenza B viruses (red bars) **(B)**. Bars indicate the percentage of the serum samples in which antibodies were detected and error bars indicate the 95% confidence intervals.

Discussion/conclusions

Using serum samples collected during Pienter 2, we were able to determine the seroprevalence of antibodies against various influenza viruses in children from 0-7 years of age during non-pandemic influenza seasons. We demonstrated that at seven years of age, all children developed antibodies against at least one of the influenza viruses tested. Furthermore, the highest attack rates calculated based on the seroprevalence of antibodies to influenza A viruses were observed in children two and three years of age. These data provide information on the age at which children experience their first infections with influenza viruses and develop immunity to these viruses. This type of information aid decision making for the implementation of vaccination strategies that aim at achieving optimal protective immunity against seasonal and pandemic influenza. Ideally, infants vaccines are used that not only induce antibodies to seasonal influenza viruses but also immunity to influenza A viruses of other subtypes. In addition, a follow up study would be of interest since the introduction of the pandemic influenza A/H1N1 virus in the human population during 2009 had a major impact on the influenza viruses that circulate among humans.

Publication

Bodewes R, de Mutsert G, van der Klis FR, Ventresca M, Wilks S, Smith DJ, Koopmans M, Fouchier RA, Osterhaus AD, Rimmelzwaan GF. **Prevalence of antibodies against seasonal influenza A and B viruses in children in Netherlands**. *Clin Vaccine Immunol.* 2011 Mar;18(3):469-76.

Contact persons: Rogier Bodewes, email: r.bodewes@erasmusmc.nl
Fiona.van.der.Klis@rivm.nl

15. Cytomegalovirus disease

Background

Cytomegalovirus (CMV) is an endemic virus, present in the whole human population. It has the capacity to establish lifelong latency following primary infection. In general, in healthy persons CMV infection does not lead to clinical symptoms. However, in immunocompromised individuals CMV infection can cause significant morbidity and mortality. In case of fetal CMV infection, symptoms at birth occur in about 12%. Long term sequelae, predominantly hearing loss, is seen in approximately 20% (both symptomatic and asymptomatic at birth). Congenital CMV infection is the result of maternal CMV infection during pregnancy and is related to maternal serological CMV-status and seroprevalence in the surrounding population.
The prevalence of CMV-antibodies varies widely among populations associated with geographic and socio-economic factors. In general the seroprevalence is higher in developing countries and among those with a lower socio-economic background. In most developed countries seroprevalence is around 50%, whereas it may be as high as 90 to 100% in developing regions.
We assessed the seroprevalence of CMV in the Netherlands using population-based sera from the Pienter 2 study. It offers the opportunity to study determinants of infection and to estimate the force of CMV infection that will be used as input for modelling scenarios to explore future vaccination programs.

Preliminary results

An overall seroprevalence of CMV in the general population (0-79 years) of 48.8% was found. The seroprevalence increases with age from around 30% in the first year up to 70% in persons > 65 year. There is a marked difference in seroprevalence between the native Dutch population (and Western immigrants) and Non-Western immigrants (Figure 1). The overall seroprevalence in the Dutch population was 42% compared to 77% in non-Western immigrants. Education and income, as markers for socio-economic status, were associated with CMV seroprevalence, with a higher seroprevalence in groups with low income (63%) and low educational levels (75%) compared to groups with high income (40%) and high educational levels (39%).
The seroprevalence of women of childbearing age (19 to 44 years) varies from 38% in women of Dutch origin to 88% in non-Western immigrants. In Dutch women of childbearing age the seroprevalence increased from 30% to 50% between the ages of 19 to 44 years. In addition, women with children have higher seroprevalence rates (46%) compared to women without children (39%).

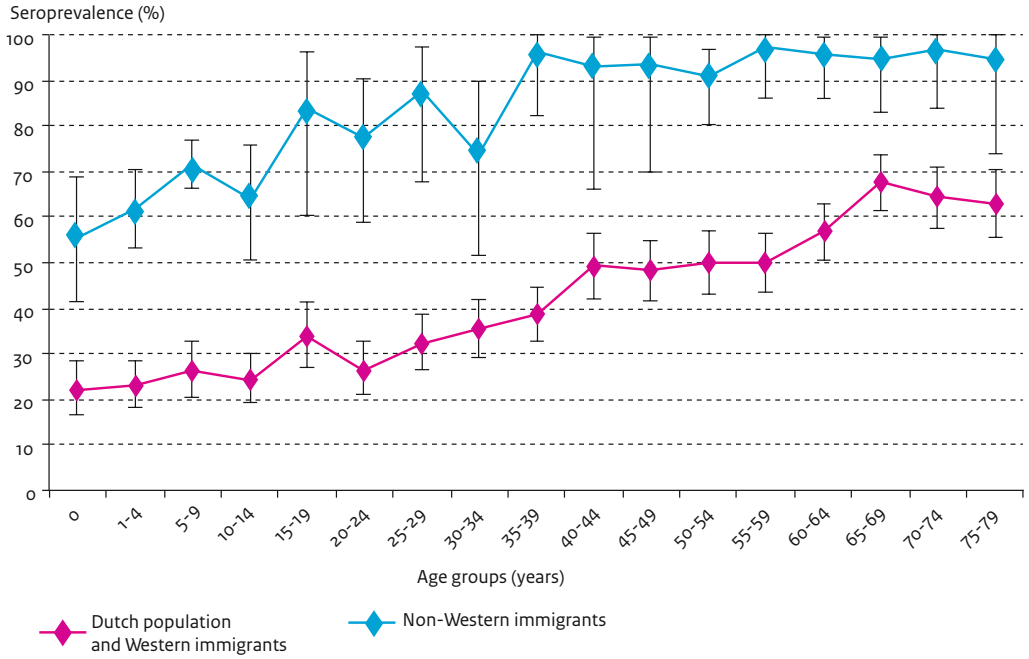

Figure 1. Age-specific prevalence of IgG antibodies against CMV.

Discussion/conclusions

CMV infection is common in the Netherlands. In almost half of the population there is serological evidence of prior infection. Furthermore, the increase in women of childbearing age shows that infections occur frequently during pregnancy. These infections might lead to congenital infections which are associated with long term sequelae, in particular hearing loss. In accordance with the literature CMV infection is even more prevalent among non-Western immigrants and those with a lower socio-economic status. These data alongside a cohort study of children will be used to assess the disease burden of congenital CMV-infection in the Netherlands. This information is necessary to evaluate on the implications for primary and secondary preventive measures.

Contact persons: Marjolein.Korndewal@rivm.nl
Hester.de.Melker@rivm.nl

16. Q fever

Background

The Netherlands has experienced large community outbreaks of Q fever between 2007 and 2010, related to goat farming. Before 2007 the yearly number of reported cases was low, 20 to 30. The questions arose what the seroprevalence was before the outbreak and whether the outbreak started earlier unnoticed. As the Pienter samples were taken just prior to the Q fever outbreaks, in 2006 - early 2007, it provided an excellent opportunity.

Pienter sera were tested for IgG phase-2 antibodies against *Coxiella burnetii* with an ELISA to estimate the seroprevalence and to identify determinants for seropositivity before the observed Q fever outbreaks. The questionnaire provided relevant epidemiological information such as contact with animals.

Results

Overall seroprevalence was 1·5% (95% CI 1.3-1.7). Corrected for confirmation with immunofluorescence results in a subset, the estimated seroprevalence was 2.4%. Seropositivity increased with age from 0.5% (95% CI 0.0-1.0) in the 0-4 years age group to 2.3% (95% CI 1.5-3.2) in the 60-79 years age group (Figure 1). Keeping ruminants, increasing age and being born in Turkey were independent risk factors for seropositivity.

Discussion/conclusions

The low seroprevalence before the start of the outbreaks supports the hypothesis that The Netherlands has been confronted with a newly emerging Q fever problem since spring 2007.
As the Q fever outbreak and the control of it had large societal and financial implications, that are still ongoing, it is important to know as exactly as possible the extent of the outbreak and the extent of the endemic situation that existed before. The analysis in the Pienter study paid an important contribution to that.

Several seroprevalence studies have been performed among certain risk groups with possibly increased risk of transmission, such as veterinarians, goat farmers and among groups with increased risk of complications, such as pregnant women and patients with vascular aneurysms. These studies indicate that the seroprevalence in the highest endemic area in Eastern North-Brabant is probably around 12 to 15%. This suggests that only a small fraction of the infected patients were actually identified and reported through regular health care.
A study with a truly random population sample, such as a new Pienter study, could give a more reliable estimate of the Q fever seroprevalence after the outbreak and in more regions of the country.

Publications

Schimmer B, Notermans DW, Harms MG, Reimerink JHJ, Bakker J, Schneeberger P, Mollema L, Teunis P, van Pelt W, van Duynhoven Y. **Low seroprevalence of Q fever in the Netherlands preceding a series of large outbreaks**. *Epid Infect* 2011; [Epub ahead of print: feb 16, 2011].

Notermans DW, Schimmer B, Harms MG, Reimerink JHJ, Bakker J, Schneeberger PM, Mollema L, Teunis PFM, van Pelt W, van Duynhoven YTHP. **Sero-epidemiologie van Q-koorts in Nederland in 2006-2007**. *Infect Bull*. 2010;21:314-6 [Dutch].

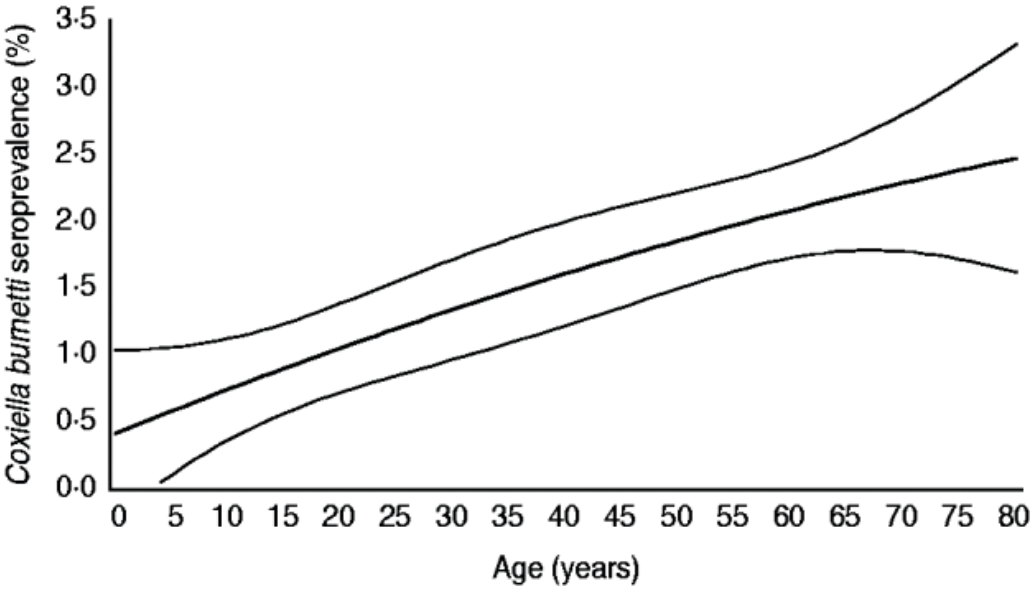

Figure 1. Age-specific weighted seroprevalence of *C. burnetii* IgG antibodies in the national sample of Pienter 2, Prevalence rates per age group were estimated using a linear model with a spline function for age (i.e., second-degree polynomial).

Contact persons: Barbara.Schimmer@rivm.nl
Daan.Notermans@rivm.nl

17. Sexually transmitted disease caused by *Chlamydia trachomatis*

Background

Chlamydia trachomatis (*C.h trachomatis*) is the most commonly reported sexually transmitted infection in the Netherlands. The often asymptomatic nature of chlamydia infections allows it to spread unknowingly. *C. trachomatis* infection is an important public health concern because it may cause severe damage to female reproductive organs. In women, persistent infections can cause pelvic inflammatory disease (PID), which in turn, may lead to infertility and ectopic pregnancy.
Programmes to control *C. trachomatis* include early diagnosis and effective treatment of infected cases and their sexual partners. Due to these prevention efforts, both the number of chlamydia tests and the number of detected and treated infections have increased in recent years. However, the evidence from randomised controlled trials supporting the effectiveness of an active testing policy to prevent long term complications is limited.
The Pienter studies are used to explore to what extent current control policy is reflected in changes in chlamydia serology data over time. The Chlamydia Antibody Test (CAT) detects chlamydial IgG antibodies in serum and is considered an intermediate marker for tubal pathology caused by a chlamydia infection.

Preliminary results

In total 3357 CAT results from both men and women aged 15- 40 years were collected (using Medac *Chlamydia trachomatis* IgG Elisa + Kit) in 1996/1997 (Pienter 1) and 2006/2007 (Pienter 2) (Table 1) and tested in collaboration with Dr Morré from the VUmc; his laboratory is responsible for the Dutch *C. trachomatis* reference tasks.

Table 1: Overview of study population and preliminary serological test results

	Pienter-1	Pienter-2
N	1647	1649
Men / Women	649 / 998	650 / 999
Mean age [Range]	27,8 [15 – 40]	27,8 [15 – 40]
Serological test results		
Positive (%)	143 (8,7%)	131 (7,9%)
Negative (%)	1493 (90,6%)	1510 (91,6%)
Grey zone (%)	11 (0,7%)	8 (0,5%)

Analysis showed a 10% decrease in the number of positive CAT results among those aged 15-40 years in the Pienter 2 study versus the Pienter 1 study. However, when stratified by gender, the CAT positivity ratio for women showed a significantly larger decrease whereas the positivity ratio among men increased.

Discussion/conclusions

Further analyses including data on age, sexual preference, region etcetera need to be conducted, as well as analyses of serum chlamydia antibody titers, to improve understanding of the dynamics in the epidemiology. We expect to gain better insight into the effects of current control policy in preventing long term consequences of chlamydia infection. Associations and hypotheses can be tested and validated with data to be obtained in future population based serosurveillance studies.

Contact person: Marianne.van.der.Sande@rivm.nl

18. Hepatitis E

Background

Hepatitis E virus (HEV) is now considered an endemic pathogen in industrialized countries, leading to acute and sometimes chronic hepatitis, mostly in vulnerable people. The endemic sources are unclear. With the Pienter 2 study it was possible to study which determinants might be associated with HEV seropositivity.

Results

The seroprevalence of HEV antibodies measured by ELISA and confirmed by immunoblot in the nationwide sample was 1.9%. The seroprevalence of anti-HEV IgG antibodies increases with age (Figure 1). Overall, among 134/7072 (1.9%) seropositives, older age ($p<0.01$), males ($p < 0.01$), working with patients ($p = 0.03$), working with animals ($p = 0.07$), recent diarrhoeal complaints ($p = 0.07$) and adhering to a religion considering pigs unclean ($p < 0.01$) were independently associated with seropositivity in multivariate analysis. Sub-analysis of 59/4022 (1.5%) anti-HEV antibody positive subjects with likely endemic exposure showed independent association with youngest member in the household <5 or 19-< 65 years ($p = 0.05$) in multivariate analysis.

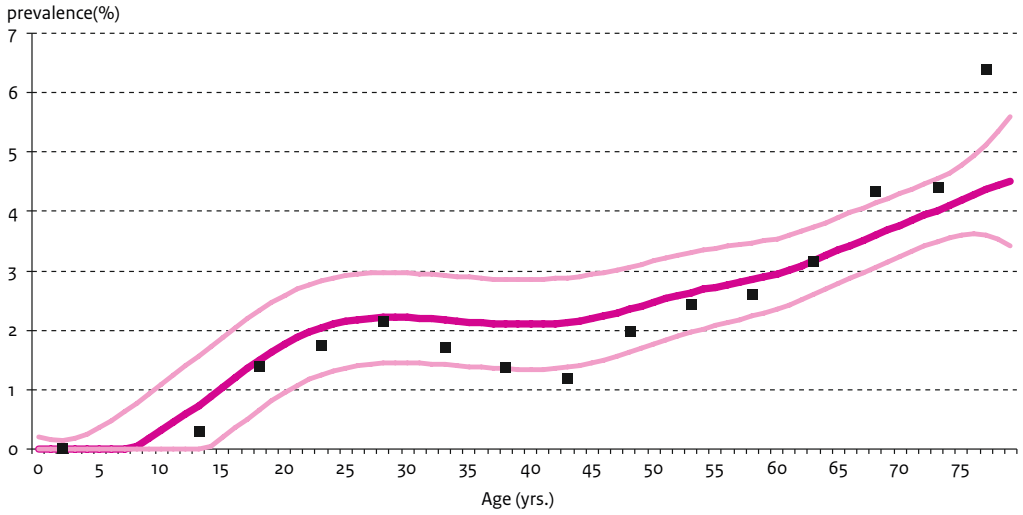

Figure 1. Weighted age prevalence estimates (mid violet line) of hepatitis E antibodies and 95% confidence intervals (outer pink lines) presented in age per year in Pienter 2 (n = 6386). Black squares represent the weighted seroprevalence estimates of 5-year age classes.

Discussion/conclusions

Although the seroprevalence seems to be lower compared to surrounding European countries, endemic exposure appears to occur with associations suggesting zoonotic potential. Several other identified potential associations, like older age and gender, are consistent with findings in other studies. Other additional associations for HEV IgG antibodies were found which may assist in further development of a risk profile for HEV infection. Given a weighted seroprevalence of 1.9% in the general population, of which 44% are people with no risk abroad and including blood donors, this indicates a potential public health risk, even though the viraemic phase is known to be short. In the absence of a clear risk profile, people at risk of HEV infection cannot be excluded from donating blood, certainly since the proportion of asymptomatic HEV infections is unclear. We advise increased surveillance and serological follow-up of HEV cases and their potentially asymptomatic HEV seropositive family members in The Netherlands in order to obtain a better understanding of the kinetics of genotype 3 infections and sources of autochthonous exposure to HEV.

Publication

Verhoef L, Koopmans M, Duizer E, Bakker J, Reimerink J, van Pelt W. **Seroprevalence of hepatitis E antibodies and risk profile of HEV seropositivity in the Netherlands, 2006-2007**. *Epidemiology and Infection*, 2012; 24:1-10.

Contact person: Linda.Verhoef@rivm.nl

19. Disease caused by Hantavirus

Background

There are many different hantaviruses circulating worldwide. Hantaviruses circulating in Europe may cause hemorrhagic fever with renal syndrome (HFRS). Most cases are caused by the Puumala serotype (PUUV). In several western European countries like Belgium, Germany, Luxembourg and France an increase in the number of hantavirus cases is observed since 2005. In the Netherlands notification of hantavirus cases is uncommon. From December 2008, when hantavirus infection became nationally notifiable, only 26 cases were reported. As the disease is only reported sporadically in the Netherlands, it is suspected that it is heavily underdiagnosed. From 19 selected municipalities of Pienter 2 (Figure 1) sera were tested for presence of hantavirus IgG antibodies to study the seroprevalence for hantavirus in the Netherlands.

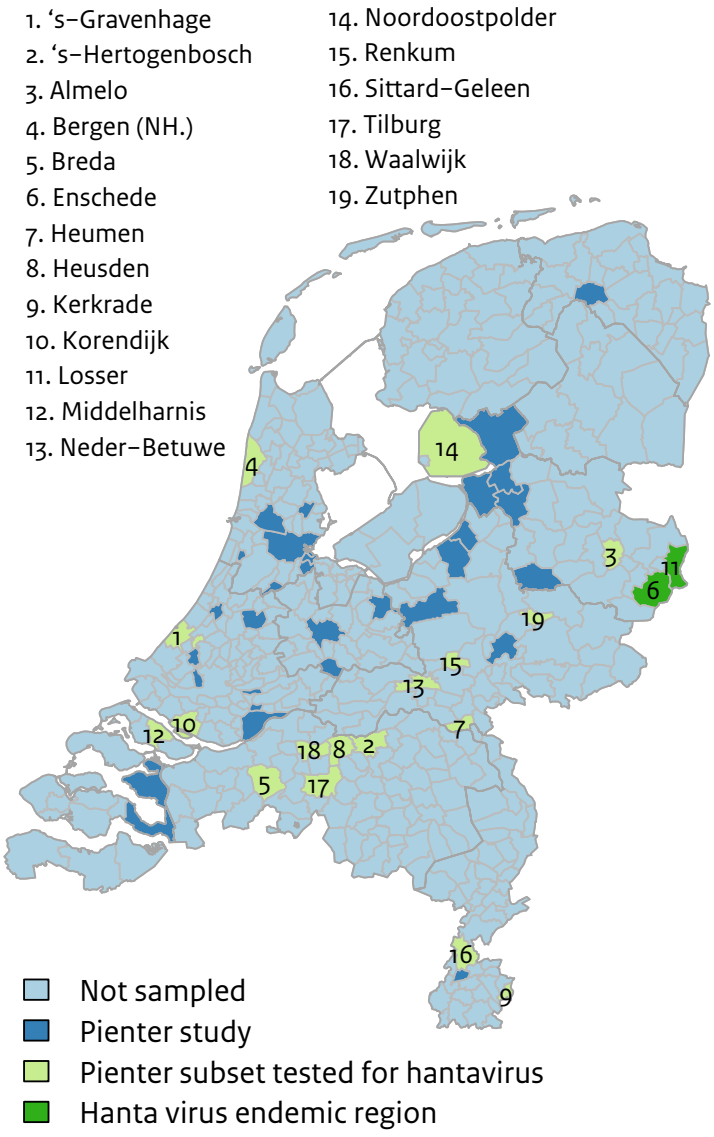

Figure 1. Municipalities participating in the Pienter 2 study, municipalities tested for presence of hantavirus IgG antibodies and hantavirus endemic regions.

Results

In total, 154 of the 2929 tested samples turned out to be ELISA-positive (5.4%). 27 out of 154 (0.9%) also tested positive in the IFA test at a dilution of 1:32 and were considered true positives. One sample of the ELISA negative subset of 119 samples also tested positive for IFA at a dilution of 1:128. When correcting for this false negative fraction a seroprevalence of 1.7% (95%CI 1.3-2.3) for the municipalities of this study was found.
The proportion seropositives among females was higher than among males (1.2% vs. 0.7% respectively) The highest percentage of seropositives was found in the age group of 15 to 40 years of age, however no significantly increased risk was associated with any of the age groups.
Univariately both municipalities from the Twente region, namely, Enschede and Losser showed an increased risk. Risk factors that remained in the multivariate model were: being female (odds ratio (OR): 1.8 (95% CI 1.2 - 2.8)), living in the municipality of Enschede (OR 5.2 (95% CI 3.7 - 7.4)), owning a dog (OR 5.0 (95% CI 2.9 - 8.9)), owning livestock (OR 4.6 (95%CI 2.8 - 7.5)) and having a net income below 1150 euros (OR 4.7 (95% CI 2.1 - 10.8)).

Discussion/conclusions

The estimated seroprevalence in this study seems fairly low compared to seroprevalence found elsewhere in Western Europe. The seroprevalence must be considered as a conservative estimate and in contrast to many other studies it is an estimate for the general population and not a selected risk population. Even with this estimate, and knowing from international literature that 30% of infected individuals develop symptoms of which 5- 10% seek medical advice, the number of cases reported yearly in the Netherlands with a population of over 16.5 million should be higher. This indicates that many cases are most likely not recognized by Dutch physicians. Especially when climate change might increase the level of hantavirus circulation and its geographic distribution in the Netherlands, it is likely that the number of infections will increase accordingly, making an increased awareness for the disease among Dutch physicians necessary.

Publication

M.G. Harms, J. Reimerink, B. Schimmer, J. Bakker, C. Reusken, W. van Pelt. **Hantavirus seroprevalence in the Netherlands**. *Submitted*.

Contact person: Margriet.Harms@rivm.nl

20. Echinococcosis (vossen- en hondenlintworminfestatie)

Background

Echinococcosis is a parasitic disease that affects both humans and other mammals. Cystic echinococcosis is caused by *Echinococcus granulosus* (a dog tapeworm) while alveolar echinococcosis is caused by *Echinococcus multilocularis* (a fox tapeworm). The parasite *Echinococcus multilocularis* was first detected in The Netherlands in 1997 and repeated studies have shown that the parasite subsequently spread in the local population of foxes in the provinces of Limburg and Groningen (Figure 1), and there are indications for further spread and increasing incidence.

Figure 1. Prevalence of *E. multilocularis* positive foxes in Nederland indicated by black dots. The white dots are investigated foxes where no *E. multilocularis* was found (source RIVM, Oost-Groningen (2000) and Zuid-Limburg (2003))

Humans function as accidental hosts, because they are usually a 'dead end' for the parasitic infection cycle. Alveolar echinococcosis is a rare but fatal disease with a long average incubation period of 10 years. Four human cases of alveolar echinococcosis have been diagnosed in The Netherlands so far, of whom 3 cases were infected abroad and one autochtonous case in the province of Limburg. It is unknown if and to what extent *Echinococcus multilocularis* infections occur in the Dutch population. The study aim was to determine the seroprevalence of *Echinococcus multilocularis* infections in persons (0-79 years) residing in several 'municipalities at risk' (i.e., with infected foxes in the region) and control municipalities in The Netherlands from the Pienter 2 study.

Results

1581 sera collected in 2006 and 2007 from 11 selected PIENTER 2 municipalities (Six 'municipalities at risk': Groningen, Sittard-Geleen, Enschede, Losser, Kerkrade, Beek and five 'control municipalities': Bergen, Korendijk, Middelharnis, 's-Gravenhage, Noordoostpolder) were investigated. Using two different ELISA tests followed by Immunoblot, no evidence was found for a specific antibody response against *E. multilocularis* in this study population.

Discussion/conclusions

Pienter 2 study was set-up to measure in the first place antibodies against the vaccine preventable diseases in the national immunization program. Municipalities were randomly selected and therefore did not have to include the risk areas for diseases such as *E. multilocularis*. This might be the reason that no antibodies were found in persons living in the municipalities included in the Pienter 2 study. To study the presence of antibodies against *E. multilocularis* infections in humans it might be better to focus on specific risk populations (i.e., inhabitants residing in areas bordering Germany and Belgium, occupational groups with an increased risk for a *E. multilocularis* infection, such as (animal) farmers, hunters, dog owners and clinically suspect patients). In a Pienter 3 study it might be thought of how this could be integrated without losing the aim of evaluating the NIP and still be able to compare the results with the former Pienter studies.

Publication

Briefrapport '**Seroprevalentieonderzoek naar de vossenlintworm (Echinococcus multilocularis) in gemeenten uit Pienter 2**' sent to Drs. S. Beukers, Directie Publieke Gezondheid/VWS, date 22 February 2010, pertaining to the product 'Rapportage serologisch onderzoek Echinococcus (V/210112/01/ZO)' delivered by the projectgroup gastro-enteritis and zoonoses of EPI/CIb/RIVM.

Contact person: Barbara.Schimmer@rivm.nl

21. Toxoplasmosis

Background

Toxoplasmosis is caused by an obligate intracellular protozoan *Toxoplasma gondii*. This parasite is able to infect various animal species as intermediate host, but only Felidae such as domestic cats shed oocysts. Intermediate hosts like cattle, sheep and pigs can be infected through ingestion of the oocysts. Humans become infected with *Toxoplasma gondii* through ingestion of tissue cysts in undercooked meat from intermediate hosts, through ingestion of oocysts that have been shed into the environment by cats, and relatively less frequently through transplantation of an organ with a tissue cyst.
Human toxoplasmosis is usually subclinical or with non-specific symptoms like fatigue and general malaise. Clinical symptoms are lymphadenopathy and ocular disease, and toxoplasmosis can be fatal in immunocompromised patients. Primary infection during pregnancy may cause spontaneous abortion or stillbirth. An unborn child exposed to *Toxoplasma gondii* in utero may develop congenital toxoplasmosis with major ocular and neurological consequences. Due to its long-term complications and the fact that *Toxoplasma gondii* is widely present in our environment, knowledge of the disease epidemiology and seroprevalence help shape health policies for prevention, particularly focused on pregnant women or women of childbearing age.
To estimate the change in the seroprevalence and risk factors for toxoplasmosis in The Netherlands the two Pienter studies were used.

Results

Testing 5541 sera for IgG antibodies against *Toxoplasma gondii* showed a marked decrease of the overall seroprevalence to 26·0% [95% confidence interval (CI) 24·0-28·0] in 2006/7, compared to 40·5% (95% CI 37·5-43·4) in 1995/1996 (see figure 1). In women of reproductive age the seroprevalence decreased from 35·2% (95% CI 32·9-38·6) in 1995/1996 to 18·5% (95% CI 16·2-20·7) in 2006/2007, leaving the majority of pregnant women susceptible to primary infection with *T. gondii* and their babies to congenital toxoplasmosis. In participants aged ≥ 20 years, toxoplasma seropositivity was associated with living in the Northwest, living in urban areas, low educational level, consumption of raw pork, keeping a cat, and not having occupational contact with clients or patients. For younger participants, risk factors were keeping sheep or cattle, consumption of raw unwashed vegetables and putting sand in the mouth (see table 1).

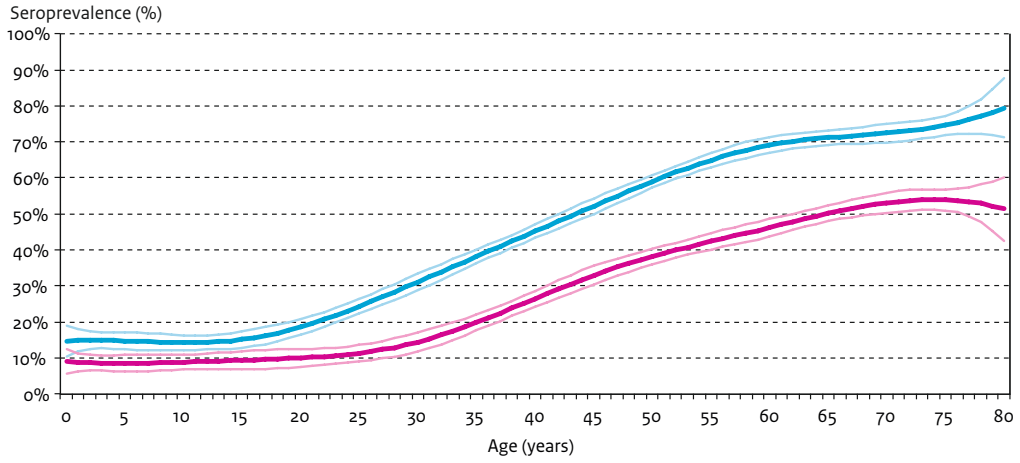

Figure 1. Age-specific prevalence of Toxoplasma gondii IgG antibodies in the first national serum bank in 1995/1996 (blue line; n = 7521) (Kortbeek2004), and in the second national serum bank in 2006/2007 (violet line; n = 5541). Prevalence rate per age class were estimated using spline functions.

Table 1. Uncorrected prevalence of specific antibodies to *Toxoplasma gondii* (%) and multivariate logistic regression analyses of risk factors associated with seropositivity in participants 0–19 and 20-79 years of age.

	Age group 0-19 years (n = 2511)			Age group 20-79 years (n = 4519)		
	N	Prevalence IgG %	Multivariate OR* (95% CI)	N	Prevalence IgG %	Multivariate OR* (95% CI)
Educational level [a]						
High	527	8%	ns	1087	29%	1.0
Medium	1272	8%		2141	31%	1.2 (1.0 - 1.4)
Low	650	10%		1242	47%	1.6 (1.3 - 1.9)
Geographical region [b]						
Southeast	294	10%	ns	627	26%	1.0
Central	555	9%		1014	35%	2.0 (1.5 - 2.5)
Northwest	582	8%		809	43%	2.3 (1.8 - 3.0)
Northeast	479	8%		987	33%	1.5 (1.2 - 1.9)
Southwest	601	10%		1084	37%	1.9 (1.5 - 2.3)
Urbanization [c]						
Rural area	1177	9%	ns	2348	37%	1.0
Urban area	1334	9%		2173	33%	1.3 (1.1 - 1.5)
Consumption of raw or undercooked pork during the past year						
No	2284	9%	ns	3478	34%	1.0
Yes	156	8%		958	38%	1.4 (1.1 - 1.6)
Occupational contact with clients or patients during the past five years						
No	2409	9%	ns	2759	41%	1.0
Yes	102	3%	1762		38%	0.8 (0.7 - 0.9)
Kept a cat during the past five years						
No	1876	9%	ns	3444	35%	1.0
Yes	594	8%	1031	35%		
Kept sheep or cattle during the past five years						
No	2375	8%	1.0	4276	35%	ns
Yes	79	16%	2.0 (1.1 - 3.9)	144	40%	

Note. OR: odds ratio, 95% CI: confidence interval, ns: not significant.

* Adjusted for age and gender.

a The following categories were used for educational level of those aged ≥15 years and of one the parents for those aged <15 years: 'low' (primary school, lower vocational or lower general secondary education), 'medium' (intermediate vocational or intermediate general secondary and higher general secondary education) and 'high' (higher vocational secondary education and university education).

b The geographical regions were based on the Dutch provinces: 'Central' (Utrecht and Gelderland), 'Southeast' (Noord-Brabant and Limburg), 'Northwest' (Noord-Holland and Flevoland), 'Southwest' (Zeeland and Zuid-Holland) and 'Northeast' (Groningen, Drenthe, Overijssel and Friesland).

c The following categories were used for level of urbanization: 'urban' (>1500 addresses/km^2) and 'rural' (<1500 addresses/km^2).

Discussion/conclusions

Along with the overall seroprevalence, the seroprevalence among women of reproductive age (15-49 years) in the Netherlands decreased from 35.2% in 1995/1996 to 18.5% in 2006/2007, leaving the majority of pregnant women susceptible to primary infection with *Toxoplasma gondii*. Therefore, education about dietary and environmental sources of toxoplasma infection remains essential to prevent toxoplasmosis and a range of other infections. Considering the fact that acquired toxoplasmosis can cause eye disease at all ages, and can be fatal to immunocompromised patients, this education may be as relevant to the general public as for pregnant women. For persons aged older than twenty, the outcomes of risk factor analyses did not point at predictors of toxoplasma seropositivity, that not are already incorporated into the education program for pregnant women. However, additional attention could focus on prevention of acquired toxoplasmosis in children through sandboxes and through consumption of raw vegetables.

Publication

Hofhuis A, van Pelt W, van Duynhoven YT, Nijhuis CD, Mollema L, van der Klis FR, Havelaar AH, Kortbeek LM. **Decreased prevalence and age-specific risk factors for Toxoplasma gondii IgG antibodies in The Netherlands between 1995/1996 and 2006/2007.** Epidemiol Infect. 2011 Apr;139(4):530-8. Epub 2010 May 24.

Contact person: Agnetha.Hofhuis@rivm.nl

22. Gastroenteritis caused by *salmonella* and *campylobacter*

Background

Estimation of the incidence of infectious diseases based on notifications of clinical cases may lead to serious underreporting. This is partly caused by the "detection pyramid" where only a small fraction of the cases in the population is diagnosed, with a laboratory confirmed specimen. Subjects exposed through food or (drinking) water may however become infected without any symptoms (or with symptoms so mild to remain unnoticed).
Infection is usually associated with seroconversion: a brisk increase in serum antibody titre, followed by a slow decline over an extended period. This happens both with symptomatic and asymptomatic infections. The antibody concentration in serum thus provides information on the time since infection: a high concentration means that that person experienced an episode a short while ago.
Using a cross-sectional sample of sera from the Dutch population an estimate can be made of the frequency of seroconversion, inclusive of any cases who never develop any symptoms. Such a procedure provides better insight into the infection pressure than may be obtained from notification data. Incidence estimates have been calculated for *Salmonella* and *Campylobacter*.

Results

The frequencies of seroconversions for *Salmonella* and *Campylobacter* estimated are much higher than those of notified cases (of serious illness). The estimated average (over all age groups) incidence of *Salmonella* (mixed ELISA, for *Enteritidis/Typhimurium* combined) is 0.12 (95% CI 0.06-0.27) per person per year (pppy), for *Campylobacter* this is 0.71 (95% CI 0.67-0.76) pppy. This means that for every symptomatic *Salmonella* case there are up to 600 unobserved infections in the population; for *Campylobacter* this ratio is even higher, about 2000.

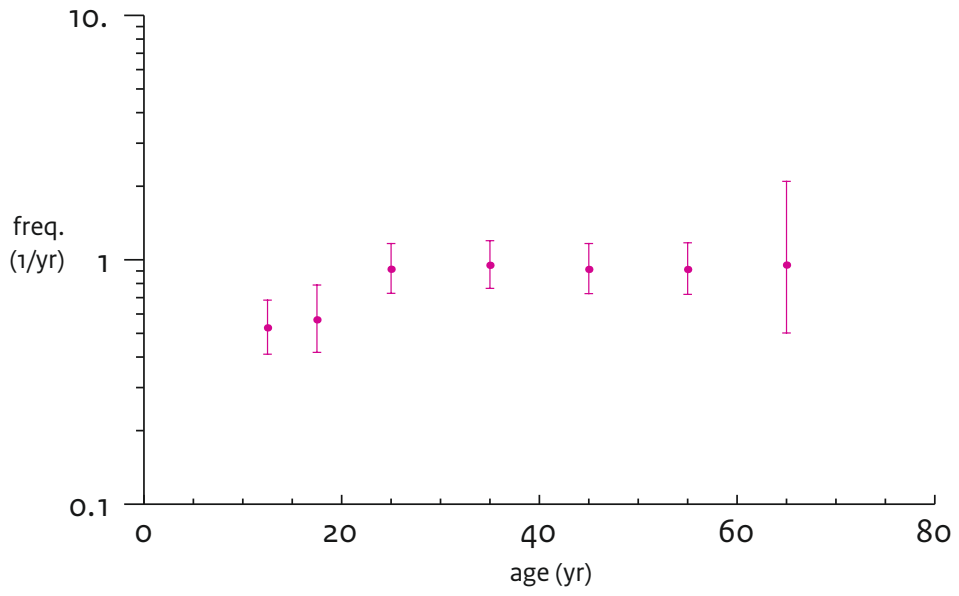

Figure 1. Sero-incidence (frequency of seroconversion on a logscale) for infection by *Campylobacter*, based on Pienter 2 data.

Discussion/conclusions

The high sero-incidence estimates imply that most infections from these two pathogens remain invisible, because they usually are associated with absence of symptoms (or mild symptoms). Note that exposure estimates of *Campylobacter* (CARMA project) led to incidence estimates consistent with these high seroincidences. Rather interestingly, the seroincidence appeared to not show seasonal variation (as studied with the same method, not using Pienter data but with data from other EU countries), while it is known that notifications are strongly seasonal. This means that illness, as a consequence of infection, is influenced by factors different from those determining infection, requiring further study.

Publication

P Teunis, J van Eijkeren, W Ang, Y van Duynhoven, J Simonsen, M Strid, W van Pelt (2012): **Biomarker dynamics: estimating infection rates from serological data.** *IStatistics in Medicine*, 2012; Mar 15 (available on line)

Contact person: Peter.Teunis@rivm.nl

Additional studies

23. Sensitization to milk, egg and peanut (voedselallergie)

Background

In developed countries allergic diseases affect 15-30% of the population and the prevalence of asthma and atopic eczema has increased in the past decades. In the last decade it has been shown that this prevalence has reached a plateau. There is evidence that food allergies are on the rise as well. In the US, a cross-sectional survey showed that from 1997 to 2007 the prevalence of self-reported food allergy increased significantly from 3.2% to 3.9%. In addition, an 11-year follow-up study from the US showed an increase in the prevalence of self-reported peanut and tree nut allergy in children younger than 18 years in the period from 1997 to 2008. For peanut allergy a rise in prevalence from 0.4% to 1.4% was demonstrated and the prevalence of tree nut allergy increased from 0.2% to 1.1%. In a birth cohort study from the UK it was shown that peanut allergy increased in 3-4 year old children from 0.4% in 1989 to 1.4% in 1996 and stabilized to 1.2% in 2002. These studies indicate that certain food allergies are increasing, thereby increasing the burden of this disease.

To investigate if there is evidence for a time trend in food allergy in the Netherlands sera were obtained from two sequential nationwide serum banks which were collected for the PIENTER studies in 1995-1996 and 2006-2007. Sensitization to food allergens was assessed with a radioallergosorbent test (RAST) and IgE titres equal to or higher than 1.2 IU/ml were used to select sensitized subjects. Sensitization was assessed in four age groups: 0-4 years (n=452), 5-18 years (n=1067), 19-40 years (n=1493) and 41-79 years (n=2006).

Results

Prevalence of cow's milk and egg sensitization

The prevalence of sensitization to cow's milk and egg was the highest in children aged 0-4 years and decreased in the older age groups. These findings were expected, since cow's milk and egg allergy are typical childhood allergies. The frequency of sensitization to cow's milk and egg did not increase from 1995-1996 to 2006-2007 (results not shown).

Prevalence of peanut sensitization

The prevalence of sensitization to peanut is calculated by using a cut-off value of 0.35 IU/ml. In Pienter 1 (1995-1996), an increase in the prevalence of peanut sensitization was seen when age group 0-4 years was compared to age group 5-18 years. Thereafter, a decline in peanut sensitization was found in the older age groups. In Pienter 2 the prevalence of peanut sensitization increased further in age group 19-40 years and then declined again. The prevalence of peanut sensitization is higher in Pienter 2 than in Pienter 1, except for the age group 5-18 years. For the age groups 19-40 years and 41-79 years this increase is statistically significantly different compared to Pienter 1.

Table 1. Prevalence of peanut sensitization

Age group	Pienter 1 (1995/1996)		Pienter 2 (2006/2007)	
	%	n[§]	%	n[§]
0 - 4 years	2.6%	6/235	5.6%	12/217
5 - 18 years	8.2%	36/440	7.5%	47/627
19 - 40 years	6.7%	37/556	9.8%*	92/937
41 - 79 years	3.6%	36/1009	5.5%*	55/997

[§] n is the number of sensitized subjects compared to the total number of subjects analyzed

* Significantly different from Pienter 1 ($p<0.05$)

Discussion/conclusions

An increase in peanut sensitization was shown from 1996-1997 to 2006-2007, which was especially observed in children aged 0-4 years and in adults aged 19-79 years but not in children aged 5-18 years. There was no increase in sensitization to cow's milk and egg. The observed increase in peanut sensitization is in line with what has been shown in other studies in children. This immunosurveillance provided cross-sectional data of children and adults. The majority of peanut allergic subjects are sensitized during childhood (before the age of 15 years). This study showed that the most significant differences were observed in the adults, which may be a reflection of events that took place during their childhood. The rise in prevalence of peanut sensitization in these individuals may be due to conditions that were apparent during their childhood.

The increased prevalence of food allergy and other allergic diseases has initiated many epidemiological studies that tried to elucidate which external factors are involved in this increase. It is well-known that besides genetic susceptibility, dietary, environmental and lifestyle factors could have an impact on the development of food allergies. Insight in external factors that are increasing or decreasing the risk on food allergy is important for the development of preventive strategies. A literature review conducted by the RIVM showed that the effects of the majority of external factors on food allergy could not be determined because there were either too few studies or the results of different studies were conflicting. One important finding is that avoidance of food allergens either during pregnancy or in early childhood does not reduce the risk on food allergy. This has lead to a paradigm shift in this area. The current hypothesis is that delayed introduction of solid foods, including food allergens during infancy increases rather than decreases the risk on food allergy. This has been explained by the fact that during infancy the immune system has to be exposed to proteins in order to develop tolerance. Currently, several human intervention studies are ongoing that are investigating this new hypothesis and information from these studies might provide more insight into this complex area. In the future this might lead to modifications of the current recommendations concerning timing of the introduction of food allergens in the infant's diet. These and other preventive strategies should aim at reducing the risk on the development of food and other allergic diseases thereby reducing the burden of allergic diseases.

Publication

Ezendam, J., van der Klis, F.R. & van Loveren, H. **Time trends in prevalence of sensitization to milk, egg and peanut in the Netherlands.** RIVM report 340350003 (2009)

Contact person: Janine.Ezendam@rivm.nl
Fiona.van.der.Klis@rivm.nl

24. Contact patterns ('dagboekje')

Background

An important question is how an infection will spread through the population when an outbreak occurs. Will the infection affect only particular age groups with a low vaccine uptake, will the infection affect age groups that are vulnerable, or will the infection affect all age groups? To answer these questions we need to know how frequently at-risk contacts occur within and between age groups. Most of the vaccine-preventable infections are transmitted by respiratory droplets and by close contact, hence a proxy for at-risk contact is having a conversation or touching. In the Pienter 2 study we asked the participants about the frequency of social contacts, stratified by age.

Results

The number of persons a participant contacts during a day depends on the participants age. Children in secondary school make most contacts. Most of these contacts are made within the same age group. Based on these data we can infer that, if an outbreak occurs in a susceptible population, a 16 year-old infective will contribute more to future spread than a 60-year old.

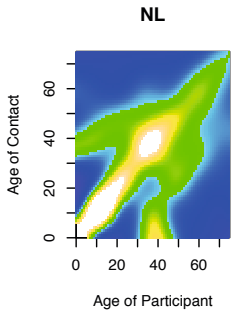

Figure 1. Smoothed contact matrix based on all reported contacts. White indicates high contact rates, green intermediate contact rates, and blue low contact rates. (from Mossong et al. PLoS Medicine 2008)

Discussion/conclusions

These results are essential to determine whether the Dutch population is protected from outbreaks of vaccine-preventable infections. When we detect in the Pienter 2 study that an age group has fewer immunes than expected, we also need to know how much this age group will contribute to the future spread of infections before we reach our conclusions about the risk for an outbreak. The contact data that is collected in the Pienter 2 study provides the necessary data to inform us on the contribution to future spread of infections.

Publication

Mossong, J., et al., **Social contacts and mixing patterns relevant to the spread of infectious diseases.** *PLoS Med*, 2008. 5(3): p. e74.

Contact person: Jacco.Wallinga@rivm.nl

Editors:
Fiona van der Klis, Liesbeth Mollema, Yolanda van Weert.

The Pienter 2 project is considered as one of the important products of the RIVM. With this project the RIVM monitors the protection of the general population against several infectious diseases primarily those included in the National Immunization Program.

In 2006 and 2007, blood material and questionnaire data were collected from more than 8000 Dutch inhabitants.

Now, most of the serological and epidemiological analyses planned have been performed and are published or will be made public in the near future.

It is now time to combine all scientific results thereby giving an overview of the broad use of the Pienter materials and data; it is an impressive list.

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National Institute for Public Health and the Environment
P.O. Box 1 | 3720 BA Bilthoven
www.rivm.nl

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