National serosurvey of vaccine preventable diseases

Report to the Ministry of Health

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Published in May 2009 by the Ministry of Health
PO Box 5013, Wellington, New Zealand

ISBN: 978-0-478-31924-8
HP 4766

Executive Summary

Aim and Objectives

The aim of the study was to estimate the sero-prevalence of a range of vaccine preventable infections in New Zealand.

Specific objectives were to estimate the sero-prevalence of the following infections in New Zealand:

- diphtheria
- hepatitis A
- hepatitis B
- measles
- mumps
- pertussis
- polio
- rubella
- tetanus
- toxoplasmosis
- varicella.

Methods

Study design

A cross-sectional sero-prevalence survey was conducted selecting:

1. consecutive children aged six to 15 years who presented to a community laboratory for a diagnostic specimen that required venepuncture
2. consecutive adults aged 16 to 70 years presenting to donate blood.

Inclusion was to cease when 600 people provided a specimen in each of the following age groups:

- Six to 10 years
- 11 to 15 years
- 16 to 24 years
- 25 to 44 years
- 45+ years.

Exclusion criteria were:

1. Received a blood transfusion in the past three months
2. Had a suspected or confirmed vaccine preventable infectious disease in the past month
3. Currently immunocompromised.

A sample stratified by area was selected in each age group. Participants were recruited at each participating community laboratory and blood donor service centre. The community laboratories were scattered throughout New Zealand although larger urban sites were over-represented (by population). Blood donor centres were geographically representative of the blood donor centres throughout the country. Each collection site was assigned target recruitment numbers based on the New Zealand population distribution.

Demographic data were collected at the same time as the serum sample. Data collected included age, gender, residential area and ethnicity. Serological tests performed varied by age group (see Table 1).

Table 1 Age groups and serological testing

Serological marker	Age (years)				
	6-10	11-15	16-24	25-44	45+
Diphtheria antibody	X	X	X	X	X
Hepatitis A Total antibody	X	X	X	X	
Hepatitis B core antibody	X	X	X	X	
Hepatitis B surface antigen	X	X			
Hepatitis B surface antibody	X	X	X	X	
Measles IgG antibody	X	X	X	X	
Mumps IgG antibody	X	X	X	X	
Pertussis IgG antibody	X	X	X	X	
Poliovirus type 1 antibody	X	X	X		
Poliovirus type 2 antibody	X	X	X		
Poliovirus type 3 antibody	X	X	X		
Rubella IgG antibody	X	X	X	X	
Tetanus antibody	X	X	X	X	X
Toxoplasma IgG antibody			X	X	
Varicella IgG antibody	X	X	X	X	

Serology

Type of assay used varied by serological marker. Methods used were:

- Diphtheria antibody: Enzyme Immunoassay (EIA)
- Hepatitis A total antibody: Chemiluminessence
- Hepatitis B core antibody: Chemiluminessence
- Hepatitis B surface antigen: Chemiluminessence
- Hepatitis B surface antibody: Chemiluminessence
- Measles IgG antibody: EIA
- Mumps IgG antibody: EIA
- Pertussis IgG antibody: EIA
- Poliovirus type 1 antibody: Neutralisation
- Poliovirus type 2 antibody: Neutralisation
- Poliovirus type 3 antibody: Neutralisation
- Rubella IgG antibody: Chemiluminessence
- Tetanus antibody: EIA
- Toxoplasma IgG antibody: Chemiluminessence
- Varicella IgG antibody: EIA

All equivocal results were re-tested once using the same method. The results of the second testing were entered.

Statistical analysis

A database was developed in Epi-Info version 6.04 for entry of the demographic data. After data cleaning, the demographic data were transfererd to STATA version 7. The laboratory results were initially entered on the laboratory's standard software package (DELPHIC™). These results and the same unique identifier used for the demographic data were then dumped into excel and then imported into STATA version 7. The demographic and laboratory databases were merged.

Age stratified levels of immunity were estimated. Crude univariate analyses were performed by cross tabulating age, ethnicity and area (District Health Board region)

with the number classified as positive to each serological marker. The Chi squared test for trend was used to evaluate the presence of any trend in seroprevalence across age strata. Potential confounding factors for the relationship between seroprevalence and age were identified by:

- comparing the potential confounding factor with age
- comparing the potential confounding factor with immune status.

Crude odds ratios (OR) between the age strata and sero-prevalence were compared with Mantel-Haenszel adjusted ORs to further assess for confounding. Stratified analyses between age strata, sex, area and ethnicity, and immune status were also conducted to identify effect modifiers.

Logistic regression was also conducted to further evaluate the effect of age on sero-prevalence. An *a priori* decision was made to include age, sex, ethnicity and area in all models.

Sensitivity analyses were conducted that:

- assumed equivocal results represented a positive result
- assumed equivocal results represented a negative result.

Amendment to methods

The proposed methods outlined above differed from the actual methods adopted in the study. The reason for this difference related to very slow recruitment in the paediatric age groups. Proposed targets for sample sizes were therefore not met in the 6-10 and 11-15 year age groups. The original proposal suggested 600 participants would participate in each of these two age groups. However, the final sample sizes were 466 and 589 respectively in these two age groups. It had also originally been proposed that North Island collection sites in these two age groups would be restricted to Auckland and Wellington. As a result of the slow recruitment it was decided to increase the number of sites (from other North Island regions).

Results

Sample

Characteristics of the study sample included:

- Male: 49%
- Self determined ethnicity (allowing multiple categories) included:
    - European: 86%
    - Maori: 10%
    - Asian: 7%
    - Pacific: 5%
    - Other: 1%
- Residential area (by District Healthy Board area):
    - Canterbury: 16%
    - Auckland 16%
    - Waitemata: 16%
    - Counties Manukau: 15%
    - Waikato: 9%
    - Capital Coast: 9%
    - All other regions under 4%.

The participation rate varied by age group:

- 6-10: 74%
- 11-15: 77%
- 16-24: 98%
- 25-44: 98%
- 45+: 99%

Crude seroprevalence estimates

Table 2 shows the crude seroprevalence estimates by age group. In this table equivocal results for all serological tests except pertussis have been treated as a positive result. For all tests except pertussis, Hepatitis B surface antigen and core antibody, a positive result represents past exposure to infection or vaccination from the relevant disease. A positive pertussis result is most likely to represent recent infection. However the test is not 100% specific and may also represent very recent vaccination to pertussis. A positive Hepatitis B surface antigen result indicates current infection with Hepatitis B virus. A positive Hepatitis B core antibody result indicates current or past infection with Hepatitis B virus.

Table 2 Crude seroprevalence by age group

Serological assay	Age group (years) % positive (95% CI)				
	6-10	11-15	16-24	25-44	45+
Diphtheria	60.5 (55.9-64.9)	77.2 (73.6-80.5)	71.4 (67.5-75.0)	48.2 (44.1-52.2)	45.6 (41.6-49.7)
Hepatitis A total	7.9 (5.6-10.7)	9.7 (7.4-12.3)	11.2 (8.8-14.0)	27.7 (24.2-31.5)	Not tested
Hepatitis B core antibody (anti-HBc)	0.4 (0.1-1.5)	0.7 (0.2-1.7)	1.8 (0.9-3.3)	8.6 (6.5-11.2)	Not tested
Hepatitis B surface antigen (HBsAg)	0.2 (0.0-1.2)	0.0 ((0.0-0.6)	Not tested	Not tested	Not tested
Hepatitis B surface antibody (anti-HBs)	40.6 (36.1-45.3)	57.8 (53.7-61.8)	65.0 (61.0-68.8)	36.0 (32.2-40.0)	Not tested
Measles	92.9 (90.2-95.1)	91.1 (88.6-93.3)	96.0 (94.1-97.4)	97.0 (95.3-98.2)	Not tested
Mumps	87.6 (84.2-90.4)	89.2 (86.4-91.5)	94.0 (91.7-95.7)	93.2 (90.9-95.1)	Not tested
Pertussis[1]	20.3 (16.8-24.3)	20.2 (17.0-23.6)	14.4 (11.7-17.5)	10.1 (7.8-12.8)	Not tested
Poliovirus type 1	97.0 (95.0-98.3)	97.4 (95.8-98.6)	96.2 (94.3-97.6)	Not tested	Not tested
Poliovirus type 2	98.3 (96.6-99.2)	98.5 (97.1-99.3)	98.6 (97.3-99.4)	Not tested	Not tested
Poliovirus type 3	91.1 (88.2-93.6)	89.4 (86.6-91.8)	85.8 (82.7-88.6)	Not tested	Not tested
Rubella	89.7 (86.6-92.3)	90.5 (87.9-92.8)	96.3 (94.5-97.7)	90.2 (87.5-92.5)	Not tested
Tetanus	85.3 (81.7-88.3)	94.1 (91.9-95.8)	94.6 (92.5-96.3)	94.5 (92.4-96.2)	88.7 (85.9-91.1)
Toxoplasma	Not tested	Not tested	20.0 (16.9-23.4)	34.1 (30.3-38.0)	Not tested
Varicella	87.3 (83.9-90.2)	94.4 (92.2-96.1)	96.6 (94.9-97.9)	97.2 (95.5-98.3)	Not tested

[1]Indicator of recent exposure to pertussis infection or vaccination

Key results by marker

Diphtheria

- There was a significant linear trend in seroprevalence to diphtheria with generally decreasing levels of seroprevalence by age group. The highest seroprevalence level was 77% in the 11-15 age group and this declined to 46% in the 45+ age group.
- Logistic regression (controlling for age, sex, ethnicity and DHB region) suggested the seroprevalence was significantly higher in the 11-15 and 16-24 age groups than the 6-10 age group but was significantly lower in the 25-44 and 45+ groups compared with 6-10 year olds. The seroprevalence was

slightly lower in Pacific people than Europeans on multivariate analysis (adjusted OR 0.62, 95% CI 0.39-0.97). Nelson-Marlborough District Health Board (DHB) residential location also had a significantly lower seroprevalence level on multivariate analysis (adjusted OR 0.52, 95% CI 0.27-0.98) compared with the Auckland DHB.

Hepatitis A

- There was a statistically significant linear trend with increasing seroprevalence levels with increasing age such that the seroprevalence was 8% in 6-10 year olds, 10% in 11-15 year olds, 11% in 16-24 and 28% in 25-44 year olds
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - Age groups 16-24 and 25-44 had significantly higher seroprevalence levels than 6-10 year olds
    - Asian and Pacific peoples had significantly higher seroprevalence levels than Europeans
    - Otago and Canterbury residents had significantly lower seroprevalence levels than Auckland residents.

Hepatitis B core antibody

- There was a statistically significant linear trend consisting of increasing proportions of positive anti-HBc results with increasing age such that the proportion with positive results was 0.4% in 6-10 year olds, 0.7% in 11-15 year olds, 1.8% in 16-24 and 8.6% in 25-44 year olds
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - There were significantly higher levels with current/past exposure to infection in the 25-44 age group compared with 6-10 year olds
    - Maori, Pacific and Asian people also had significantly higher levels of current/past exposure than Europeans
    - Bay of Plenty and Counties Manukau residents had significantly higher levels of current/past exposure than Europeans.

Hepatitis B surface antibody

- There was variation in the level with anti-HBs positive results by age such that the age group with the highest level of positive results were those aged 16-24 (65%). The 25-44 age group had the lowest estimated proportion of positive results (36%) though this wasn't significantly lower than the 6-10 age group.
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - There were significantly higher levels with positive anti-HBs results in the 11-15 and 16-24 age group than the 6-10 age group
    - Significantly more Asians were classified as being immune to hepatitis B than Europeans.

Measles

- There was a statistically significant but clinically small linear trend indicating increasing levels of protection with increasing age
- Logistic regression (controlling for age, sex, ethnicity and DHB region) suggested the seroprevalence was significantly higher in the 25-44 age group than the 6-10 age group and the seroprevalence was low in Otago (when compared with Auckland).

Mumps

- Seroprevalence ranged from 87.6% (6-10 age group) up to 94.0% (16-24 age group)
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - Seroprevalence was significantly higher in the 16-24 and 25-44 year age groups compared with the 6-10 year age group
    - Classification as more than one ethnic group (in the sole/combined data) was associated with a significantly higher seroprevalence compared with Europeans.

Pertussis

- There was a statistically significant linear trend consisting of decreasing seroprevalence levels with increasing age such that the seroprevalence was 15% in 6-10 year olds, 15% in 11-15 year olds, 10% in 16-24 and 6% in 25-44 year olds. A positive pertussis result is most likely to represent recent infection. However the test is not 100% specific and may also represent very recent vaccination to pertussis.
- On logistic regression, controlling for age, sex, ethnicity and DHB region 16-24 year olds and 25-44 year olds had a significantly lower seroprevalence than the 6-10 age group.

Poliovirus type 1

- Seroprevalence for type 1 ranged between 96% and 97% in the three age groups tested (6-10, 11-15 and 16-24 years)
- There were no significant associations detected on logistic regression analyses for poliovirus type 1.

Poliovirus type 2

- Seroprevalence for type 2 ranged between 98% and 99% in the three age groups tested (6-10, 11-15 and 16-24 years)
- There were no significant associations detected on logistic regression analyses for poliovirus type 2.

Poliovirus type 3

- Seroprevalence declined with increasing age for type 3 with 91% being seropositive in the 6-10 age group, 89% in the 11-15 age group and 86% in the 16-24 age group.
- For poliovirus type 3 the following significant associations were observed:
    - The 16-24 age group had significantly lower levels of immunity than the reference category (6-10 years)
    - Self determining as belonging to more than one ethnic group was associated with significantly lower levels of immunity than the reference category (Europeans)
    - Three regions had significantly lower seroprevalence estimates when compared with the reference category of Auckland. These were:
        - Hawkes Bay (OR 0.30, 95% CI 0.11-0.84)
        - Hutt Valley (OR 0.04, 95% CI 0.00-0.44)
        - Nelson-Marlborough (OR 0.31, 95% CI 0.11-0.88).

Rubella

- Seroprevalence estimates were 90% and over across the four age groups studied (up to 44 years) and there was no significant linear trend
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - The 16-24 age group had significant higher seroprevalence levels than the 6-10 age group
    - Males had significantly lower seroprevalence levels than females
    - There was interaction between age and gender with males having lower seroprevalence estimates in all four age groups though this only reached statistical significance in the oldest age group (25-44 years).

Tetanus

- There was an inverted U shape for seroprevalence by age with 94-95% seroprevalence in the 11-15, 16-24 and 25-44 age groups but only 85% and 89% in age groups 6-10 and 45+ respectively
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - Maori and Pacific peoples had significant lower seroprevalence levels than the European population
    - Males had significantly higher seroprevalence levels than females
    - Counties-Manukau residents had a significantly lower adjusted OR when compared with Auckland.

Toxoplasma

- Twenty per cent of participants aged 16-24 and 34% of participants aged 25-44 years had past contact with toxoplasma.
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - The 16-24 age group had significantly lower past contact with toxoplasma
    - Males had a significantly higher level of past contact than females.

Varicella

- There was a statistically significant linear trend consisting of increasing seroprevalence levels with increasing age such that the seroprevalence was 87% in 6-10 year olds, 94% in 11-15 year olds, and 97% in 16-24 and 25-44 year olds
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - 6-10 year olds had a significantly lower seroprevalence than the other three age groups
    - Males had a significantly lower seroprevalence than females
    - Otago residents had a significantly lower seroprevalence than Auckland residents.

Discussion

Potential sources of bias exist in the estimates detailed above. Sources of selection bias included:

- The use of a blood donor population for the adult age groups
- The participation rate of 74-77% in the paediatric age group

- The restriction on use of community laboratories in defined geographical regions for the collection of paediatric samples.

Sources of information bias also exist. Most notably, a positive assay does not necessarily mean protection against the relevant disease. This limitation is particularly relevant to the pertussis assay but applies to all assays. There was also uncertainty concerning the interpretation of equivocal results. In relation to modelling the seroprevalence data to predict the potential for outbreaks, a further limitation related to the prolonged time over which samples were collected for serological testing in the paediatric age groups. A change in the level of seroprevalence may have occurred during this collection time (16 months) resulting in difficulties estimating a true point prevalence at a defined point in time.

Conclusions

While seroprevalence estimates were low for some of the diseases tested when compared with others tested, some of these results were consistent with overseas literature. However, the estimates in this study found poliovirus type 3 results were lower than those in a Swedish study and comparisons were conflicting for rubella (New Zealand estimates were lower than those in Australia and Luxembourg but higher than USA findings). Diphtheria results were similar to those found in Australia and Sweden.

When comparing with herd immunity thresholds:

- age specific estimates for measles ranged between 91% and 97% with the two youngest age groups being slightly below the herd immunity threshold of 95%
- age specific estimates for mumps ranged between 88% and 94% with the two youngest age groups being slightly below the herd immunity threshold of 90%-92%
- rubella estimates approximated the herd immunity threshold

There is some uncertainty about levels for herd immunity associated with varicella and it is unclear whether the level estimated in the 6-10 age group is above the herd immunity threshold. These comparisons with herd immunity thresholds should be considered in light of variable levels of seroprevalence by geographical region in the current study.

Acknowledgements

This work could not have been conducted without the help of a large number of people.

Participants were recruited and samples were collected by numerous staff in the New Zealand Blood Donor Service and community laboratories around New Zealand. Within the New Zealand Blood Donor Service Peter Flanagan and Krishna Badami provided valuable assistance with setting the project up and Olive Utiera provided assistance during sample collection. Community laboratories involved were Diagnostic MedLab, MedLab South, Medical Laboratory, MedLab Hamilton and MedLab Central. Particular assistance was provided by Daphne Fairfoot, Steven Martin and Glenis Stokes (Diagnostic MedLab), Heather Muir (MedLab South), Fiona Massey and Helen Lagan (Medical Laboratory), Steve Soufflot and Sarah Dixon (MedLab Hamilton), and Jane Kendall (MedLab Central).

Diphtheria and tetanus testing was performed by Myfanwy Spellerburg and assistance with interpretation of these results was provided by John O'Donnell (Canterbury Health Laboratories). The testing for poliovirus types 1, 2 and 3 was performed by ESR.

The Ministry of Health funded this research.

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Abbreviations

95% CI	95% confidence interval
anti-HBc	Hepatitis B core antibody
anti-HBs	Hepatitis B surface antibody
DHB	District Health Board
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbent assay
GP	General Practitioner
HBsAg	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
NHANES	National Health and Nutritional Examination Survey program
OR	odds ratio
PNT	plaque neutralisation test
USA	United States of America
VAPP	vaccine associated paralytic poliomyelitis

Background

Introduction

This research was developed in response to the Ministry of Health seeking tenders for the design, planning, implementation, analysis and reporting of a national serosurvey of vaccine preventable diseases. The following services were requested by the Ministry in their request for tenders document:

- Design and implementation of a national serosurvey for the vaccine preventable diseases identified as a priority for this survey (measles, diphtheria, hepatitis B, tetanus, rubella and pertussis) and other vaccine preventable diseases (varicella, mumps, hepatitis A and polio). The survey was to include both child and adult groups, appropriate to the antigen under study. The study proposal requested options for age groups to be sampled.
- The serosurvey was to include collection of basic demographic information on samples including age, sex, geographic location and ethnicity. Vaccination status or other clinical information was not required.
- Management of the process of Ethics Committee(s) approval
- The proposed method of collaboration with community and hospital laboratories in order to collect samples and implement the survey
- The analysis and reporting of the serosurvey data
- Estimated costings, by antigen, including a breakdown of specimen collection and storage costs
- Options for extending such a survey to include other antigens or to be repeated on a regular basis. It was envisaged that a number of antigens could be included in this survey, with the potential for storage of samples for future testing and/or the methodology used for further surveys.

Uses of serosurveillance

Serosurveillance complements disease surveillance. In New Zealand, many vaccine preventable diseases are notifiable. Disease notification data are necessary to detect outbreaks and to provide timely information on the patterns of disease. However, notification data may be biased due to misclassification of the disease and due to being incomplete. Serosurveillance measures immunity which can result from vaccination or past infection (including subclinical infection). Such information is particularly helpful for mathematical modelling to determine the potential for future epidemics, and potential age groups at risk. This knowledge can help to determine the need for public health intervention. Serosurveillance can also be used to evaluate specific vaccination campaigns. For instance, a before and after serosurvey noted the proportion of schoolchildren who were immune to measles rose from 85% to 90% following a measles control campaign (Gilbert et al. 2001).

Serosurveillance methods

There are two main methods that can be used to obtain sera for a serosurvey. Ideally, a nationally representative sample would be randomly selected from the study population and the participation rate would be 100%. The alternative is to use a convenience sample of sera that have been collected for another purpose. Both

approaches have been used internationally. For example, the population-based approach has been used in the USA as part of their National Health and Nutritional Examination Survey program (NHANES). This program uses an approach similar to the New Zealand Health Survey, conducted by the Ministry of Health, in that a multistage design was used to select the sample (Gergen et al. 1995). In the Netherlands, a cluster sampling technique is used to select a sample specifically for serosurveillance (De Melker and Conyn-van Spaendonck 1998). In contrast, convenience samples have been selected for serosurveys conducted in England and Wales and in Australia amongst other locations (Gidding 2003; Osborne et al. 2000).

Advantages and disadvantages of population-based serosurveys

Population-based serosurveillance programs have fewer concerns about selection bias than do convenience samples. However, low participation rates can result in significant selection bias. Participation in a population-based survey that involves the collection of blood is likely to be low. For instance, a serosurvey of adults in Christchurch resulted in a participation rate of 25% (Chapman et al. 2000). Other studies have found higher participation rates. For example, a study conducted in Victoria that used a three stage random cluster sample from school age children had a response rate between 32 and 39% (Kelly et al. 2002). Participation in the NHANES III study was higher at 77%, although participation among children aged 6 to 11 years was 57% (Gergen et al. 1995). The collection of blood from children is likely to be particularly problematic. Considerable efforts were used to encourage participation in a serosurvey in Sweden that used a multistage sampling approach. Those selected to take part were informed by their county medical officer, information about the study was spread in a national media campaign and local investigators were responsible for obtaining a limited number of samples only. The response rate was 71% in this group of people aged 18 years and over (Svensson et al. 1998).

A major advantage of population-based sampling is the ability to collect detailed demographic information from the study sample. Such information allows the appropriate targeting of public health interventions in groups with low levels of immunity. In contrast, convenience samples rely on the demographic data collected at the time of venepuncture. Over-sampling of particular risk groups (such as specific ethnic groups) can also be used for appropriate sub-analyses in population-based samples.

The major disadvantages of population-based sampling are that they are more costly and time consuming than convenience samples. For example, an Australian based study estimated the cost of a random cluster sample was about eleven times that of a convenience sample (Kelly et al. 2002). A population-based serosurveillance program conducted in the Netherlands used a two stage cluster sampling design and selected 380 individuals from each of eight municipalities. Data were collected from October 1995 to December 1996 in that study (De Melker and Conyn-van Spaendonck 1998). Fifty-five percent of those invited completed the questionnaire and had a blood sample taken. There were differences between responders and nonresponders that probably led to a biased estimate of immunity. For example, participation was low in both the youngest age group and the oldest age group. Age is

likely to be associated with immune status. Non-response was also associated with a belief that vaccination was not necessary.

Advantages and disadvantages of convenience samples

Studies using convenience samples can be conducted relatively quickly and cheaply. However, they are prone to selection bias. The degree of such bias is likely to be determined by the source of sample. The following is a potential list of sources of sera:

- Sera collected as part of a national, randomly selected, population-based survey
- Blood donor groups
- Diagnostic specimens collected from a community laboratory
- Diagnostic specimens collected from a hospital setting
- Diagnostic specimens from a reference laboratory.

It is suggested the sources towards the bottom of the list are likely to be more prone to selection bias. For example, community samples are likely to be collected following presentation of the participant to their general practitioner (GP). In contrast, sera collected from the hospital setting will probably be derived from people who have first attended their GP and have then been referred to the hospital setting. Serosurveillance conducted in Australia was designed to reduce this selection bias by enrolling most major laboratories in the country with the majority of samples from ambulatory rather than hospitalized patients (Gidding 2003). Blood donor groups are also prone to bias and blood borne viruses will be underestimated in this group. The health conscious behaviour of blood donors would suggest they are more likely to be vaccinated than the general population. Since less is known about participants in convenience samples, potential biases can be more difficult to identify and control.

Comparison of population-based and convenience samples

Estimates of immunity to measles, mumps, rubella, hepatitis B and varicella zoster viruses were compared in Victorian school children using samples from diagnostic laboratories with those from a population-based sample that used a three stage sampling design (Kelly et al. 2002). There were no significant differences in immunity to any of these viruses with the exception of rubella. The population-based sample estimated higher immunity to rubella in both primary (98.7% versus 93.6%, $P = 0.002$) and secondary school children (98.4% versus 93.2%, $P = 0.003$). It was also noted that there was a difference in cost of collection and retrieval per sample ($AUD3.39 for the convenience sample versus $AUD38.71 for the population-based sample). However, the volume of serum available was larger in the population-based sample. Sufficient serum was available in this sample for all five antibody tests of interest. This contrasted with the community samples where there was sufficient serum for three tests only. Sera were collected from both samples in 1999 and participating laboratories were asked to exclude sera from subjects known to have been immunocompromised, multiply transfused, infected with HIV or recently infected with measles. Results for both samples may be biased. There was non-participation at both the school level and the student level in the population-based sample. Selection biases are also likely to be present in the convenience sample although sera were obtained from most major laboratories in the region. The study may not have had sufficient power to detect significant differences in immunity

although it had 80% power to detect a 7% difference in both measles and rubella immunity, assuming 95% of primary schoolchildren would have evidence of immunity following a measles control program.

International approaches

Both population-based sampling and convenience sampling have been used internationally. Sero-prevalence surveys have examined single diseases or have examined multiple diseases within the same survey. The studies have been conducted amongst differing age groups and with different sample sizes and over different periods of study duration.

Population approaches

Prospective, population-based approaches have been conducted both in conjunction with other population-based studies and using a population-based approach in the context of a sero-prevalence survey alone.

In the United States, the population-based sero-prevalence surveys have been conducted as part of NHANES. The last NHANES survey was conducted between 1988 and 1994 (NHANES III). Samples were tested for immunity to tetanus, measles, rubella, hepatitis B and C, and human immunodeficiency virus (HIV). As an example, rubella was tested in 21,288 of the 30,930 persons aged six years and over who were eligible for the survey (69% response), (Dykewicz et al. 2001). Study results were broken down to the following age groups (years):

- 6-11
- 12-19
- 20-29
- 30-39
- 40-49
- 50-59
- 60+.

In Sweden, population-based studies have been conducted with the sole purpose of estimating sero-prevalence to various diseases of interest. For example, a random sample of the Swedish population was carried out in 1968 to evaluate a vaccination program against polio. Subsequently, a survey was conducted in 1990 and 1991 to estimate the level of immunity against diphtheria, tetanus and polio. Both studies used a population-based approach (Svensson et al. 1998). In the latter, 4800 people were randomly selected in a stratified two stage plan, with the primary stage selecting study region and the secondary stage using a systematic sampling approach ordered by date of birth. Age groups selected were from 18 years and up. The results were stratified according to the following date of births (with the study being conducted during 1990-1):

- 1966-1972
- 1956-1965
- 1946-1955
- 1931-1945
- ≤1930.

Population-based sampling, with the sole purpose of conducting a sero-prevalence study, has also been conducted in The Netherlands.

Convenience samples

Convenience samples have also been used internationally. The following are some examples where this approach has been adopted.

Australia's first national serosurvey collected samples between 1996 and 1999. Diseases and age groups tested are shown in Table 3 (Gidding 2003).

Table 3 Diseases examined in Australia's first national serosurvey, including collection dates, age ranges and sample sizes.

Infection	Collection date	Age groups tested (years)	Sample size
Diphtheria	1997-1999	5-70+	1,953
Hepatitis A	1998	1-60+	3,043
Hepatitis B	1998-1999	1-18	1,735
Hepatitis C	1996-1998	1-70+	2,800
Measles	1996-1998	1-49	5,062
Measles	1999	1-18	2,918
Mumps	1996-1998	1-59	2,787
Mumps	1999	1-18	1,249
Pertussis	1998	1-65+	1,022
Polio	1998	1-65+	1,816
Rubella	1996-1998	1-49	4,288
Rubella	1999	1-18	2,947
Tetanus	1999	5-70+	2,884
Varicella	1996-1998	1-49	2,027

The Australian approach was based on methods used in England and Wales. Participating laboratories distributed throughout the region were asked to provide aliquots of serum from residues following completion of microbiological and/or biochemical investigations. Sera from immunocompromised people were excluded. Since 1996 sera submitted for HIV and hepatitis B testing have also been excluded. Each year, target numbers of sera, stratified by age and sex, are collected in the age group 0-24 years. Every five years sera are collected across the entire age range. Testing has been conducted for measles, mumps, rubella, diphtheria, varicella, and Hepatitis A and B. Other studies proposed include testing for *Helicobacter pylori* and *Toxoplasma gondii* (Osborne et al. 2000).

A serosurvey was conducted to estimate the sero-prevalence of varicella in Italy. One hundred samples were required in each one year interval in the age range 0-19 years and 200 samples for each of the following age groups: 20-24, 25-29, 30-34, 35-39, 40-49, 50-59 and 60+). Residual sera were used from routine laboratory testing. Although the investigators excluded samples from individuals known to be immunosuppressed, had an acute infectious disease or had undergone a recent blood transfusion, the sera selected may not have been representative of the general population. For instance, collection of sera may have been associated with socio-economic status and varicella immunity may also be associated with socio-economic status potentially distorting the estimated sero-prevalence. It is unclear, whether such an association exists and, if it does, the magnitude of the effect on varicella sero-prevalence. It is also unclear how rigorously the exclusion criteria were applied. For

instance, in New Zealand, these details are likely to be partially documented at best on request forms. Thus, interpretation of the result is uncertain (Gabutti et al. 2001).

Summary of sero-prevalence survey methods

Two main methods have been used in the design of sero-prevalence surveys: population-based sampling and convenience samples. Provided the participation rate is high, population-based approaches would give less biased results. However, these studies are time consuming and expensive. Considerable effort is required to ensure an adequate participation rate. There has been considerable variation in participation rates and the best rates have been in the order of 70-80%. Typically, these studies have used multiple techniques to ensure adequate participation. A recent New Zealand based sero-prevalence survey only had 25% participation (Chapman et al, 2000).

Both diagnostic specimens and samples from blood donors have been used in convenience samples. Both are potentially susceptible to selection bias, although the magnitude of this bias is likely to be larger and more uncertain in diagnostic specimens. Investigators have attempted to reduce this bias by excluding certain groups from the sample, including: recent blood transfusion recipients, immunosuppressed or people with evidence of a recent infectious disease. However, it is not clear to what extent that information was available in the study population. These exclusions are important. For example, exclusion of people with a recent infectious disease reduces the potential bias in the estimated sero-prevalence by the following mechanism. People with a vaccine preventable infectious disease are less likely to be vaccinated against that specific disease and therefore other vaccine preventable diseases. Therefore, sero-prevalence is likely to be underestimated in this group. This is a particular problem with the use of residual sera as a proportion of sera would have been originally collected for the diagnosis of a vaccine preventable infectious disease. People who have recently been transfused may have passive antibodies from the donor, thus resulting in misclassification of immune status. Immunosuppressed individuals may also have their immune status misclassified due to an inability to mount an immune response. Convenience samples also have the difficulty that the collection of data is outside the investigators control hence, there may be misclassification in variables of interest and some variables of interest may not be collected at all. However, convenience sampling is quicker and cheaper.

Impact of the diseases of interest in New Zealand

Diphtheria

The last case of diphtheria in New Zealand occurred in 1998 in an unimmunised child. Sporadic cases of non-toxigenic *Corynebacterium diphtheriae* continue to occur. There were 29 such cases in 2006 (26 isolates from a cutaneous source, two from blood and one respiratory isolate), (Anonymous 2007).

Hepatitis A

Since the mid 1980s, the incidence of hepatitis A has been low in New Zealand. The most common sources of infection are food borne outbreaks, overseas travel and

secondary household transmission. There were 122 notifications in 2006 (2.9 notifications per 100,000 population which was significantly higher than the rate of 1.2 notifications per 100,000 in 2005), (Anonymous 2007).

During 2006 the notification rate was evenly distributed between sexes. The highest age specific rate was 7.1 per 100,000 population in the 1-4 years age group followed by 6.2 per 100,000 population in the 5-9 years age group. Europeans had the highest number of notifications (53 cases) followed by Pacific people (42 cases), (Anonymous 2007).

Hepatitis B

A survey of 5,510 police and customs personnel estimated that Maori adults had a carrier prevalence of 5.4% (95% CI 3.1-8.8), Pacific adults 4.4% (95% CI 1.7-9.4) and European adults 0.4% (95% CI 0.2-0.7), (Blakely et al. 1998). Ethnicity was self-assigned. It is possible that these data underestimate the true carrier prevalence since participants who were hepatitis B surface antibody (anti-HBs) positive were not screened for HBsAg status and hence a proportion of this population may have been carriers (Shiels et al. 1987). The participation rate was 77%. This survey potentially provides the most accurate estimate of the prevalence of hepatitis B carriage in the New Zealand population.

Hepatitis B notifications peaked in 1984 at 604 cases. Vaccination against hepatitis B was introduced in New Zealand incrementally from1985 until universal infant vaccination with four doses in 1988 and then three doses in 1989 (Moyes 1994). Hepatitis B notifications have declined since 1984 although the number of cases was slightly higher in 2005 and 2006 compared with 2004. The notification rate was 1.5 per 100,000 population in 2006 (Anonymous 2007). However, the accuracy of notification needs to be considered in the context of incomplete reporting. It could be expected that the reporting rate would increase following the introduction of vaccination with the heightened awareness resulting from that policy.

Measles

The measles vaccine was introduced to New Zealand in 1969. However, major measles epidemics still occur. Since 1980, major epidemics occurred in 1985, 1991 and 1997. In 1997 there were 1,984 cases notified compared with 66 notifications during 2003. The vaccination schedule for mumps, measles and rubella (MMR) was changed in 2001 as a mathematical model predicted that measles epidemics would continue to occur if the MMR vaccine continued to be given at 15 months and 11 years (Roberts and Tobias 2000). It is now recommended at 15 months and four years. It is therefore difficult to predict if, and when, a further measles epidemic may occur. In the past, epidemics have occurred every five to seven years but the change in vaccination schedule and the large vaccination programme associated with the 1997 epidemic may have changed this pattern (Mansoor et al. 1998).

Notification rates for measles vary by age. There were 20 measles notifications in 2006. Highest notification rates occur in the young age groups As an example, the estimated incidence of measles, based on notification data, was 14.0 per 100,000

population in the under one age group and 4.9 per 100,000 in the 1-4 year age group in 2006. These were the two highest age specific rates in 2006 (Anonymous 2007).

Mumps

Mumps epidemics typically occurred every four years until 1990 when the MMR vaccine was introduced. From 1992, two doses have been given. Initially these were given at 15 months and 11 years but the schedule was altered in 2001 with the second dose now being given at four years. Since introduction of the vaccination, there has only been one epidemic (in 1994). Mumps became notifiable in 1996. There were 48 cases notified during 2006 (Anonymous 2007).

Highest notification rates occurred in the 1-4 age group (4.9 per 100,000) followed by the 5-9 age group (3.8 per 100,000) in 2006 (Anonymous 2007).

Pertussis

Pertussis epidemics typically occur every four to five years in New Zealand. The last epidemic peaked in 2004 and the incidence remained higher than baseline in 2005 and 2006. There were 1122 cases notified during 2006, representing a rate of 27.1 cases per 100,000 (Anonymous 2007).

There has been a change to the age distribution infected with pertussis during the latest epidemic. While the highest age specific rate occurred in children under one year of age (66.5 per 100,000) the next highest rate was in 50-59 year olds (33.3 per 100,000), (Anonymous 2007).

Polio

The last case of wild-type poliovirus infection occurred in New Zealand in 1962. The last imported case occurred in 1976. Cases of vaccine-associated paralytic poliomyelitis (VAPP) occurred in 1970, 1977, 1990 and 1998. The inactivated polio vaccine was introduced in 2002 to avoid cases of VAPP (Anonymous 2004).

Rubella

There was an outbreak of rubella in New Zealand in 1995 with in excess of 1500 laboratory reported cases. Rubella became notifiable in June 1996. There were 8 notifications recorded in 2006 and no cases of congenital rubella (Anonymous 2007). Age specific rates were highest in the 1-4 year age group (1.8 per 100,000).

Tetanus

Sporadic cases of tetanus occur in New Zealand. Between 1980 and 2003 there have been 71 cases (mean three per year, range zero to eight per year). This disease typically occurs in older New Zealanders (Anonymous 2004). There was one non-fatal case of tetanus in an unvaccinated nine year old in 2006 (Anonymous 2007).

Varicella

Varicella is not notifiable in New Zealand so there is limited epidemiological information available. Based on primary diagnosis coding of hospital discharge data, there were 281 hospitalisations for varicella in 2002. This was the highest in 15 years (Sneyd and Baker 2003).

Vaccination coverage

The last National Childhood Immunisation Coverage Survey was conducted in 2005. The overall coverage level for being fully immunised at age two years was 77% (95% CI 75-80). Maori children had lower coverage levels (69%, 95% CI 64-74) compared with European/other children (80%, 95% CI 77-83). Pacific children had the highest coverage levels although this was not statistically significant from the European/Other group (Ministry of Health 2007).

If these vaccination coverage results are followed through to sero-prevalence estimates, we would expect lower sero-prevalence among Maori than New Zealand Europeans.

Research significance

Sero-prevalence surveys complement disease surveillance and vaccine coverage surveillance. Vaccine coverage studies estimate the proportion of a population group who have been vaccinated either at a point in time or the proportion who were vaccinated over a period of time. Vaccine coverage surveys cannot estimate the proportion of a population who are immune to a specific vaccine preventable disease since:

1. natural infection is not considered
2. failed vaccination is not considered.

Selected vaccine preventable diseases are notifiable in New Zealand. Notification of these diseases is useful for:

1. identifying health problems such as epidemics and providing timely information on the patterns of disease;
2. facilitating control of health problems (such as the prevention of secondary transmission of hepatitis A);
3. evaluating prevention and control programmes.

However, notification data may be biased due to incomplete and inaccurate reporting. Some vaccine preventable diseases, such as rubella and hepatitis B, are frequently sub-clinical or difficult to recognise. For example, less than 10% of children with acute hepatitis B will become jaundiced (Chin 2000).

In contrast, sero-surveillance measures immunity from both vaccination and natural infection (including sub-clinical infection). Sero-prevalence data can be used to:

1. model future patterns of disease, including the prediction of epidemics;
2. determine the need for public health intervention on the basis of this modelling;
3. evaluate vaccination campaigns.

In New Zealand, notifiable diseases are under-reported. As an example, information on laboratory reported cases and notified cases could be examined for the degree of overlap between sources. For instance, during 2002, of the six laboratory reported measles cases only three were notified and of the four laboratory reported cases of rubella only one was notified. While these numbers are very small they do indicate under-notification with only 40% (95% CI: 12-74) of these laboratory confirmed cases also being notified. Therefore, notifiable diseases are prone to bias resulting from under-notification. While the introduction of laboratory notification will result in improved notification rates, it is expected that notification will underestimate the incidence of disease. This under-notification will predominantly result from lack of laboratory testing for notifiable diseases. In addition, sero-prevalence data are more useful for predicting future patterns of diseases. For example, while measles epidemics have occurred in New Zealand every five to six years historically the recent change in the vaccination schedule may have altered this pattern. On that basis, notification data can not be used to predict if or when the next measles epidemic will occur. A sero-prevalence study is able to identify specific groups with low levels of immunity and this information can be used to predict both the timing and the groups at risk from future epidemics. Therefore, vaccination programmes could be developed to avoid such epidemics.

The baseline sera collected in this study could also be stored and used to:

- evaluate future vaccination programs
- study the sero-prevalence of other pathogens of interest
- study the sero-prevalence of newly discovered pathogens.

Aim and objectives

The aim of the study was to estimate the sero-prevalence of a range of vaccine preventable diseases in New Zealand.

Specific objectives were to estimate the sero-prevalence of the following infections in New Zealand:

- measles
- diphtheria
- hepatitis B
- tetanus
- rubella
- pertussis
- varicella
- mumps
- hepatitis A
- polio.

The sero-prevalence of the non-vaccine preventable disease toxoplasmosis was also estimated.

The sero-prevalence of these diseases were estimated by age, ethnicity and region.

Methods

The proposed methods differed from the actual methods adopted in the study. The reason for this difference related to very slow recruitment in the paediatric age groups. Proposed targets for sample sizes were therefore not met in the 6-10 and 11-15 year age groups. The original proposal suggested 600 participants would participate in each of these two age groups. However, the final sample sizes were 466 and 589 respectively. It had also originally been proposed that North Island collection sites in these two age groups would be restricted to Auckland and Wellington. As a result of the slow recruitment it was decided to increase the number of sites (from other North Island regions).

The original methods set out in the final proposal are repeated below for clarity.

Study description

Study design

A cross-sectional sero-prevalence survey was proposed selecting:

1. consecutive children aged six to 15 years who presented to a community laboratory for a diagnostic specimen that required venepuncture
2. consecutive adults presenting to donate blood.

Selection of study population

Inclusion criteria

Inclusion criteria were:

1. Any child aged six to 15 years inclusive who presented to a community laboratory for a diagnostic specimen that required venepuncture and who had not previously provided a specimen for this study
2. Any adult aged 16+ who presented to the New Zealand Blood Donor Service and had not previously provided a specimen for this study.

Inclusion was to cease when 600 people have provided a specimen in each of the following age groups:

- Six to 10 years
- 11 to 15 years
- 16 to 24 years
- 25 to 44 years
- 45+ years

Exclusion criteria

Exclusion criteria were:

1. Received a blood transfusion in the past three months
2. Had a suspected or confirmed vaccine preventable infectious disease in the past month
3. Currently immunocompromised.

Sampling

A sample stratified by area was selected in each age group. The proposed sample selection for age groups 16 to 24, 25 to 44 and 45+ is shown in Table 4 (collected from blood donor centres). The proposed sample selection for age groups six to 10 and 11 to 15 is shown in Table 5 (collected from community laboratories).

Table 4 Study sample from blood donor centres

Area	Age (years)		
	16-24	25-44	45+
North Shore	81	86	81
Auckland	176	187	177
Manukau	81	86	81
Hamilton	34	27	27
Tauranga	16	19	24
Napier	24	26	33
Palmerston North	22	17	17
Wellington	43	46	39
Nelson	9	9	12
Christchurch	77	73	81
Dunedin	37	24	28
Total	600	600	600

Table 5 Study sample from community laboratories

Laboratory	Age (years)	
	6-10	11-15
Diagnostic MedLab (Northern North Island)	300	300
Medical Laboratory (Southern North Island)	150	150
MedLab South (South Island)	150	150
Total	600	600

Study procedures

Procedures at sample selection

Children

The parent/guardian of children presenting to a participating community laboratory were approached regarding participation in the study. If the child fulfilled the selection criteria, the parent/guardian was asked for informed consent at two levels:

1. informed consent for testing of the specimen for the current sero-prevalence study
2. informed consent to store the specimen and use at a later date.

An information sheet was provided as part of the informed consent process. If competent, the child was also asked for informed consent.

If informed consent was obtained for participation in this seroprevalence study, the child was enrolled in the study. The child was given a unique identifier, which was included on the informed consent form.

An option was provided to send the serological results to the child's GP. This necessitated documentation of the participant (and GP's) name on the consent form.

Adults

Any adult aged 16+ presenting to the New Zealand Blood Donor Service was approached regarding participation in the study. If they fulfilled the selection criteria, they were asked for informed consent at two levels:

1. informed consent for testing of the specimen for the current sero-prevalence study
2. informed consent to store the specimen and use at a later date.

An information sheet was provided as part of the informed consent process.

If informed consent was obtained at either level, the person was enrolled in the study. They were given a unique identifier, which was included on the informed consent form.

An option was also provided to send the serological results to the participant's GP. This necessitated documentation of the participant (and GP's) name on the consent form.

Measurement of demographic variables

A form was designed to accompany the serum sample and included measurement of the following variables:

- Age (initially measured as a continuous variable but recoded as a categorical variable in the analysis: 6-10, 11-15, 16-24, 25-44, 45+)
- Gender (coded as a categorical variable)
- Area (DHB region)
- Ethnicity (coded using the categories available in the 2001 Census ethnicity question).

The study questionnaire is included in Appendix 1.

Measurement of immune status

Specific serological markers and the type of assay used for the proposed diseases are shown in Table 6. Three markers were used for hepatitis B: core and surface antibody and surface antigen. Surface antibody is typically positive either following natural infection or vaccination against hepatitis B. Core antibody is positive following natural infection but not vaccination. The use of both markers rather than hepatitis B surface antibody alone is advisable due to false negative results resulting from reliance on hepatitis B surface antibody alone. A positive surface antigen indicates current infection.

All equivocal results were re-tested once using the same method. The results of the second testing were entered and no further testing occurred even if the repeat test was equivocal.

Table 6 Serological markers of immunity and type of assay for each disease

Infection	Assay	Source
Diphtheria	EIA	CHLabs In-house
Hepatitis A Total	Chemiluminessence	Vitros ECi, HAV IgG, OrthoClinical Diagnostics, New York, USA
Hepatitis B core antibody	Chemiluminessence	Vitros ECi, HBc IgG, OrthoClinical Diagnostics, New York, USA

Hepatitis B surface antibody	Chemiluminessence	Vitros ECi, HBs IgG, OrthoClinical Diagnostics, New York, USA
Measles IgG	EIA	Enzygnost-Anti Measles IgG, Dade Behring, Marburg GmbH
Mumps IgG	EIA	Enzygnost-Anti Mumps IgG, Dade Behring, Marburg GmbH
Pertussis IgG	EIA	Pertussis Toxin Elisa IgG/IgA test-kit, Genzyme Virotech GmbH.
Poliovirus type 1	Neutralisation	ESR In-house
Poliovirus type 2	Neutralisation	ESR In-house
Poliovirus type 3	Neutralisation	ESR In-house
Rubella IgG	Chemiluminessence	Vitros ECi, Rubella IgG, OrthoClinical Diagnostics, New York, USA
Tetanus	EIA	CHLabs In-house
Toxoplasmosis	Chemiluminessence	Vitros ECi, Toxoplasma IgG, OrthoClinical Diagnostics, New York, USA
Varicella IgG	EIA	Enzygnost-Anti VZ IgG, Dade Behring, Marburg GmbH

Specific serological tests varied by age group. Testing patterns are shown in Table 7.

Table 7 Age groups and serological testing

Infection	Age (years)				
	6-10	11-15	16-24	25-44	45+
Diphtheria	X	X	X	X	X
Hepatitis A Total	X	X	X	X	
Hepatitis B core antibody	X	X	X	X	
Hepatitis B surface antigen	X	X			
Hepatitis B surface antibody	X	X	X	X	
Measles IgG	X	X	X	X	
Mumps IgG	X	X	X	X	
Pertussis IgG	X	X	X	X	
Poliovirus type 1	X	X	X		
Poliovirus type 2	X	X	X		
Poliovirus type 3	X	X	X		
Rubella IgG	X	X	X	X	
Tetanus	X	X	X	X	X
Toxoplasmosis			X	X	
Varicella IgG	X	X	X	X	

Sample size

A sample of 600 people in each age group was proposed. This resulted in 95% confidence intervals of +/- 0.04 when the true probability was 0.50. When the true probability was 0.80 the corresponding 95% confidence interval was +/- 0.03.

The power to detect a difference in sero-prevalence of 5.0%, 7.5% and 10.0% between age strata at the 5% level of significance at various baseline sero-prevalence levels is shown in Figure 1 assuming there are 600 participants in each age stratum. These power curves are symmetrical about 50% sero-prevalence.

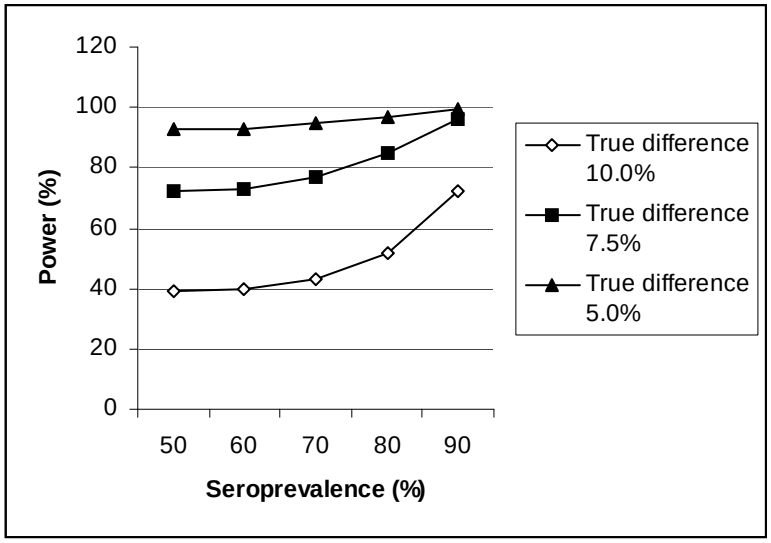

Figure 1 **Power to detect a true difference of 10.0% 7.5% and 5.0% by seroprevalence level with 600 in each comparison group**

Data management

Demographic data

A database was developed in Epi-Info version 6.04 for entry of the demographic data (Dean et al. 1995). Variables included in the database included a unique identifier for each participant, age, gender, DHB region and ethnicity. After data cleaning, the demographic data were transfererd to STATA version 7 (StataCorp 2001).

Laboratory data

The laboratory results were initially entered on the laboratory's standard software package (DELPHIC™). These results and the same unique identifier used for the demographic data was then dumped into excel and then imported into STATA version 7 (StataCorp 2001). The demographic and laboratory databases were merged.

Data entry

The demographic data were entered by an experienced data entry clerk who was familiar with Epi-Info. The laboratory data were entered by laboratory staff who routinely enter and check data on a daily basis.

Analysis

The following analyses were conducted for each disease tested. All analyses were performed using STATA version 7 (StataCorp 2001).

Univariate age stratified analysis

All categorical variables were summarised in tabular form. Age stratified levels of immunity were estimated by:

$$\frac{\text{Number classified as positive by laboratory testing in each age stratum}}{\text{Total number in each age stratum}}$$

Crude univariate analyses were performed by cross tabulating the exposure variables (age, ethnicity and area) against the number classified as positive to each disease. Sero-prevalence and 95% confidence intervals were estimated by strata of the exposure variables. All confidence intervals were estimated using exact binomial methods.

Trends in immunity by age group

Comparison of sero-prevalence levels by age strata were conducted using two approaches:

1. Cross age group comparisons of seroprevalence were conducted using the chi squared test provided the expected value was at least five in each cell. Otherwise, Fisher's exact test was used.
2. Chi squared test for trend was used to evaluate the presence of any linear trend in sero-prevalence across age strata.

Potential confounding factors

Potential confounding factors for the relationship between sero-prevalence and age were identified by:

- comparing the potential confounding factor with age
- comparing the potential confounding factor with serological result.

Variables associated with both age and immune status at the 10% significance level were considered as potential confounders. Gender, area and ethnicity were assessed as potential confounders.

Adjusted age stratified analyses

Crude odds ratios (OR) between the age strata and sero-prevalence were compared with Mantel-Haenszel adjusted ORs to further assess for confounding. Stratified analyses between age strata, sex, area and ethnicity, and immune status were also conducted to identify effect modifiers.

Logistic regression was also conducted to further evaluate the effect of age on sero-prevalence. Age, gender, ethnicity and residential area were entered in all logistic regression models as explanatory variables.

Sensitivity analyses

Sensitivity analyses were conducted that:

- assumed equivocal results represented a positive result
- assumed equivocal results represented a negative result.

Ethical Approval

Ethics approval was granted by the Multi-region Ethics Committee.

Amended methods

The proposed methods outlined above differed from the actual methods adopted in the study. The reason for this difference related to very slow recruitment in the paediatric age groups. Proposed targets for sample sizes were therefore not met in the 6-10 and 11-15 year age groups. The original proposal suggested 600 participants would participate in each of these two age groups. However, the final sample sizes were 466 and 589 respectively in these two age groups. It had also originally been proposed that North Island collection sites in these two age groups would be restricted to Auckland and Wellington. As a result of the slow recruitment it was decided to increase the number of sites (from other North Island regions). More details of the sample are shown in the results.

Results

Sample characteristics

As discussed in the methods the actual sample differed from the proposed sample. These differences are shown in Tables 8 (for adult age groups) and 9 (for paediatric age groups).

Table 8 Study sample from blood donor centres

Area	Proposed sample			Actual sample		
	Age (years)			Age (years)		
	16-24	25-44	45+	16-24	25-44	45+
North Shore	81	86	81	69	92	81
Auckland	176	187	177	168	183	159
Manukau	81	86	81	82	86	81
Hamilton	34	27	27	33	28	29
Tauranga	16	19	24	16	19	26
Napier	24	26	33	23	26	33
Palmerston North	22	17	17	22	17	17
Wellington	43	46	39	43	45	38
Nelson	9	9	12	9	10	12
Christchurch	77	73	81	90	72	97
Dunedin	37	24	28	39	24	28
Diagnostic MedLab (Northern North Island)				1		
MedLab Hamilton				1		
Medlab Central				1		
Total	600	600	600	597	602	601

Table 9 Study sample from community laboratories

Laboratory	Proposed sample		Actual sample	
	Age (years)		Age (years)	
	6-10	11-15	6-10	11-15
Diagnostic MedLab (Northern North Island)	300	300	146	218
Medical Laboratory (Southern North Island)	150	150	60	83
MedLab South (South Island)	150	150	162	172
MedLab Hamilton			75	83
Medlab Central			25	35
Total	600	600	468	591

Demographics of the actual sample are summarised in Table 10. The median age was 21 years (range 6-71 years).

Table 10 Summary of sample characteristics from the participating population

	Number (%)
Age group (years)	
6-10	468 (16)
11-15	591 (21)
16-24	597 (21)
25-44	602 (21)
45+	601 (21)
Gender	
Male	1398 (49%)
Female	1459 (51%)
Ethnicity (level 1 total ethnicity data)	
European	2436 (86%)
Maori	282 (9.9%)
Pacific	152 (5.4%)
Asian	183 (6.5%)
Middle East/ Latin America/ Africa	15 (0.5%)
Other	18 (0.6%)
Ethnicity (by level 1 sole/combined)	
European	2225 (78.4%)
Maori	106 (3.7%)
Pacific	94 (3.3%)
Asian	159 (5.6%)
Middle East/ Latin America/ Africa	13 (0.5%)
Other	15 (0.5%)
> 1 ethnic group	225 (7.9%)
District Health Board	
Auckland	445 (15.7%)
Bay of Plenty	62 (2.2%)
Canterbury	458 (16.2%)
Capital Coast	252 (8.9%)
Counties Manukau	434 (15.3%)
Hawkes Bay	82 (2.9%)
Hutt Valley	9 (0.3%)
Lakes	13 (0.5%)
Midcentral	93 (3.3%)
Nelson Marlborough	50 (1.8%)
Northland	4 (0.1%)
Otago	89 (3.1%)
South Canterbury	59 (2.1%)
Southland	54 (1.9%)
Taranaki	2 (0.1%)
Waikato	245 (8.6%)
Wairapapa	16 (0.6%)
Waitemata	460 (16.2%)
Whanganui	8 (0.3%)

Participation rate

The participation rate varied by age group:

- 6-10: 74%
- 11-15: 77%
- 16-24: 98%
- 25-44: 98%
- 45+: 99%

Diphtheria

Crude age-specific results for diphtheria are shown in Table 11.

Table 11 Crude age-specific diphtheria results

Age group	Positive	Negative	Prevalence
6-10	283	185	60.5% (55.9-64.9)
11-15	456	135	77.2% (73.6-80.5)
16-24	426	171	71.4% (67.5-75.0)
25-44	290	312	48.2% (44.1-52.2)
45+	274	327	45.6% (41.6-49.7)

Significant linear trend, $P<0.0001$

There was a significant linear trend in seroprevalence with the predominant pattern being declining seroprevalence with increasing age, although noting the seroprevalence was higher in the 11-15 and 16-24 age groups than the 6-10 year age group (see Table 11). The only comparison by age where a statistically significant difference in seroprevalence did not exist was that between the 25-44 and 45+ age groups (see Table 12).

Table 12 Comparison of age specific diphtheria prevalence estimates, univariate analysis

Age group 1	Age group 2	Prevalence, *p* value
45+	25-44	45.6% v 48.2%, p=0.37
45+	16-24	45.6% v 71.4%, p<0.0001
45+	11-15	45.6% v 77.2%, p<0.0001
45+	6-10	45.6% v 60.5%, P<0.0001
25-44	16-24	48.2% v 71.4%, P<0.0001
25-44	11-15	48.2% v 77.2%, P<0.0001
25-44	6-10	48.2% v 60.5%, P=0.0001
16-24	11-15	71.4% v 77.2%, P=0.02
16-24	6-10	71.4% v 60.5%, P=0.0002
11-15	6-10	77.2% v 60.5%, P<0.0001

The seroprevalence was similar in males and females (see Table 13).

Table 13 Diphtheria status by sex, univariate analysis

Sex	Positive	Negative	Prevalence
Male	853	545	61.0% (58.4-63.6)
Female	876	583	60.0% (57.5-62.6)

Ethnicity was considered using two methods. These were:

1. total ethnicity (where people can be counted in more than one ethnic group such that all the participants and groups combined can produce over 100% recording)
2. Sole/combined (where participants self-determining as a sole ethnic group are categorised to the group and participants self-determining to more than one ethnic group are categorised in a multiple group).

In both cases, level 1 ethnicity classifications were used (using the criteria set out by Statistics New Zealand). The diphtheria univariate results are presented in Tables 14 and 15. The estimated seroprevalence was low in the European, Maori and Pacific groups in both analyses.

Table 14 Diphtheria status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	1461	975	60.0% (58.0-61.9)
Maori	166	116	58.9% (52.9-64.7)
Pacific	93	59	61.2% (53.0-69.0)
Asian	135	48	73.8% (66.8-80.0)
Middle East/ Latin America/ Africa	11	4	73.3% (44.9-92.2)
Other	13	5	72.2% (46.5-90.3)

Table 15 Diphtheria status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	1321	904	59.4% (57.3-61.4)
Maori	56	50	52.8% (42.9-62.6)
Pacific	53	41	56.4% (45.8-66.6)
Asian	116	43	73.0% (65.3-79.7)
Middle East/ Latin America/ Africa	10	3	76.9% (46.2-95.0)
Other	10	5	66.7% (38.4-88.2)
> 1 ethnic group	149	76	66.2% (38.4-88.2)

Participants were classified into DHB regions based on their residential address. Univariate analyses for diphtheria by DHB region are presented in Table 16. These are ordered by prevalence but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 16 Diphtheria status by DHB region

DHB	Positive	Negative	Prevalence
Lakes	11	2	84.6% (54.6-98.1)
Hawkes Bay	55	27	67.1% (55.8-77.1)
Midcentral	62	31	66.7% (56.1-76.1)
Waikato	162	83	66.1% (59.8-72.0)
South Canterbury	39	20	66.1% (52.6-77.9)
Auckland	281	164	63.2% (58.5-67.6)
Wairapapa	10	6	62.5% (35.4-84.8)
Whanganui	5	3	62.5% (24.5-91.5)
Canterbury	282	176	61.6% (56.9-66.0)
Southland	33	21	61.1% (46.9-74.1)
Bay of Plenty	37	25	59.7% (46.4-71.9)
Capital Coast	149	103	59.1% (52.8-65.3)
Waitemata	269	191	58.5% (53.8-63.0)
Hutt Valley	5	4	55.6% (21.2-86.3)
Counties Manukau	242	192	55.8% (50.9-60.5)
Otago	47	42	52.8% (41.9-63.5)
Northland	2	2	50.0% (6.8-93.2)
Taranaki	1	1	50.0% (1.3-98.7)
Nelson Marlborough	23	27	46.0% (31.8-60.7)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 17 where ethnicity has been classified based on the sole/combined classification of ethnicity. Age groups 11-15

and 16-24 had significantly higher seroprevalence levels than the 6-10 age group; age groups 25-44 and 45+ had significantly lower seroprevalence levels than the 6-10 age group; Pacific peoples had significantly lower seroprevelnce levels than Europeans and Nelson-Marlborough residents had significantly lower seroprevalence levels than Auckland residents.

Table 17 Multivariate analysis of factors contributing to the seroprevalence of diphtheria

Variable	Categories	Odds ratio (95% CI)
Age	6-10	1
	11-15	2.31 (1.76-3.06)
	16-24	1.64 (1.24-2.17)
	25-44	0.58 (0.44-0.76)
	45+	0.51 (0.39-0.67)
Ethnicity	European	1
	Maori	0.66 (0.44-1.01)
	Pacific	0.62 (0.39-0.97)
	Asian	1.41 (0.96-2.08)
	Middle East	1.67 (0.45-6.27)
	Other	1.10 (0.34-3.57)
	>1group	1.12 (0.82-1.52)
Sex	Female	1
	Male	1.13 (0.96-1.32)
DHB	Auckland	1
	Bay of Plenty	1.20 (0.69-2.11)
	Canterbury	0.91 (0.68-1.22)
	Capital Coast	0.73 (0.52-1.02)
	Counties Manukau	0.85 (0.64-1.13)
	Hawkes Bay	1.66 (0.99-2.79)
	Hutt Valley	0.83 (0.21-3.30)
	Lakes	2.66 (0.55-12.91)
	Midcentral	1.10 (0.67-1.81)
	Nelson Marlborough	0.52 (0.27-0.98)
	Northland	0.57 (0.07-4.52)
	Otago	0.76 (0.47-1.24)
	South Canterbury	0.73 (0.40-1.35)
	Southland	0.61 (0.33-1.12)
	Taranaki	0.59 (0.03-10.80)
	Waikato	0.99 (0.70-1.41)
	Wairapapa	0.54 (0.18-1.59)
	Waitemata	0.89 (0.67-1.18)
	Whanganui	0.66 (0.15-2.88)

Hepatitis A

Crude age-specific results for Hepatitis A are shown in Table 18.

Table 18 Crude age-specific Hepatitis A results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
6-10	32	431	5	6.8% (4.7-9.5)	7.9% (5.6-10.7)
11-15	47	533	10	8.0% (5.9-10.5)	9.7% (7.4-12.3)
16-24	61	530	6	10.2% (7.9-12.9)	11.2% (8.8-14.0)
25-44	157	435	10	26.1% (22.6-29.8)	27.7% (24.2-31.5)

[1] Significant linear trend, $P<0.0001$
[2] Significant linear trend, $P<0.0001$

Under 1.5% of the results were in the equivocal range so results for Hepatitis A were similar irrespective of the method of analysing equivocal results. There was a

significant linear trend demonstrating increasing seroprevalence with increasing age (see Table 18). The seroprevalence was also significantly higher in the 25-44 age group compared with the 16-24 age group but comparisons between age groups 16-24 and 11-15, and 11-15 with 6-10 did not reach statistical significance (see Table 19).

Table 19 Comparison of age specific Hepatitis A prevalence estimates, univariate analysis

Age group 1	Age group 2	equivocal=nonimmune	equivocal=immune
25-44	16-24	26.1 v 10.2, *P*<0.0001	27.7 v 11.2, *P*<0.0001
25-44	11-15	26.1 v 8.0, *P*<0.0001	27.7 v 9.7, *P*<0.0001
25-44	6-10	26.1 v 6.8, *P*<0.0001	27.7 v 7.9, *P*<0.0001
16-24	11-15	10.2 v 8.0, *P*=0.18	11.2 v 9.7, *P*=0.38
16-24	6-10	10.2 v 6.8, *P*=0.05	11.2 v 7.9, *P*=0.07
11-15	6-10	8.0 v 6.8, *P*=0.49	9.7 v 7.9, *P*=0.32

The seroprevalence was similar in males and females (see Table 20).

Table 20 Hepatitis A status by sex, univariate analysis

Sex	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Male	142	908	13	13.4% (11.4-15.6)	14.6% (12.5-16.8)
Female	155	1019	18	13.0% (11.1-15.0)	14.5% (12.6-16.6)

Ethnicity was considered using two methods as previously described. The Hepatitis A univariate results are presented in Tables 21 and 22. The estimated seroprevalence was high in Asian (*P*<0.0001 compared with European group under assumption that equivocal is not immune in the sole/combined analysis) and, to a lesser extent, Pacific people (*P*=0.0006 compared with European group under assumption that equivocal is not immune in the sole/combined analysis).

Table 21 Hepatitis A status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	219	1618	23	11.8% (10.3-13.3)	13.0% (11.5-14.6)
Maori	24	227	3	9.4% (6.1-13.7)	10.6% (7.1-15.1)
Pacific	26	119	4	17.4% (11.7-24.5)	20.1% (14.0-27.5)
Asian	46	127	4	26.0% (19.7-33.1)	28.2% (21.8-35.5)
Middle East/ Latin America/ Africa	2	12	0	14.3% (1.8-42.8)	14.3% (1.8-42.8)
Other	2	11	1	14.3% (1.8-42.8)	21.4% (4.7-50.8)

Table 22 Hepatitis A status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	199	1447	21	11.9% (10.4-13.6)	13.2% (11.6-14.9)
Maori	5	90	0	5.3% (1.7-11.9)	5.3% (1.7-11.9)
Pacific	22	67	2	24.2% (15.8-34.3)	26.4% (17.7-36.7)
Asian	44	106	4	28.8% (21.7-36.6)	31.4% (24.1-39.4)
Middle East/ Latin America/ Africa	2	11	0	15.4% (1.9-45.4)	15.4% (1.9-45.4)
Other	2	9	1	16.7% (2.1-48.4)	25.0% (5.5-57.2)
> 1 ethnic group	22	182	3	10.6% (6.8-15.6)	12.1% (8.0-17.3)

Participants were classified into DHB regions based on their residential address. Univariate analyses for Hepatitis A by DHB region are presented in Table 23. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 23 Hepatitis A status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Northland	1	2	0	33.3% (0.8-90.6)	33.3% (0.8-90.6)
Hutt Valley	2	6	0	25.0% (3.2-65.1)	25.0% (3.2-65.1)
Auckland	70	273	8	19.9% (15.9-24.5)	22.2% (18.0-26.9)
Bay of Plenty	8	28	0	22.2% (10.1-39.1)	22.2% (10.1-39.2)
Counties Manukau	52	260	4	16.5% (12.5-21.0)	17.7% (13.7-22.4)
Lakes	2	11	0	15.4% (1.9-45.4)	15.4% (1.9-45.4)
Hawkes Bay	7	42	0	14.3% (5.9-27.2)	14.3% (5.9-27.2)
Waitemata	48	303	1	13.6% (10.2-17.7)	13.9% (10.5-18.0)
Capital Coast	26	188	4	11.9% (7.9-17.0)	13.8% (9.5-19.1)
Nelson Marlborough	5	33	0	13.2% (4.4-28.1)	13.2% (4.4-28.1)
Southland	4	47	3	7.4% (2.1-17.9)	13.0% (5.4-24.9)
Midcentral	7	66	2	9.3% (3.8-18.3)	12.0% (5.6-21.6)
Otago	7	55	0	11.3% (4.7-21.9)	11.3% (4.7-21.9)
Waikato	19	193	4	8.8% (5.4-13.4)	10.6% (6.9-15.5)
Canterbury	33	325	4	9.1% (6.4-12.6%)	10.2% (7.3-13.8)
South Canterbury	3	53	1	5.3% (1.1-14.6)	7.0% (1.9-17.0)
Wairapapa	1	15	0	6.3% (0.2-30.2)	6.3% (0.2-30.2)
Whanganui	0	8	0	0.0% (0-36.9)	0.0% (0-36.9)
Taranaki	0	1	0	0.0% (0-97.5)	0.0% (0-97.5)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 24 where ethnicity has been classified based on the sole/combined classification for ethnicity. Results were similar whether equivocal results were treated as immune or non-immune except for some variation on seroprevalence by DHB region. The age groups 16-24 and 25-44 had significantly higher levels of immunity than the reference category (6-10 years), Asian and Pacific people had significantly higher levels of immunity (compared with Europeans), and when equivocal results were treated as immune, both Otago and Canterbury residents had significantly lower seroprevalence estimates than the reference group of Auckland residents.

Table 24 Multivariate analysis of factors contributing to the seroprevalence of Hepatitis A

Variable	Categories	Odds ratio (95% CI)	
		Equivocal=non-immune	Equivocal=immune
Age	6-10	1	1
	11-15	1.20 (0.74-1.95)	1.27 (0.81-2.00)
	16-24	1.73 (1.06-2.82)	1.75 (1.10-2.77)
	25-44	5.86 (3.73-9.23)	5.84 (3.79-8.99)
Ethnicity	European	1	1
	Maori	0.43 (0.17-1.11)	0.38 (0.15-0.96)
	Pacific	2.90 (1.66-5.10)	2.92 (1.70-5.04)
	Asian	3.89 (2.52-6.02)	3.96 (2.59-6.05)
	Middle East	2.05 (0.42-9.88)	1.89 (0.39-9.10)
	Other	2.05 (0.41-10.36)	3.10 (0.77-12.53)
	>1group	1.09 (0.67-1.78)	1.12 (0.71-1.78)
Sex	Female	1	1
	male	0.99 (0.77-1.29)	0.97 (0.75-1.24)
DHB	Auckland	1	1
	Bay of Plenty	1.10 (0.46-2.61)	0.96 (0.40-2.27)
	Canterbury	0.64 (0.40-1.03)	0.63 (0.40-0.99)
	Capital Coast	0.88 (0.52-1.48)	0.89 (0.55-1.46)
	Counties Manukau	0.73 (0.48-1.12)	0.70 (0.46-1.06)
	Hawkes Bay	0.64 (0.27-1.53)	0.56 (0.23-1.33)
	Hutt Valley	1.32 (0.25-7.09)	1.13 (0.21-6.08)
	Lakes	1.98 (0.40-9.80)	1.76 (0.36-8.63)
	Midcentral	0.64 (0.27-1.52)	0.73 (0.34-1.60)
	Nelson Marlborough	0.98 (0.35-2.75)	0.84 (0.30-2.34)
	Northland	2.50 (0.20-31.06)	2.19 (0.18-27.00)
	Otago	0.52 (0.22-1.23)	0.45 (0.19-1.06)
	South Canterbury	0.69 (0.20-2.37)	0.79 (0.27-2.35)
	Southland	1.03 (0.35-3.09)	1.62 (0.67-3.91)
	Waikato	0.73 (0.41-1.29)	0.77 (0.45-1.32)
	Wairapapa	0.88 (0.11-6.98)	0.74 (0.09-5.84)
	Waitemata	0.66 (0.43-1.01)	0.58 (0.38-0.88)

Hepatitis B

Three markers were used that examined aspects of Hepatitis B seroprevalence: HBsAg, anti-HBc and anti-HBs. The anti-HBc and anti-HBs markers were used in the following age groups:

- 6-10
- 11-15
- 16-24
- 25-44.

However, since the two older age groups were selected from blood donors analysis of HBsAg was restricted to the 6-10 and 11-15 age groups.

A positive HBsAg result indicates current infection, a positive anti-HBc result indicates past or present Hepatitis B infection and a positive anti-HBs indicates immunity to infection either from vaccination or past infection.

Crude age-specific results for HBsAg, anti-HBc and anti-HBs are shown in Tables 25, 26 and 27 respectively.

Table 25 Crude age-specific HBsAg results

Age group	Positive	Negative	Equivocal	Prevalence
6-10	1	465	0	0.2% (0.0-1.2)
11-15	0	587	0	0.0% (0-0.6)

Table 26 Crude age specific anti-HBc results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
6-10	1	466	1	0.2% (0.0-1.2)	0.4% (0.1-1.5)
11-15	2	586	2	0.3% (0.0-1.2)	0.7% (0.2-1.7)
16-24	8	586	3	1.3% (0.6-2.6)	1.8% (0.9-3.3)
25-44	51	550	7	8.5% (6.4-11.0)	8.6% (6.5-11.2)

[1] Significant linear trend, $P<0.0001$
[2] Significant linear trend, $P<0.0001$

Table 27 Crude age specific anti-HBs results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
6-10	162	276	27	34.8% (30.5-39.4)	40.6% (36.1-45.3)
11-15	309	249	32	52.4% (48.3-56.5)	57.8% (53.7-61.8)
16-24	370	209	18	62.0% (57.9-65.9)	65.0% (61.0-68.8)
25-44	205	385	12	34.1% (30.3-38.0)	36.0% (32.2-40.0)

[1] Test for linear trend, $P=0.94$
[2] Test for linear trend, $P=0.12$

There were no equivocal HBsAg results, under 1% of the anti-HBc results were in the equivocal range but close to 4% of the anti-HBs results were equivocal. There was a significant linear trend by age consisting of increasing proportions positive to anti-HBc with increasing age (see Table 26). The proportion with a positive anti-HBc result was significantly higher while the presence of a positive anti-HBs result was significantly lower in the 25-44 age group compared with the 16-24 age group. There was also a significantly higher proportion of positive anti-HBs results in the 11-15 age group than the 6-10 age group (see Tables 28-29). There was no significant difference in seroprevalence of HBsAg between the 6-10 and 11-15 age group ($P=0.26$).

Table 28 Comparison of age specific anti-HBc results, univariate analysis

Age group 1	Age group 2	equivocal=nonimmune	equivocal=immune
25-44	16-24	8.5 v 1.3, $P<0.0001$	8.6 v 1.8, $P<0.0001$
25-44	11-15	8.5 v 0.3, $P<0.0001$	8.6 v 0.7, $P<0.0001$
25-44	6-10	8.5 v 0.2, $P<0.0001$	8.6 v 0.4, $P<0.0001$
16-24	11-15	1.3 v 0.3, $P=0.06$	1.8 v 0.7, $P=0.07$
16-24	6-10	1.3 v 0.2, $P=0.05$	1.8 v 0.4, $P=0.04$
11-15	6-10	0.3 v 0.2, $P=0.70$	0.7 v 0.4, $P=0.59$

Table 29 Comparison of age specific anti-HBs results, univariate analysis

Age group 1	Age group 2	equivocal=nonimmune	equivocal=immune
25-44	16-24	34.1 v 62.0, $P<0.0001$	36.0 v 65.0, $P<0.0001$
25-44	11-15	34.1 v 52.4, $P<0.0001$	36.0 v 57.8, $P<0.0001$
25-44	6-10	34.1 v 34.8, $P=0.79$	36.0 v 40.6, $P=0.13$
16-24	11-15	62.0 v 52.4, $P=0.0008$	65.0 v 57.8, $P=0.01$
16-24	6-10	62.0 v 34.8, $P<0.0001$	65.0 v 40.6, $P<0.0001$
11-15	6-10	52.4 v 34.8, $P<0.0001$	57.8 v 40.6, $P<0.0001$

The single positive HBsAg result was in a female participant. The proportion with a positive anti-HBc result was similar in males and females (see Table 30) but a lower

proportion of males had positive anti-HBs results (see Table 31, *P*=0.004 when equivocal was treated as immune and *P*=0.01 when equivocal was treated as non-immune).

Table 30 anti-HBc status by sex, univariate analysis

Sex	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Male	28	1030	5	2.6% (1.8-3.8)	3.1% (2.1-4.3)
Female	34	1156	2	2.9% (2.0-4.0)	3.0% (2.1-4.2)

Table 31 anti-HBs status by sex, univariate analysis

Sex	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Male	463	561	38	43.6% (40.6-46.6)	47.2% (44.1-50.2)
Female	582	557	51	48.9% (46.0-51.8)	53.2% (50.3-56.1)

The single positive HBsAg was in a person self-classifying as European. Anti-HBc univariate results by ethnicity are presented in Tables 32 and 33. Both Maori and Pacific people had higher proportions with positive anti-HBc results compared with Europeans (*P*=0.0004 and *P*=0.0003 when equivocal results assumed to be non-exposed to hepatitis B infection in the sole/combined ethnicity classification). Anti-HBs results by ethnicity are presented in Tables 34 and 35. Levels were similar across the ethnic groups.

Table 32 anti-HBc status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	41	1814	5	2.2% (1.6-3.0)	2.5% (1.8-3.3)
Maori	16	237	1	6.3% (3.6-10.0)	6.7% (3.9-10.5)
Pacific	9	139	1	6.0% (2.8-11.2)	6.7% (3.3-12.0)
Asian	7	169	1	4.0% (1.6-8.0)	4.5% (2.0-8.7)
Middle East/ Latin America/ Africa	0	14	0	0.0% (0-23.2)	0.0% (0-23.2)
Other	0	14	0	0.0% (0-23.2)	0.0% (0-23.2)

Table 33 anti-HBc status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	32	1631	4	1.9% (1.3-2.7)	2.2% (1.5-3.0)
Maori	7	88	0	7.4% (3.0-14.6)	7.4% (3.0-14.6)
Pacific	7	83	1	7.7% (3.1-15.2)	8.8% (3.9-16.6)
Asian	6	147	1	3.9% (1.5-8.3)	4.6% (1.9-9.2)
Middle East/ Latin America/ Africa	0	13	0	0.0% (0-24.7)	0.0% (0-24.7)
Other	0	12	0	0.0% (0-26.5)	0.0% (0-26.5)
> 1 ethnic group	10	196	1	4.8% (2.3-8.7)	5.3% (2.7-9.3)

Table 34 anti-HBs status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	855	932	71	46.0% (43.7-48.3)	49.8% (47.5-52.1)
Maori	115	128	11	45.3% (39.0-51.6)	49.6% (43.3-55.9)
Pacific	66	70	13	44.3% (36.2-52.7)	53.0% (44.7-61.2)
Asian	87	81	7	49.7% (42.1-57.4)	53.7% (46.0-61.3)
Middle East/ Latin America/ Africa	8	6	0	57.1% (28.9-82.3)	57.1% (28.9-82.3)
Other	6	7	1	42.9% (17.7-71.1)	50.0% (23.0-77.0)

Table 35 anti-HBs status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	772	835	59	46.3% (43.9-48.8)	49.9% (47.5-52.3)
Maori	41	50	4	43.2% (33.0-53.7)	47.4% (37.0-57.9)
Pacific	44	41	6	48.4% (37.7-59.1)	55.0% (44.2-65.4)
Asian	83	66	4	54.6% (46.3-62.7)	57.2% (49.0-65.2)
Middle East/ Latin America/ Africa	7	6	0	53.8% (25.1-80.8)	53.8% (25.1-80.8)
Other	5	6	1	41.7% (15.2-72.3)	50.0% (21.1-78.9)
> 1 ethnic group	89	105	12	43.2% (36.3-50.3)	49.0% (42.0-56.1)

Participants were classified into DHB regions based on their residential address. The single HBsAg positive participant was located in the Waitemata DHB. Univariate analyses for anti-HBc and anti-HBs by DHB region are presented in Tables 36 and 37 respectively. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 36 anti-HBc status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Bay of Plenty	4	32	0	11.1% (3.1-26.1)	11.1% (3.1-26.1)
Counties Manukau	26	289	1	8.2% (5.4-11.8)	8.5% (5.7-12.2)
Hawkes Bay	2	46	1	4.1% (0.5-14.0)	6.1% (1.3-16.9)
Waitemata	11	340	1	3.1% (1.6-5.5)	3.4% (1.8-5.9)
Auckland	7	342	2	2.0% (0.8-4.1)	2.6% (1.2-4.8)
Waikato	4	211	1	1.9% (0.5-4.7)	2.3% (0.8-5.3)
Canterbury	6	356	0	1.7% (0.6-3.9)	1.7% (0.6-3.6)
Capital Coast	2	216	0	0.9% (0.1-3.3)	0.9% (0.1-3.3)
Hutt Valley	0	8	0	0.0% (0-36.9)	0.0% (0-36.9)
Lakes	0	13	0	0.0% (0-24.7)	0.0% (0-24.7)
Midcentral	0	75	0	0.0% (0-4.8)	0.0% (0-4.8)
Nelson Marlborough	0	37	1	0.0% (0-9.3)	2.6% (0.1-13.8)
Northland	0	3	0	0.0% (0-70.8)	0.0% (0-70.8)
Otago	0	62	0	0.0% (0-5.8)	0.0% (0-5.8)
South Canterbury	0	57	0	0.0% (0-6.3)	0.0% (0-6.3)
Southland	0	54	0	0.0% (0-6.6)	0.0% (0-6.6)
Taranaki	0	1	0	0.0% (0-2.5)	0.0% (0-2.5)
Wairapapa	0	16	0	0.0% (0-20.6)	0.0% (0-20.6)
Whanganui	0	8	0	0.0% (0-36.9)	0.0% (0-36.9)

Table 37 anti-HBs status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Northland	3	0	0	100.0% (29.2-100)	100.0% (29.2-100)
Otago	37	25	0	59.7% (46.4-71.9)	59.7% (46.4-71.9)
Capital Coast	110	93	12	51.2% (44.3-58.0)	56.7% (49.8-63.5)
Hutt Valley	4	4	0	50.0% (15.7-84.3)	50.0% (15.7-84.3)
Whanganui	4	3	1	50.0% (15.7-84.3)	62.5% (24.5-91.5)
Canterbury	179	162	21	49.4% (44.2-54.7)	55.2% (50.0-60.4)
Auckland	172	166	13	49.0% (43.7-54.4)	52.7% (47.3-58.0)
Bay of Plenty	16	20	0	44.4% (27.9-61.9)	44.4% (27.9-61.9)
Southland	24	27	3	44.4% (30.9-58.6)	50.0% (36.1-63.9)
Waitemata	156	183	13	44.3% (39.1-49.7)	48.0% (42.7-53.4)
Waikato	95	114	7	44.0% (37.3-50.9)	47.2% (40.4-54.1)
Counties Manukau	138	168	10	43.7% (38.1-49.3)	46.8% (41.2-52.5)
South Canterbury	24	31	2	42.1% (29.1-55.9)	45.6% (32.4-59.3)
Midcentral	31	40	4	41.3% (30.1-53.3)	46.7% (35.0-58.6)
Wairapapa	6	10	0	37.5% (15.2-64.6)	37.5% (15.2-64.6)
Hawkes Bay	18	31	0	36.7% (23.4-51.7)	36.7% (23.4-51.7)
Nelson Marlborough	13	23	2	34.2% (19.6-51.4)	39.5% (24.0-56.6)
Lakes	2	10	1	15.4% (1.9-45.4)	23.1% (5.0-53.8)
Taranaki	0	1	0	0.0% (0-97.5)	0.0% (0-97.5)

Logistic regression analyses were produced for anti-HBc and anti-HBs. Results are presented in Table 38 for anti-HBc where ethnicity has been classified based on the sole/combined classification for ethnicity. A similar approach has been used for anti-HBs (results presented in Table 39).

The anti-HBc results were similar irrespective of whether equivocal results were treated as immune or non-immune. The age group 25-44 had significantly higher levels of exposure to Hepatitis B than the reference category (6-10 years); Maori, Asian and Pacific people had significantly higher levels of exposure (compared with

Europeans), and both Bay of Plenty and Counties Manukau residents had significantly higher exposure levels than the reference group of Auckland residents.

The results were slightly different for anti-HBs. Both the 11-15 and 16-24 age groups had significantly higher levels of immunity to Hepatitis B than the reference category (6-10 years) as did Asians when compared with Europeans.

Table 38 Multivariate analysis of factors contributing to anti-HBc

Variable	Categories	Odds ratio (95% CI)	
		Equivocal=non-immune	Equivocal=immune
Age	6-10	1	1
	11-15	1.88 (0.17-21.02)	1.86 (0.34-0.34)
	16-24	5.47 (0.67-44.94)	3.84 (0.82-17.92)
	25-44	45.27 (6.06-338.25)	22.85 (5.34-97.76)
Ethnicity	European	1	1
	Maori	5.04 (1.93-13.13)	4.29 (1.70-10.83)
	Pacific	5.26 (1.92-14.41)	5.28 (2.08-13.41)
	Asian	2.87 (1.07-7.73)	2.88 (1.15-7.19)
	>1group	3.86 (1.75-8.52)	3.73 (1.76-7.87)
Sex	Female	1	1
	male	0.95 (0.55-1.64)	1.07 (0.64-1.78)
DHB	Auckland	1	1
	Bay of Plenty	5.52 (1.43-21.36)	4.32 (1.18-15.82)
	Canterbury	1.53 (0.49-4.84)	1.15 (0.39-3.40)
	Capital Coast	0.79 (0.16-4.03)	0.59 (0.12-2.85)
	Counties Manukau	3.30 (1.35-8.06)	2.71 (1.21-6.08)
	Hawkes Bay	1.66 (0.31-8.74)	2.13 (0.53-8.64)
	Nelson Marlborough	NR[1]	1.92 (0.22-16.57)
	Waikato	2.06 (0.55-7.69)	1.89 (0.58-6.08)
	Waitemata	1.76 (0.65-4.78)	1.50 (0.60-3.73)

[1]NR – dropped from the final model as it predicted success perfectly.

Table 39 Multivariate analysis of factors contributing to anti-HBs

Variable	Categories	Odds ratio (95% CI)	
		Equivocal=non-immune	Equivocal=immune
Age	6-10	1	1
	11-15	2.07 (1.60-2.68)	2.07 (1.60-2.67)
	16-24	3.23 (2.45-4.26)	2.94 (2.23-3.87)
	25-44	1.01 (0.76-1.34)	0.89 (0.67-1.17)
Ethnicity	European	1	1
	Maori	0.99 (0.63-1.53)	1.01 (0.65-1.56)
	Pacific	1.03 (0.66-1.63)	1.18 (0.75-1.87)
	Asian	1.56 (1.08-2.24)	1.47 (1.02-2.12)
	Middle East	1.48 (0.48-4.60)	1.23 (0.40-3.82)
	Other	0.85 (0.24-3.02)	1.05 (0.31-3.62)
	>1group	0.91 (0.67-1.24)	1.00 (0.74-1.36)
Sex	Female	1	1
	male	0.86 (0.72-1.02)	0.83 (0.70-0.99)
DHB	Auckland	1	1
	Bay of Plenty	0.85 (0.41-1.76)	0.76 (0.37-1.58)
	Canterbury	1.10 (0.80-1.51)	1.17 (0.85-1.61)
	Capital Coast	1.21 (0.85-1.74)	1.26 (0.87-1.80)
	Counties Manukau	0.83 (0.60-1.14)	0.81 (0.59-1.12)
	Hawkes Bay	0.58 (0.30-1.12)	0.52 (0.27-1.00)
	Hutt Valley	0.99 (0.23-4.31)	0.87 (0.20-3.82)
	Lakes	0.23 (0.05-1.09)	0.33 (0.08-1.27)
	Midcentral	0.78 (0.46-1.33)	0.82 (0.49-1.39)
	Nelson Marlborough	0.64 (0.30-1.34)	0.61 (0.29-1.28)
	Otago	1.42 (0.79-2.57)	1.28 (0.71-2.30)
	South Canterbury	0.87 (0.48-1.59)	0.81 (0.45-1.48)
	Southland	1.01 (0.55-1.86)	1.02 (0.56-1.87)
	Waikato	0.92 (0.64-1.32)	0.87 (0.60-1.25)
	Wairapapa	0.87 (0.29-2.57)	0.69 (0.23-2.03)
	Waitemata	0.86 (0.63-1.17)	0.86 (0.63-1.18)
	Whanganui	0.85 (0.20-3.58)	1.20 (0.27-5.29)

Measles

Crude age-specific results for measles are shown in Table 40.

Table 40 Crude age-specific measles results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
6-10	385	33	48	82.6% (78.9-85.9)	92.9% (90.2-95.1)
11-15	455	52	82	77.3% (73.7-80.6)	91.1% (88.6-93.3)
16-24	507	24	66	84.9% (81.8-87.7)	96.0% (94.1-97.4)
25-44	554	18	30	92.0% (89.6-94.1)	97.0% (95.3-98.2)

[1] Significant linear trend, $P<0.0001$
[2] Significant linear trend, $P=0.0001$

Overall 10% of the results were in the equivocal range, therefore seroprevalence estimates varied significantly by the method of accounting for these equivocal results (whether assuming equivocal results can be considered as non-immune or immune to measles infection). This also resulted in different levels of significance when comparing different age groups as shown in Table 41. Specifically, the statistical significance of age group comparisons was different for the following, depending on the method of dealing with equivocal results:

- 25-44 versus 16-24
- 16-24 versus 6-10
- 11-15 versus 6-10.

Table 41 Comparison of age specific measles prevalence estimates, univariate analysis

Age group 1	Age group 2	equivocal=nonimmune	equivocal=immune
25-44	16-24	92.0% v 84.9%, P=0.0001	97.0 v 96.0, *P*=0.33
25-44	11-15	92.0% v 77.3%, *P*<0.0001	97.0 v 91.1, *P*<0.0001
25-44	6-10	92.0% v 82.6%, *P*<0.0001	97.0 v 92.9, *P*=0.002
16-24	11-15	84.9% v 77.3%, *P*=0.0007	96.0 v 91.1, *P*=0.0007
16-24	6-10	84.9% v 82.6%, *P*=0.31	96.0 v 92.9, *P*=0.03
11-15	6-10	77.3% v 82.6%, *P*=0.03	91.1 v 92.9, *P*=0.30

There was no significant difference in seroprevalence of measles by sex whichever method was used for analysing the equivocal results (see Table 42).

Table 42 Measles status by sex, univariate analysis

Sex	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Male	884	69	108	83.3% (80.9-85.5)	93.5% (91.8-94.9)
Female	1015	58	118	85.2% (83.1-87.2)	95.1% (93.7-96.3)

Ethnicity was considered using two methods as previously described. These univariate results are presented in Tables 43 and 44.

Table 43 Measles status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	1572	102	185	84.6% (82.8-86.2)	94.6% (93.5-95.6)
Maori	221	14	19	87.0% (82.2-90.9)	94.5% (90.9-96.9)
Pacific	134	6	8	90.5% (84.6-94.7)	95.9% (91.4-98.5)
Asian	134	12	30	76.1% (69.1-82.2)	93.2% (88.4-96.4)
Middle East/ Latin America/ Africa	12	0	2	85.7% (57.2-98.2)	100.0% (76.8-100.0)
Other	13	1	0	92.9% (66.1-99.8)	92.9% (66.1-99.8)

Table 44 Measles status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	1403	94	169	84.2% (82.4-85.9)	94.4% (93.1-95.4)
Maori	83	7	5	87.4% (79.0-93.3)	92.6% (85.4-97.0)
Pacific	80	6	4	88.9% (80.5-94.5)	93.3% (86.1-97.5)
Asian	116	10	27	76.3% (68.8-82.8)	93.4% (88.2-96.8)
Middle East/ Latin America/ Africa	11	0	2	84.6% (54.6-98.1)	100.0% (75.3-100)
Other	11	1	0	91.7% (61.5-99.8)	91.7% (61.5-99.8)
> 1 ethnic group	182	8	17	87.9% (82.7-92.0)	96.1% (92.5-98.3)

In both analyses, the estimated seroprevalence was low in the Asian group when analysing under the assumption that equivocal results should be treated as non-immune. For example, when comparing sole Asian with sole European groups and assuming equivocal results were consistent with lack of immunity, the prevalence in the sole Asian group was significantly lower than in the sole European group

(P=0.007). These results were not replicated when equivocal results were treated as immune.

Participants were classified into DHB regions based on their residential address. Univariate analyses for measles by DHB region are presented in Table 45. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 45 Measles status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Hawkes Bay	45	0	4	91.8% (80.4-97.7)	100.0% (92.7-100)
Bay of Plenty	32	0	4	88.9% (73.9-96.9)	100.0% (90.3-100)
Hutt Valley	8	0	0	100.0% (63.1-100.0)	100.0% (63.1-100)
Northland	3	0	0	100.0% (29.2-100.0)	100.0% (29.2-100)
Taranaki	1	0	0	100.0% (2.5-100.0)	100.0% (2.5-100)
Nelson Marlborough	32	1	5	84.2% (68.7-94.0)	97.4% (86.2-99.9)
Waitemata	296	13	43	84.1% (79.8-87.8)	96.3% (93.8-98.0)
Counties Manukau	277	12	26	87.9% (83.8-91.3)	96.2% (93.4-98.0)
Auckland	294	17	40	83.8% (79.5-87.5)	95.2% (92.4-97.2)
Capital Coast	186	12	19	85.7% (80.3-90.1)	94.5% (90.5-97.1)
Wairarapa	14	1	1	87.5% (61.7-98.4)	93.8% (69.8-99.8)
Midcentral	65	5	5	86.7% (76.8-93.4)	93.3% (85.1-97.8)
South Canterbury	42	4	11	73.7% (60.3-84.5)	93.0% (83.0-98.1)
Southland	47	4	3	87.0% (75.1-94.6)	92.6% (82.1-97.9)
Canterbury	298	28	36	82.3% (78.0-86.1)	92.3% (89.0-94.8)
Lakes	10	1	2	76.9% (46.2-95.0)	92.3% (64.0-99.8)
Otago	47	5	10	75.8% (63.3-85.8)	91.9% (82.2-97.3)
Waikato	179	20	17	82.9% (77.2-87.6)	90.7% (86.1-94.3)
Whanganui	7	1	0	87.5% (47.3-99.7)	87.5% (47.3-99.7)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 46 where ethnicity has been classified based on the sole/combined method. The same two factors reached statistical significance whether equivocal results were treated as immune or non-immune. The age group 25-44 had a significantly higher seroprevalence for measles than the 6-10 age group and the seroprevalence was low in the Otago DHB (Auckland was used as the reference). When assuming equivocal results could be considered as immune the odds ratio of immunity in the 25-44 group was 2.12 (95% CI 1.13-3.97) compared with the reference category of 6-10 year olds. Likewise, the OR for Otago was 0.38 (95% CI 0.13-1.11) when compared with Auckland as the reference.

Table 46 Multivariate analysis of factors contributing to the seroprevalence of measles

Variable	Categories	Odds ratio (95% CI)	
		Equivocal=non-immune	Equivocal=immune
Age	6-10	1	1
	11-15	0.75 (0.55-1.03)	0.81 (0.51-1.30)
	16-24	1.33 (0.93-1.90)	1.75 (0.97-3.15)
	25-44	2.70 (1.79-4.06)	2.12 (1.13-3.97)
Ethnicity	European	1	1
	Maori	1.43 (0.74-2.76)	0.86 (0.36-2.07)
	Pacific	1.59 (0.80-3.19)	0.72 (0.29-1.77)
	Asian	0.68 (0.44-1.04)	0.83 (0.40-1.70)
	Middle East	1.29 (0.28-5.99)	NR[1]
	Other	1.88 (0.24-15.1)	0.77 (0.09-6.35)
	>1group	1.54 (0.98-2.44)	1.82 (0.82-4.02)
Sex	Female	1	1
	Male	0.86 (0.68-1.09)	0.73 (0.50-1.05)
DHB	Auckland	1	1
	Bay of Plenty	0.98 (0.33-2.95)	NR[1]
	Canterbury	0.92 (0.60-1.40)	0.62 (0.32-1.20)
	Capital Coast	1.27 (0.77-2.09)	0.99 (0.45-2.17)
	Counties Manukau	1.16 (0.73-1.83)	1.18 (0.55-2.55)
	Hawkes Bay	1.44 (0.49-4.27)	NR[1]
	Lakes	0.63 (0.16-2.48)	0.68 (0.08-5.79)
	Midcentral	1.23 (0.58 (2.61)	0.71 (0.25-2.07)
	Nelson Marlborough	0.90 (0.35-2.32)	1.67 (0.21-13.20)
	Otago	0.42 (0.21-0.84)	0.38 (0.13-1.11)
	South Canterbury	0.81 (0.40-1.62)	0.94 (0.29-3.04)
	Southland	1.75 (0.73-4.17)	0.91 (0.28-2.94)
	Waikato	1.05 (0.65-1.71)	0.60 (0.29-1.22)
	Wairapapa	1.66 (0.36-7.69)	0.93 (0.11-7.68)
	Waitemata	0.88 (0.58-1.34)	1.19 (0.56-2.52)
	Whanganui	1.49 (0.18-12.57)	0.36 (0.04-3.32)

[1]NR – dropped from the final model as it predicted success perfectly.

Mumps

Crude age-specific results for mumps are shown in Table 47.

Table 47 Crude age-specific mumps results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
6-10	345	58	63	74.0% (69.8-78.0)	87.6% (84.2-90.4)
11-15	432	64	94	73.2% (69.5-76.8)	89.2% (86.4-91.5)
16-24	506	36	55	84.8% (81.6-87.5)	94.0% (91.7-95.7)
25-44	513	41	48	85.2% (82.1-88.0)	93.2% (90.9-95.1)

[1] Significant linear trend, $P<0.0001$
[2] Significant linear trend, $P=0.0001$

Overall 12% of the results were in the equivocal range and seroprevalence estimates varied significantly by the method of accounting for these equivocal results (whether assuming equivocal results represented non-immunity or immunity to mumps infection). However, comparisons between age groups resulted in the identification of statistically significant results in the same comparisons, irrespective of the classification of equivocal results (see Table 48).

Table 48 Comparison of age specific mumps prevalence estimates, univariate analysis

Age group 1	Age group 2	equivocal=nonimmune	equivocal=immune
25-44	16-24	85.2% v 84.8%, *P*=0.82	93.2% v 94.0%, *P*=0.58
25-44	11-15	85.2% v 73.2%, *P*<0.0001	93.2% v 89.2%, *P*=0.01
25-44	6-10	85.2% v 74.0%, *P*<0.0001	93.2% v 87.6%, *P*=0.002
16-24	11-15	84.8% v 73.2%, *P*<0.0001	94.0% v 89.2%, P=0.003
16-24	6-10	84.8% v 74.0%, P<0.0001	94.0% v 87.6%, *P*=0.003
11-15	6-10	73.2% v 74.0%, *P*=0.77	89.2% v 87.6%, *P*=0.42

When assuming equivocal results represented lack of immunity, the seroprevalence was lower in males than females (*P*=0.0003). No such difference existed when equivocal results were treated as immune. See Table 49 for details.

Table 49 Mumps status by sex, univariate analysis

Sex	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Male	812	106	144	76.5% (73.8-79.0)	90.0% (88.1-91.8)
Female	983	93	115	82.5% (80.3-84.7)	92.2% (90.5-93.7)

Ethnicity was considered using two methods as previously described. The mumps univariate results are presented in Tables 50 and 51. No specific variation was noted by ethnic group.

Table 50 Mumps status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	1492	156	212	80.2% (78.3-82.0)	91.6% (90.3-92.8)
Maori	207	19	28	81.5% (76.2-86.1)	92.5% (88.5-95.4)
Pacific	120	9	19	81.1% (73.8-87.0)	93.9% (88.8-97.2)
Asian	137	20	19	77.8% (71.0-83.7)	88.6% (83.0-92.9)
Middle East/ Latin America/ Africa	13	0	1	92.9% (66.1-99.8)	100.0% (76.8-100)
Other	11	1	2	78.6% (49.2-95.3)	92.9% (66.1-99.8)

Table 51 Mumps status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	1327	148	192	79.6% (77.6-81.5)	91.1% (89.7-92.4)
Maori	73	11	11	76.8% (67.1-84.9)	88.4% (80.2-94.1)
Pacific	70	7	13	77.8% (67.8-85.9)	92.2% (84.6-96.8)
Asian	118	20	15	77.0% (69.5-83.4)	86.8% (80.4-91.8)
Middle East/ Latin America/ Africa	12	0	1	92.3% (64.0-99.8)	100.0% (75.3-100)
Other	9	1	2	75.0% (42.8-94.5)	91.7% (61.5-99.8)
> 1 ethnic group	176	9	22	85.0% (79.4-89.6)	95.7% (91.9-98.0)

Participants were classified into DHB regions based on their residential address. Univariate analyses for mumps by DHB region are presented in Table 52. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some

age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 52 Mumps status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Hutt Valley	8	0	0	100.0% (63.1-100)	100.0% (63.1-100)
Northland	3	0	0	100.0% (29.2-100)	100.0% (29.2-100)
Taranaki	1	0	0	100.0% (2.5-100)	100.0% (2.5-100)
South Canterbury	49	2	6	86.0% (74.2-93.7)	96.5% (87.9-99.6)
Bay of Plenty	31	2	3	86.1% (70.5-95.3)	94.4% (81.3-99.3)
Auckland	283	25	43	80.6% (76.1-84.6)	92.9% (89.7-95.3)
Waitemata	292	25	35	83.0% (78.6-86.7)	92.9% (89.7-95.4)
Counties Manukau	262	24	30	82.9% (78.3-86.9)	92.4% (88.9-95.1)
Lakes	10	1	2	76.9% (46.2-95.0)	92.3% (64.0-99.8)
Midcentral	57	6	12	76.0% (64.7-85.1)	92.0% (83.4-97.0)
Canterbury	283	35	44	78.2% (73.6-82.3)	90.3% (86.8-93.2)
Capital Coast	170	21	26	78.3% (72.3-83.6)	90.3% (85.6-93.9)
Otago	51	7	4	82.3% (70.5-90.8)	88.7% (78.1-95.3)
Hawkes Bay	40	6	3	81.6% (68.0-91.2)	87.8% (75.2-95.4)
Wairapapa	10	2	4	62.5% (35.4-84.8)	87.5% (61.7-98.4)
Whanganui	5	1	2	62.5% (24.5-91.5)	87.5% (47.3-99.7)
Waikato	158	28	30	73.1% (66.7-78.9)	87.0% (81.8-91.2)
Southland	42	7	5	77.8% (64.4-88.0)	87.0% (75.1-94.6)
Nelson Marlborough	26	5	7	68.4% (51.3-82.5)	86.8% (71.9-95.6)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 53 where ethnicity has been classified based on sole/combined classification of ethnicity. Results were similar whether equivocal results were treated as immune or non-immune except for some variation on seroprevalence by sex. In both models age groups 16-24 and 25-44 had significantly higher levels of immunity (6-10 age group as the reference), and classification as more than one ethnic group was also associated with higher seroprevalence estimates. When equivocal results were treated as non-immune, males had lower seroprevalence estimates but this was not replicated when equivocal results were considered to be non-immune.

Table 53 Multivariate analysis of factors contributing to the seroprevalence of mumps

Variable	Categories	Odds ratio (95% CI)	
		Equivocal=non-immune	Equivocal=immune
Age	6-10	1	1
	11-15	0.97 (0.73-1.30)	1.19 (0.80-1.76)
	16-24	1.94 (1.39-2.71)	2.32 (1.44-3.74)
	25-44	2.00 (1.43-2.80)	2.02 (1.27-3.22)
Ethnicity	European	1	1
	Maori	0.93 (0.55-1.56)	0.90 (0.45-1.81)
	Pacific	0.85 (0.50-1.47)	1.02 (0.45-2.32)
	Asian	0.93 (0.61-1.42)	0.64 (0.37-1.10)
	Middle East	3.62 (0.46-28.32)	NR[1]
	Other	0.89 (0.23-3.48)	1.29 (0.16-10.40)
	>1group	1.69 (1.11-2.56)	2.30 (1.14-4.63)
Sex	Female	1	1
	Male	0.70 (0.57-0.89)	0.80 (0.59-1.08)
DHB	Auckland	1	1
	Bay of Plenty	1.09 (0.40-2.95)	0.88 (0.20-3.97)
	Canterbury	0.97 (0.65-1.42)	0.74 (0.42-1.32)
	Capital Coast	1.01 (0.65-1.56)	0.77 (0.41-1.46)
	Counties Manukau	1.11 (0.74-1.66)	0.83 (0.46-1.51)
	Hawkes Bay	0.79 (0.36-1.75)	0.38 (0.14-1.01)
	Lakes	0.93 (0.24-3.61)	1.07 (0.13-8.87)
	Midcentral	0.78 (0.42-1.45)	0.82 (0.32-2.13)
	Nelson Marlborough	0.59 (0.27-1.32)	0.56 (0.18-1.74)
	Otago	0.79 (0.38-1.64)	0.40 (0.16-1.01)
	South Canterbury	2.15 (0.95-4.86)	2.78 (0.63-12.39)
	Southland	1.25 (0.61-2.57)	0.70 (0.27-1.77)
	Waikato	0.77 (0.50-1.18)	0.55 (0.30-1.01)
	Wairarapa	0.65 (0.21-2.02)	1.26 (0.16-10.20)
	Waitemata	1.09 (0.74-1.62)	0.89 (0.50-1.61)
	Whanganui	0.42 (0.10-1.85)	0.52 (0.06-4.52)

[1]NR – dropped from the final model as it predicted success perfectly.

Pertussis

Crude age-specific results for *B. pertussis* are shown in Table 54.

Table 54 Crude age-specific pertussis results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
6-10	70	372	25	15.0% (11.9-18.6)	20.3% (16.8-24.3)
11-15	90	471	29	15.3% (12.4-18.4)	20.2% (17.0-23.6)
16-24	58	511	28	9.7% (7.5-12.4)	14.4% (11.7-17.5)
25-44	38	541	23	6.3% (4.5-8.6)	10.1% (7.8-12.8)

[1] Significant linear trend, $P<0.0001$
[2] Significant linear trend, $P<0.0001$

Table 55 Comparison of age specific pertussis prevalence estimates, univariate analysis

Age group 1	Age group 2	equivocal=nonimmune	equivocal=immune
25-44	16-24	6.3% v 9.7%, $P=0.03$	10.1% v 14.4%, $P=0.02$
25-44	11-15	6.3% v 15.3%, $P<0.0001$	10.1% v 20.2%, $P<0.0001$
25-44	6-10	6.3% v 15.0%, $P<0.0001$	10.1% v 20.3%, $P<0.0001$
16-24	11-15	9.7% v 15.3%, $P=0.004$	14.4% v 20.2%, $P=0.009$
16-24	6-10	9.7% v 15.0%, $P=0.009$	14.4% v 20.3%, $P=0.01$
11-15	6-10	15.3% v 15.0%, $P=0.91$	20.2% v 20.3%, $P=0.95$

Overall 4% of the results were in the equivocal range. However, comparisons between age groups resulted in the identification of statistically significant results in the same comparisons, irrespective of the classification of equivocal results (see Table 55). There was a significant linear trend in seroprevalence with increasing age in both groups (see Table 54). Examination of the trends reveals the trend is in the opposite direction to that encountered for most of the other vaccine preventable diseases in this study. This is most likely to be due to a difference in interpretation of a positive pertussis result when compared with the other tests used. Specifically, a positive result is indicative of recent infection. Pebody et al (2005) measured pertussis toxin levels in a seroprevalence study in Western Europe. They indicated a positive pertussis toxin antibody result was predictive of recent infection with *B. pertussis*. This approach of focussing on high titre results (indicative of recent infection) was consistent with the approach used in standardising pertussis assay results in the European Sero-Epidemiology Network (Giammanco et al. 2003). The investigators are not aware of any further advances on the ability to predict susceptibility to pertussis infection since the above publications so will follow the same approach henceforth. Therefore, equivocal results will be treated as a negative result below.

The seroprevalence was similar in males and females (see Table 56).

Table 56 Pertussis status by sex, univariate analysis, equivocal results treated as negative

Sex	Positive	Negative	Equivocal	Prevalence
Male	125	884	54	11.0% (9.3-12.9)
Female	131	1010	50	11.8% (9.9-13.8)

Ethnicity was considered using two methods as previously described. The pertussis univariate results are presented in Tables 57 and 58. The estimated seroprevalence was similar across ethnic groups, taking into consideration the small sample sizes in some categories.

Table 57 Pertussis status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence
European	209	1560	91	11.2% (9.8-12.8)
Maori	31	218	5	12.2% (8.4-16.9)
Pacific	22	121	6	14.8% (9.5-21.5)
Asian	19	145	12	10.8% (6.6-16.3)
Middle East/ Latin America/ Africa	3	11	0	21.4% (4.7-50.8)
Other	1	13	0	7.1% (0.2-33.9)

Table 58 Pertussis status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence
European	182	1400	85	10.9% (9.5-12.5)
Maori	9	85	1	9.5% (4.4-17.2)
Pacific	14	75	2	15.4% (8.7-24.5)
Asian	14	130	9	9.2% (5.1-15.0)
Middle East/ Latin America/ Africa	2	11	0	15.4% (1.9-45.4)
Other	0	12	0	0.0% (0.0-26.5)
> 1 ethnic group	30	170	7	14.5% (10.0-20.0)

Participants were classified into DHB regions based on their residential address. Univariate analyses for pertussis by DHB region are presented in Table 59. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 59 Pertussis status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence
Wairarapa	5	11	0	31.3% (11.0-58.7)
Southland	10	38	6	18.5% (9.3-31.4)
South Canterbury	10	42	5	17.5% (8.7-29.9)
Hawkes Bay	7	41	1	14.3% (5.9-27.2)
Counties Manukau	41	262	13	13.0% (9.5-17.2)
Waikato	27	178	11	12.5% (8.4-17.7)
Whanganui	1	7	0	12.5% (0.3-52.7)
Canterbury	44	302	16	12.2% (9.0-16.0)
Auckland	38	302	11	10.8% (7.8-14.6)
Midcentral	7	63	5	9.3% (3.8-18.3)
Waitemata	32	303	17	9.1% (6.3-12.6)
Capital Coast	19	186	12	8.8% (5.4-13.3)
Nelson Marlborough	3	33	2	7.9% (1.7-21.4)
Lakes	1	12	0	7.7% (0.2-36.0)
Bay of Plenty	2	33	1	5.6% (0.7-18.7)
Otago	3	56	3	4.8% (1.0-13.5)
Hutt Valley	0	7	1	0.0% (0.0-36.9)
Northland	0	3	0	0.0% (0.0-70.6)
Taranaki	0	1	0	0.0% (0.0-97.5)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 60 where ethnicity has been classified based on the sole/combined classification of ethnicity. The age groups 16-24 and 25-44 had significantly lower levels of exposure to recent infection than the reference category (6-10 years). There were no other statistically significant differences.

Table 60 Multivariate analysis of factors contributing to the seroprevalence of pertussis

Variable	Categories	Odds ratio (95% CI)
Age	6-10	1
	11-15	1.09 (0.77-1.56)
	16-24	0.64 (0.42-0.96)
	25-44	0.40 (0.25-0.63)
Ethnicity	European	1
	Maori	0.80 (0.39-1.63)
	Pacific	1.23 (0.65-2.31)
	Asian	0.76 (0.42-1.38)
	Middle East	1.35 (0.29-6.26)
	>1group	1.23 (0.79-1.89)
Sex	Female	1
	male	0.98 (0.74-1.28)
DHB	Auckland	1
	Bay of Plenty	0.71 (0.16-3.12)
	Canterbury	1.04 (0.63-1.70)
	Capital Coast	0.71 (0.39-1.30)
	Counties Manukau	1.39 (0.85-2.26)
	Hawkes Bay	2.02 (0.82-4.95)
	Lakes	0.68 (0.08-5.54)
	Midcentral	0.83 (0.35-1.98)
	Nelson Marlborough	0.75 (0.21-2.61)
	Otago	0.59 (0.17-2.03)
	South Canterbury	1.26 (0.57-2.79)
	Southland	1.39 (0.63-3.09)
	Waikato	0.99 (0.57-1.72)
	Wairapapa	2.80 (0.89-8.81)
	Waitemata	0.92 (0.55-1.53)
	Whanganui	1.03 (0.12-8.73)

Polio

Crude age-specific results for polioviruses types 1-3 are shown in Tables 61-63 respectively. All results were classified as positive or negative (i.e. there were no equivocal results) so columns for equivocal results are excluded from all tables in this section.

Table 61 Crude age-specific poliovirus type 1 results

Age group	Positive	Negative	Prevalence[1]
6-10	448	14	97.0% (95.0-98.3)
11-15	571	15	97.4% (95.8-98.6)
16-24	558	22	96.2% (94.3-97.6)

[1] No linear trend, P=0.44

Table 62 Crude age-specific poliovirus type 2 results

Age group	Positive	Negative	Prevalence[1]
6-10	453	8	98.3% (96.6-99.2)
11-15	577	9	98.5% (97.1-99.3)
16-24	571	8	98.6% (97.3-99.4)

[1] No linear trend, P=0.65

Table 63 Crude age-specific poliovirus type 3 results

Age group	Positive	Negative	Prevalence[1]
6-10	421	41	91.1% (88.2-93.6)
11-15	524	62	89.4% (86.6-91.8)
16-24	497	82	85.8% (82.7-88.6)

[1] Significant linear trend, *P*=0.007

Seroprevalence was uniformly high for poliovirus types 1 and 2 across the age groups and there was no significant linear trend in proportion positive by age group for these two viruses (see tables 61 and 62). However, seroprevalence was lower for poliovirus type 3 and showed a significant linear trend with declining seroprelence by increasing age. The seroprevalence of poliovirus type 3 was significantly lower in the 16-24 age group than in the 6-10 age group (85.8% versus 91.1%, *p*=0.009) (see table 66). There were no significant differences between the age groups for poliovirus types 1 and 2 (see tables 64 and 65).

Table 64 Comparison of age specific poliovirus type 1 prevalence estimates, univariate analysis

Age group 1	Age group 2	Prevalence
16-24	11-15	96.2% v 97.4%, P=0.23
16-24	6-10	96.2% v 97.0%, P=0.50
11-15	6-10	97.4% v 97.0%, P=0.64

Table 65 Comparison of age specific poliovirus type 2 prevalence estimates, univariate analysis

Age group 1	Age group 2	Prevalence
16-24	11-15	98.6% v 98.5%, P=0.83
16-24	6-10	98.6% v 98.3%, P=0.65
11-15	6-10	98.5% v 98.3%, P=0.80

Table 66 Comparison of age specific poliovirus type 3 prevalence estimates, univariate analysis

Age group 1	Age group 2	Prevalence
16-24	11-15	85.8% v 89.4%, P=0.06
16-24	6-10	85.8% v 91.1% P=0.009
11-15	6-10	89.4% v 91.1%, P=0.36

The seroprevalence was similar in males and females for all three polio viruses (see Tables 67-69).

Table 67 Poliovirus type 1 status by sex, univariate analysis

Sex	Positive	Negative	Prevalence
Male	747	25	96.8% (95.3-97.9)
Female	828	26	97.0% (95.6-98.0)

Table 68 Poliovirus type 2 status by sex, univariate analysis

Sex	Positive	Negative	Prevalence
Male	761	10	98.7% (97.6-99.4)
Female	838	15	98.2% (97.1-99.0)

Table 69 Poliovirus type 3 status by sex, univariate analysis

Sex	Positive	Negative	Prevalence
Male	679	92	88.1% (85.6-90.3)
Female	762	92	89.2% (87.0-91.2)

Ethnicity was considered using two methods as previously described. Again there were no significant differences by ethnicity for poliovirus types 1 and 2. However, for poliovirus type 3, seroprevalence appeared to be lower in European, Maori and Pacific peoples compared with the Asian group for both total ethnicity data and, to a lesser extent the sole/combined ethnicity data. These results are summarised in tables 70-75.

Table 70 Poliovirus type 1 status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	1272	42	96.8% (95.7-97.7)
Maori	196	3	98.5% (95.7-99.7)
Pacific	120	2	97.6% (93.0-99.5)
Asian	139	4	97.2% (93.0-99.2)
Middle East/ Latin America/ Africa	12	0	100.0% (73.5-100)
Other	8	0	100.0% (63.1-100)

Table 71 Poliovirus type 2 status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	1292	20	98.5% (97.7-99.1)
Maori	195	4	98.0% (94.9-99.4)
Pacific	122	1	99.2% (95.6-100.0)
Asian	142	0	100.0% (97.4-100)
Middle East/ Latin America/ Africa	12	0	100.0% (73.5-100)
Other	8	0	100.0% (63.1-100)

Table 72 Poliovirus type 3 status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	1155	158	88.0% (86.1-89.7)
Maori	171	28	85.9% (80.3-90.4)
Pacific	103	20	83.7% (76.0-89.8)
Asian	138	5	96.5% (92.0-98.9)
Middle East/ Latin America/ Africa	12	0	100.0% (73.5-100)
Other	7	1	87.5% (47.3-99.7)

Table 73 Poliovirus type 1 status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	1118	41	96.5% (95.2-97.4)
Maori	69	2	97.2% (90.2-99.7)
Pacific	71	3	95.9% (88.6-99.2)
Asian	120	4	96.8% (91.9-99.1)
Middle East/ Latin America/ Africa	11	0	100.0% (71.5-100)
Other	8	0	100.0% (63.1-100)
> 1 ethnic group	165	1	99.4% (96.7-100.0)

Table 74 Poliovirus type 2 status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	1140	18	98.4% (97.6-99.1)
Maori	70	2	97.2% (90.3-99.7)
Pacific	73	1	98.6% (92.7-100.0)
Asian	123	0	100.0% (97.0-100)
Middle East/ Latin America/ Africa	11	0	100.0% (71.5-100)
Other	8	0	100.0% (63.1-100)
> 1 ethnic group	163	2	98.8% (95.7-99.9)

Table 75 Poliovirus type 3 status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	1025	133	88.5% (86.5-90.3)
Maori	63	8	88.7% (79.0-95.0)
Pacific	65	9	87.8% (78.2-94.3)
Asian	119	5	96.0% (90.8-98.7)
Middle East/ Latin America/ Africa	11	0	100.0% (71.5-100)
Other	7	1	87.5% (47.3-99.7)
> 1 ethnic group	139	27	83.4% (77.2-89.0)

Participants were classified into DHB regions based on their residential address. Univariate analyses for poliovirus types 1-3 are presented by DHB region in Tables 76-78 respectively. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 76 Poliovirus type 1 status by DHB region

DHB	Positive	Negative	Prevalence
South Canterbury	56	0	100.0% (93.6-100)
Bay of Plenty	17	0	100.0% (80.5-100)
Wairarapa	16	0	100.0% (79.4-100)
Lakes	12	0	100.0% (73.5-100)
Hutt Valley	4	0	100.0% (39.8-100)
Northland	2	0	100.0% (15.8-100)
Taranaki	1	0	100.0% (2.5-100)
Waikato	179	3	98.4% (95.3-99.7)
MidCentral	55	1	98.2% (90.4-100.0)
Capital Coast	170	4	97.7% (94.2-99.4)
Auckland	230	7	97.0% (94.0-98.8)
Counties Manukau	192	6	97.0% (93.5-98.9)
Waitemata	212	8	96.4% (93.0-98.4)
Nelson Marlborough	26	1	96.3% (81.0-99.9)
Southland	51	2	96.2% (87.0-99.5)
Canterbury	274	12	95.8% (92.8-97.8)
Hawkes Bay	22	1	95.7% (78.1-99.9)
Otago	33	3	91.7% (77.5-98.2)
Whanganui	7	1	87.5% (47.3-99.7)

Table 77 Poliovirus type 2 status by DHB region

DHB	Positive	Negative	Prevalence
South Canterbury	55	0	100.0% (93.5-100)
Nelson Marlborough	28	0	100.0% (87.7-100)
Hawkes Bay	23	0	100.0% (85.2-100)
Bay of Plenty	17	0	100.0% (80.5-100)
Wairarapa	16	0	100.0% (79.4-100)
Hutt Valley	4	0	100.0% (39.8-100)
Northland	2	0	100.0% (15.8-100)
Taranaki	1	0	100.0% (2.5-100)
Auckland	236	1	99.6% (97.7-100.0)
Waikato	180	2	98.9% (96.1-99.9)
Canterbury	281	4	98.6% (96.4-99.6)
Waitemata	217	3	98.6% (96.1-99.7)
MidCentral	55	1	98.2% (90.4-100.0)
Southland	52	1	98.1% (89.9-100.0)
Counties Manukau	194	4	98.0% (94.9-99.4)
Capital Coast	170	4	97.7% (94.2-99.4)
Otago	35	1	97.2% (85.5-99.9)
Lakes	11	1	91.7% (61.5-99.8)
Whanganui	7	1	87.5% (47.3-99.7)

Table 78 Poliovirus type 3 status by DHB region

DHB	Positive	Negative	Prevalence
Northland	2	0	100.0% (15.8-100)
Taranaki	1	0	100.0% (2.5-100)
Wairarapa	15	1	93.8% (69.8-99.8)
Counties Manukau	182	16	91.9% (87.2-95.3)
South Canterbury	51	5	91.1% (80.4-97.0)
Auckland	215	22	90.7% (86.3-94.1)
Waikato	164	18	90.1% (84.8-94.0)
Capital Coast	156	18	89.7% (84.1-93.8)
Canterbury	255	31	89.2% (85.0-92.5)
MidCentral	49	6	89.1% (77.8-95.9)
Southland	47	6	88.7% (77.0-95.7)
Waitemata	195	25	88.6% (83.7-92.5)
Whanganui	7	1	87.5% (47.3-99.7)
Lakes	10	2	83.3% (51.6-97.9)
Otago	29	7	80.6% (64.0-91.8)
Nelson Marlborough	21	6	77.8% (57.7-91.4)
Bay of Plenty	12	5	70.6% (44.0-89.7)
Hawkes Bay	16	7	69.6% (47.1-86.8)
Hutt Valley	1	3	25.0% (0.6-80.6)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Tables 79-81 where ethnicity has been classified based on the sole/combined classification of ethnicity. There were no statistically significant differences noted for poliovirus types 1 and 2. However, there were statistically significant differences in the poliovirus type 3 results:

- The 16-24 age group had significantly lower levels of immunity than the reference category (6-10 years)
- Self determining as belonging to more than one ethnic group was associated with significantly lower levels of immunity than the reference category (Europeans)

- Three regions had significantly lower seroprevalence estimates when compared with the reference category of Auckland. These were:
    - Hawkes Bay (OR 0.30, 95% CI 0.11-0.84)
    - Hutt Valley (OR 0.04, 95% CI 0.00-0.44)
    - Nelson-Marlborough (OR 0.31, 95% CI 0.11-0.88).

Table 79 Multivariate analysis of factors contributing to the seroprevalence of poliovirus type 1

Variable	Categories	Odds ratio (95% CI)
Age	6-10	1
	11-15	1.49 (0.68-3.23)
	16-24	1.04 (0.49-2.21)
Ethnicity	European	1
	Maori	2.26 (0.30-17.0)
	Pacific	0.87 (0.25-3.05)
	Asian	1.10 (0.37-3.31)
	>1group	5.47 (0.74-40.4)
Sex	Female	1
	Male	0.96 (0.54-1.72)
DHB	Auckland	1
	Canterbury	0.75 (0.28-2.04)
	Capital Coast	1.28 (0.36-4.59)
	Counties Manukau	1.03 (0.34-3.18)
	Hawkes Bay	0.78 (0.09-6.96)
	Midcentral	1.60 (0.19-13.7)
	Nelson Marlborough	0.74 (0.08-6.45)
	Otago	0.42 (0.10-1.83)
	Southland	0.77 (0.15-4.05)
	Waikato	1.75 (0.43-7.12)
	Waitemata	0.82 (0.29-2.33)
	Whanganui	0.26 (0.03-2.50)

Table 80 Multivariate analysis of factors contributing to the seroprevalence of poliovirus type 2

Variable	Categories	Odds ratio (95% CI)
Age	6-10	1
	11-15	1.27 (0.43-3.74)
	16-24	1.56 (0.49-4.99)
Ethnicity	European	1
	Maori	1.40 (0.17-11.46)
	Pacific	1.13 (0.14-9.33)
	>1group	1.04 (0.23-4.70)
Sex	Female	1
	Male	1.62 (0.65-4.02)
DHB	Auckland	1
	Canterbury	0.42 (0.04-3.90)
	Capital Coast	0.23 (0.02-2.17)
	Counties Manukau	0.23 (0.03-2.13)
	Lakes	0.05 (0.00-0.89)
	Midcentral	0.30 (0.02-5.04)
	Otago	0.15 (0.01-2.77)
	Southland	0.32 (0.02-5.47)
	Waikato	1.05 (0.06-17.33)
	Waitemata	0.53 (0.05-5.90)
	Whanganui	0.04 (0.00-0.77)

Table 81 Multivariate analysis of factors contributing to the seroprevalence of poliovirus type 3

Variable	Categories	Odds ratio (95% CI)
Age	6-10	1
	11-15	0.77 (0.50-1.20)
	16-24	0.62 (0.39-0.99)
Ethnicity	European	1
	Maori	0.97 (0.43-2.19)
	Pacific	0.84 (0.38-1.85)
	Asian	2.46 (0.97-6.28)
	Other	0.63 (0.07-5.39)
	>1group	0.58 (0.36-0.93)
Sex	Female	1
	Male	0.81 (0.59-1.12)
DHB	Auckland	1
	Bay of Plenty	0.32 (0.10-1.01)
	Canterbury	0.83 (0.45-1.51)
	Capital Coast	0.86 (0.44-1.70)
	Counties Manukau	1.29 (0.65-2.56)
	Hawkes Bay	0.30 (0.11-0.84)
	Hutt Valley	0.04 (0.00-0.44)
	Lakes	0.45 (0.09-2.26)
	Midcentral	0.91 (0.34-2.41)
	Nelson Marlborough	0.31 (0.11-0.88)
	Otago	0.50 (0.19-1.31)
	South Canterbury	0.96 (0.34-2.74)
	Southland	0.75 (0.28-2.02)
	Waikato	1.05 (0.52-2.11)
	Wairapapa	1.53 (0.19-12.46)
	Waitemata	0.84 (0.45-1.56)
	Whanganui	0.75 (0.09-6.47)

Rubella

Crude age-specific results for rubella are shown in Table 82.

Table 82 Crude age-specific rubella results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
6-10	399	48	21	85.3% (81.7-88.3)	89.7% (86.6-92.3)
11-15	504	56	30	85.4% (82.3-88.2)	90.5% (87.9-92.8)
16-24	564	22	11	94.5% (92.3-96.2)	96.3% (94.5-97.7)
25-44	523	59	20	86.9% (83.9-89.5)	90.2% (87.5-92.5)

[1] Significant linear trend, P=0.03
[2] Significant linear trend, P=0.22

Overall 4% of the results were in the equivocal range. However, comparisons between age groups resulted in the identification of statistically significant results in the same comparisons, irrespective of the classification of equivocal results (see Table 83). Seroprevalence was significantly lower in the 25-44 age group than in the 16-24 age group (P<0.0001).

Table 83 Comparison of age specific rubella prevalence estimates, univariate analysis

Age group 1	Age group 2	equivocal=nonimmune	equivocal=immune
25-44	16-24	86.9% v 94.5%, *P*<0.0001	90.2% v 96.3%, *P*<0.0001
25-44	11-15	86.9% v 85.4%, *P*=0.47	90.2% v 90.5%, *P*=0.86
25-44	6-10	86.9% v 85.3%, *P*=0.45	90.2% v 89.7%, *P*=0.81
16-24	11-15	94.5% v 85.4%, *P*<0.0001	96.3% v 90.5%, *P*=0.0001
16-24	6-10	94.5% v 85.3%, *P*<0.0001	96.3% v 89.7%, *P*<0.0001
11-15	6-10	85.4% v 85.3%, *P*=0.94	90.5% v 89.7%, *P*=0.68

The seroprevalence was lower in males than females (see Table 84). This difference was highly statistically significant whether equivocal represents were assumed to be immune or non-immune (*P*<0.0001).

Table 84 Rubella status by sex, univariate analysis

Sex	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Male	896	127	40	84.3% (82.0-86.4)	88.1% (85.9-89.9)
Female	1092	58	42	91.6% (89.9-93.1)	95.1% (93.6-96.3)

Ethnicity was considered using two methods as previously described. The rubella univariate results are presented in Tables 85 and 86. No significant variation was noted by ethnic group although participants classified as Asian overall had the lowest seroprevalence estimates irrespective of the method of classification used.

Table 85 Rubella status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	1635	155	70	87.9% (86.3-89.4)	91.7% (90.3-92.9)
Maori	231	16	7	90.9% (86.7-94.2)	93.7% (90.0-96.4)
Pacific	138	6	5	92.6% (87.2-96.3)	96.0% (91.4-98.5)
Asian	152	20	5	85.9% (79.9-90.6)	88.7% (83.1-93.0)
Middle East/ Latin America/ Africa	14	0	0	100.0% (76.8-100)	100.0% (76.8-100)
Other	13	0	1	92.9% (66.1-99.8)	100.0% (76.8-100)

Table 86 Rubella status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	1460	142	65	87.6% (85.9-89.1)	91.5% (90.0-92.8)
Maori	86	7	2	90.5% (82.8-95.6)	92.6% (85.4-97.0)
Pacific	83	5	3	91.2% (83.4-96.1)	94.5% (87.6-98.2)
Asian	132	17	5	85.6% (79.0-90.8)	88.9% (82.8-93.4)
Middle East/ Latin America/ Africa	13	0	0	100.0% (75.3-100)	100.0% (75.3-100)
Other	11	0	1	91.7% (61.5-99.8)	100.0% (73.5-100)
> 1 ethnic group	188	13	6	90.8% (86.0-94.4)	93.7% (89.5-96.6)

Participants were classified into DHB regions based on their residential address. Univariate analyses for rubella by DHB region are presented in Table 87. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but

note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 87 Rubella status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Hutt Valley	8	0	0	100.0% (63.1-100)	100.0% (63.1-100)
Northland	3	0	0	100.0% (29.2-100)	100.0% (29.2-100)
Taranaki	1	0	0	100.0% (2.5-100)	100.0% (2.5-100)
Hawkes Bay	47	1	1	95.9% (86.0-99.5)	98.0% (89.1-99.9)
Capital Coast	199	9	10	91.3% (86.7-94.7)	95.9% (92.3-98.1)
Canterbury	328	22	12	90.6% (87.1-93.4)	93.9% (90.9-96.2)
Waikato	193	14	9	89.4% (84.5-93.1)	93.5% (89.4-96.4)
Midcentral	68	5	2	90.7% (81.7-96.2)	93.3% (85.1-97.8)
Auckland	310	27	14	88.3% (84.5-91.5)	92.3% (89.0-94.9)
Lakes	12	1	0	92.3% (64.0-99.8)	92.3% (64.0-99.8)
South Canterbury	51	5	1	89.5% (78.5-96.0)	91.2% (80.7-97.1)
Waitemata	299	35	18	84.9% (80.8-88.5)	90.1% (86.4-93.0)
Nelson Marlborough	30	4	4	78.9% (62.7-90.4)	89.5% (75.2-97.1)
Counties Manukau	276	34	6	87.3% (83.2-90.8)	89.2% (85.3-92.4)
Wairapapa	14	2	0	87.5% (61.7-98.4)	87.5% (61.7-98.4)
Whanganui	7	1	0	87.5% (47.3-99.7)	87.5% (47.3-99.7)
Otago	53	8	1	85.5% (74.2-93.1)	87.1% (76.1-94.3)
Southland	43	8	3	79.6% (66.5-89.4)	85.2% (72.9-93.4)
Bay of Plenty	30	6	0	83.3% (67.2-93.6)	83.3% (67.2-93.6)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 88 where ethnicity has been classified based on the sole/combined classification of ethnicity. Results were similar whether equivocal results were treated as immune or non-immune except for some variation on seroprevalence by DHB region. In both models the age group 16-24 had significantly higher levels of immunity than the reference category (6-10 years), and males had significantly lower seroprevalence estimates. When equivocal results were treated as immune, Otago DHB had lower seroprevalence estimates but this was not replicated when equivocal results were considered to be non-immune.

Table 88 Multivariate analysis of factors contributing to the seroprevalence of rubella

Variable	Categories	Odds ratio (95% CI)	
		Equivocal=non-immune	Equivocal=immune
Age	6-10	1	1
	11-15	1.03 (0.72-1.47)	1.09 (0.71-1.68)
	16-24	3.35 (2.09-5.38)	3.51 (1.99-6.21)
	25-44	1.40 (0.94-2.07)	1.30 (0.82-2.06)
Ethnicity	European	1	1
	Maori	1.62 (0.76-3.46)	1.43 (0.60-3.42)
	Pacific	1.82 (0.81-4.10)	1.75 (0.68-4.53)
	Asian	1.05 (0.63-1.75)	0.89 (0.50-1.59)
	Middle East		
	Other	2.08 (0.26-16.85)	
	>1group	1.57 (0.94-2.62)	1.57 (0.86-2.89)
Sex	Female	1	1
	Male	0.49 (0.37-0.64)	0.38 (0.27-0.53)
DHB	Auckland	1	1
	Bay of Plenty	0.52 (0.20-1.39)	0.32 (0.12-0.89)
	Canterbury	1.40 (0.84-2.33)	1.33 (0.72-2.47)
	Capital Coast	1.65 (0.91-2.99)	2.19 (0.98-4.86)
	Counties Manukau	0.84 (0.52-1.36)	0.63 (0.37-1.10)
	Hawkes Bay	2.46 (0.56-10.75)	3.16 (0.41-24.33)
	Lakes	1.81 (0.22-14.86)	1.15 (0.14-9.65)
	Midcentral	1.39 (0.58-3.31)	1.16 (0.42-3.21)
	Nelson Marlborough	0.53 (0.21-1.32)	0.83 (0.23-2.99)
	Otago	0.51 (0.23-1.16)	0.35 (0.14-0.86)
	South Canterbury	1.74 (0.68-4.45)	1.25 (0.44-3.53)
	Southland	0.76 (0.35-1.67)	0.65 (0.26-1.60)
	Waikato	1.28 (0.72-2.25)	1.29 (0.64-2.60)
	Wairapapa	1.29 (0.27-6.08)	0.75 (0.16-3.64)
	Waitemata	0.70 (0.44-1.09)	0.68 (0.40-1.18)
	Whanganui	0.79 (0.09-6.97)	0.43 (0.05-3.89)

However, there was a significant interaction between age and gender. As shown in Table 89, when equivocal results were treated as non-immune males generally had significantly lower seroprevalence estimates in each age group. When equivocal results were treated as immune, while males again had lower seroprevalence estimates this only reached statistical significance in the 25-44 age group.

Table 89 Effect of gender on age specific seroprevalence estimates for rubella, adjusted odds ratio

Age group	Sex	Equivocal=non-immune	Equivocal=immune
6-10	Female	1	1
	Male	0.57 (0.33-0.99)	0.71 (0.37-1.33)
11-15	Female	1	1
	Male	0.75 (0.47-1.21)	0.68 (0.38-1.21)
16-24	Female	1	1
	Male	0.41 (0.20-0.85)	0.46 (0.19-1.10)
25-44	Female	1	1
	Male	0.29 (0.17-0.49)	0.08 (0.03-0.19)

Adjusted for ethnicity (sole/combined coding), and area of residence

Tetanus

Crude age-specific results for tetanus are shown in Table 90.

Table 90 Crude age-specific tetanus results

Age group	Positive	Negative	Prevalence
6-10	399	69	85.3% (81.7-88.3)
11-15	556	35	94.1% (91.9-95.8)
16-24	565	32	94.6% (92.5-96.3)
25-44	569	33	94.5% (92.4-96.2)
45+	533	68	88.7% (85.9-91.1)

Key results in the tetanus seroprevalence by age group were:

- the significantly lower seroprevalence in the 6-10 age group than that in age groups 11-15, 16-24 and 25-44
- the significantly lower seroprevalence in the 45+ age group than that in age groups 11-15, 16-24 and 25-44 (see Table 91).

Table 91 Comparison of age specific tetanus prevalence estimates, univariate analysis

Age group 1	Age group 2	Prevalence, *p* value
45+	25-44	88.7% v 94.5%, p=0.0003
45+	16-24	88.7% v 94.6%, p=0.0002
45+	11-15	88.7% v 94.1%, p=0.0009
45+	6-10	88.7% v 85.3%, p=0.10
25-44	16-24	94.5% v 94.6%, p=0.93
25-44	11-15	94.5% v 94.1%, p=0.74
25-44	6-10	94.5% v 85.3%, p<0.0001
16-24	11-15	94.6% v 94.1%, p=0.67
16-24	6-10	94.6% v 85.3%, p<0.0001
11-15	6-10	94.1% v 85.3%, p<0.0001

The seroprevalence was similar in males and females irrespective of the method of analysing equivocal results (see Table 92).

Table 92 Tetanus status by sex, univariate analysis

Sex	Positive	Negative	Prevalence
Male	1293	105	92.5% (91.0-93.8)
Female	1328	131	91.0% (89.4-92.4)

Ethnicity was considered using two methods as previously described. The tetanus univariate results are presented in Tables 93 and 94. The estimated seroprevalence was low in the Maori and Pacific groups in the sole/combined analysis but was only low in the Pacific group in the total ethnicity analysis.

Table 93 Tetanus status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	2250	186	92.4% (91.2-93.4)
Maori	255	27	90.4% (86.4-93.6)
Pacific	126	26	82.9% (76.0-88.5)
Asian	169	14	92.3% (87.5-95.8)
Middle East/ Latin America/ Africa	15	0	100.0% (78.2-100)
Other	18	0	100.0% (81.5-100)

Table 94 Tetanus status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Prevalence
European	2054	171	92.3% (91.1-93.4)
Maori	89	17	84.0% (75.6-90.4)
Pacific	77	17	81.9% (72.6-89.1)
Asian	146	13	91.8% (86.4-95.6)
Middle East/ Latin America/ Africa	13	0	100.0% (75.3-100)
Other	15	0	100.0% (78.2-100)
> 1 ethnic group	209	16	92.9% (88.7-95.9)

Participants were classified into DHB regions based on their residential address. Univariate analyses for tetanus by DHB region are presented in Table 95. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 95 Tetanus status by DHB region

DHB	Positive	Negative	Prevalence
Lakes	13	0	100.0% (75.3-100)
Hutt Valley	9	0	100.0% (66.4-100)
Whanganui	8	0	100.0% (63.1-100)
Northland	4	0	100.0% (39.8-100)
Taranaki	2	0	100.0% (15.8-100)
Hawkes Bay	81	1	98.8% (93.4-100.0)
Canterbury	430	28	93.9% (91.3-95.9)
Auckland	417	28	93.7% (91.0-95.8)
Midcentral	87	6	93.5% (86.5-97.6)
Otago	83	6	93.3% (85.9-97.5)
Waikato	226	19	92.2% (88.2-95.3)
Nelson Marlborough	46	4	92.0% (80.8-97.8)
Capital Coast	230	22	91.3% (87.1-94.4)
Waitemata	417	43	90.7% (87.6-93.2)
South Canterbury	53	6	89.8% (79.2-96.2)
Counties Manukau	384	50	88.5% (85.1-91.3)
Wairapapa	14	2	87.5% (61.7-98.4)
Bay of Plenty	54	8	87.1% (76.1-94.3)
Southland	45	9	83.3% (70.7-92.1)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 96 where ethnicity has been classified based on the sole/combined classification of ethnicity. As with the univariate results, seroprevalence was significantly higher in age groups 11-15, 16-24 and 25-44 compared with the 6-10 age group. The adjusted seroprevalence was also significantly higher in males than females. As with the univariate results, the adjusted seroprevalence was significantly lower in sole Maori and Pacific groups compared with sole Europeans. Seroprevalence was also significantly lower in participants resident in Counties-Manukau and Southland DHBs.

Table 96 Multivariate analysis of factors contributing to the seroprevalence of tetanus

Variable	Categories	Odds ratio (95% CI)
Age	6-10	1
	11-15	3.01 (1.92-4.73)
	16-24	3.45 (2.12-5.59)
	25-44	3.11 (1.93-5.01)
	45+	1.25 (0.82-1.90)
Ethnicity	European	1
	Maori	0.49 (0.28-0.88)
	Pacific	0.32 (0.17-0.58)
	Asian	0.90 (0.48-1.68)
	>1group	1.16 (0.66-2.05)
Sex	Female	1
	Male	1.47 (1.11-1.94)
DHB	Auckland	1
	Bay of Plenty	0.42 (0.18-1.00)
	Canterbury	1.08 (0.61-1.90)
	Capital Coast	0.72 (0.39-1.32)
	Counties Manukau	0.55 (0.34-0.91)
	Hawkes Bay	5.21 (0.69-39.23)
	Midcentral	0.89 (0.35-2.26)
	Nelson Marlborough	1.03 (0.30-3.63)
	Otago	0.74 (0.29-1.89)
	South Canterbury	0.66 (0.25-1.74)
	Southland	0.39 (0.16-0.94)
	Waikato	0.92 (0.48-1.75)
	Wairapapa	1.03 (0.13-8.43)
	Waitemata	0.62 (0.37-1.03)

Toxoplasma

The toxoplasma analyses were limited to two age groups: 16-24 and 25-44. Crude age-specific results for toxoplasma are shown in Table 97. The seroprevalence was significantly higher in the 25-44 age group compared with the 16-24 age group ($P<0.0001$). Under 1% of the results were in the equivocal range.

Table 97 Crude age-specific toxoplasma results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
16-24	118	476	1	19.8% (16.7-23.3)	20.0% (16.9-23.4)
25-44	198	397	7	32.9% (29.1-36.8)	34.1% (30.3-38.0)

The seroprevalence was higher in males than females (see Table 98, $P=0.05$ when equivocal results classified as immune and $P=0.03$ when equivocal results classified as non-immune).

Table 98 Toxoplasma status by sex, univariate analysis

Sex	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Male	153	365	5	29.3% (25.4-33.4)	30.2% (26.3-34.3)
Female	163	508	3	24.2% (21.0-27.6)	24.6% (21.4-28.1)

Ethnicity was considered using two methods as previously described. The toxoplasma univariate results are presented in Tables 99 and 100. There were no statistically significant differences in seroprevelance by ethnicity for toxoplasma.

Table 99 Toxoplasma status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	272	745	7	26.6% (23.9-29.4)	27.3% (24.5-30.1)
Maori	38	79	0	32.5% (24.1-41.8)	32.5% (24.1-41.8)
Pacific	15	55	1	21.1% (12.3-32.4)	22.5% (13.5-34.0)
Asian	10	55	0	15.4% (7.6-26.5)	15.4% (7.6-26.5)
Middle East/ Latin America/ Africa	0	5	0	0.0% (0-52.2)	0.0% (0-52.2)
Other	4	2	0	66.7% (22.3-95.7)	66.7% (22.3-95.7)

Table 100 Toxoplasma status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	247	684	7	26.3% (23.5-29.3)	27.1% (24.3-30.0)
Maori	16	28	0	36.4% (22.4-52.2)	36.4% (22.4-52.2)
Pacific	10	38	1	20.4% (10.2-34.3)	22.5% (11.8-36.6)
Asian	9	50	0	15.3% (7.2-27.0)	15.3% (7.2-27.0)
Middle East/ Latin America/ Africa	0	5	0	0.0% (0-52.2)	0.0% (0-52.2)
Other	2	2	0	50.0% (6.8-93.2)	50.0% (6.8-93.2)
> 1 ethnic group	27	65	0	29.3% (20.3-39.8)	29.3% (20.3-39.8)

Participants were classified into DHB regions based on their residential address. Univariate analyses for toxoplasma by DHB region are presented in Table 101. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 101 Toxoplasma status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Lakes	3	1	0	75.0% (19.4-99.4)	75.0% (19.4-99.4)
Whanganui	2	2	0	50.0% (6.8-93.2)	50.0% (6.8-93.2)
Nelson Marlborough	7	12	0	36.8% (16.3-61.6)	36.8% (16.3-61.6)
Northland	1	2	0	33.3% (0.8-90.6)	33.3% (0.8-90.6)
Waikato	23	46	0	33.3% (22.4-45.7)	33.3% (22.4-45.7)
Counties Manukau	69	151	3	30.9% (24.9-37.5)	32.3% (26.2-38.9)
Midcentral	11	25	0	30.6% (16.3-48.1)	30.6% (16.3-48.1)
Auckland	60	146	1	29.0% (22.9-35.7)	29.5% (23.4-36.2)
Otago	15	45	1	24.6% (14.5-37.3)	26.2% (15.8-39.1)
Capital Coast	19	58	1	24.4% (15.3-35.4)	25.6% (16.4-36.8)
Waitemata	53	178	2	22.7% (17.5-28.7)	23.6% (18.3-29.6)
Hawkes Bay	11	38	0	22.4% (11.8-36.6)	22.4% (11.8-36.6)
Canterbury	34	129	0	20.9% (14.9-27.9)	20.9% (14.9-27.9)
Bay of Plenty	7	29	0	19.4% (8.2-36.0)	19.4% (8.2-36.0)
Hutt Valley	1	7	0	12.5% (0.3-52.7)	12.5% (0.3-52.7)
Southland	0	1	0	0.0% (0-97.5)	0.0% (0-97.5)
Taranaki	0	1	0	0.0% (0-97.5)	0.0% (0-97.5)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 102 where ethnicity has been classified based on the sole/combined classification for ethnicity. Results were similar whether equivocal results were treated as immune or non-immune except for some variation on seroprevalence by DHB region. The age group 16-24 had significantly lower levels of immunity than the reference category (25-44 years), Asians had significantly lower levels of immunity compared with Europeans.

Table 102 Multivariate analysis of factors contributing to the seroprevalence of toxoplasma

Variable	Categories	Odds ratio (95% CI)	
		Equivocal=non-immune	Equivocal=immune
Age	16-24	0.49 (0.37-0.64)	0.47 (0.35-0.61)
	25-44	1	1
Ethnicity	European	1	1
	Maori	1.52 (0.78-2.95)	1.46 (0.75-2.84)
	Pacific	0.67 (0.32-1.41)	0.72 (0.35-1.49)
	Asian	0.43 (0.20-0.91)	0.41 (0.19-0.86)
	Other	2.82 (0.37-21.41)	2.67 (0.35-20.42)
	>1group	1.26 (0.77-2.05)	1.22 (0.75-1.98)
Sex	Female	1	1
	male	1.30 (0.99-1.70)	1.33 (1.02-1.74)
DHB	Auckland	1	1
	Bay of Plenty	0.56 (0.23-1.37)	0.54 (0.22-1.33)
	Canterbury	0.68 (0.41-1.11)	0.66 (0.40-1.09)
	Capital Coast	0.79 (0.43-1.45)	0.82 (0.45-1.50)
	Counties Manukau	1.17 (0.76-1.81)	1.22 (0.79-1.87)
	Hawkes Bay	0.69 (0.33-1.46)	0.67 (0.32-1.42)
	Hutt Valley	0.37 (0.04-3.15)	0.36 (0.04-3.08)
	Lakes	8.67 (0.85-88.28)	8.61 (0.84-87.70)
	Midcentral	1.17 (0.53-2.56)	1.14 (0.52-2.51)
	Nelson Marlborough	1.27 (0.44-3.62)	1.23 (0.43-3.52)
	Northland	1.15 (0.10-13.41)	1.13 (0.10-13.20)
	Otago	0.92 (0.47-1.80)	0.98 (0.50-1.91)
	Waikato	1.24 (0.67-2.27)	1.21 (0.66-2.23)
	Waitemata	0.71 (0.46-1.11)	0.73 (0.47-1.13)
	Whanganui	4.04 (0.54-30.33)	3.95 (0.53-29.52)

Varicella

Crude age-specific results for varicella are shown in Table 103.

Table 103 Crude age-specific varicella results

Age group	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)[1]	Prevalence (assuming equivocal=immune)[2]
6-10	403	59	3	86.7% (83.2-89.6)	87.3% (83.9-90.2)
11-15	552	33	4	93.7% (91.4-95.5)	94.4% (92.2-96.1)
16-24	574	20	3	96.1% (94.3-97.5)	96.6% (94.9-97.9)
25-44	583	17	2	96.8% (95.1-98.1)	97.2% (95.5-98.3)

[1] Significant linear trend, $P<0.0001$
[2] Significant linear trend, $P<0.0001$

Under 1% of the results were in the equivocal range so results for varicella were similar irrespective of the method of analysing equivocal results. There was a significant linear trend in seroprevalence with increasing age (see Table 103). The seroprevalence was also significantly higher in the 11-15 age group compared with

the 6-10 age group but comparisons between age groups 16-24 and 11-15, and 25-44 with 16-24 did not reach statistical significance (see Table 104).

Table 104 Comparison of age specific varicella prevalence estimates, univariate analysis

Age group 1	Age group 2	equivocal=nonimmune	equivocal=immune
25-44	16-24	96.8 v 96.1, *P*=0.51	97.2 v 96.6, *P*=0.60
25-44	11-15	96.8 v 93.7, *P*=0.01	97.2 v 94.4, *P*-=0.02
25-44	6-10	96.8 v 86.7, *P* < 0.0001	97.2 v 87.3, *P*<0.0001
16-24	11-15	96.1 v 93.7, *P*=0.06	96.6 v 94.4, *P*=0.06
16-24	6-10	96.1 v 86.7, *P*<0.0001	96.6 v 87.3, *P*<0.0001
11-15	6-10	93.7 v 86.7, *P*=0.0001	94.4 v 87.3, *P*=0.0001

The seroprevalence was lower in males than females (see Table 105). This association applied when equivocal results were treated as non-immune (*P*=0.01) and as immune (*P*=0.03).

Table 105 Varicella status by sex, univariate analysis

Sex	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Male	980	73	8	92.4% (90.6-93.9)	93.1% (91.4-94.6)
Female	1130	56	4	95.0% (93.6-96.1)	95.3% (93.9-96.4)

Ethnicity was considered using two methods as previously described. The varicella univariate results are presented in Tables 106 and 107. The estimated seroprevalence was low in the Asian group in both analyses. For example, when comparing sole Asian with sole European groups, the prevalence in the sole Asian group was significantly lower than in the sole European group (P<0.0001).

Table 106 Varicella status by level 1 total ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	1761	90	7	94.8% (93.7-95.7)	95.2% (94.1-96.1)
Maori	239	15	0	94.1% (90.4-96.7)	94.1% (90.4-96.7)
Pacific	142	5	1	95.9% (91.4-98.5)	96.6% (92.3-98.9)
Asian	145	27	3	82.9% (76.4-88.1)	84.6% (78.4-89.6)
Middle East/ Latin America/ Africa	12	1	1	85.7% (57.2-98.2)	92.9% (66.1-99.8)
Other	13	1	0	92.9% (66.1-99.8)	92.9% (66.1-99.8)

Table 107 Varicella status by level 1 sole/combined ethnicity data

Ethnicity	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
European	1579	80	7	94.8% (93.6-95.8)	95.2% (94.1-96.2)
Maori	91	4	0	95.8% (89.6-98.8)	95.8% (89.6-98.8)
Pacific	85	4	1	94.4% (87.5-98.2)	95.6% (89.0-98.8)
Asian	125	25	3	82.2% (75.2-88.0)	84.2% (77.4-89.6)
Middle East/ Latin America/ Africa	11	1	1	84.6% (54.6-98.1)	92.3% (64.0-99.8)
Other	11	1	0	91.7% (61.5-99.8)	91.7% (61.5-99.8)
> 1 ethnic group	194	12	0	94.2% (90.0-97.0)	94.2% (90.0-97.0)

Participants were classified into DHB regions based on their residential address. Univariate analyses for varicella by DHB region are presented in Table 108. These are ordered by prevalence for the analysis where equivocal is assumed to be immune but note the sample sizes are very small in some DHBs. It should also be noted that some age groups are not represented in some regions so these univariate results should be treated with a high level of caution.

Table 108 Varicella status by DHB region

DHB	Positive	Negative	Equivocal	Prevalence (assuming equivocal=nonimmune)	Prevalence (assuming equivocal=immune)
Hawkes Bay	49	0	0	100.0% (92.7-100)	100.0% (92.7-100)
Bay of Plenty	36	0	0	100.0% (90.3-100)	100.0% (90.3-100)
Wairarapa	16	0	0	100.0% (79.4-100)	100.0% (79.4-100)
Lakes	13	0	0	100.0% (75.3-100)	100.0% (75.3-100)
Whanganui	8	0	0	100.0% (63.1-100)	100.0% (63.1-100)
Northland	3	0	0	100.0% (29.2-100)	100.0% (29.2-100)
Taranaki	1	0	0	100.0% (2.5-100)	100.0% (2.5-100)
Waitemata	339	12	1	96.3% (93.8-98.0)	96.6% (94.1-98.2)
Capital Coast	207	8	1	95.8% (92.2-98.1)	96.3% (92.8-98.4)
Southland	52	2	0	96.3% (87.3-99.5)	96.3% (87.3-99.5)
Midcentral	71	4	0	94.7% (86.9-98.5)	94.7% (86.9-98.5)
Canterbury	339	21	2	93.6% (90.6-95.9)	94.2% (91.3-96.4)
Counties Manukau	294	19	2	93.3% (90.0-95.8)	94.0% (90.7-96.3)
Waikato	203	13	0	94.0% (89.9-96.8)	94.0% (89.9-96.8)
Auckland	322	23	6	91.7% (88.3-94.4)	93.5% (90.3-95.8)
Hutt Valley	7	1	0	87.5% (47.3-99.7)	87.5% (47.3-99.7)
Nelson Marlborough	33	5	0	86.8% (71.9-95.6)	86.9% (71.9-95.6)
South Canterbury	49	8	0	86.0% (74.2-93.7)	86.0% (74.2-93.7)
Otago	52	10	0	83.9% (72.3-92.0)	83.9% (72.3-92.0)

Logistic regression analyses were conducted with the inclusion of age, ethnicity, sex and DHB in each model. Results are presented in Table 109 where ethnicity has been classified based on the sole/combined classification of ethnicity. Results were similar whether equivocal results were treated as immune or non-immune except for some variation on seroprevalence by DHB region. The age groups 11-15, 16-24 and 25-44 had significantly higher levels of immunity than the reference category (6-10 years), males and Otago residents had significantly lower seroprevalence estimates than their reference categories (females and Auckland respectively).

Table 109 Multivariate analysis of factors contributing to the seroprevalence of varicella

Variable	Categories	Odds ratio (95% CI)	
		Equivocal=non-immune	Equivocal=immune
Age	6-10	1	1
	11-15	2.07 (1.32-3.24)	2.18 (1.27-3.47)
	16-24	4.61 (2.57-8.29)	5.29 (2.82-9.93)
	25-44	5.13 (2.84-9.28)	5.50 (2.94-10.28)
Ethnicity	European	1	1
	Maori	1.23 (0.43-3.55)	1.13 (0.39-3.26)
	Pacific	0.97 (0.37-2.57)	1.06 (0.36-3.09)
	Asian	0.32 (0.19-0.56)	0.32 (0.18-0.56)
	Middle East	0.35 (0.07-1.72)	0.70 (0.08-5.84)
	Other	0.53 (0.06-4.45)	0.50 (0.06-4.21)
	>1group	1.03 (0.54-1.96)	0.92 (0.48-1.77)
Sex	Female	1	1
	male	0.66 (0.45-0.95)	0.69 (0.47-1.01)
DHB	Auckland	1	1
	Canterbury	1.22 (0.65-2.29)	1.03 (0.52-2.03)
	Capital Coast	2.10 (0.93-4.74)	1.84 (0.77-4.39)
	Counties Manukau	0.94 (0.50-1.75)	0.78 (0.40-1.53)
	Hutt Valley	0.20 (0.02-1.76)	0.15 (0.02-1.31)
	Midcentral	1.30 (0.42-4.04)	1.00 (0.32-3.18)
	Nelson Marlborough	0.48 (0.15-1.55)	0.37 (0.11-1.22)
	Otago	0.18 (0.08-0.44)	0.13 (0.05-0.32)
	South Canterbury	0.71 (0.29-1.76)	0.55 (0.22-1.41)
	Southland	3.17 (0.70-14.34)	2.48 (0.54-11.45)
	Waikato	1.62 (0.77-3.42)	1.27 (0.58-2.76)
	Waitemata	1.62 (1.04-4.39)	1.75 (0.81-3.76)

Discussion

This section is divided into firstly, general comments and secondly, discussion about specific diseases. The general comments are relevant to the discussion of each specific disease.

Selection characteristics

There were potential sources of selection bias in the sample selected. These sources included:

- Use of a blood donor population for the three adult age groups, which is likely to select a healthier population than the general population. If the parents of blood donors were also more health conscious than parents of non-blood donors at the time their children were due to receive their childhood vaccinations then these donors (i.e. the current adult participants) are likely to have come from a population with higher childhood vaccination coverage levels and may therefore result in overestimation of seroprevalence due to high vaccination rates. However, a very high participation rate was achieved in this population (98-99%) across the three adult age groups minimising the effect of non-participation as a source of bias. It should also be observed that groups who were not vaccinated in childhood may be more likely to have natural active immunity from exposure to the disease, which may result in longer lasting immunity potentially negating any high childhood vaccination coverage levels amongst the blood donors.
- Use of the community laboratories as a source of samples in the paediatric age groups. There may be differences between the general population and children presenting for a diagnostic blood sample that resulted in bias. However, we chose this approach to maximise participation, recognising the low likelihood of interest in participating if a needle was required specifically for the study. The estimated participation rate was 74% and 77% in the two paediatric age groups so, while there was some non-participation this was much lower than would have resulted from alternative approaches.
- While our reported participation rate was 74% and 77% in the two paediatric age groups, these estimates were reliant on accurate reporting by the participating laboratories. It is therefore possible that these estimates are inaccurate. Some of the participating laboratories were paid a sum of money for the work required with people who didn't participate, thus there was no particular incentive for those laboratories to under-report participation. Balancing this, the participation rate was based on information provided in weekly 'tally sheets' provided from each participating laboratory. It is possible that some eligible people who did not participate were not recorded on the tally sheet, thus resulting in an overestimation of the participation rate.

General information biases

There were a number of potential sources of measurement error for the variables of interest in the study. Such misclassification may have resulted in bias in the data collected by questionnaire (age, sex, ethnicity, DHB region) and in the serological analysis. General sources of bias that may have applied across all diseases included:

- Incorrect classification of demographic details through incorrect completion of the form and coding errors in data entry
- Misclassification of the serological status.

The magnitude of misclassification of demographic details is likely to be small. Misclassification of serological status is more complicated and is likely to vary by disease. This is therefore discussed in more detail under each specific disease.

Effects of slow recruitment

Recruitment was slow in the paediatric age groups. It is possible the true seroprevalence of the diseases investigated changed over time. For example, given New Zealand's history of cyclical epidemics from some vaccine preventable diseases (e.g. pertussis), it is implied that the seroprevalence declines over time for such epidemics to occur. Thus, the seroprevalence estimates for the two paediatric age groups may not represent a true point prevalence at a defined point in time since collection occurred between October 2005 and February 2007. This limitation does not apply to the adult age groups since collection occurred over a more limited time (September 2005 to January 2006).

As a result of the slow recruitment, samples were obtained from a wider range of geographical locations than initially planned. Thus, the participants were more representative of New Zealand's population given the use of a wider catchment population for the study.

The actual sample size was smaller than originally targeted in the 6-10 year age group (actual size was 468 compared with target of 600) resulting in less precise estimates than originally planned.

Diphtheria

The laboratory that conducted the diphtheria testing in the present study normally uses both the diphtheria and tetanus assays to assess ability to mount an immune response by comparing levels pre and post vaccination. As the serosurvey assessed status in one-off samples (and the purpose was to assess seroprevalence using single samples) a different approach was required to the interpretation of results compared with the usual interpretation process. On this occasion results were categorised as positive or negative by reference to:

1. cut off levels using similar techniques used in other serum surveys
2. an understanding of the performance characteristics of the actual test used by the testing laboratory.

The key paper used to determine the cut-off examined the level of antibody that protected against tetanus. They found a cut-off of 0.16IU/ml was reliably predictive of protective antibody activity in serum (Simonsen et al. 1986). This cut off was rounded up to 0.2IU/ml for the current serum survey to keep within the accuracy of the test used. Thus, any results of 0.2IU/ml or higher were categorised as positive and assumed to represent immunity for tetanus. The same level was used as the cut off for diphtheria as other serum surveys have used the same cut-off for tetanus and

diphtheria (Gidding et al. 2005). Gidding et al considered results to be positive at a level of 0.1IU/ml and above for both diphtheria and tetanus.

Keeping the uncertainty about the relationship between measured levels of diphtheria antibodies and immunity in mind the findings suggested:

- There was a significant linear trend in seroprevalence to diphtheria with generally decreasing levels of seroprevalence by age group. The highest seroprevalence level was 77% in the 11-15 age group and this declined to 46% in the 45+ age group.
- Univariate analyses suggested higher seroprevalence levels in the group self-determining as Asian but this difference was not sustained on multivariate analysis.
- Logistic regression (controlling for age, sex, ethnicity and DHB region) suggested the seroprevalence was significantly higher in the 11-15 and 16-24 age groups than the 6-10 age group but was significantly lower in the 25-44 and 45+ groups compared with 6-10 year olds. The seroprevalence was slightly lower in Pacific people than Europeans on multivariate analysis (adjusted OR 0.62, 95% CI 0.39-0.97). Nelson-Marlborough DHB residential location also had a significantly lower seroprevalence level on multivariate analysis (adjusted OR 0.52, 95% CI 0.27-0.98) compared with the Auckland DHB.

The age categories used in the Australian study differed from those in our present study. However, the 93% categorised as immune in the 5-9 age group was higher than the 61% in the 6-10 year age group in our current study. Seventy percent were immune in the 10-19 age group in the Australian study compared with 77% in the 11-15 age group and 71% in the 16-24 age group in our current study. The seroprevalence levels appeared to be similar in the 25-44 age group in the two studies and tended to be higher in the 45+ group in New Zealand compared with the Australian data (New Zealand 46% in the 45+ group compared with 45%, 33% and 29% in the 40-49, 50-59 and 60-69 year age groups respectively in Australia). The Australian study had a further category labelled as partially immune in their study but this category did not correspond to any category in our study so has been ignored in the present comparison. The cut-off for the immune category was lower in the Australian study than our study (0.1IU/ml compared with 0.2IU/ml in our study) so a higher proportion of immune participants would have been expected in the Australian study (Gidding et al. 2005).

A study set in Sweden found significant variation in diphtheria seroprevalence by gender (Bottiger et al. 1998). The results are presented in Table 110.

Table 110 Immunity to diphtheria by sex and age group: Sweden, 1990-1991

Age group (years)[1]	Women (%)	Men (%)
18-25	87.2	93.4
25-35	77.6	87.3
35-45	40.4	61.1
45-60	45.4	64.8
60+	25.8	24.6

[1]Age groups overlap as the original results are presented by date of birth with sample collection occurring over two years.

Herd immunity is thought to occur when approximately 80-85% are vaccinated against diphtheria (Anderson and May 1990). The age specific seroprevalence levels were below this threshold in all groups.

Hepatitis A

Under 1.5% of the hepatitis A results were classified as equivocal so the method of managing these results did not influence the overall results. Under the assumption that equivocal results were consistent with protection, the key results were:

- There was a statistically significant linear trend with increasing seroprevalence levels with increasing age such that the seroprevalence was 8% in 6-10 year olds, 10% in 11-15 year olds, 11% in 16-24 and 28% in 25-44 year olds
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - Age groups 16-24 and 25-44 had significantly higher seroprevalence levels than 6-10 year olds
    - Asian and Pacific peoples had significantly higher seroprevalence levels than Europeans
    - Otago and Canterbury residents had significantly lower seroprevalence levels than Auckland residents

Chapman et al (2000) studied the seroprevalence of viral hepatitis (HAV, HBV and HCV) in Christchurch in the late 1990s. The investigators randomly selected participants from the electoral roll but unfortunately only obtained a participation rate of 30%. Age-specific HAV results were presented in a figure but appeared to have lower positivity rates than in our present study (the seroprevalence in 18-25 year olds was well under 10%, in 26-35 year olds was well under 20%, and in 36-45 year olds was under 20%). This may be due to the selection of a healthier population in the study by Chapman et al. Alternatively, increased use of the Hepatitis A vaccine may have contributed to the higher positivity rates in the present study.

Hepatitis B

Three hepatitis B markers were measured (HBsAg, anti-HBc and anti-HBs). Both anti-HBc and anti-HBs were measured in four age groups (encompassing 6-44 year olds) and HBsAg was measured in the two youngest age groups (6-10 and 11-15). The highest level of equivocal results occurred in the measurement of anti-HBs (4%), with negligible equivocal results for anti-HBc and no equivocal results for HBsAg. Henceforth, it will be assumed the equivocal results represent protection as current thinking is that people who mounted an immune response post HBV vaccination and now have undetectable anti-HBs levels may be protected against hepatitis B infection. There was only one positive HBsAg result giving a seroprevalence of 0.2% (95% CI 0.0-1.2) in the 6-10 age group. Key results for anti-HBc were:

- There was a statistically significant linear trend with an increasing proportion of positive anti-HBc results with increasing age such that the proportion with positive results was 0.4% in 6-10 year olds, 0.7% in 11-15 year olds, 1.8% in 16-24 and 8.6% in 25-44 year olds
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - There were significantly higher levels with positive anti-HBc results in the 25-44 age group compared with 6-10 year olds

    - Maori, Pacific and Asian people also had significantly higher levels of positive anti-HBc results than Europeans
    - Bay of Plenty and Counties Manukau residents had significantly higher levels of positive anti-HBc results than Europeans

It is likely that the results in the adult age groups underestimate the true prevalence of positive anti-HBc results given the use of a blood donor population in these age groups.

Key results for anti-HBs were:

- There was variation in the level with anti-HBs positive results by age such that the age group with the highest level of positive results were those aged 16-24 (65%). The 25-44 age group had the lowest estimated proportion of positive results (36%) though this wasn't significantly lower than the 6-10 age group.
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - There were significantly higher levels with positive anti-HBs results in the 11-15 and 16-24 age group than the 6-10 age group
    - Significantly more Asians were classified as being immune to hepatitis B than Europeans.

A study of New Zealand police and customs personnel conducted in 1987 estimated an HBsAg carrier prevalence of 1.02% in those under 30 years and 0.72% in those aged 30 or over (Blakely et al. 1998). The current study could not produce similar estimates due to the use of blood donors in the adult participants.

Measles

The seroprevalence of measles varied depending on whether equivocal results were treated as immune or non-immune. If equivocal results were treated as immune all four age groups (6-10, 11-15, 16-24 and 25-44) had seroprevalence estimates over 90% with a significant linear trend indicating increasing levels of immunity with increasing age. A similar trend was observed when equivocal results were considered as non-immune but the estimates by age ranged between 77% and 92%.

Recent work suggests that equivocal results obtained by EIA are likely to be coded as positive (representing the presence of neutralising antibodies) when tested by the plaque neutralisation test (PNT) (Tischer et al. 2007b). Others have also used the PNT to arbitrate on equivocal EIA results (Gilbert et al. 2001) and some have indicated that equivocal levels usually indicate low, but not necessarily protective levels of vaccine induced antibodies (MacIntyre et al. 2002). Thus, there is some uncertainty concerning whether equivocal results represent protection from infection but most agree that they indicate the presence of low levels of antibody.

An Australian study compared the use of a convenience sample with a random cluster sample (Kelly et al. 2002). Both samples produced similar results. In the random sample 95% of children aged 6-12 and 93% of 14-16 year olds were classified as immune to measles. Under the assumption that equivocal results represent immunity, these levels were similar to those found in our study.

In the UK susceptibility to measles rose from 6% to 9.2% in 7-14 year olds between 1986 and 1991. A mass campaign was subsequently conducted to immunise all

school aged children (Osborne et al. 2000). The impact of the 1998 Australian Measles Control Campaign was assessed. Amongst individuals aged 1-18 years, the proportion immune to measles rose from 85% before to 90% after the campaign (Gilbert et al. 2001). An additional 3% of children had equivocal results both before and after the campaign.

A seroprevalence study in Luxembourg collected samples from school aged children and adults (Mossong et al. 2003). Results directly adjusted to the 2000 Luxembourg population found 96.6% of people were positive and 1.6% were equivocal for anti-measles virus antibodies.

Further investigation of the results where it is assumed equivocal indicates immunity suggest the following findings:

- There is a statistically significant but clinically small linear trend indicating increasing levels of protection with increasing age
- There was no difference by sex
- There was no difference by ethnicity (both in the total ethnicity classification and the sole/combined classification)
- Logistic regression (controlling for age, sex, ethnicity and DHB region) suggested the seroprevalence was significantly higher in the 25-44 age group than the 6-10 age group and the seroprevalence was low in Otago (when compared with Auckland).

It has been estimated that herd immunity is achieved when approximately 95% of the population is immune to measles (Gay 2004). Even when it is assumed equivocal results represent immunity, the estimated seroprevalence levels in this study indicate borderline achievement of the necessary levels with 91% and 93% seroprevalence in the 11-15 and 6-10 age groups respectively and 96% and 97% seroprevalence in the 16-24 and 25-44 age groups respectively.

Mumps

There were similarities between the results for measles and for mumps. Specifically there was a statistically significant linear trend with increasing seroprevalence with increasing age. However, unlike with measles, cross tabulations and results of logistic regression had similar statistically significant findings whether equivocal results were treated as immune or non-immune. There was support for equivocal results by EIA being likely to be classified as positive by PNT. For example, Backhouse et al (2006) found 88% of results equivocal by the Enzygnost enzyme linked immunosorbent assay (ELISA) were positive by PNT and 70% of the Microimmune equivocal results were positive by PNT. The European Sero-Epidemiology Network standardised mumps assays (Tischer et al. 2007a). In that study, equivocal results were assumed to be positive and the authors noted that this contributed to the imperfect specificity estimated with three equivocal and two positive sera on EIA being negative by PNT (specificity was 87.1%). The estimated sensitivity was 92.8%. Based on the findings and approaches of the two studies above it seems more appropriate to rely on the results that assume equivocal results represent immunity. Based on that assumption, key results for mumps were:

- Seroprevalence ranged from 87.6% (6-10 age group) up to 94.0% (16-24 age group)

- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - Seroprevalence was significantly higher in the 16-24 and 25-44 year age groups compared with the 6-10 year age group
    - Classification as more than one ethnic group (in the sole/combined data) was associated with a significantly higher seroprevalence compared with Europeans.

An Australian study compared the use of a convenience sample with a random cluster sample (Kelly et al. 2002). In the random sample 92% of children aged 6-12 and 87% of 14-16 year olds were classified as immune to mumps. Under the assumption that equivocal results represent immunity, these levels were similar to those found in our study (88% immune in the 6-10 age group and 89% in the 11-15 age group).

The seroprevalence study of school aged children set in Luxembourg described under measles also tested for mumps (Mossong et al. 2003). Results directly adjusted to the 2000 Luxembourg population found 75.4% of people were positive and 14.1% were equivocal for anti-measles virus antibodies. These results are therefore similar to the current results in this New Zealand study.

A study of Israeli military recruits aged 18-19 found a seroprevalence of 83.3% in 1999 which compared with 94% in 1987 (Huerta et al. 2006). Equivocal results were treated as negative in both of these studies. The 1999 result was similar to that of our present study.

It has been estimated that herd immunity is achieved when approximately 90-92% of the population is immune to mumps (Anderson and May 1990). Comparing the age specific results for mumps with the estimated levels required for herd immunity reveals a similar pattern to that for measles with the estimates for the two younger age groups being slightly below the herd immunity threshold and those in the two older age groups being slightly above it (assuming equivocal results represent immunity).

Pertussis

Various groups have focussed on high titre results when conducting seroprevalence surveys of pertussis (Giammanco et al. 2003; Pebody et al. 2005). The same approach has been used in analysing the results in this New Zealand serosurvey. High titres of pertussis toxin antibody are predictive of recent infection with *B. pertussis*. There do not currently appear to be any acceptable methods of assessing the level of immunity to pertussis. Therefore, the focus of the analysis was on the results categorised as positive by serology (implying recent infection). Key results were:

- There was a statistically significant linear trend consisting of decreasing seroprevalence levels with increasing age such that the seroprevalence was 15% in 6-10 year olds, 15% in 11-15 year olds, 10% in 16-24 year olds and 6% in 25-44 year olds
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - 16-24 year olds and 25-44 year olds had a significantly lower seroprevalence than the 6-10 age group.
    - There were no significant differences by gender, ethnicity or residential area.

However, note that these results refer to recent infection, so appear consistent with increasing levels of protection with increasing age.

Pebody et al (2005) measured pertussis toxin levels in a seroprevalence study in Western Europe. Pertussis toxin antibody is predictive of recent infection with *Bordetella pertussis*. In countries with high pertussis vaccination coverage the prevalence of high-titre sera was higher in the 10-19 age group than the 3-9 year age group. For example, in Finland 7% of the 10-19 group and 2% of the 3-9 age group were positive with the corresponding data for France being 5% and 1%, The Netherlands being 2% and 1%, and East Germany being 6% and 3%. This contrasted with low coverage countries: England and Wales 3% in both age groups, and West Germany 5% in 3-9 year olds and 3% in the 10-19 age group.

This approach of focussing on high titre results (indicative of recent infection) was consistent with the approach used in standardising pertussis assay results in the European Sero-Epidemiology Network (Giammanco et al. 2003).

Herd immunity is thought to occur when approximately 92-95% are vaccinated against pertussis (Anderson and May 1990). Since the results suggest ongoing infection it appears unlikely that this level of the population is protected against pertussis.

Polio

All results were classified as positive or negative (i.e. there were no equivocals) for the three polioviruses. Seroprevalence estimates were higher and showed less variation by age group, ethnicity and DHB region for poliovirus types 1 and 2 than for type 3. Key results were:

- Seroprevalence for type 1 ranged between 96% and 97% in the three age groups tested (6-10, 11-15 and 16-24 years)
- Seroprevalence for type 2 ranged between 98% and 99% in the three age groups tested (6-10, 11-15 and 16-24 years)
- Seroprevalence declined with increasing age for type 3 with 91% being seropositive in the 6-10 age group, 89% in the 11-15 age group and 86% in the 16-24 age group.
- There were no significant associations detected in the logistic regression analyses for poliovirus types 1 and 2.
- For poliovirus type 3 the following significant associations were observed:
    - The 16-24 age group had significantly lower levels of immunity than the reference category (6-10 years)
    - Self determining as belonging to more than one ethnic group was associated with significantly lower levels of immunity than the reference category (Europeans)
    - Three regions had significantly lower seroprevalence estimates when compared with the reference category of Auckland. These were:
        - Hawkes Bay (OR 0.30, 95% CI 0.11-0.84)
        - Hutt Valley (OR 0.04, 95% CI 0.00-0.44)
        - Nelson-Marlborough (OR 0.31, 95% CI 0.11-0.88).

A study set in Sweden found some variation in polio seroprevalence by gender (Bottiger et al. 1998). The results are presented in Table 111. Overall, the results by age group were similar in this Swedish study and our New Zealand study for poliovirus types 1 and 2. However, seroprevalence estimates were lower for poliovirus type 3 in our New Zealand study.

Table 111 Immunity to poliovirus types 1, 2 and 3 by sex and age group: Sweden, 1990-1991

Age group (years)[1]	Women (%)	Men (%)
Type 1		
18-25	99.6	98.8
25-35	98.8	95.8
35-45	97.6	95.4
45-60	99.1	95.9
60+	96.8	93.0
Type 2		
18-25	99.7	99.7
25-35	99.3	99.1
35-45	99.6	99.6
45-60	99.7	95.7
60+	96.5	93.8
Type 3		
18-25	98.8	97.3
25-35	97.5	97.6
35-45	95.5	89.7
45-60	99.1	94.4
60+	96.4	90.0

[1]Age groups overlap as the original results are presented by date of birth with sample collection occurring over two years.

Rubella

Only 4% of the results were equivocal in rubella testing and this had little impact on the cross tabulation comparisons. In keeping with the approach for measles and mumps, under the assumption that equivocal results represented protection, key results were:

- Seroprevalence estimates were 90% and over across the four age groups studied (up to 44 years) and there was no significant linear trend
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - The 16-24 age group had significant higher seroprevalence levels than the 6-10 age group
    - Males had significantly lower seroprevalence levels than females
    - There was interaction between age and gender with males having lower seroprevalence estimates in all four age groups though this only reached statistical significance in the oldest age group (25-44 years).

An Australian study compared the use of a convenience sample with a random cluster sample (Kelly et al. 2002). In the random sample 99% of children aged 6-12 and 98% of 14-16 year olds were classified as immune to rubella. These levels were higher than those found in our study even when equivocal results were treated as immune (90% immune in the 6-10 age group and 91% in the 11-15 age group).

The seroprevalence study in Luxembourg described earlier also tested for rubella (Mossong et al. 2003). Results directly adjusted to the 2000 Luxembourg population found 95.7% of people were positive and 0.6% were equivocal for anti-rubella virus

antibodies. These results provide slightly higher seroprevelance estimates than those found in our New Zealand study.

The impact of the 1998 Australian Measles Control Campaign was also assessed for its effect on levels of rubella immunity. Amongst individuals aged 1-18 years, the proportion immune to rubella rose from 83% before to 91% after the campaign (Gilbert et al. 2001). An additional 1% of children had equivocal results both before and after the campaign. The post campaign estimates were similar to those in our present New Zealand study.

A seroepidemiology study was conducted in association with the third NHANES study in the USA during 1988-1994. Rubella seropositivity rates in this study were:

- 6-11 years: 92%
- 12-19 years: 83%
- 20-29 years: 85%
- 30-39 years: 89%
- 40+ years: ≥93% (Dykewicz et al. 2001).

These results were based on the assumption that serum rubella IgG antibody levels 10 IU or higher represented a positive result. In the present New Zealand study results in the range 10-15 IU were treated as equivocal and higher levels were treated as positive. On that basis, the estimated levels of protection were higher in the New Zealand study in the 11-15 and 16-24 age groups.

A study of rubella status in school girls and pregnant women set in Turkey found 92.5% of the schoolgirls were rubella seropositive (Karakoc et al. 2003).

Herd immunity is thought to occur when approximately 85-87% are vaccinated against rubella (Anderson and May 1990). All age specific estimates were above this level when it was assumed equivocal results represented immunity and either approximated or were above the threshold level when equivocal results were treated as non-immune.

Tetanus

The considerations applied to setting a threshold for protection to tetanus by antibody specific levels were outlined under diphtheria. Keeping the uncertainty about the relationship between measured levels of tetanus antibodies and immunity in mind the findings suggest:

- An inverted U shape for seroprevalence by age with 94-95% seroprevalence in the 11-15, 16-24 and 25-44 age groups but only 85% and 89% in age groups 6-10 and 45+ respectively
- Univariate analyses suggested lower seroprevalence levels in Pacific peoples and, to a lesser extent, Maori than the European population.
- Logistic regression (controlling for age, sex, ethnicity and DHB region) was consistent with the univariate results for age group and ethnicity (significantly lower levels in Maori and Pacific peoples) but males had a significantly higher adjusted OR than one (consistent with increased seroprevelance amongst males). Counties-Manukau had a significantly lower adjusted OR when compared with Auckland (OR 0.55, 95% CI 0.34-0.91).

The age categories used in the Australian study differed from those in our present study. Bearing in mind the limitation of the different age categories a higher proportion of participants in the New Zealand study were immune in the respective ages than the Australian study. With the exception of the 6-10 category this absolute difference approximated 15-20%. The Australian study had a further category labelled as partially immune in their study but this category did not correspond to any category in our study so has been ignored in the present comparison. The cut-off for the immune category was lower in the Australian study than our study (0.1IU/ml compared with 0.2IU/ml in our study) so a higher proportion of immune participants would have been expected in the Australian study (Gidding et al. 2005).

Serum samples were tested for immunity to tetanus from the NHANES III study conducted in the USA (Gergen et al. 1995). The samples were collected during 1988-1991. The seroprevalence varied by age group but was over 80% in the 6-11, 12-19, 20-29 and 30-39 groups but with declining levels thereafter (down to 28% in the 70+ age group.

A study set in Sweden found significant variation in tetanus seroprevalence by gender (Bottiger et al. 1998). The results are presented in Table 112. Again the New Zealand estimates were higher than those in the Swedish study.

Table 112 Immunity to tetanus by sex and age group: Sweden, 1990-1991

Age group (years)[1]	Women (%)	Men (%)
18-25	98.6	98.6
25-35	88.3	95.4
35-45	53.5	89.9
45-60	44.3	83.2
60+	30.3	50.1

[1]Age groups overlap as the original results are presented by date of birth with sample collection occurring over two years.

Toxoplasma

The toxoplasma analyses were restricted to participants in the 16-24 and 25-44 age groups. Under 1% of the toxoplasma results were classified as equivocal so the method of managing these results did not influence the overall results. Under the assumption that equivocal results were consistent with protection, the key results were:

- Twenty per cent of participants aged 16-24 and 34% of participants aged 25-44 years had past contact with toxoplasma.
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - The 16-24 age group had significantly lower past contact with toxoplasma (OR 0.47, 95% CI 0.35-0.61)
    - Males had a significantly higher level of past contact than females (OR 1.33, 95% CI 1.02-1.74)

Varicella

Under 1% of the varicella results were classified as equivocal so the method of managing these results did not influence the overall results. Under the assumption that equivocal results were consistent with protection, the key results were:

- There was a statistically significant linear trend consisting of increasing seroprevalence levels with increasing age such that the seroprevalence was 87% in 6-10 year olds, 94% in 11-15 year olds, and 97% in 16-24 and 25-44 year olds
- On logistic regression, controlling for age, sex, ethnicity and DHB region
    - 6-10 year olds had a significantly lower seroprevalence than the other three age groups
    - Males had a significantly lower seroprevalence than females
    - Otago residents had a significantly lower seroprevalence than Auckland residents

An Australian study compared the use of a convenience sample with a random cluster sample (Kelly et al. 2002). In the random sample 76% of children aged 6-12 and 82% of 14-16 year olds were classified as immune to varicella. These levels were lower than those found in our study even when equivocal results were treated as immune (87% immune in the 6-10 age group and 94% in the 11-15 age group). Seroprevalence levels were also lower in an Italian study where 62% of 5-9 year olds and 82% of 10-14 year olds had positive results (Gabutti et al. 2001).

The level of protection required for herd immunity to varicella is less clear than for other vaccine preventable diseases. Data from US surveillance documented an approximate 80% decline in varicella incidence in association with 75% vaccination coverage. The US Public Health Service goal for varicella immunisation levels among young and school-aged children are 90% and 95% respectively (Frenkel 2004). Based on the age specific seroprevalence estimates in this study, the 6-10 year age group may be below the necessary threshold but other groups appear satisfactory.

Conclusions

Overall, seroprevalence estimates were lower for some vaccine preventable diseases than others. On direct comparison between diseases the seroprevalence was low for:

- tetanus in the 6-10 age group
- varicella in the 6-10 age group
- diphtheria (particularly the 25-44 and 45+ age groups)
- rubella in the 6-10 and 11-15 age groups
- poliovirus type 3 (particularly in the 25-44 age group)

However, comparisons between the current study and overseas studies revealed the results for:

- tetanus found higher levels of seroprevalence in the current New Zealand study than other studies set in Australia, the USA and Sweden
- varicella found higher levels of seroprevalence in the current New Zealand study than an Australian study
- diphtheria were similar to those found in Australia and Sweden

- rubella found lower levels of seroprevalence in the current New Zealand study than other studies set in Australia and Luxembourg but higher levels when compared with a USA study
- poliovirus type 3 found lower levels of seroprevalence in the current New Zealand study than a Swedish study.

Furthermore, when comparing with accepted herd immunity thresholds:

- the rubella estimates approximated the herd immunity threshold
- given uncertainty about levels for herd immunity associated with varicella it is unclear whether the level estimated in the 6-10 age group is above the herd immunity threshold (age specific estimates were higher in the older age groups).

There were also some diseases with higher seroprevalence estimates that were close to herd immunity thresholds:

- age specific estimates for measles ranged between 91% and 97% with the two youngest age groups being slightly below the herd immunity threshold of 95%
- age specific estimates for mumps ranged between 88% and 94% with the two youngest age groups being slightly below the herd immunity threshold of 90%-92% (though similar results have been found in Australia, Luxembourg and Israel).

Further considerations apply to herd immunity thresholds. The estimates discussed above relate to overall data across New Zealand. Logistic regression analyses revealed some variation by region. For example, Otago residents had lower levels of seroprevalence to both measles and varicella when compared with the reference standard district (Auckland).

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Appendix 1 Test request form

Canterbury Health Laboratories

NATIONAL SEROSURVEY TEST REQUEST FORM

[PLACE LABEL HERE] Participant Identifier			Sample date (dd/mm/yy) Time	Delphic #
DOB (dd / mm / yy)	**M / F** Sex	Community Labs: NHI # BTS: Donor #		Serology ID #
Residential Suburb & Nearest Town		**RS305** Project Code	Collected by (Initials)	

Lab Use only

INSTRUCTIONS: **Collecting Site**: Please refer to Instruction Sheet. **CHLabs:** Do not number, separate or register. Forward to Serology for processing.	**Send Completed Forms & Specime** Canterbury Health Laboratories Cnr Hagley Ave & Tuam Street PO Box 151 CHRISTCHURCH

Which ethnic group or groups do you belong to?

(You may indicate more than one answer)

Circle the number or numbers beside each answer that applies.

1. New Zealand European
2. Maori
3. Samoan
4. Cook Island Maori
5. Tongan
6. Niuean
7. Chinese
8. Indian
9. Other (Please State) _____