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A Study of Respiratory Infections in a Healthy Adult Population During the 1987 Australian Winter

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During the 1987 Australian winter, respiratory illness patterns were studied in a population of 454 healthy adults, aged 18–59, over a period of 45 days. These patterns were matched with data obtained from laboratory diagnoses for respiratory viruses, *Mycoplasma pneumoniae* and bacteria. Influenza B/1/86 was by far the most prevalent pathogen but other viruses including influenza A, paramyxoviruses, respiratory syncytial virus and coronavirus OC-43 were also present, either alone or in combination during the sampling period. Overall, 92 males and 101 females experienced one episode, 12 males and 22 females experienced two episodes and four females experienced three episodes. However, there were only 52 instances of viral or *M. pneumoniae* infections, of which 37 had a defined aetiology, while the remainder were clinically silent. No bacterial pathogens could be detected from throat swabs taken from 15 of 37 volunteers in whom a viral infection was detected, or from 43 of 70 volunteers who did not experience such infections. The study indicates that major deficiencies in our understanding of the aetiology of respiratory viral illness are probably due to methodological problems in obtaining laboratory diagnoses for many respiratory viruses, and that great difficulties exist in establishing an aetiology for respiratory infections based upon clinical symptoms alone.

INTRODUCTION

Although respiratory viral and mycoplasmal infections are major causes of morbidity in healthy adults, it is surprising how few comprehensive aetiological data are available, even at the times of greatest incidence which, in temperate climates, are the winter months. In clinical practice, finding the cause for such infections is rarely undertaken because classical diagnostic procedures (isolation in cell culture and/or serology) usually do not yield diagnostic results until the illness has subsided. Large-scale studies to determine the aetiology and patterns of infection have been uncommon and are usually undertaken to determine responses to antiviral chemotherapy. They require extensive collaboration between patients, nursing staff, physicians and laboratory workers, and comprehensive computer analysis.[1–3]

During the Australian winter of 1987 we planned a clinical trial to determine the efficacy of the antiviral drug rimantadine for the prophylaxis of influenza A. As it happened, influenza A viruses were not sufficiently prevalent to justify administration of the drug. This report is an intensive survey of a population of 454 healthy adults who volunteered for the rimantadine trial, to document the aetiology for each respiratory tract illness by standard laboratory procedures.

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MATERIALS AND METHODS

Plan of Study

The time for the study was determined from the incidence of influenza A in Australia over the previous 10 years, as reported in the *Communicable Diseases Intelligence Bulletin* (Commonwealth Department of Health, Canberra, ACT, Australia). Based upon these data, the period of 20 July–28 August was planned for medication, but observation and serum samples were continued for a total of 45 days to detect late infections.

In order to obtain advance information of any impending influenza epidemic, a surveillance programme was commenced 5 weeks before the time for the study. Surveillance was carried out on patients who were not

part of the study cohort but who presented with influenza symptoms at the surgeries of groups of general practitioners (GPs) (a total of 27 physicians) located throughout the region and was continued throughout the time of the study. Attempts to identify influenza A and B viruses from nasal washings from the sentinel population yielded 1 influenza A and 19 influenza B viruses from a total of 46 specimens. This rate of infection for influenza A viruses was too low to commence medication with rimantadine in the larger volunteer population.

Volunteer Population

Our subjects were 232 males and 222 females aged 18–59 from a range of occupations; they included students, office workers, manual workers, unemployed and pensioners from the Newcastle-Lake Macquarie region of New South Wales. Recruitment commenced in May and was achieved in response to widespread advertising throughout the region. Four clinics were set up at different locations to cover the test population. The final volunteer population of 454 from whom informed consent was obtained was selected from 682 individuals who indicated an interest in the study. Criteria for exclusion included pregnancy or inadequate contraception, concurrent treatment or adverse experience with amantadine, recent experience of influenza-like symptoms, recent experience of neurologic symptoms or vaccination against influenza. A range of antibiotics was used by 11% of the final test population during the sampling period for the treatment of respiratory or non-respiratory illnesses (including lacerations, enteric and other infections) and were prescribed independently by regular physicians to the volunteers. Approval for the study was obtained from the Human Research Ethics Committee of the University of Newcastle.

Acute Specimens

Patients experiencing influenza-like symptoms were required to report to one of four clinics that were set up for the purposes of the study where nasal and throat washings were collected, together with blood for serum preparation and a throat swab for attempted culture of bacterial pathogens. For the purposes of the study, influenza-like symptoms were defined as the presence of fever (>38°C for at least 24 hours) plus at least one respiratory sign or symptom (runny nose, sneezing, stopped up nose, sore scratchy throat, hoarseness, cough) and one systemic sign or symptom (chills, headache, muscle ache, nausea, vomiting, dizziness, diarrhoea, chest pain, fatigue and/or malaise). A total of 85 acute blood samples, nasal washings and throat washings were obtained and this figure included 11 specimens that were obtained from volunteers on two occasions. A total of 58 acute throat swabs were processed for bacteriology from acute samples. Base-line pre-trial blood samples were obtained from all volunteers.

Outcome Measurements

These included symptoms of upper and lower respiratory tract infections, virus isolation in acute specimens and seroconversion over the sampling period for the entire population. Symptoms were recorded by each volunteer on a severity scale of 0–3 for 45 days, which encompassed the intended period for medication. Symptom cards were examined by nurses during weekly visits to the clinics. At these visits volunteers were also required to provide additional nasal and throat wash specimens and blood samples for serology in order to maximize the chances of detecting a viral infection around the time of an illness when acute samples were taken (see above). The range of illness categories was designed to include both upper and lower respiratory tract illness and symptoms caused by previously described side effects of rimantadine, which included anxiety, insomnia, light headedness, difficulty in concentrating and irritability.[4]

Upper respiratory tract episodes (URTEs) and generalized influenza episodes (GIEs) were determined objectively by a symptom complex algorithm modified from one previously described in a study on the prophylaxis of common cold viruses with interferon.[3] Briefly, the ratings for all symptoms were added to produce a daily total symptom score (DTSS). In order to allow for many individuals experiencing persistent symptoms during winter that were apparently unrelated to infections (i.e. continuous 'runny' nose), the DTSS for each type of episode was reduced by the whole number of the daily average score for the relevant symptoms over the trial period. Thus, if an individual was assigned a score of '1' for 'runny nose' over the whole trial period interspersed with an occasional sneeze to produce an average daily score of 1.1, the scores for both URTEs and GIEs were reduced by 1 on each day.

Any adjusted DTSS above zero for a particular day was added to that for the next 2 days. If the combined score was 4 for URTE symptoms or 6 for GIE, an episode was considered to have started. An episode was considered to have ended when the adjusted DTSS was zero and was followed by an adjusted DTSS of <2 on the following day. URTE and GIE are not mutually exclusive categories[5] and, for most analyses, were used interchangeably.

Data were entered on a weekly basis from individual symptom cards using an IBM XT personal computer. Episodes were then determined from a specially written programme. A profile of symptoms experienced by one volunteer is shown in Figure 1.

Laboratory Procedures

Bacteriology. Throat swabs for the detection of bacterial pathogens were obtained immediately before the trial and, as far as possible, whenever acute samples were collected. Swabs were placed in transport medium (Ames) and were streaked onto two horse blood agar plates in the laboratory. One was incubated

RIMANTADINE TRIAL 1987 - PATIENT SYMPTOMS SUMMARY SHEET

PATIENT NUMBER 518 PATIENT INITIALS IHC

Symptom	Week/Day :---1---:---2---:---3---:---4---:---5---:---6---:---7---: 1234567123456712345671234567123456712345671234567
Daily Record Completed	**********
Nasal	
Dry or Itchy Nose	1
Runny Nose	2221 11
Continuous Sneezing	111
Nasal Congestion	11111131 111
Throat	
Sore Throat	111111333 1111
Cough with Phlegm	1111113332221
Dry Cough	
Hoarse/Irritated	11111121
General	
Dry Mouth	1111
Fever/Chill	1 21
Sore Eyes	1111112111
Loss Appetite	11111121
Headache	111111211 1
Muscular Ache	11111121 11 1
Nausea	111111111
Vomiting	
Diarrhoea	1
Dizziness/Giddiness	11111111
Other Symptoms : :	
Fever (38 or over)	*****
Suggested Episodes	
Cold Like	********** ***
Influenza Like	********** ****
Ill at Home	YYYYYYYY
Medication Purpose	
Ongoing	YYYYYY
Cold/Influenza	Y
Other	YYYYYY Y
Symptoms in Contacts	
Nasal	YYYY
Throat	YYYY
General	YYYY

Other Recorded Symptoms include :-

Viral Findings : Antibody Rise : Influenza B/Ann Arbor

FIGURE 1 *Illness profile of a volunteer participating in the study. Data were abstracted from weekly patient symptom cards. The degree of severity of symptoms is graded numerically. Cold-like episodes (URTE) or generalized influenza episodes (GIE) are denoted (*) and are defined according to the algorithm (see Materials and methods)*

aerobically and the other anaerobically at 37°C for 48 hours. Group A β-haemolytic streptococci were identified by standard procedures. Other streptococci were identified according to Lancefield grouping using a commercial streptococcus identification kit (Oxoid). Other micro-organisms were identified according to criteria of Cowan.[5] Base-line data obtained from volunteers before the commencement of the study are given in Table 1.

Virology. Virus-specific immunofluorescence (IFA) in cells present in nasal washings was used to detect influenza A and B viruses. For attempted virus isolation, aliquots consisting of 0.1 ml of throat washings were inoculated to cell cultures of the Madin-Darby canine kidney (MDCK), BSC-1, HEp-2 and MRC-5 lines. After adsorption for 30 minutes, 1 ml of maintenance medium consisting of a 1:1 mixture of Leibovitz L15 medium with non-essential amino acids and Eagle's minimal essential medium was added to each culture. Maintenance medium for MDCK cells also contained 1 μg trypsin/ml to facilitate the recovery of influenza viruses.[6] The cultures were rolled at 0.5 r.p.m. at 34°C and examined microscopically for cytopathic effects

(CPEs) on alternate days up to 2 weeks. When a CPE was noted, the culture was retained for characterization. Negative cultures were passaged up to three times and then discarded. Additional aliquots of both nasal and throat washings from volunteers who seroconverted to respiratory syncytial virus (RSV) were re-inoculated to the same cells in a further attempt at isolation. Re-culture was also attempted on nasal and throat washings in MDCK cells in volunteers who were initially IFA positive for influenza A or B viruses but culture negative. In addition to acute samples, attempts to culture virus during the prodromal period were also made from routine nasal washings collected the week before the recorded occurrence of fever ($\geq$38.5°C), to maximize the chances of isolation.

TABLE 1 *Base-line bacteriological profile of participants before the commencement of the study*

Bacteriological result	Number[a]	Percentage
Normal flora alone	415	86
Scant-profuse β-haemolytic streptococci group G[b]	27	6
Scant-profuse *Streptococcus agalactiae*[b]	17	4
Scant-profuse β-haemolytic streptococci group C[b]	9	2
Moderate *Streptococcus pneumoniae*[b]	1	< 1
Scant β-haemolytic streptococci	1	< 1
Moderate *Haemophilus influenzae*[b]	5	1
Scant *Staphylococcus aureus*[b]	3	< 1
Moderate *Pseudomonas aeruginosa*[b]	1	< 1
Total number of samples	479	100

[a]Includes data from volunteers who were subsequently excluded.
[b]In addition to normal flora.

Two techniques were used for viral serology on pre-trial, post-trial and acute and convalescent serum samples: haemagglutination inhibition tests for influenza A/Mississippi/1/85 (A/Miss/1/85) (H3N2), influenza A/Taiwan/1/86 (H1N1), influenza B/Ann Arbor/1/86 (B/AA/1/86) parainfluenza 1, 2 and 3 and human coronavirus α-43 using 4 haemagglutinating units/virus serum mixture;[7] complement fixation tests for adenoviruses, RSV and *Mycoplasma pneumoniae* were carried out by the method of Bradstreet and Taylor.[8] For either test, 4-fold or greater increases in titre were the criteria for an antibody response.

RESULTS

Age-distribution and Sequence of Episodes of Respiratory Illness Within the Test Population

Figure 2 shows the age distribution of episodes of respiratory illness (URTE and/or GIE) as defined by the algorithm described above. Volunteers younger than 20 and between 41 and 50 years old comprised the greatest proportion of the population; the younger than 20 cohort comprised almost twice as many males as females, while for the 41–50 cohort this ratio was reversed. Overall, 92 males (39.7%) and 101 females (45.5%) of a total population of 232 and 222 experienced one episode; 12 males and 22 females experienced two episodes and four females experienced three episodes during the sampling period of 45 days. The percentage experiencing one episode ranged from 69% for females in the younger than 20 cohort to 33% for males in the 36–40 age cohort. Except for the small over 50 female group, where two from a total of 10 volunteers experienced more than one episode, the percentage experiencing two episodes ranged from 0% in the 26–30 and 41–50 year male cohorts to 13% in the 26–30 year female cohorts. Females in the younger than 20 (20%) and 31–35 (37%) age cohorts comprised the smallest groups that did not experience episodes of infection. For the remaining groups the range was 42–69% (mean 53.7%).

The cumulative occurrence of episodes throughout the sampling period is shown in Figure 3, which indicates that most first episodes had occurred during the first 36 days of the study and that activity had declined by days 40–45, 29 August–2 September, which was the end of the Australian winter. There were no significant differences in the numbers of episodes experienced by non-smokers and smokers and no trends among smokers according to the extent of their habit (Table 2).

Viral and M. pneumoniae Infections in the Trial Population

Results of tests for the detection of respiratory viruses and *M. pneumoniae* by isolation and/or serology are shown in Table 3. Clearly the predominant pathogen during the trial period was influenza B/AA/1/86. Table 2 also indicates that many other respiratory pathogens were present, some of which were present concurrently and some which occurred sequentially with others. An aetiology was regarded as confirmed if a virus was isolated from an acute specimen collected at the time of an episode and/or an antibody rise occurred within 1–3 weeks of the episode, irrespective of whether or not virus isolation was attempted. Overall, some 52 instances of infection could be detected in the trial population, of which 37 were associated with defined episodes of illness, while the remainder were clinically silent; isolation and/or detection of virus by IFA from acute nasal or throat washings was only achieved in 13 of these specimens, which demonstrated the value of carrying out weekly

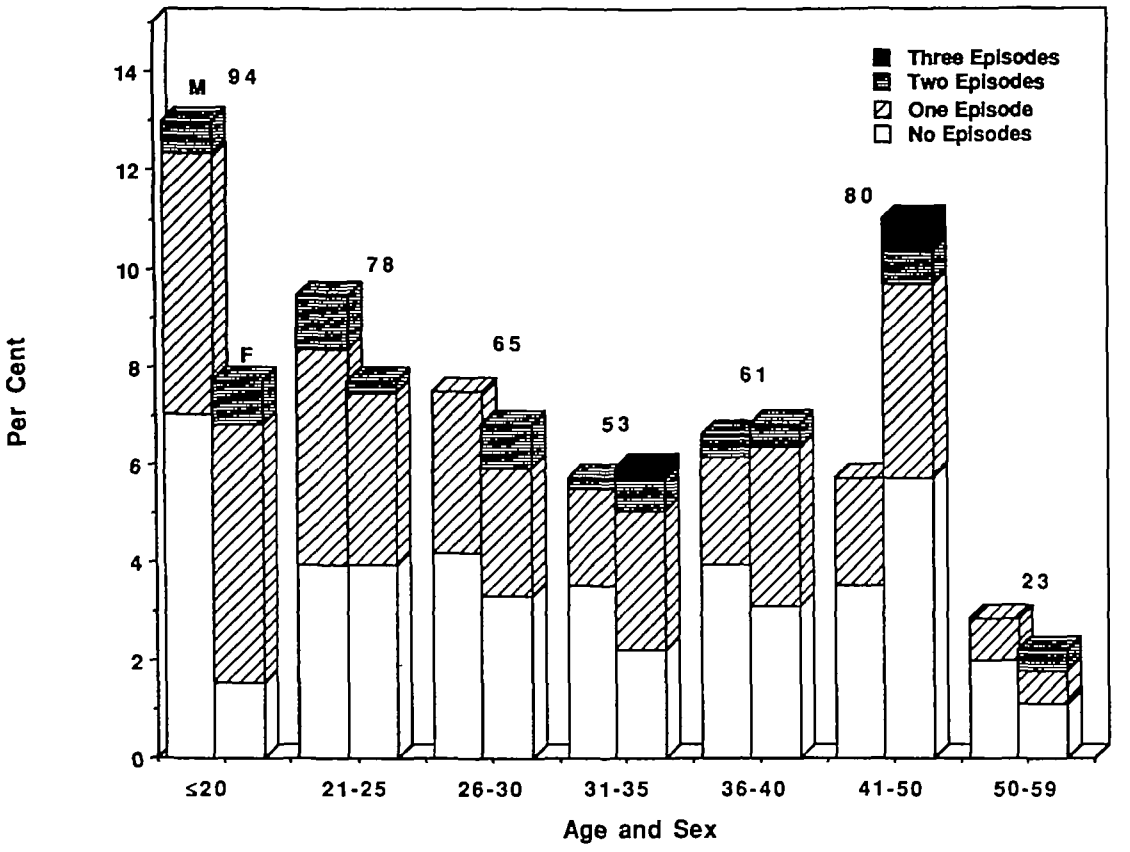

FIGURE 2 *Profile of study population, showing the number of episodes according to age and sex over the sampling period. Episodes are listed, irrespective of whether they were defined as upper respiratory tract episodes (URTEs) or generalized influenza episodes (GIEs), according to the algorithm. Total numbers per age cohort are given*

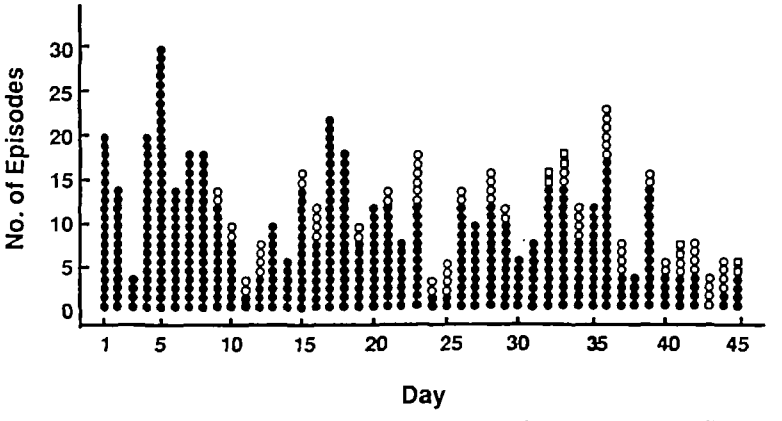

FIGURE 3 *Cumulative occurrence of first, second or third episodes in individual volunteers throughout the sampling period.* (●) *first episode,* (○) *second episode,* (□) *third episode*

TABLE 2 *Incidence of episodes of respiratory illness in smokers and non-smokers*

Status	No. episodes experienced				Totals
	0	1	2	3	
Non-smoker	63 (52)[a]	49 (40)	10 (8)	0	122
< 10/day	44 (47)	43 (46)	6 (6)	1 (1)	94
10–15/day	116 (49)	101 (42)	18 (8)	3 (1)	238

[a]Per cent of total.

bleeds to define the aetiology of an episode by retrospective serology. No isolates were made from prodromal nasal and throat washings collected within the week prior to an episode. Of the 22 individuals with infections of defined aetiology for B/AA/1/86, 20 experienced both URTE and GIE while two experienced URTE alone. For A/Taiwan/1/86, RSV and parainfluenza 3, the corresponding figures for both URTE and GIE, and URTE alone, are 3 and 0, 2 and 1, and 1 and 1, respectively. It was, therefore, not possible to distinguish between episodes that were caused by the different viruses according to our algorithm. Only 52 infections by respiratory viruses and *M. pneumoniae* were detected despite the occurrence of a total of 231 episodes (URTE and/or GIE). The mean ages ± SEM of volunteers experiencing defined episodes were 29.5 ± 2.1 years for B/AA/1/86, 24.0 ± 3.6 for A/Taiwan/1/86 and 44.7 ± 4.7 for RSV.

Results of Bacteriology from Symptomatic Volunteers With or Without a Defined Aetiology for Viral Infections

Of the 37 volunteers with episodes of illness for which a viral aetiology was defined, 15 provided samples at acute visits. Table 4 summarizes the results of the bacteriology from these volunteers, seven of whom experienced fever. Clearly, there was no evidence of infection by pathogenic bacteria, and the pattern is similar to that observed in acute specimens taken at acute visits corresponding with a defined episode from a further 43 volunteers without proven viral or mycoplasmal infections (Table 5). One volunteer from whom *Haemophilus influenzae* was isolated in significant amounts had also experienced swollen glands, a purulent sore throat and dental problems and was shown retrospectively by serology to have experienced a cytomegalovirus (CMV) infection. CMV serology was not carried out on other acute specimens. Both patterns are similar to that obtained from samples taken from 479 participants or intended participants before entry to the study (Table 1).

TABLE 3 *Viral and M. pneumoniae infections in the trial population*

Agent(s)	All volunteers			Volunteers with defined aetiology			
	No.	Number asymptomatic	Mean no. of episodes ± SEM in symptomatic volunteers	No.	No. with fever (>38.5°C)	Mean time ± SEM for episode no:[a]	
						1	2
B/AA/1/86	28	6	1.23 ± 0.11	22	10	7.09 ± 0.83	—
B/AA/1/86 and A/Miss/1/85	1	0	1	1	0	6	—
A/Taiwan/1/86 and B/AA/1/86	1	0	2	1	1[c]	4	3[b,c]
OC-43 and B/AA/1/86	1	0	2	1	1[c]	4	10[b,c]
A/Taiwan/1/86	3	0	1.33 ± 0.33	3	1	17.30 ± 8.88	6[d]
A/Miss/1/85 and parainfluenza 1	1	0	1	1	0	35[e]	—
Parainfluenza 1	2	1	1	1	0	11	—
Parainfluenza 2	2	1	1	1	0	10	—
Parainfluenza 3	4	0	1.25 ± 0.25	2	0	4.50 ± 2.50	—
RSV	3	0	1	3	0	7.00 ± 1.53	—
α-43	1	0	1	1	0	9	—
M. pneumoniae	5	4[f]	1	0	0	—	—

[a]Days.
[b]In mixed infections of defined aetiology, agents are listed in order of occurrence.
[c]Fever occurred with B/AA/1/86.
[d]No defined aetiology for second episode.
[e]Concurrent infection.
[f]An episode of undetermined aetiology occurred in one volunteer after *M. pneumoniae* seroconversion.

TABLE 4 *Results of bacteriology from 15 of 37 symptomatic volunteers with a defined aetiology for viral infections*

Virus(es)	No. of episodes	Defined aetiology for episode no.	Fever for episode no.	Bacteriological results
B/AA/1/86	1	1		normal flora
	2	2	2	profuse normal flora[a]
	1	1		scant β-haemolytic strep. group G[b]
	1	1	1	profuse normal flora[c]
	2	1	1	moderate normal flora
	1	1		moderate normal flora
	1	1		moderate normal flora
	1	1	1	profuse normal flora
	2	2		scant β-haemolytic strep. group C[a,b]
	1	1	1	profuse normal flora
	1	1		moderate normal flora
	1	1	1	profuse normal flora
	1	1		profuse normal flora
OC-43 and B/AA/1/86	2	1,2	2	profuse β-haemolytic strep. group G (1)[b] scant β-haemolytic strep. group C (2)[b]
A/Taiwan/1/86	1			profuse normal flora

[a]Bacteriology carried out on sample from second episode.
[b]In addition to normal flora.
[c]A 2 week convalescent sample showed profuse *Streptococcus pyogenes.*

TABLE 5 *Summarized results of bacteriology from 43 symptomatic volunteers without proven viral or mycoplasmal infections*

Clinical result	Scant-profuse normal flora alone	Scant-profuse β-haemolytic strep. group C[a]	Scant-profuse β-haemolytic strep. group G[a]	Profuse *Haemophilus influenzae*[a]
Single episode without fever	27	4	2	0
Single episode with fever	2	0	0	1[b]
Two episodes, bacteriology done on one	3	1	0	0
Two episodes, bacteriology done on both	3	0	0	0

[a]In addition to normal flora.
[b]Volunteer also seroconverted to cytomegalovirus.

DISCUSSION

The present study demonstrates the problems in defining aetiological relationships for respiratory viral illnesses. Many more episodes of respiratory illness were detected in the test population than could be accounted for by the diagnostic tests used in the study. Influenza infections were the primary focus of the study and influenza B/AA/1/86 was by far the most predominant pathogen. No attempts were made to screen for *Chlamydia pneumoniae* (TWAR), which is now recognized as a major cause of respiratory morbidity,[9,10] or human coronavirus 229E, which is a significant upper respiratory tract pathogen.[11] No rhinoviruses or adenoviruses were isolated and nor were other less common respiratory pathogens such as echoviruses, Coxsackie viruses or enteroviruses. Eight of 47 viral infections were asymptomatic, as were all four proven infections due to *M. pneumoniae.* A greater rate of seroconversion in symptomatic volunteers without evidence of infection would probably

have been detected if a serological test more sensitive than the complement fixation procedure had been used for RSV, adenoviruses and *M. pneumoniae*. Infections due to RSV occurred in three older volunteers, which is consistent with suggestions from other studies that suggest that RSV is a cause of significant respiratory morbidity in adults[12,13] and severe pneumonia in the elderly.[14] We were able to detect viruses by culture or IFA in only 13 of 37 acute nasal and/or throat washings in volunteers who had experienced a clinically defined illness with aetiology demonstrated by retrospective serology. Our isolation rate would have undoubtedly been improved by the application of more sensitive procedures that do not involve cell culture, including the use of the polymerase chain reaction and time-resolved fluoroimmunoassays that were not available at the time of this study.

Female volunteers appeared to experience more episodes of infection than males, especially in the younger than 20 and 31–35 year cohorts (Figure 2). This could be due to greater exposure to infection in households through contact with children although, surprisingly, there were no trends in incidence according to the number of children in the households of either male or female volunteers (unpublished observations). There was no substantial difference in the incidence of episodes experienced by non-smokers (comprising 27% of the study population) and smokers with differing habits. However, these data are unlikely to be representative of either smokers or non-smokers, which is reflected in the occupational status of the subjects and the prevalence of smoking which is approximately twice a recently reported Australian level.[15] Previous comparative studies have indicated an increased incidence of respiratory infections among smokers.[16] Of interest is the range of single and dual infections by viral pathogens that appear to produce clinically indistinguishable symptoms. These findings do not bode well for the widespread therapeutic application of newer respiratory antiviral drugs, which are likely to be highly specific in their mode of action for particular respiratory viruses, most of which have relatively short phases of acute illness. Such antiviral drugs could not be used without major advances in the accessibility of rapid viral diagnostic techniques (within 12–24 hours) and similar considerations apply to evaluations of the efficacy of influenza vaccines according to clinical outcomes. Although we obtained bacteriological results from 58 individuals with defined episodes of respiratory illness, of whom 15 had an established viral infection, our study also provides no basis for the prophylactic use of antibiotics in normal adult populations. The profile of bacteria from acute samples taken from volunteers with and without concurrent viral infection was no different from that of others before entry to the study and there was no evidence of synergism involving changes to the bacterial flora of the respiratory tract following viral infection. The widespread and often inappropriate use of antibiotics for respiratory viral infections is a severe impost of modern health care budgets and it is especially interesting that, despite the limitations of our bacteriological sampling, no group A β-haemolytic streptococci were isolated.

Above all, our study demonstrates that for the Australian winter of 1987 where the infection was predominantly caused by influenza B/AA/1/86, the cause of the illness could not be detected according to clinical symptoms alone. In a large study on the efficacy of interferon[3] we were also unable to show such differences. In that study the presence of concurrent or sequential episodes of respiratory illness that were caused by differing viruses was detected even in the presence of a major influenza A epidemic. Data from descriptive studies such as this are extremely difficult to obtain but do provide some indication as to the magnitude of the problem, and further studies should be carried out in different situations.

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