AUSTRALIA'S NATIONAL SEROSURVEILLANCE PROGRAM

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Serosurveillance is an important component of any comprehensive surveillance system for vaccine preventable diseases. Disease notification data are necessary to detect outbreaks and can provide timely epidemiological profiles of a disease. However, the incidence of a disease is often under-estimated by notifications, especially when a proportion of cases are asymptomatic. If this proportion changes with age, or if cases are clinically misdiagnosed, then notification data may present biased information. Serosurveillance is therefore the gold standard for measuring immunity in a population, and complements disease surveillance. The data from serosurveillance are also an essential contribution to mathematical modelling, which can predict the potential for cases in the future, and thus when—and in which age groups—intervention is required to prevent an epidemic. This article describes Australia's national serosurveillance program, which is an important source of information for public health action.

CONDUCTING SEROSURVEILLANCE

There are two methods that can be used to obtain sera for a serosurvey. The ideal method is to collect sera from subjects randomly selected to represent the population. The second more pragmatic approach is to use a sample of sera submitted for diagnostic testing that would otherwise have been discarded. The national serosurveillance program in Australia uses the latter approach.

In the first serosurvey conducted in 1999, 52 public and private diagnostic laboratories throughout Australia were invited to provide samples of residual sera. Approximately 13,000 sera from patients aged one year to over 90 years were collected from 45 laboratories.

Serosurveillance using a convenience sample

Our methodology was modelled on that used for serosurveillance in England and Wales, which began in 1986–87 to coincide with the introduction of the measles–mumps–rubella (MMR) vaccine in 1988. Each year since then they have collected sera from specific age groups, and every five years sera are collected from across the entire age range.[1]

Serosurveillance using a population-based random sample

The alternative approach to convenience sampling is population-based random sampling. In the USA sera are collected as part of the National Health and Nutritional Examination Survey program (NHANES). This program includes periodic national surveys based on a multistage probability-sample design and involves a household interview and physical examination.[2] The last survey (NHANES III) was conducted in 1988–1994, when approximately 40,000 sera from persons aged two months and over were collected and used to determine immunity to tetanus, measles, rubella, hepatitis B and C, and HIV.[2–7] In The Netherlands, a population-based random sample of sera, designed specifically for serosurveillance, is collected using a two-stage cluster sampling technique.[8] The first such collection was in 1995–96, when nearly 10,000 sera were obtained to examine for immunity to diphtheria, poliomyelitis, measles, mumps, rubella, *Haemophilus influenzae* type b, and hepatitis A, B and C.[8–15]

Advantages and disadvantages of population-based sampling

Serosurveillance programs in both the USA and The Netherlands collect detailed information about risk factors and the vaccination status of participants. This is a major advantage of the population-based random sampling methodology over the opportunistic collection method. It also permits over-sampling of particular at-risk groups, so that appropriate sample sizes are obtained for subgroup analyses. In addition, risk factors associated with low levels of immunity can be identified allowing vaccination programs to be targeted appropriately. For example, in the USA, susceptibility to tetanus was associated with certain sub-populations.[2]

The major disadvantage with random sampling is that it is more costly and time consuming than collecting a convenience sample. A study in Victoria by Kelly et al. estimated the cost of retrieval and storage per antibody tested using a random cluster sample to be about seven times more than the equivalent cost for a convenience sample.[16]

Comparing serosurveys

To allow comparisons between laboratory methods and differing immunisation programs, a European Sero-Epidemiology Network (ESEN) was established in 1996.[17] The network aims to coordinate and standardise serosurveillance for vaccine preventable diseases in Europe. Panels of sera representing a range of immunity levels are prepared by a designated reference laboratory. These are then tested by each country using their usual testing method and calibrated against the reference laboratory's results by way of a standardisation equation. This equation is then applied to the serum bank collected in each country to convert the results into standard reference laboratory units. The first ESEN project looked at five vaccine preventable diseases (measles, mumps, rubella, pertussis and diphtheria) and involved six countries (Denmark, France, Germany, Italy, The Netherlands and the United Kingdom). The results for measles, mumps and rubella have been published and show promise although there is still some residual lack of comparability after standardisation.[18]

A second ESEN project (ESEN2) is now under way and will include more countries and additional vaccine-preventable diseases (varicella and hepatitis A and B). In Australia, the National Centre for Immunisation Research

TABLE 1

DISEASES EXAMINED IN THE FIRST AUSTRALIAN NATIONAL SEROSURVEY TOGETHER WITH THE COLLECTION DATES, AGE RANGES, SAMPLE SIZES AND STATUS OF EACH SURVEY

Disease	Collection date	Age groups tested (years)	Sample size *N*	Status of survey (publication)
Diphtheria	May 1997–Jan 1999	5–70+	1,953	Completed
Hepatitis A	1998	1–60+	3,043	Completed [25]
Hepatitis B	March 1998–May 1999	1–18	1,735	Completed[17]
Hepatitis C	Jun 1996–Dec 1998	1–70+	2,800	Completed
Measles	1996–98	1–18	2,936	Completed[21–23]
		19–49	2,126	
Measles	Jan–Jun 1999	1–18	2,918	Completed.[17,21,22]
Mumps	1996–98	1–59	2,787	Completed
Mumps	Jan–Jun 1999	1–18	1,249	Completed[17]
Pertussis	1998	1–65+	1,022	Completed
Polio	1998	1–65+	1,816	Completed
Rubella	1996–98	1–18	2,859	Completed[21,22]
		19–49	1429	
Rubella	Jan–Jun 1999	1–18	2,947	Completed[17,21,22]
Tetanus	Feb 1997–Mar 1999	5–70+	2,884	Completed
Varicella	1996–98	1–49	2,027	Completed[17]

Source: National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases.

and Surveillance of Vaccine Preventable Diseases (NCIRS), in collaboration with the Institute of Clinical Pathology and Medical Research (ICPMR), will be participating in ESEN2.

BIASES IN SEROSURVEYS

Biases in population-based random samples

To be confident that a sample is unbiased, a well-randomised, population-based sample with a 100 per cent response rate is required. In practice, however, this is impossible to achieve and non-participation is usually quite high in randomised surveys. In a Victorian study using a three-stage random cluster sample from school-aged children, the school response rate was 59 per cent and the rate for students consenting to provide sera was between 32 and 39 per cent.[16] Participation in the NHANES III survey was higher at 77.4 per cent, although rates were much lower for children aged 6–11 years (52.7 per cent).[2] In The Netherlands serosurvey, the participation rate was 55 per cent.[8]

Low response rates may lead to non-participation bias if participation is related to disease immunity. In The Netherlands serosurveys, some demographic characteristics were available from a municipal database on all eligible individuals.[19] These were examined: non-participants were more likely to be unmarried, not Dutch in origin, and live in highly urbanised areas. The latter two factors could to be related to immune status and thus lead to a biased estimate of immunity. The risk of infection may be greater in urban areas compared with small towns, due to the higher population density. Nationalities other than Dutch are also likely to have differing immunity because of variations in vaccination programs and disease incidence between countries. Some adjustment can be made by over sampling the under-represented groups and weighting the seroprevalence estimates.[2,19] However this does not always reduce the bias, especially when there are other unmeasured differences between participants and non-participants.

Biases in convenience samples

As with random samples, convenience samples may also be biased. However, because less is known about participants in convenience samples compared with those from a random sample, any potential biases are more difficult to identify and control for when estimating seroprevalence.[1,20] In Australia, we reduced the potential for selection bias by enrolling most (86.5 per cent) major laboratories in the country with the majority of samples from ambulatory subjects rather than hospitalised patients. We have also been able to demonstrate that our convenience sample of sera gave similar results to those obtained from a prospectively collected, random sample from school-aged children in Victoria for immunity levels to measles, mumps, rubella, hepatitis B and varicella.[16] However, for some diseases (such as hepatitis C) for which seropositive individuals may be over-sampled due to increased diagnostic testing, opportunistic collections may not yield accurate estimates of immunity, although the distribution of immunity by age and over time may still provide useful information.

AUSTRALIA'S FIRST SEROSURVEY

Methodology

Australia's first national serosurvey aimed to provide a national picture of immunity to each disease examined within each age group surveyed. In each age group for both males and females, states and territories were sampled proportionally to their 1997 population size. Sample sizes

TABLE 2

EXAMPLES OF DISEASES, AGE RANGES AND SAMPLE SIZES THAT MAY BE EXAMINED IN THE SECOND AUSTRALIAN NATIONAL SEROSURVEY TOGETHER WITH THE RATIONALE FOR EACH SURVEY

Disease	Age range (years)	Sample size *N*	Rationale
CMV	1–59	3,593	Precise estimate of immunity
EBV	1–59	3,655	Precise estimate of immunity
Helicobacter	1–59	2,410	Precise estimate of immunity
Hepatitis A*	1–59	2,605	Update (precise estimate) of immunity
Hepatitis B core antibody*	1–59	1,760	Update (precise estimate) of immunity
Hepatitis B surface antibody*	1–59	2,580	1–2 years—effect of universal infant vaccination (compare with first serosurvey) 12–17 years—compare states with and without school-based programs Other age groups—update (precise estimate) of immunity
Measles*	1–34	3,560	18–34 years—effect of young adult campaign (compare with first serosurvey) 1–18 years—update (precise estimate) of immunity
Rubella*	1–34	3,605	As per measles
Varicella*	1–5	380	Effect of vaccine pre funding (compare with first serosurvey)

*also tested in the first serosurvey

CMV: cytomegalovirus EBV: Epstein-Barr virus.

Source: National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases.

were calculated to achieve confidence intervals of approximately +/- five per cent for each age group, based on the expected level of immunity to each disease. This provided a precise estimate of immunity for Australia as a whole in each age group; however, the sample sizes calculated did not provide appropriate power for a precise estimate of immunity in each individual state and territory.

Except for the pertussis serology (which was performed in Italy) all testing was performed at the Centre for Infectious Diseases and Microbiology (CIDM), Institute of Clinical Pathology and Medical Research (ICPMR) at Westmead Hospital, Westmead.

Timing

The main reason for the timing of the first national serosurveys was to contribute to the evaluation of the Measles Control Campaign (MCC), which was conducted in the second half of 1998.[21] Two convenience samples of sera were collected to do this. The first comprised 9,341 sera collected from individuals aged from one year to over 90 years in the two years prior to the campaign. The second was of 3,513 sera from 1–18-year-old children collected between January and May 1999. Participating laboratories were requested to exclude sera from subjects who were known to be immunocompromised; to have received multiple transfusions in the previous three months; be infected with human immunodeficiency virus; or to have had serum collected for diagnosis of measles. Only one sample from any subject was tested.

Results

The first serosurvey has provided a wealth of information. Not only were we able to provide a qualitative measure of the success of the MCC,[20] we have been able to determine the age-specific immunity to several diseases (Table 1). These serological profiles demonstrate the effect of past and current vaccination policies, as well as natural infection on immunity. In addition the profiles have been used to determine those age groups now most at risk of infection, which has enabled appropriately targeted interventions. For example, the MCC measles serology identified a cohort of young adults with a low level of immunity.[22] To improve their immunity, this group were targeted by a MMR vaccination campaign conducted in 2001.[23]

Data from the first serosurvey have and will continue to be a particularly valuable ingredient in the mathematical modelling conducted by NCIRS. The measles serology results have been used to predict when another epidemic may occur and what must be achieved to prevent it from occurring.[25] The pre-vaccination serosurvey on varicella immunity has provided data to calculate epidemiological parameters such as the age-specific force of infection (that is, the incidence rate in the non-immune population), the average age of infection and the average number of susceptibles infected per case in a completely susceptible population. These data provide us with a better understanding of the current epidemiology of varicella and also with the information needed to model the impact of different vaccination scenarios. Modelling is now under way based on the methodology used in Canada and the United Kingdom.[26]

THE FUTURE OF SEROSURVEILLANCE IN AUSTRALIA

The first national serosurveys have been extremely useful but only provide a snapshot of immunity at one time. For diseases whose epidemiology does not change over time, a one-off serosurvey may be sufficient. However, after

vaccination is introduced, or when the incidence of infection is changing over time, ongoing serosurveillance is required. In addition, we would like to examine immunity to other diseases and the serum bank is now severely depleted. With these points in mind, collection is currently under way for the second serosurvey. We are using the same methodology as that used in the first serosurvey and estimate that at least 5,000 serum specimens need to be collected from ages 1–59 years to perform tests that are currently under consideration (Table 2).

Given the results of the first serosurvey, the second is likely to provide us with just as many interesting findings to guide vaccination activity and policy development. Looking further ahead, NCIRS plans to conduct regular serosurveys as part of its surveillance program.

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REFERENCES

1. Osborne K, Gay N, Hesketh L, Morgan-Capner P, Miller E. Ten years of serological surveillance in England and Wales: Methods, results, implications and action. *Int J Epidemiol* 2000;29:362–8.
2. Gergen PJ, McQuillan GM, Kiely M, et al. A population based serologic survey of immunity to tetanus in the United States. *New Engl J Med* 1995;332:761–6.
3. McQuillan GM, Khare M, Karon JM, Schable CA, Vlahov D. Update on the seroepidemiology of human immunodeficiency virus in the United States household population: NHANES III, 1988-1994. *J Acquir Immune Defic Syndr Human Retrov* 1997;14:355–60.
4. Hutchins SS, Redd SC, Schrag S, et al. National serologic survey of measles immunity among persons 6 years of age or older, 1988–1994. *Med Gen Med* 2001;E5.
5. Dykewicz CA, Kruszon-Moran D, McQuillan GM, et al. Rubella seropositivity in the United States, 1988–1994. *Clin Infect Dis* 2001;33:1279–86.
6. Williams I. Epidemiology of hepatitis C in the United States. *Am J Med* 1999;107:S2–S9.
7. McQuillan GM, Coleman PJ, Kruszon-Moran D, Moyer LA, Lambert SB, Margolis HS. Prevalence of hepatitis B virus infection in the United States: the National Health and Nutrition Examination Surveys, 1976 through 1994. *Am J Public Health* 1999;89:14–18.
8. de Melker HE, Conyn-Van Spaendonck MA. Immunosurveillance and the evaluation of national immunization programmes: A population-based approach. *Epidemiol Infect* 1998;121:637–43.
9. Conyn-Van Spaendonck MA, de Melker HE, Abbink F, Elzinga-Gholizadea N, Kimman TG, van Loon T. Immunity to poliomyelitis in the Netherlands. *Am J Epidemiol* 2001;153:207–14.
10. van Den HS, de Melker HE, Berbers GA, Der Kraak PH, Spaendonck MA. Antibodies to *Haemophilus influenzae* serotype b in the Netherlands a few years after the introduction of routine vaccination. *Clin Infect Dis* 2001;32:2–8.
11. Termorshuizen F, Dorigo-Zetsma JW, de Melker HE, van Den HS, Conyn-Van Spaendonck MA. The prevalence of antibodies to hepatitis A virus and its determinants in The Netherlands: a population-based survey. *Epidemiol Infect* 2000;124:459–66.
12. van Den HS, Berbers GA, de Melker HE, Conyn-Van Spaendonck MA. Sero-epidemiology of measles antibodies in the Netherlands, a cross-sectional study in a national sample and in communities with low vaccine coverage. *Vaccine* 1999;18:931–40.
13. de Haas R, van Den HS, Berbers GA, de Melker HE, Conyn-Van Spaendonck MA. Prevalence of antibodies against rubella virus in The Netherlands 9 years after changing from selective to mass vaccination. *Epidemiol Infect* 1999;123:263–70.
14. de Melker HE, Berbers GA, Nagelkerke NJ, Conyn-Van Spaendonck MA. Diphtheria antitoxin levels in the Netherlands: A population-based study. *Emerg Infect Dis* 1999;5:694–700.
15. de Melker HE, van Den HS, Berbers GA, Nagelkerke NJ, Rumke HC, Conyn-Van Spaendonck MA. A population-based study on tetanus antitoxin levels in The Netherlands. *Vaccine* 1999;18:100–8.
16. Kelly H, Riddell MA, Gidding HF, Nolan T, Gilbert GL. A random cluster survey and a convenience sample give comparable estimates of immunity to vaccine preventable diseases in children of school age in Victoria, Australia. *Vaccine* 2002;20:3130–6.
17. Osborne K, Weinburg J, Miller E. The European Sero-Epidemiology Network. *Euro Surveill* 1997;2:29–31.
18. Andrews N, Pebody RG, Berbers G, et al. The European Sero-Epidemiology Network: standardizing the enzyme immunoassay results for measles, mumps and rubella. *Epidemiol Infect* 2000; 25: 127–41.
19. de Melker HE, Nagelkerde NJ, Conyn-Van Spaendonck MA. Non-participation in a population-based seroprevalence study of vaccine-preventable diseases. *Epidemiol Infect* 2000;124:255–62.
20. Gilbert GL, Escott RG, Gidding HF, et al. Impact of the Australian Measles Control Campaign on immunity to measles and rubella. *Epidemiol Infect* 2001;127:297–303.
21. Turnbull FM, Burgess MA, McIntyre PB, et al. The Australian Measles Control Campaign, 1998. *Bull World Health Organ* 2001;79:882–8.
22. Gidding HF, Gilbert GL. Measles immunity in young Australian adults. *Commun Dis Intell* 2001;25:133–6.
23. Campbell M. Young adult measles vaccination [editorial]. *Commun Dis Intell* 2000;24:241–2.
24. Amin J, Gilbert GL, Escott RG, Heath TC, Burgess MA. Hepatitis A epidemiology in Australia: National seroprevalence and notifications. *Med J Aust* 2001;174:338–41.
25. MacIntyre CR, Gay NJ, Gidding HF, Hull BP, Gilbert GL, McIntyre PB. A mathematical model to measure the impact of the Measles Control Campaign on the potential for measles transmission in Australia. *Int J Infect Dis* 2002;6:277–281.
26. Brisson M, Edmunds WJ, Gay NJ, Law B, De Serres G. Modelling the impact of immunization on the epidemiology of varicella zoster virus. *Epidemiol Infect* 2000;125:651–69.
27. Australian Measles Conytrol Campaign 1998—Evaluation Report at http://immunise.health.gov.au/metadata/measeval.htm pages 8–9