Epidemiol. Infect. (2010), **138**, 426–433. © Cambridge University Press 2009
doi:10.1017/S0950268809990707 Printed in the United Kingdom

The utility of seroepidemiology for tracking trends in pertussis infection

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(*Accepted 3 August 2009; first published online 1 September 2009*)

SUMMARY

Comparing pertussis epidemiology over time and between countries is confounded by differences in diagnostic and notification practices. Standardized serological methods applied to population-based samples enhance comparability. Population prevalence of different levels of pertussis toxin IgG (PT IgG) antibody, measured by standardized methods, were compared by age group and region of Australia between 1997/1998 and 2002. The proportion of 5- to 9-year-olds with presumptive recent pertussis infection (based on IgG levels $\geqslant$62·5 ELISA units/ml) significantly decreased in 2002, consistent with notification data for the same period and improved uptake of booster vaccines following the schedule change from whole-cell to acellular vaccine. In contrast, recent presumptive infection significantly increased in adults aged 35–49 years. Population-based serosurveillance using standardized PT IgG antibody assays has the potential to aid interpretation of trends in pertussis incidence in relation to vaccine programmes and between countries.

Key words: Epidemiology, pertussis (whooping cough), serosurvey, vaccine.

INTRODUCTION

Australia, in common with a number of other developed countries with long-established pertussis immunization programmes (Table 1), experienced substantial increases in the numbers of notified pertussis cases in the 1990s [1]. This increase coincided with the widespread availability of serological diagnosis and the introduction of direct notification of pertussis cases by laboratories as well as by clinicians [2]. Hospitalizations recorded as due to pertussis also increased [3], suggesting a true underlying increase in pertussis disease activity, not simply increased testing, since serology makes little contribution to pertussis diagnosis in infants, whereas infants account for most hospitalizations. Nevertheless, physician practice and diagnostic test availability confound the interpretation of trends in pertussis rates, both within and between countries. Population-based, cross-sectional seroepidemiology potentially offers a less biased means of comparison of age-specific patterns of pertussis infection at the national and international level, subject to acceptable standardization and reproducibility

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Table 1. *Significant events in pertussis immunisation practice in Australia*

Year	Vaccine type	Event
1953	DTPw	Diphtheria-tetanus-pertussis (DTPw) infant based schedule of three doses introduced – varied by state and territory
1975	DTPw	National vaccination schedule recommended for infants aged 3, 4 and 5 months
	DTPw	4th dose introduced as a booster for infants aged 15–18 months
1978	DTPw	4th dose removed from schedule
1982	DTPw	National vaccination schedule changed to a primary series at ages 2, 4 and 6 months
1985	DTPw	4th dose re-introduced at age 18 months due to an increase in pertussis incidence in 4- to 5-year-olds
1994	DTPw	5th dose at age 4–5 years added to the recommend vaccination schedule
1997	DTPa	Diphtheria-tetanus-acellular pertussis (DTPa) recommended for the 4th and 5th doses of vaccination
1999	DTPa	All five scheduled doses of DTPw replaced with DTPa
2000	DTPa	Second booster dose recommended at 4 years instead of 4–5 years
2003	DTPa	4th dose, previously given at age 18 months, removed from schedule
	DTPa	4th dose recommended at age 4 years
	dTpa	Adolescent/adult formula diphtheria-tetanus-acellular pertussis (dTpa) recommended as a booster dose at age 15–17 years
	dTpa	dTpa available as a single boosting dose for adults

of serological tests and the ability to extrapolate these results to estimates of symptomatic cases.

The European Sero-Epidemiology Network (ESEN) standardized the use of a serological criterion for recent infection, measured in IgG-PT ELISA units (EU) [4] between participating laboratories using different serological methods. This allowed meaningful comparison of *B. pertussis* seroepidemiology in six European countries [5, 6], as well as a within-country comparison at several time points [7]. We applied this standardized method to sera collected in Australia in 1997/1998 in a nationally representative serosurvey. In that study, the highest prevalence of recent pertussis infection was in the 5–9 years age group, corresponding to notification patterns for the same period [8]. Since then, a number of important changes in pertussis and pertussis vaccine use have occurred in Australia. First, the pertussis notification rate in the 5–9 years age group declined with a progressive increase in vaccine coverage [9, 10]. Second, acellular vaccines replaced the locally made whole-cell vaccine, initially for booster doses and then for all doses in the pertussis vaccine schedule (Table 1) [11]. Third, peak notification rates progressively moved to the adolescent and older age groups, beginning in 1999 [10, 12].

This study examined the cross-sectional seroepidemiological profile of pertussis in 2002, using the same laboratory methods as for the 1997 study, in consultation with the Italian reference laboratory [8]. The aim was to evaluate age-specific patterns of presumptive recent pertussis infection in the context of changes to the vaccine schedule and pertussis notifications.

METHODS

Population and study design

The sera used in this study were selected from a bank of about 7700 sera collected opportunistically from a geographically representative group of 37 diagnostic laboratories receiving samples from hospitalized and ambulant persons throughout Australia in 2002, as part of a national serosurveillance programme [13]. Opportunistic sampling has been shown to yield results comparable to population-based cluster sampling for the measurement of measles immunity [14]. The sera in the opportunistic sample were residual from specimens submitted for diagnostic testing and would otherwise have been discarded. Residual sera from subjects who were immunosuppressed, had received multiple or recent (within 3 months) blood transfusions, or were known to be infected with HIV were excluded by staff at the diagnostic laboratory. The immunization status of subjects was unknown. Sera were identified by a medical record number (MRN), sex, age, state/territory of origin and a unique identifier, to ensure that only one sample from any subject was tested. Approval for the serosurvey was obtained from the Western Sydney Area Health Service Human Research Ethics Committee.

In all age groups the sample size was calculated to achieve a point estimate of seroprevalence with 95%

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confidence intervals (CI) of ±4%, based on the expected level of seroprevalence for that age group. There were equal numbers of males and females within each age group.

Testing and serological criteria for recent pertussis infection

A total of 1999 sera randomly selected from those available in each age group were tested using an ELISA method adapted from Giammanco *et al.* [15]. The method was validated in our laboratory against a panel of sera previously tested by the ESEN reference laboratory at the Department of Hygiene and Microbiology, Palermo University, Italy.

Microtitre plate wells (Greiner Bio-one, Germany) were coated with 100 μl of PT (GlaxoSmithKline, Belgium) diluted to 1 μg/ml in carbonate buffer (pH 9·6) (Sigma-Aldrich, USA). The sealed plates were incubated at 28 °C for 16–18 h. The wells were then aspirated and rinsed with wash buffer (0·145 M NaCl, 0·05% Tween-20; Sigma-Aldrich).

Reference, positive and negative control sera with defined levels of anti-PT IgG and test sera pre-diluted in serum incubation buffer (PBS; Oxoid Ltd, UK), 0·5% BSA (Sigma-Aldrich) and 0·5% Tween-20 were then added to the appropriate wells of each plate. The reference serum [US reference pertussis antiserum (human), lot 3] has an assigned value of 200 EU/ml of anti PT IgG.

In each plate, eight doubling dilutions (100 μl/well) were made, of the reference serum prediluted to 1:200, and the high and low positive controls prediluted to 1:60. The remaining wells contained doubling dilutions of test sera commencing with a 1:60 dilution.

Plates were incubated at 28 °C for 2 h. Following incubation they were rinsed with wash buffer and 100 μl of alkaline phosphatase-conjugated anti-human goat IgG (KPL Inc., USA), diluted 1:5000 in conjugate incubation buffer (serum incubation buffer with 2% foetal calf serum), was added to each well and incubated at 28 °C overnight (16–24 h). The plates were then washed with wash buffer and 100 μl of 1 mg/ml substrate (*p*-nitrophenyl phosphate, Sigma) was added to each well. After 30 min incubation at room temperature, the reaction was stopped by the addition of 50 μl of 5 N NaOH. The optical density (OD) was read at 405/630 nm using a LP400 (Diagnostics Pasteur, France) plate reader.

OD readings for the reference sera were used to produce a standard curve for each plate, and antibody levels (EU/ml) for the test sera were calculated using Multicalc version 2.60 (Wallac Oy, Finland). The minimum level of detection, defined as the minimum amount of antibody that must be present for the serum to have at least one OD value within the linear range of the reference serum response curve, was estimated to be 2 EU/ml. Anti-PT IgG levels were divided into four categories, previously described by the ESEN study group as suggestive of pertussis infection within certain time periods [6]: <5 EU/ml (undetectable), 5 to <62·5 EU/ml (infection >1 year previously), 62·5 to <125 EU/ml (infection within 12 months) and ⩾125 EU/ml (infection within 6 months).

Statistical analysis

Proportions of sera with anti-PT IgG levels in each of the categories described above were calculated by age group and by geographical area. Odds ratios (ORs) were calculated to compare groups and the χ^2 test statistic used to calculate CIs. Rate ratios were used to compare proportions in relevant age groups by collection year and region of residence. *P* values <0·05 were considered significant. Analyses were performed using Microsoft Excel, SAS version 9.1.3 and EpiInfo 3.3.2.

For analysis by region of residence, data from New South Wales (NSW) and the Australian Capital Territory (ACT) were pooled, based on their geographical proximity, as were data from the Northern Territory, Tasmania and Western Australia based on similar patterns of pertussis notification, presumed to be associated with population density and relative isolation [10].

RESULTS

PT IgG by age group

The proportions of anti-PT IgG levels ⩾62·5 EU/ml, as a proxy for pertussis infection in the previous 12 months [6] are shown in Table 2 for the 1997/1998 [8] and 2002 national serosurveys. Using the 1–4 years age group as a comparator, in 1997/1998, the only groups in which the proportion of PT IgG levels ⩾62·5 EU/ml was significantly higher were those aged 5–9 and 15–19 years, although the higher proportion in the 10–14 years group almost reached statistical significance. By 2002, this age-specific pattern had substantially changed. Compared to the 1–4 years

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Table 2. *Proportion of anti-PT IgG levels ⩾62·5 EU/ml, by age group in the 1997/1998 and 2002 national serosurveys*

	1997/1998 collection			2002 collection			
Age group (years)	*n*	Anti-PT IgG ⩾62·5 EU/ml (%)	OR* (95% CI)	*n*	Anti-PT IgG ⩾62·5 EU/ml (%)	OR* (95% CI)	Anti-PT IgG ratio† (95% CI)
1–4	163	12	1	165	9	1	0·8 (0·4–1·5)
5–9	253	30	3·3 (1·9–5·9)	231	14	1·7 (0·8–3·4)	0·48 (0·3–0·8)
10–14	314	18	1·7 (0·9–3·1)	742	11	1·2 (0·7–2·3)	0·59 (0·3–1·0)
15–19	109	22	2·1 (1·1–4·4)	306	11	1·2 (0·6–2·4)	0·49 (0·3–0·9)
20–24	45	18	1·6 (0·6–4·4)	160	16	1·9 (0·9–3·9)	0·9 (0·5–1·4)
25–34	32	9	0·8 (0·2–3·1)	205	14	1·7 (0·8–3·4)	1·5 (0·9–2·5)
35–44	54	6	0·5 (0·1–1·7)	140	19	2·4 (1·2–5·0)	3·4 (2·2–5·4)
45–59	36	3	0·2 (0·0–1·6)	50	14	1·6 (0·6–4·6)	5 (3·0–8·4)

OR, Odds ratio; CI, confidence interval.
* χ^2 analysis comparing anti-PT IgG ⩾62·5 EU/ml in the 1–4 years age group with other age groups in the same collection year.
† Comparing the 2002 collection year to the 1997/1998 collection year, by age group.

age group, those aged 5–19 years had similar proportions of PT IgG levels ⩾62·5 EU/ml and the 35–44 years group had significantly higher proportions. The magnitude of the increase in high levels in this age group is more apparent when the ratios of anti-PT IgG levels in 2002 to those in 1997/1998 are examined (Table 2). Compared to 1997/1998, the proportion with anti-PT IgG levels ⩾62·5 EU/ml decreased significantly in 2002 as measured by the rate ratios, in the 5–9 and 15–19 years age groups ($P<0·01$ for each age group); the rate ratio was similar but just failed to reach statistical significance in the 10–14 years age group. In contrast, the rate ratio for 2002 *vs*. 1997/1998 was significantly higher in all adult groups aged ⩾24 years, with an almost linear increase in rate ratio from age 20 years.

Similar, but non-significant, differences were observed in the proportions of anti-PT IgG levels ⩾125 EU/ml, associated with infection within the previous 6 months [6] by age between 1997/1998 and 2002 and are shown in Figure 1. In 2002, the highest proportion of anti-PT IgG levels ⩾125 EU/ml was seen in the 35–44 years age group, followed by those aged 20–24 and 25–34 years (Fig. 1). Those aged ⩾20 years had a significantly greater proportion of PT IgG levels ⩾125 EU/ml when compared to persons aged <20 years (OR 1·9, 95% CI 1·4–2·6, $P=0·0002$). The proportion of non-immune persons (<5 EU/ml) was greatest in those aged <20 years.

Figure 2 shows the distribution of anti-PT IgG levels by age. In those children not yet eligible for a pre-school booster vaccine (aged 1–3 years), the highest proportion of anti-PT IgG levels ⩾125 EU/ml was seen in 1-year-olds. A similar pattern was observed in the 1997/1998 serosurvey (data not shown). In both the 2002 and 1997/1998 serosurveys, the 4-year-old age group, who were eligible to receive a DTPa preschool booster vaccine, had comparable proportions of anti-PT IgG levels ⩾125 EU/ml of 12% and 10%, respectively. In the 2002 serosurvey, all children aged 5–9 years were eligible to receive a DTPa preschool booster vaccine. The proportion of anti-PT IgG levels ⩾125 EU/ml ranged from 5% in those aged 6, 7 and 9 years to 16% in 8-year-olds (Fig. 2). In contrast to this, proportions of anti-PT IgG levels ⩾125 EU/ml ranging from 21% to 29% were seen in those aged 5, 6, 7 and 9 years in the 1997/1998 serosurvey. In this earlier serosurvey only 5-year-olds were eligible for a DTPa booster vaccine and those aged 6–9 years were eligible for a DTPw booster vaccine.

PT IgG levels by geographical area

As the sera were sampled proportionate to population, the majority came from the more populous eastern states of NSW, Victoria and Queensland which account for about 80% of the total population. NSW/ACT and Queensland had the largest proportions of high anti-PT IgG levels, for both ⩾125 EU/ml and ⩾62·5 EU/ml. Queensland had a pertussis epidemic in the year of collection, and three other states and territories (NSW, the Northern Territory and South Australia) had experienced epidemics in 2001, the year preceding collection.

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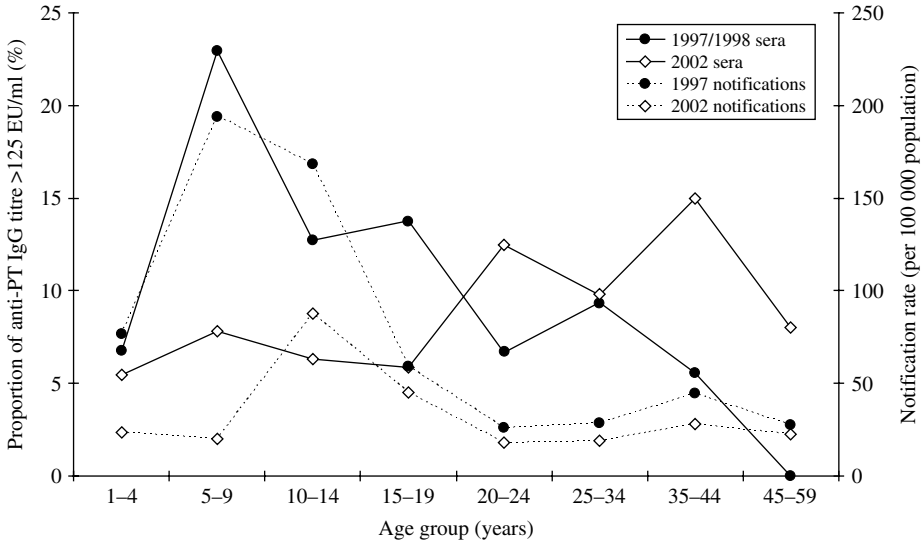

Fig. 1. Cross-sectional proportion of anti-PT IgG levels ⩾125 EU/ml and notification rate in 2002 compared to 1997/1998.

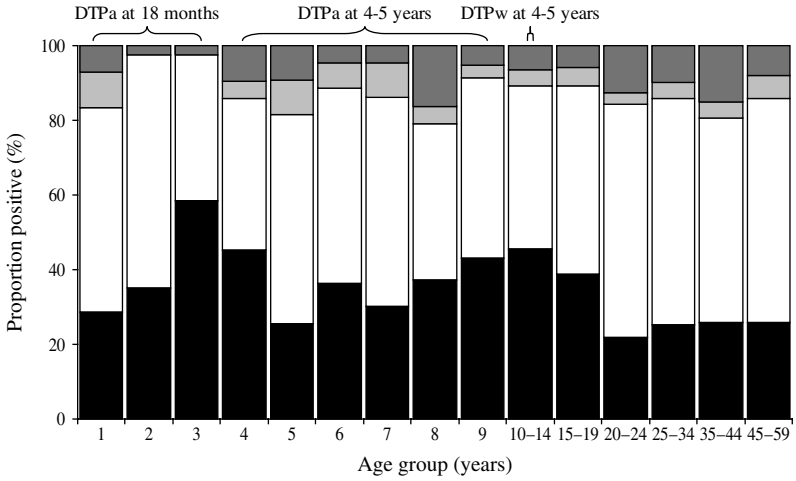

Fig. 2. Cross-sectional distribution of anti-PT IgG levels by age (1–9 years) and age group (⩾10 years). ■, <5 EU/ml; □, 5 to <62·5 EU/ml; ▫, 62·5 to <125 EU/ml; ▪, ⩾125 EU/ml.

There were striking differences in the anti-PT IgG proportions between NSW and other regions of Australia, with the proportion of anti-PT IgG levels ⩾125 EU/ml varying more markedly by age (Fig. 3). In groups aged >20 years, the proportion of anti-PT IgG levels ⩾125 EU/ml ranged from 17% to 34%, significantly higher than the 2–7% range in other states and territories (OR 13·3, 95% CI 6·5–27·9, $P<0·0001$). Similarly, the proportion with anti-PT IgG levels ⩾62·5 EU/ml in adults aged ⩾20 years was significantly higher than in other states and territories (OR 7·5, 95% CI 4·4–13·0, $P<0·0001$).

In contrast to other states and territories, in NSW the proportion of anti-PT IgG ⩾62·5 EU/ml was significantly higher in those aged 20–24, 25–34 and 35–44 years, using the group aged 1–4 years as a comparator. In the 1997/1998 serosurvey the age-specific patterns in NSW were also different than in other regions of Australia; however, the difference was significant only in those aged 15–19 years (data not shown). No other state or territory had as striking a difference in the distribution of anti-PT IgG levels, when compared individually with the remainder of Australia (data not shown).

DISCUSSION

Comparing trends in the epidemiology of pertussis over time in different geographic areas is problematic because of variations in physician awareness [16],

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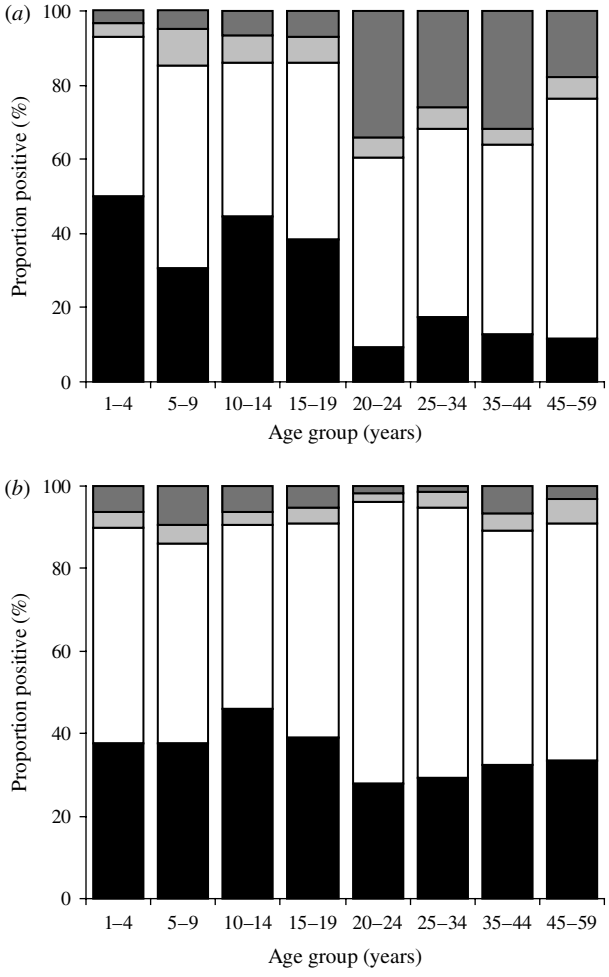

Fig. 3. Cross-sectional distribution of anti-PT IgG levels by age group, in (*a*) NSW compared to (*b*) all other states and territories. ■, <5 EU/ml; □, 5 to <62·5 EU/ml; ▫, 62·5 to <125 EU/ml; ▪, ⩾125 EU/ml.

diagnostic testing practices, and test performance, with no appropriate gold standard for diagnosis [17]. Although a high level anti-PT IgG due to immunization cannot be distinguished from that due to infection, the latter persists for longer and the former affects only certain age groups [4]. Therefore an increase in the prevalence of high level anti-PT IgG over time in age groups not targeted for vaccination can be reasonably interpreted as indicating higher rates of pertussis infection, some of which will have been symptomatic. This study compares patterns of high-level anti-PT IgG by age and geographic area between two time periods and, given the limitations of other methods of monitoring trends in pertussis, has the potential for wide and ongoing application.

The previous pertussis serological survey conducted in Australia in 1997/1998 identified that the highest rates of pertussis infection were in persons aged 5–25 years and those aged ⩾65 years [8]. The concentration of high anti-PT IgG levels in 5- to 9-year-olds reflects the notification data for the same period. Although the high PT IgG levels may have been in part due to recent vaccination in this age group [6], vaccination was likely to be only a minor contributor as it was largely limited to a whole-cell vaccine with low estimated uptake [18]. In the 2002 serological survey, the proportion of high anti-PT IgG levels in 5- to 9-year-olds was significantly lower, consistent with the decrease in notifications seen after the introduction of DTPa for all five vaccine doses in 1999 [10, 12, 19]. It is extremely unlikely that this decrease in the proportion of high anti-PT IgG levels is due to the schedule change from whole-cell to acellular booster vaccines, as a previous study has demonstrated that the anti-PT IgG level after an acellular booster vaccine is likely to be greater than after a whole-cell booster vaccine [6]. If vaccination was having any measurable effect on the anti-PT IgG levels in this study, we would expect to see an increase in the proportion of high anti-PT IgG levels in the 5–9 years age group, due to the change in vaccine type and improved vaccination coverage.

In the 1997/1998 survey, adolescents aged 10–19 years had a high proportion of high anti-PT IgG levels, which corresponded with high notification rates in this age group [1, 10]. The relatively high rate of notification in this age group continued, reaching a peak in notifications in 10- to 19-year-olds in 2001 at 132/100 000 [10]. This rate fell to 62·2/100 000 in 2002, corresponding to the 2002 serosurvey finding of a significantly lower proportion of high anti-PT IgG levels in the 10–19 years age group when compared to the 1997/1998 survey. In the 10–14 years age group, lower IgG levels may be attributable to more children who had received the scheduled preschool booster dose moving into this cohort.

A notable feature of the present survey was the significantly higher proportion of high anti-PT IgG levels in adults aged 20–59 years compared to 1997/1998. The pertussis notification rate in adults has remained relatively low and stable over the past decade, only beginning to rise after 2003 [10, 12]. This may suggest that substantial numbers of pertussis cases, some of which are symptomatic, have actually been occurring in Australian adults for some time, but have only been detected in more recent notifications due to an increasing awareness about adult pertussis among general practitioners resulting in more

laboratory testing of potential cases. This increase in awareness may in part be due to the availability of an adult formulation pertussis vaccine in Australia since 2003 [11].

The proportion of high anti-PT IgG levels varied considerably by region, as in the 1997/1998 serological survey. In the 2002 serosurvey, higher levels of recent pertussis activity were detected in NSW/ACT and Queensland, consistent with notification data for this period, with NSW experiencing an outbreak in 2001 and Queensland in 2002 [10]. Much of the observed increase in anti-PT IgG levels in adult age groups appears to be attributable to high levels in NSW. The explanation for this pattern in not clear but it may relate to past vaccination history, with NSW's historical reliance on general practice to deliver childhood immunization perhaps contributing to relative under-immunization against pertussis, and thus different historical cycles of pertussis infection by age group and a differing pattern in waning of immunity at the population level.

There were several limitations in this study. The sera used in this study were opportunistically collected, rather than randomly sampled. We tried to minimize any biases by obtaining sera that were submitted for a range of diagnostic tests (excluding HIV) to major laboratories throughout Australia that serviced mainly ambulatory populations. We cannot exclude the possibility of selection bias toward a more sick population. No information regarding the clinical status of individuals whose sera were used here was collected and we did not exclude sera that were submitted for the diagnosis of pertussis. However, the method used here has been validated for measles against a prospective, cluster-sampling method and was found to be representative [14]. The immunization status of subjects was unknown. Whilst it is possible to estimate immunization coverage for the childhood population in this study, it is largely unknown for the older age groups. Sample size calculations were calculated to be proportional to the population size of each state and territory, resulting in a small number of samples being collected from regions such as the ACT, Tasmania and the Northern Territory. This restricted the analysis that could be performed at a regional level.

The significant changes in the age-specific prevalence of high anti-PT IgG levels, over time and by geographic area, demonstrated in this study have some important implications. The impact of vaccination programes is likely to explain the significant decrease in the proportion of high levels seen in children. The significant increase in the proportion of high levels in adults aged 35–59 years not targeted by vaccine programmes may well have shown pertussis to be a problem in this age group prior to evidence from notification data. Seroepidemiological data have value as a method for tracking trends in pertussis over time, and could provide a means for more readily comparable assessment of the impact of vaccination programmes in adolescents.

ACKNOWLEDGEMENTS

NCIRS is supported by The Australian Government Department of Health and Ageing, the NSW Department of Health and The Children's Hospital at Westmead. CIDM-Public Health is supported by an infrastructure grant from the New South Wales Department of Health. We acknowledge the staff of the laboratories that provided the sera, Diana Arulanandam (ICPMR) for assistance in the processing and testing of sera and Dr Amy Glasswell (NCIRS) for assistance in preparation of the manuscript. GlaxoSmithKline provided funding for the testing of sera, but all testing and analysis was conducted at NCIRS.

DECLARATION OF INTEREST

None.

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