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Antibody levels to *Mycoplasma pneumoniae* in sera collected from healthy blood donors of Wellington, New Zealand, during 1976–80

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SUMMARY

The sera of healthy blood donors from the Wellington area of New Zealand, collected between 1976 and 1980, were analysed by the complement fixation test for antibody to *Mycoplasma pneumoniae*. A high prevalence of antibody to this organism was demonstrated and the occurrence of an *M. pneumoniae* epidemic in New Zealand within the survey period was shown to be reflected in the immune status of this healthy adult population. This would suggest that during an epidemic many people within the Wellington community may have *M. pneumoniae* infections involving little overt illness.

INTRODUCTION

Infections with *Mycoplasma pneumoniae* are normally endemic in the populations of temperate climates. However it has been noted in many countries that cyclic increases resulting in prolonged epidemics occur at approximately 5-yearly intervals (Foy *et al.* 1979). Most infections with *M. pneumoniae* are mild or even subclinical with the predominant symptoms being cough, fever and headache. More severe infections may proceed to pneumonia. It has been found that a non-specific febrile illness has been more commonly reported in adults than in children (Communicable Diseases Report, 1982).

Foy *et al.* (1979) demonstrated from the results of a 12-year study of *M. pneumoniae* infections in Seattle, USA, that infection rates in that population varied from approximately 2% in endemic years to 35% in epidemic periods.

In New Zealand there have been no long-term studies of the incidence of infection with *M. pneumoniae*. A report on 100 general practice patients with bronchitis or pneumonia over the period 1967–8 (Gallagher, 1970) claimed that 14% of these were due to the organism. In 1978 a survey of the results from New Zealand laboratories was undertaken by the National Health Institute (NHI) Markham, 1979) which indicated the occurrence of an *M. pneumoniae* epidemic in New Zealand during 1978. In this survey 40% of the cases catalogued were children under 13 years of age.

The National Serum Bank (NSB), maintained by the NHI, provides a basis for long-term epidemiological research in New Zealand. To date it has been used mainly to investigate infections of zoonotic importance such as the studies on antibody levels to *Brucella abortus*, *Toxoplasma gondii*, and *Leptospirae* (Metcalfe *et al.* 1981). Levels of antibody to *Legionella pneumophila* have also been

investigated (Bettelheim, Metcalfe & Sillars, 1982). In the present study the NSB has been used to determine the prevalence of *M. pneumoniae* antibodies in New Zealand over the 5-year period from 1976–80. As sera have been collected annually from Wellington, these collections are particularly suited to a study on the possible cyclic nature of *M. pneumoniae* infections.

MATERIALS AND METHODS

Sera investigated

The standard methods of collecting sera for the NSB were used. Samples were taken by the New Zealand Blood Transfusion Service with the assistance of staff from the NHI. Each serum sample was tested by the Blood Transfusion Service for hepatitis B surface antigen and only non-reactors were accepted into the serum bank. All serum samples were divided into six portions, two to be kept for posterity, one for restricted research, two for general research, and one for the present study. The five serum collections studied in this report were all sampled in the Wellington district in the spring and early summer (August–December) of the years from 1976–80 inclusive. All sera had been kept frozen at −90 °C from the time of collection until the present study.

Mycoplasma pneumoniae *complement fixation test*

All serum specimens were tested by a *M. pneumoniae* complement fixation test (CFT) based on the method of McSwiggan & Taylor (1972). The tests were performed in U-bottomed microtitre plates (Cooke Dynatitre) with a total test volume of 0·1 ml employing antigen, haemolysin and complement manufactured by Behringwerke. Antigen having the same lot number (414029A) was used for all tests. Two units (2 minimal haemolytic doses, MHD) of complement, 2 units (2 optimal sensitizing doses, OSD) of amboceptor, 1·25 units of antigen, and 3% sheep erythrocytes were used. All dilutions were performed in veronal buffered saline, pH 7·2 (Oxoid). Complement fixation took place at 4 °C overnight and the final incubation with the sensitized cells was at 37 °C for 30 min. The lowest serum dilution tested was 1:8. For the purposes of this study a serum showing a titre of $\geqslant 64$ was regarded as positive and a titre of $\geqslant 256$ was regarded as a high positive.

Control sera

Four control sera, which had been obtained from human donors, were included with each batch of sera tested. The results with these controls demonstrated very good reproducibility between batches, the titres being within the ranges obtained by the reference laboratories (Centers for Disease Control, USA, and Public Health Laboratory Services, Colindale, London). The reference ranges for the four sera were < 8, 8–32, 32–128 and > 512. In our hands these ranges were < 8, 16–32, 64–128 and 1024–4096 respectively.

RESULTS

Over the 5-year study period it was possible to examine 1461 sera from the Wellington region. The results, which have been grouped by the year of collection sex and age group of the donor, are presented in Tables 1 and 2. There was a

Table 1. Mycoplasma pneumoniae *complement-fixing titres in males from Wellington*

Date of collection	Age group (years)	Percentage of sera giving titres of								Sera tested: total number
		< 8	8	16	32	64	128	256	⩾ 512	
1976	16–19	13·6	9·1	40·9	27·3	9·1	0·0	0·0	0·0	22
1977		16·7	0·0	44·4	27·8	11·1	0·0	0·0	0·0	18
1978		0·0	11·1	33·3	22·2	22·2	0·0	22·2	0·0	9
1979		0·0	7·7	30·8	38·5	23·1	0·0	0·0	0·0	13
1980		0·0	0·0	60·0	40·0	0·0	0·0	0·0	0·0	5
All collections		9·0	6·0	40·3	29·9	13·4	0·0	1·5	0·0	67
1976	20–29	13·0	4·3	47·8	15·2	10·9	6·5	0·0	2·2	46
1977		0·0	0·0	49·0	36·7	10·2	0·0	0·0	0·0	49
1978		0·0	0·0	24·3	32·4	40·5	2·7	0·0	0·0	37
1979		3·9	17·6	29·4	39·2	5·9	3·9	0·0	0·0	51
1980		2·6	12·8	48·7	23·1	12·8	0·0	0·0	0·0	39
All collections		4·1	7·2	40·1	29·7	14·9	3·6	0·0	0·5	222
1976	30–39	0·0	0·0	39·1	52·2	8·7	4·1	0·0	0·0	23
1977		4·1	4·1	49·0	34·7	6·1	2·0	0·0	0·0	49
1978		0·0	5·6	33·3	44·4	11·1	5·5	0·0	0·0	36
1979		5·1	13·6	23·7	37·3	11·9	8·5	0·0	0·0	59
1980		9·5	11·1	44·4	25·4	7·9	1·6	0·0	0·0	63
All collections		4·8	8·3	37·8	36·1	9·1	3·9	0·0	0·0	230
1976	40–49	4·3	8·7	30·4	43·5	13·0	0·0	0·0	0·0	23
1977		7·9	5·3	36·8	44·7	5·3	0·0	0·0	0·0	38
1978		3·3	16·7	36·7	23·3	16·7	3·3	0·0	0·0	30
1979		7·7	12·8	35·9	20·5	5·1	15·4	2·6	0·0	39
1980		6·7	8·9	57·8	17·8	4·4	4·4	0·0	0·0	45
All collections		6·3	10·3	41·1	28·6	8·0	5·1	0·6	0·0	175
1976	50–65	4·0	4·0	52·0	32·0	4·0	4·0	0·0	0·0	25
1977		3·8	3·8	50·0	25·0	7·7	0·0	0·0	0·0	26
1978		0·0	10·3	37·9	31·0	17·2	0·0	3·4	0·0	29
1979		10·5	18·4	34·2	21·1	15·8	0·0	0·0	0·0	38
1980		10·2	10·2	40·8	22·4	16·3	0·0	0·0	0·0	49
All collections		6·6	10·2	41·9	26·9	13·2	0·6	0·6	0·0	167
1976	All	7·9	5·0	43·2	30·9	9·4	2·9	0·0	0·7	139
1977	males	5·0	2·8	46·1	36·7	7·8	1·7	0·0	0·0	180
1978		0·7	7·8	32·6	32·6	22·0	2·8	1·4	0·0	141
1979		6·0	15·0	30·0	31·5	10·5	6·5	0·5	0·0	200
1980		7·5	10·4	47·8	22·9	10·0	1·5	0·0	0·0	201
All collections	All males	5·6	8·6	40·1	30·7	11·5	3·1	0·3	0·1	861

Table 2. Mycoplasma pneumoniae *complement-fixing titres in females from Wellington*

Date of collection	Age group (years)	Percentage of sera giving titres of								Sera tested: total number
		< 8	8	16	32	64	128	256	⩾ 512	
1976	16–19	0·0	0·0	64·3	35·7	0·0	0·0	0·0	0·0	14
1977		0·0	4·3	39·1	34·8	17·4	4·3	0·0	0·0	23
1978		12·5	0·0	12·5	25·0	12·5	25·0	12·5	0·0	8
1979		0·0	12·5	37·5	0·0	37·5	0·0	12·5	0·0	8
1980		0·0	0·0	62·5	12·5	12·5	12·5	0·0	0·0	8
All collections		1·6	3·3	44·3	26·2	14·8	6·6	3·3	0·0	61
1976	20–29	3·1	12·5	40·6	21·9	15·6	6·3	0·0	0·0	32
1977		10·0	4·0	48·0	24·0	10·0	4·0	0·0	0·0	50
1978		0·0	4·0	28·0	36·0	20·0	12·0	0·0	0·0	25
1979		0·0	4·8	33·3	38·1	23·8	0·0	0·0	0·0	21
1980		0·0	3·8	38·5	38·5	11·5	7·7	0·0	0·0	26
All collections		3·9	5·8	39·6	29·9	14·9	5·8	0·0	0·0	154
1976	30–39	4·3	4·3	30·4	34·8	17·4	8·7	0·0	0·0	23
1977		2·1	4·3	42·6	36·2	10·6	4·3	0·0	0·0	47
1978		0·0	3·7	37·0	33·3	18·5	3·7	0·0	3·7	27
1979		5·7	8·6	31·4	42·9	5·7	5·7	0·0	0·0	35
1980		10·7	10·7	28·6	28·6	14·3	7·1	0·0	0·0	28
All collections		4·4	6·3	35·0	35·6	12·5	5·6	0·0	0·6	160
1976	40–49	13·3	0·0	40·0	33·3	13·3	0·0	0·0	0·0	15
1977		4·2	2·1	56·3	31·3	6·3	0·0	0·0	0·0	48
1978		0·0	9·1	36·4	36·4	13·6	4·5	0·0	0·0	22
1979		0·0	18·9	40·5	29·7	10·8	0·0	0·0	0·0	37
1980		4·5	0·0	27·3	40·9	22·7	4·5	0·0	0·0	22
All collections		3·5	6·9	43·0	33·3	11·8	1·4	0·0	0·0	144
1976	50–65	36·4	0·0	45·5	9·1	9·1	0·0	0·0	0·0	11
1977		11·1	7·4	44·4	37·0	0·0	0·0	0·0	0·0	27
1978		6·3	18·8	50·0	12·5	12·5	0·0	0·0	0·0	16
1979		13·6	36·4	18·2	22·7	4·5	4·5	0·0	0·0	22
1980		0·0	60·0	0·0	40·0	0·0	0·0	0·0	0·0	5
All collections		13·6	19·8	35·8	24·7	4·9	1·2	0·0	0·0	81
1976	All	8·4	5·3	42·1	27·4	12·6	4·2	0·0	0·0	95
1977	females	5·6	4·1	47·2	31·8	8·7	2·6	0·0	0·0	195
1978		2·0	7·1	34·7	30·6	16·3	7·1	1·0	1·0	98
1979		4·1	16·3	32·5	31·7	12·2	2·4	0·8	0·0	123
1980		4·5	7·9	32·6	33·7	14·6	6·7	0·0	0·0	89
All collections	All females	5·0	7·8	39·2	31·2	12·2	4·2	0·3	0·2	600

overall reactor rate (titre ⩾ 8) of 95 % in these collections. Table 3 shows that 15·8 % of sera tested were positive, having titres of ⩾ 64. The year of 1977 showed the lowest number of positive sera in the Wellington population (10·4 %), while the succeeding years of 1978 showed the highest, with 25·9 % of sera being positive. Table 4 gives the percentage of sera giving positive and high positive titres in different age groups.

Table 3. *Percentage of sera giving positive and high positive titres in the complement fixation test from the five collections*

Year of collection	Percentage positive (⩾ 64)			Percentage high positive (⩾ 256)		
	Males	Females	Total	Males	Females	Total
1976	13·0	16·8	14·5	0·7	0·0	0·4
1977	9·5	11·3	10·4	0·0	0·0	0·0
1978	26·2	25·4	25·9	1·4	2·0	1·7
1979	17·5	15·4	16·7	0·5	0·8	0·6
1980	11·5	21·3	14·5	0·0	0·0	0·0
Total	15·0	16·9	15·8	0·3	0·5	0·5

Table 4. *Percentage of sera from donors of various age groups giving positive and high positive titres in the complement-fixation test*

Age (years)	Percentage positive (⩾ 64)			Percentage high positive (⩾ 256)		
	Males	Females	Total	Males	Females	Total
16–19	14·9	21·4	19·5	1·5	3·3	2·3
20–29	19·0	20·7	19·7	0·5	0·0	0·3
30–39	13·0	18·1	15·4	0·0	0·6	0·3
40–49	13·7	13·2	13·5	0·6	0·0	0·3
50–65	14·4	6·1	11·7	0·6	0·0	0·4

DISCUSSION

The acquisition of detectable complement fixing (CF) titres to *M. pneumoniae* has been shown by Ponka & Ukkonen (1983) to begin with babies of approximately 4 months of age. Whether this represents a specific antibody response to the *M. pneumoniae* organism or a non-specific response to various environmental glycolipids that are very similar to *M. pneumoniae* antigens has not yet been determined. However it appears that this antibody response in young people consists primarily of IgM and that with time the CF antibody in an individual demonstrates an increasing proportion of IgG.

High titres of complement fixing antibodies to *M. pneumoniae* may last less than 1 year (Foy *et al.* 1979) or they may last as long as 4 years (Biberfield, 1971) after infection before falling to pre-infection levels. With only a single serum specimen from each donor it is therefore impossible to determine accurately when an infection may have occurred.

The presence of a detectable titre in an individual does not necessarily protect that person from *M. pneumoniae* infections since it has been shown that reinfections are common even in people with circulating antibody (Fernald, Collier & Clyde, 1975). Resistance to infection appears to be more dependent on the local immunological defences of the respiratory tract. Moreover there have been suggestions that disease caused by *M. pneumoniae* may be in part immunologically determined in a host sensitized by prior infections with this organism (Fernald, Clyde & Denny, 1981).

The results presented in Tables 1 and 2 show that there is an appreciable percentage of sera exhibiting positive titres. However only a few (< 0·1 % males, < 0·2 % females) have titres of ⩾ 512. It is also noteworthy that approximately 70 % of all sera have titres of 16 or 32. This is related in the results for both sexes and all collections. Low stable titres (such as 16 and 32) are normally regarded as resulting from past infections with *M. pneumoniae*.

Table 3 shows that there was a distinct increase in the percentage of positive (⩾ 64) and of high positive (⩾ 256) titres in the Wellington Serum Bank collection for 1978. During that year *M. pneumoniae* epidemics were reported in many countries from both the northern and southern hemispheres (Communicable Diseases Report, 1982; Communicable Disease Intelligence, 1978), including New Zealand (Markham, 1979).

The collection with the lowest level of antibody in this series was the 1977 collection, that of the year preceding the epidemic. It is generally believed that epidemics of fairly common organisms can be triggered off when the level of immunity to that organism reaches a critically low level in the population. This is the theory behind herd immunity in vaccination. While the study of circulating antibody instead of local respiratory tract antibody may not be the ideal way to investigate this question, it is interesting to determine what happens after immunity in the 'normal healthy' population falls to very low levels.

Table 4 shows the percentage of positive and high positive sera for donors of various age groups. In general the trend, as expected, is towards a high number of positive sera in the younger people and a decreasing rate with age. However, it is particularly interesting to note the differences between males and females in this area. The lowest percentage positive (⩾ 64) for males is 13 %, found in the 30–39 years age group, and the highest is 19 % found in the 20–29-year-olds. On the whole there is not much variation in the percentage positive rates for the age groups in males. There is a different picture, however, for the female blood donors. The highest rate of positive reactions (21·4 %) is found in the 16–19-year-olds and there is a clear trend of falling antibody levels with age down to a level of 6·1 % positive for females in the 50–65 years age group. Foy *et al.* (1979) noted that rates of pneumonia due to *M. pneumoniae* were higher for females than males in the 30–39 years age group, presumably as a result of close contact with children. In the other adult age groups up to 65 years, the sex distribution for infection is normally about the same (Noah, 1976; Foy *et al.* 1979). The difference in NSB results between males and females was therefore unexpected and needs to be investigated further.

This study demonstrates a high percentage of reactors to *M. pneumoniae* in the Wellington area of New Zealand. A reactor rate of 95 % for the adult population of Wellington is much greater than that of approximately 55 % reported by Ponka & Ukkonen (1983) for Finland. However the frequency of high antibody titres (⩾ 128) is fairly similar in these two populations.

The majority of studies on *M. pneumoniae* have been carried out on sera from patients suffering from various respiratory diseases. The investigation of the immune status of healthy people such as those who have contributed to the New Zealand NSB may throw a different light on the patterns of *M. pneumoniae* disease. Various authors (Communicable Diseases Report, 1982; Lind & Bentzon, 1976)

have suggested on the basis of their work on clinical specimens that *M. pneumoniae* epidemics have recently been increasing in size. Our unpublished observations on specimens from clinical cases submitted to the NHI suggest that this may also be true to New Zealand. The study of sera from the NSB has the advantage of being unselective with respect to respiratory tract infections. Whether there is really an increased incidence or merely an increased index of suspicion could be tested by maintaining a continuing surveillance on healthy blood donors. With the demonstration of a peak response to *M. pneumoniae* in 1978, it has been established that the NSB is a useful tool to study herd immunity baselines in New Zealand. Therefore the investigation of further collections may be of value in the elucidation of points such as this.

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