Epidem. Inf. (1987), **98**, 47–63
Printed in Great Britain

Seroepidemiological studies on the occurrence of common respiratory infections in paediatric student nurses and medical technology students

By H.-J. GERTH, CH. GRÜNER,

Hygiene-Institut der Universität Tübingen, Abt. für Med. Virologie und Epidemiologie der Viruskrankheiten, D7400 Tübingen, FRG

R. MÜLLER

Universitäts-Kinderklinik, D7400 Tübingen, FRG

AND K. DIETZ

Institut für Med. Biometrie der Universität Tübingen, D7400 Tübingen, FRG

(*Accepted 15 September 1986*)

SUMMARY

The occupational risk of acquiring minor respiratory infections for paediatric student nurses was estimated by performing serological examinations with influenza A, B, C, parainfluenza, mumps, respiratory syncytial virus, adenovirus and *Mycoplasma pneumoniae* at 6-month intervals over a period of 4 years in paediatric student nurses at two schools of nursing and students at one school of medical technology. Titre increases against all tested agents occurred 1·86 times more often in the student nurses than in the medical technology students, the most frequent agents in both groups being influenza A and B. No difference in the relative distribution of the agents could be verified in the two occupational groups. Data on the protective value of pre-infectious antibody levels for influenza A, B, and coronavirus OC43 and on the importance of the spread of single agents among classmates are presented.

INTRODUCTION

In the companion paper (Gerth *et al.* 1987) the occupational risk of acquiring minor respiratory and gastrointestinal infections for paediatric student nurses was estimated by comparing their disease experience at two schools of paediatric nursing with that of students at a school of medical technology over a 3-year period. The investigation was based on an evaluation of symptoms which were reported by the students on questionnaires.

The risk of acquiring minor respiratory and gastrointestinal illnesses was significantly higher in the student nurses, presumably because of their occupational exposure to children.

This paper, which substantiates and differentiates the findings obtained by subjective reporting, presents our objective serological investigations for common

respiratory pathogens. The relative significance of these pathogens on the different occupational groups and the importance of spread among classmates of some of the agents were estimated.

MATERIALS AND METHODS

Study population

The participants were paediatric student nurses at two schools of nursing in Southern Germany (FRG), in Tübingen and in Reutlingen. The control group consisted of medical technology students in Tübingen. While 50–60% of the training of the student nurses was spent in direct contact with children, the medical technology students had little patient contact. New students were admitted to the schools in April and October. The student nurses train for 3 years; the medical technology students for 2 years (for detailed information on the study population see the companion paper: Gerth *et al.* 1987).

The seroepidemiological study was carried out for 1 year longer (April 1975–March 1979) than the questionnaire-based investigation.

Blood samples were obtained from the participants between the end of March and the beginning of April and between the end of September and the beginning of October. On average, venepunctures were done on 29 March and 16 October for the Tübingen student nurses, on 6 April and 1 October for the Reutlingen student nurses, and 28 March and 7 October for the medical technology students.

Students who had completed their training were venepunctured before their final examinations at the end of February or the beginning of March.

Compliance of the student nurses (94%) and the medical technology students (93%) was high during the first 3 years of the study, but declining during the year following conclusion of the questionnaire study (student nurses 85%; medical technology students 67%). The numerical breakdown of the participants is presented in Table 1.

A total of 241·5 person-years were evaluated for the Tübingen student nurses; 212 person-years for the Reutlingen nurses, and 437 person-years for the medical technology students.

Serological procedures

The complement fixation test (CFT) was done by a standard micromethod employed by the Viral and Rickettsial Disease Laboratory of the California State Department of Health (Hawkes, 1979). Standard micromethods were used for the haemagglutination inhibition test (HIT) (Dowdle, Kendal & Noble, 1979). Sera for influenza A- and B-HIT were pretreated with *Vibrio cholerae* neuraminidase (Behringwerke AG, Marburg/FRG) as described by Dowdle, Kendal & Noble (1979).

Antigen preparation

Group-specific adenovirus CF-antigen was prepared in HEp-2 cells (Kasel, 1979). The seed virus adenovirus type 2 (Adenoid 6), and human reference sera were supplied by Professor R. Wigand, National Adenovirus Centre, Homburg/Saar, FRG.

Coronavirus OC43 seed was obtained from Dr Sylvia Reed, MRC Common Cold Unit, Harvard Hospital, Salisbury, UK. Antigen was prepared by i.c. inoculation of 3-day-old NMRI-mice. Brains were harvested approximately 60 h after inoculation and homogenized. A 10% suspension (w/v) was made up in phosphate-buffered saline and the suspension cleared by low-speed centrifugation. 4 ml aliquots of the supernate, the HI-titres of which ranged from 32 to 64 with 0·5% chicken erythrocytes, were stored in screw-capped plastic tubes at −70 °C until use. Internal standardization was carried out using a hyperimmune serum prepared in weanling NMRI mice (McIntosh *et al.* 1967).

Influenza A and B CF- and HI-antigen were prepared in embryonated eggs according to routine methods. Influenza A/Victoria/3/75 seed virus was supplied by Dr H. Willers, WHO Influenza Reference Laboratory Hannover, FRG. In 1977 an influenza A/Texas/1/77-like strain was isolated from one of the participating student nurses and identified in our laboratory. The identity of the strain, which has been used since September 1977 as HI-antigen, was confirmed by Professor W. Lange, National Influenza Reference Centre, Robert Koch Institute, Berlin-West.

Mycoplasma pneumoniae antigen, influenza C antigen, and the respective immune sera were purchased from Behringwerke AG, Marburg, FRG.

Mumps virus antibodies were assayed exclusively by HIT. Mumps virus antigen was prepared in embryonated eggs by standard methods (Hopps & Parkman, 1979). An egg-adapted mumps virus strain, which we originally isolated from the spinal fluid of a child with mumps meningitis, served as seed virus. To enhance the sensitivity of the HI-antigen, infected allantoic fluids were treated with Tween 80 and ether (Hopps & Parkman, 1979). An immune serum prepared in guinea-pigs served as internal standard.

Parainfluenza 1 antigen (Sendai) for CF was prepared in embryonated hen's eggs. The seed virus was originally obtained from Professor R. Rott, Max Planck Institute for Virus Research, Tübingen, FRG.

Parainfluenza 1 (HAV-2) and parainfluenza 2 (Grear) antigens for HIT were purchased from Flow Laboratories GmbH, Bonn, FRG.

Parainfluenza 3 (HAV-1) seed virus was obtained from Professor R. Wigand, Department of Virology, Homburg/Saar, FRG. Virus was propagated in Vero cells. HI-antigen was prepared by treatment with Tween 80/ether as described for mumps antigen. Immune sera and respiratory syncytial virus (RSV)-antigen were purchased from Flow Laboratories GmbH, Bonn/FRG.

Statistical evaluation

To compare infection rates in two populations, we assumed a Poisson distribution for the number of events observed in any given time interval. In the following equation n_i represents the number of events in the two samples; T_i, the observation times in the two samples, and $i = 1$ or 2, when events occur according to the rates λ_i in the two samples, then

$$T_1(n_2+0{\cdot}5)\,\lambda_1/T_2(n_1+0{\cdot}5)\,\lambda_2$$

has approximately an F distribution with $2n_1+1$ and $2n_2+1$ D.F. (Cox, 1953).

https://doi.org/10.1017/S0950268800061707 Published online by Cambridge University Press

If n_1 is large enough, the test statistic

$$T_1 n_2 / T_2 n_1,$$

and the F distribution with $2n_1$ or $2n_2$ D.F. can be used.

RESULTS

During the 4-year study, a total of 147 fourfold titre increases were observed with all antigens used: 97 titre increases (21·25/100 person-years) in the student nurses, 50 (11·45/100 person-years) in the medical technology students. The ratio of titre increases in the student nurses to those in the medical technology students was 1·86:1.

For reference, our original results are presented in Table 1*a–c*. Listed are the number of participating students per semester and the number of titre increases for each agent by period of observation.

The number of titre increases for influenza A and B includes all cases with increases in CFT and/or HIT. For influenza C only fourfold titre increases in CFT are listed.

Group specific hexon antigen was used in the CFT for adenovirus. No attempt at a further specification of the type of infecting adenovirus was made.

In the tables, parainfluenza titre increases for type 1 with CFT and types 1–3 and for mumps with HIT were reported together.

Three antibody titre increases each for parainfluenza 1 and 2 were found in the student nurses. Presumably, one other nurse had a parainfluenza 2 infection, but titres in HIT to parainfluenza 3 and in CFT to parainfluenza 1 did also increase. Mumps meningitis was diagnosed in one student nurse; fourfold antibody titre increases for mumps in CFT and HIT and concomitant titre rises against parainfluenza 2 and 3 in HIT were detected. Three of the eight parainfluenza titre increases in the medical technology students were probably caused by parainfluenza 1 infections and four by parainfluenza 2. One case was not classifiable.

The incidence of titre rises with the single agents for the total 4-year surveillance period is summarized in Table 2. The two schools of nursing are presented separately. While the exposure to single agents differed considerably, infections with all agents were found in all three schools. The number of all serologically demonstrated infections for each school of nursing was significantly higher than for the school of medical laboratory technology, number of titre increases in Tübingen student nurses/number of titre increases in medical technology students = 1·66 ($P \leqslant 0{\cdot}01$); number of titre increases in Reutlingen student nurses/number of titre increases in medical technology students = 2·07 ($P \leqslant 0{\cdot}01$). The difference between the student nurses at the two schools was not statistically significant.

Fig. 1 shows the seasonal distribution of the titre increases for influenza A, B, and non-influenza infections. The student nurses had 2·38 times more serologically demonstrated infections in the winter semester than did the medical technology students, while in the summer semester there was practically no difference between the groups. Probably in medical technology students the infections peaked later than in student nurses, and therefore relatively more titre increases in medical

technology students were detected in the summer semester. This is in keeping with the distribution of the influenza cases. Titre increases to the faster-spreading influenza A were detected 5·5 times to the slower-spreading influenza B only 1·6 times more often in the winter than in the summer semester. Influenza A and B infections were present each year. Outstanding is the winter semester 1976–7 when, with the exception of one case of *Mycoplasma pneumoniae* infection, all 19 other titre rises were influenza A.

Table 3 was prepared to demonstrate the contribution of single infectious agents or virus groups to the total of titre rises. The antigens are ranked according to the frequency of the titre rises observed.

The most frequent serologically proven infection was influenza A which accounted in both groups for almost the same proportion of the total number of titre increases (35·1 % in student nurses and 36·0 % in medical technology students) followed by influenza B (16·5 % in student nurses and 20·0 % in medical technology students). Differences were found on the third rank taken by RSV in student nurses (12·4 %) and the parainfluenza-mumps group in the medical technology students (16·0 %). Given the low numbers, possibility of spread within one class and other vagaries of epidemiology, the ranking variations cannot be considered evidence for a differing contribution of the tested agents to the total of infections.

Clustering of infections

The frequency of spread for the rarer agents within one class of medical technology students can be estimated from Table 4. Since the risk of introduction of the frequent influenza A and B infections through sources outside the class is high, it is impossible to estimate classmate-to-classmate spread. The data on influenza A and B, therefore, have not been included in this study. The same applies to the other agents in the student nurses. The probability that medical technology students acquired the rarer infections from outside contacts, appears much lower. But actually, the distribution of the cases to class and season is compatible with a Poisson distribution as a consequence of random introduction.

However, classmate-to-classmate spread of parainfluenza viruses is nevertheless suggested: 2 of the 8 cases of parainfluenza 1 occurred in one class of medical technology students and in another class 3 of the 5 cases of parainfluenza 2 each time in the same respiratory season. In general, the spread of the rarer agents – if any – was confined to one to two classmates. Table 4 shows that there were at least 16 introductions but at most 6 secondary cases.

Time dependency of mean antibody titres and titre increases for influenza A (H3N2), B and OC43 viruses

Influenza A

The geometrical mean (GM) HI antibody titres against A/Victoria/1/75 (H3N2) antigen between October 1977 and March 1979 as well as the number of titre increases in the different student groups are shown in Fig. 2.

At the beginning of the study in 1975 the GM influenza A (H3N2) antibody titres of the student nurses and the medical technology students were almost the same. A major influenza epidemic documented by frequent virus isolation (Willers & Höpken, 1979) and excess mortality (Pöhn, 1977) occurred in the Federal

Table 1*a*. *Number of antibody titre increases to different agents by surveillance period for paediatric student nurses*

Period	*n**	Adenov.	Infl. A	Infl. B	Infl. C	Mycopl.	OC43	Parainfl.	RSV	Total
Apr. 1975–Sep. 1975	115	—	1	1	1	1	1	1	—	6
Oct. 1975–Mar. 1976	114	—	6	6	2	1	1	1	3	20
Apr. 1976–Sep. 1976	126	—	—	2	2	—	—	—	—	4
Oct. 1976–Mar. 1977	120	—	15	—	—	—	1	2	—	18
Apr. 1977–Sep. 1977	126	1	2	2	—	—	1	—	—	6
Oct. 1977–Mar. 1978	119	3	7	—	—	—	3	3	5	21
Apr. 1978–Sep. 1978	99	—	—	2	—	1	—	—	—	3
Oct. 1978–Mar. 1979	90	4	3	3	1	2	1	1	4	19
Apr. 1975–Mar. 1979	909	8	34	16	6	5	8	8	12	97

Table 1*b*. *Number of antibody titre increases to different agents by surveillance period for medical technology students*

Period	*n**	Adenov.	Infl. A	Infl. B	Infl. C	Mycopl.	OC43	Parainfl.	RSV	Total
Apr. 1975–Sep. 1975	112	1	1	1	1	—	—	1	—	5
Oct. 1975–Mar. 1976	120	—	1	2	—	—	1	—	—	4
Apr. 1976–Sep. 1976	117	—	—	1	—	2	—	1	—	4
Oct. 1976–Mar. 1977	116	—	4	—	—	—	—	—	—	4
Apr. 1977–Sep. 1977	124	1	2	1	—	1	—	1	—	6
Oct. 1977–Mar. 1978	117	—	8	—	—	—	2	3	—	13
Apr. 1978–Sep. 1978	92	—	2	—	—	1	—	2	—	5
Oct. 1978–Mar. 1979	76	—	—	5	—	—	1	—	3	9
Apr. 1975–Mar. 1979	874	2	18	10	1	4	4	8	3	50

Table 1c. *Number of antibody titre increases by surveillance period for paediatric student nurses and medical technology students*

Period	*n**	Adenov.	Infl. A	Infl. B	Infl. C	Mycopl.	OC43	Parainfl.	RSV	Total
Apr. 1975–Sep. 1975	227	1	2	2	2	1	1	2	—	11
Oct. 1975–Mar. 1976	234	—	7	8	2	1	2	1	3	24
Apr. 1976–Sep. 1976	243	—	—	2	2	2	—	1	—	7
Oct. 1976–Mar. 1977	236	—	19	1	—	—	1	2	—	23
Apr. 1977–Sep. 1977	250	2	4	3	—	1	1	1	—	12
Oct. 1977–Mar. 1978	236	3	15	1	—	—	5	6	5	35
Apr. 1978–Sep. 1978	191	—	2	1	—	2	—	2	—	7
Oct. 1978–Mar. 1979	166	4	3	8	1	2	2	1	7	28
Apr. 1975–Mar. 1979	1783	10	52	26	7	9	12	16	15	147

* Number of person-half-years.

Table 2. *Number and incidence of infections, as indicated by at least fourfold titre increases according to schools*

School	Adenov.	Infl. A	Infl. B	Infl. C	Mycopl.	OC43	Parainfl.	RSV	Total	Person-years‡
Medical technology	2*	18	10	1	4	4	8	3	50	437·0
	0·46†	4·12	2·29	0·23	0·92	0·92	1·83	0·69	11·45	
Tübingen student nurses	1	16	11	4	2	6	3	3	46	241·5
	0·41	6·63	4·55	1·66	0·83	2·48	1·24	1·24	19·05	
Reutlingen student nurses	7	18	5	2	3	2	5	9	51	212·0
	3·26	8·37	2·33	0·93	1·40	0·93	2·33	4·19	23·72	
Tübingen and Reutlingen student nurses	8	34	16	6	5	8	8	12	97	453·5
	1·75	7·45	3·50	1·31	1·10	1·75	1·75	2·63	21·25	

* Number of titre increases observed.
† Number of titre increases/100 person-years.
‡ Number of person-years of observation.

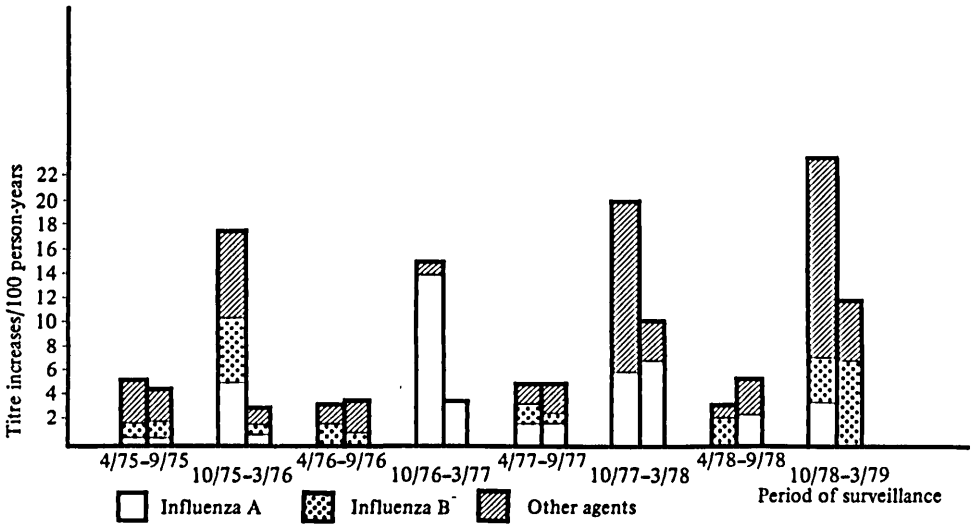

Fig. 1. Summary of all titre increases observed by HI and/or CFT by calendar half-year, 1975–9. First column: student nurses; second column, medical technology students.

Table 3. *Contribution of different agents to total number of titre increase*

		Student nurses	med. technology students		Student nurses and med. technology students	
	*n**...	97		50		147
Rank	Agent	%†	Agent	%	Agent	%
1	Infl. A	31·1	Infl. A	36·0	Infl. A	35·4
2	Infl. B	16·5	Infl. B	20·0	Infl. B	17·7
3	RSV	12·4	Parainfl.	16·0	Parainfl.	10·9
4	Adenov.	8·2	OC43	8·0	RSV	10·2
	OC43	8·2				

* Total number of titre increases.
† Percent of total number of titre increases in this occupational group.

Table 4. *Clusters of titre increases in medical technology students*

	Class-years* by numbers of titre increases			
Agent	0†	1	2	3
Influenza C	15‡	1	0	0
Adenov.	14	2	0	0
Mycoplasma pneumoniae	13	2	1	0
RSV	14	1	1	0
Parainfl. 1	14	1	1	0
Parainfl. 2	13	2	0	1
Parainfl. 3	16	0	0	0
OC43	13	2	1	0

* Epidemiological years starting October; classes comprise between 25 and 34 students.
† Titre increases/class-years.
‡ Number of class-years.

https://doi.org/10.1017/S0950268800061707 Published online by Cambridge University Press

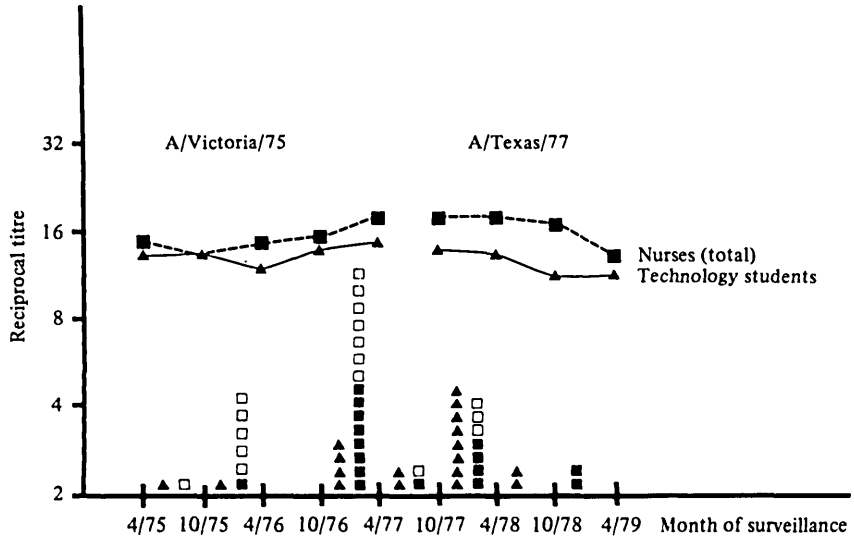

Fig. 2. Influenza A: HI-titre GM values and titre increases by HIT or/and CFT during the surveillance period. From April 1975 to April 1977 with A/Victoria/75, thereafter A/Texas/1/77 antigen was used. Each single symbol signifies one titre increase. ■, Student nurses at Tübingen; □, student nurses at Reutlingen; ▲, medical technology students.

Republic of Germany during the winter of 1974–5. The high degree of immunization in the entire population (Umbach, 1980) could explain the virtually identical HI GM antibody titres of the student nurses and the medical technology students in 1975. In the succeeding years of lower influenza activity, the gap between the lower GM values of the medical technology students and the higher values of the student nurses widened. During the first two influenza seasons, we observed proportionally more titre increases in the student nurse groups. In the winter of 1976–7, only 6 (26%) out of 23 titre increases for influenza A (A Victoria/3/75) (H3N2) occurred in the medical technology students. In the following season 1977–8 (A/Texas/1/77 (H3N2)), the titre increases of the medical technology students outnumbered those of the student nurses, the ratio being 10:7.

Influenza A activity was extremely low in 1978–9. One of the three observed cases (not included in the tables) showed an increase in CFT and in HIT only to A/USSR/90/77 (H1N1). This was the only increase for H1N1-antigen found by HIT screening all sera with at least a twofold CF-titre increase without concomitant HI titre increases for H3N2-viruses to A/USSR/90/77 (H1N1).

Influenza B

One influenza B strain, which was isolated from a child admitted to the Tübingen children's hospital in February 1976, was typed as B/Wellington/1/75-like. B/Wellington/1/75 antigen, therefore, was used for HIT, even though its antibody avidity was low. As Fig. 3 shows, the low GM antibody titres do not reflect the peaks of increased influenza B activity.

In addition to scattered antibody rises to influenza B, there were two periods of increased influenza B activity: early 1976 and early 1979 (Fig. 3). During the

https://doi.org/10.1017/S0950268800061707 Published online by Cambridge University Press

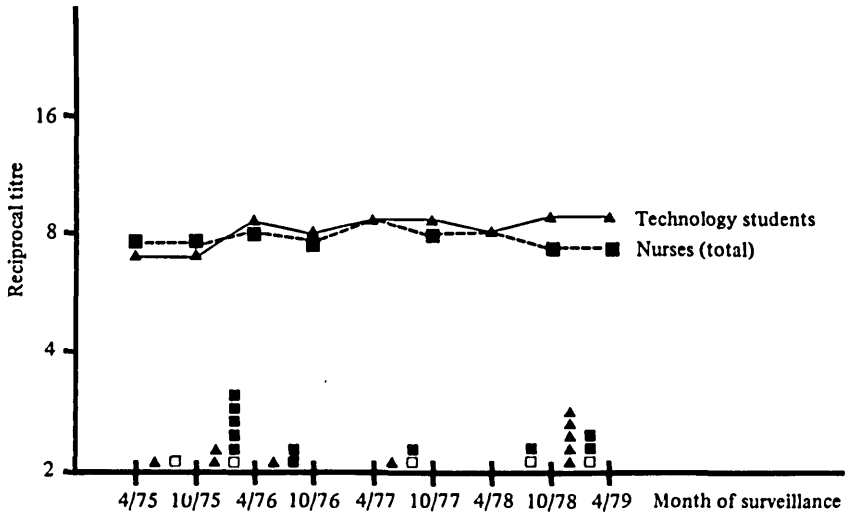

Fig. 3. Influenza B: HI-titre GM values and titre increases (single symbols). Antigen: B/Wellington/1/75. ■, Student nurses at Tübingen; □, student nurses at Reutlingen; ▲, medical technology students.

first epidemic period in 1976 9 of a total of 12 titre increases were observed in the student nurses. The higher incidence of influenza B titre increases in the student nurses (3/50/100 person-years) compared to the medical technology students (2·29/100 person-years) is due exclusively to the higher number of cases in the Tübingen student nurses, the incidence for the Reutlingen student nurses (2·32/100 person-years) equalled that of the medical technology students. The outbreaks in 1976 and 1979 seemed to be confined to student nurses and medical technology students in Tübingen; considerable influenza B activity, however, was observed in both years throughout the Federal Republic of Germany (Willers, 1981; Knocke & Kamolz, 1979). No significant difference could be established between the influenza B HI antibody mean titres of the student nurses and the medical technology student groups.

All sera were also examined by CFT. After exclusion of titre increases at the end and the beginning of the observation period and one serum with anticomplementary activity, 23 serum pairs remained for evaluation of the comparative sensitivity of CFT and HIT. In 14 pairs, fourfold titre increases were found with both tests; 6 pairs showed significant increases only with HIT and 3 pairs only with CFT. Maximal titres observed after titre increases in HIT remained comparatively low (GMT 40.47).

Coronavirus OC43

GM HI-antibody titres for OC43 during the observation periods are shown in Figure 4. The values for the Tübingen and the Reutlingen student nurses were plotted separately. The GM values of both student nurse groups are consistently higher than those of the medical technology students. Although the titre increases are scattered over the entire observation period, virus activity was slightly higher

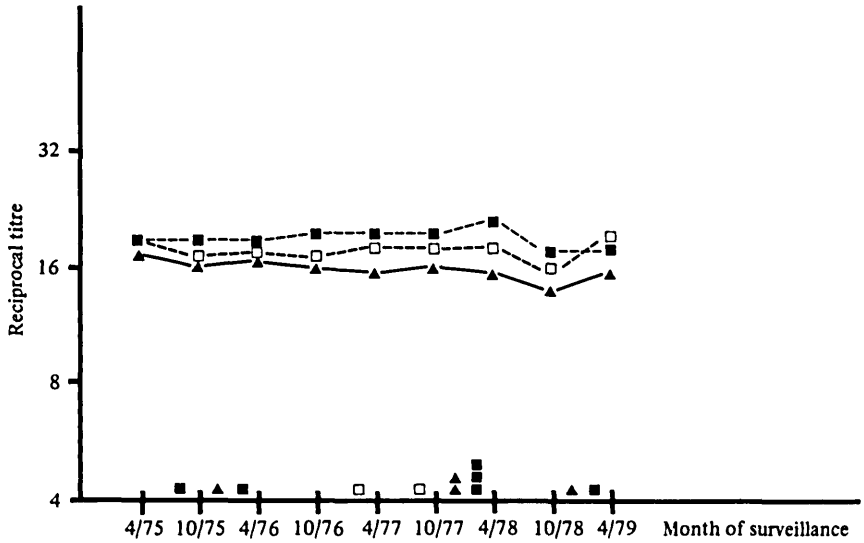

Fig. 4. Coronavirus OC43: HI-titre GM values and titre increases (single symbols). ■, Student nurses at Tübingen; □, student nurses at Reutlingen; ▲, medical technology students.

Table 5. *Immunity, as measured by HIT, and risk of infection for influenza A (H3N2)*

	Student nurses				Medical technology students			
HI-titre before infection	Person-years at risk	Titre increases*			Person-years at risk	Titre increases*		
		Number	Incidence†			Number	Incidence†	
< 8	27·5	4	14·5	19·6	57·5	10	17·4	8·2
8	105·0	22 (1)	21·0		125·5	5	4·0	
16	192·5	4 (2)	2·1		143·0	2	1·4	
32	96·0	0 (1)	—		85·5	—	—	
⩾ 64	26·5	—	—		25·0	—	—	

* Number of fourfold titre increases with HIT, CF or both. Titre increases at beginning of the observation period were excluded when initial titres were > 8 (number in parentheses).
† Per 100 person-years.

in winter 1977–8 with 5 of the 10 observed increases occurring in Tübingen (medical technology students 2; student nurses 3), suggesting a community outbreak.

Basic immunity and risk of infection

Titre increases for H3N2-viruses are correlated with the titre before infection in Table 5. Like other authors, we found that a titre of ⩾ 32 offered complete protection. The risk of acquiring a serologically demonstrated infection for participants with titres of 16 was approximately 10 times lower in the student nurses and 5·9 times lower in the medical technology students than in the participants with lower titres. The risk of infection for student nurses with titres of ⩽ 8 was almost 20% per person-year while that of the medical technology students was only 8·2%.

https://doi.org/10.1017/S0950268800061707 Published online by Cambridge University Press

Table 6. *Immunity, as measured by HIT, and risk of infection for influenza B/Wellington/1/75*

HI-titre before infection	Student nurses			Medical technology students		
	Person-years at risk	Titre increases*		Person-years at risk	Titre increases*	
		Number	Incidence†		Number	Incidence†
< 8	187·0	12	6·4	201·0	8	4·0
8	151·5	4	2·6	115·5	1	0·9 } 1·0
16	60·0	—	—	76·0	1	1·3 }
32	44·0	—	—	23·5	—	—
⩾ 64	10·5	—	—	20·5	—	—

* Number of fourfold titre increases with HIT, CF or both. Titre increases at beginning of the observation period were excluded when initial titres were 8 (number in parentheses).
† Per 100 person-years.

Table 7. *Immunity, as measured by HIT, and risk of infection for OC43*

HI-titre before infection	Student nurses			Medical technology students		
	Person-years at risk	Titre increases*		Person-years at risk	Titre increases*	
		Number	Incidence†		Number	Incidence†
< 8	6·5	3	46·2 } 12·7	16·5	2	12·1 } 4·3
8	33·0	2	6·1 }	77·5	2	2·6 }
16	249·5	3	1·2	260·5	—	—
32	127·0	—		66·0	—	—
⩾ 64	26·5	—		16·0	—	—

* Number of fourfold titre increases with HIT.
† Per 100 person-years.

The corresponding data for influenza B are given in Table 6. The risk of infection was already considerably reduced by a preinfectious titre of 8 ($P < 0{\cdot}01$ %). In spite of the low sensitivity of HIT these data also show a correlation between antibody titre and protection for influenza B.

The prognostic value of antibody titres for the risk of infection with OC43-like agents are presented in Table 7. Like with influenza A, the correlation between protection and antibody titres is good.

DISCUSSION

The antibody titre increases detect only a portion of all infections by the respective agents. This is particularly true for CF-antibodies, whose comparatively short biological half-life presents additional difficulties (Hawkes, 1979), but it also has been repeatedly demonstrated that the actual number of infections may also be grossly underestimated by HIT. Frank *et al.* (1980), for example, showed HIT to be only half as sensitive for the detection of influenza B titre increases as a microneutralization test. Similar results were obtained in a corresponding study for human coronavirus OC43 (Gerna *et al.* 1979). Poor HI-antibody responses were

https://doi.org/10.1017/S0950268800061707 Published online by Cambridge University Press

also described after infection with the new influenza A (H1N1)-strains (Ksiazek *et al.* 1980; Layde *et al.* 1980; Foy *et al.* 1981).

These shortcomings, however, do not detract from the usefulness of titre increases as an objective means of establishing the relative risk of acquiring infections. Over the 4-year surveillance period, the incidence of all serologically demonstrated infections in our study was 21·25 for the student nurses per 100 person-years and 11·45 for the medical technology students ($P < 0{\cdot}01$). The relative risk for student nurses compared with that for the medical technology students was 1·86:1.

In the companion questionnaire-based study (Gerth *et al.* 1987) the risk ratio of all respiratory and gastrointestinal illnesses for student nurses compared with that of medical technology students was calculated as 1·28:1. However, with the more reliable 'lower respiratory syndrome (LRS) plus respiratory and gastrointestinal syndrome (RGS)', the corresponding risk ratio reached 1·55:1.

Evidence for the good reproducibility of syndrome reporting is presented in the companion study. Our serological findings do not contradict the results presented in the companion study. One titre increase corresponds to 8·4 reported LR and RG syndromes in the student nurses and 10·5 in the medical technology students. Titre increases to all 12 employed antigens were found in both the student nurses and the medical technology students. The most frequently observed infections in both groups were influenza A and B, their contribution to the total number of infections being similar, i.e. 35·1% in the student nurses *vs.* 36·1% in the medical technology students for influenza A and 16·5% *vs.* 20·0% for influenza B. RSV ranked third in the student nurses (12·4%), and the parainfluenza group third in the medical technology students (16·0%). The preponderance of RSV in the student nurses appears plausible, considering the special importance of this virus for nosocomial infections in paediatric wards (Hall *et al.* 1978). However, in our study the numbers below the level of influenza B are too small to permit conclusions.

We tried to estimate the contribution of spread among classmates to the total number of infections by looking for clusters of infections in one class during the same respiratory season. Given the comparatively high probability of multiple introductions of all agents in the nurses' group and of influenza in all groups, a reasonable estimate should be limited to the less frequent non-influenza infections in the medical technology students. The number of possible secondary cases in this group was very low. Considering the high population immunity, the absence of spread for most introduced agents is not surprising.

The study gave us an opportunity to follow immunity, as reflected by antibody titres in the student nurses and the medical technology students.

Figs. 2–4 show the temporal course of HI GM titres for three agents during the surveillance period: influenza A (H3N2), influenza B, and human coronavirus OC43.

Influenza A (H3N2) HI GM titres in the student nurses were consistently higher than in the medical technology students, even though the incidence of influenza A infections was low (7·09 serologically demonstrated influenza A infections/100 person-years in the student nurses and 3·89 in the medical technology students).

The Bundesgesundheitsamt (Federal Office of Health) of the FRG reported two

periods of increased H3N2 influenza activity for the FRG during our surveillance period: early 1976 and early 1978. In both periods, excess mortality was low but definite (Pöhn, 1981). The first epidemic was caused by the spread of the new drift variant A/Victoria/3/75. In 1976 we found only 6 titre increases for all students (1 medical technology student, 5 student nurses). The low incidence in 1976 could be explained by local epidemiological factors that may well not apply to the Federal Republic as a whole. In 1974–5, a major outbreak of influenza, due to A/Port Chalmers/1/73 (H3N2), occurred which certainly led to high population immunity. According to a serological survey in samples obtained from 100 blood donors and 100 pregnant women, living in or around Tübingen, the percentage with antibodies to influenza A/Scotland/840/74 (H3N2) with HI titres of 32 rose from 14% in 1974 to 74% in 1975 (Umbach, 1980).

It is remarkable that the titre increases in early 1977 were found mainly in student nurses. Fifteen of the 19 titre increases were in the student nurses group. According to Pöhn (1981) 1977 was a non-epidemic year with no excess mortality. Cases of influenza A, however, were reported from laboratories throughout the Federal Republic (Knocke & Kamolz, 1979). The 17 titre increases in the first half of 1978 corresponded to the reported time of spread for A/Texas/1/77-like strains.

In spite of the rather low influenza A activity during the observation period the risk of acquiring a serologically demonstrated influenza A infection remained high for the student nurses with HI titres of $\leqslant 8$ (19·6/100 person-years). In student nurses with titres of 16, the risk was reduced to 2·1/100 person-years. The corresponding values for the medical technology students were 8·2 and 1·4/100 person-years.

In accordance with the results of Hobson *et al.* (1972), Pyhälä & Aho (1975), Delem & Jovanovic (1978) as well as Meiklejohn (1983) and in contrast to earlier studies (Bashe *et al.* 1964; Maynard *et al.* 1968) protection correlated well with the preinfectious titre, the protective titre being $\geqslant 32$. It should be stressed that the immunity of our subjects was naturally acquired. The correlation may not be quite so strict with vaccination-induced antibodies (Tyrrel, 1980).

In contrast to influenza A, influenza B HI GM mean antibody titres for the student nurses and the medical technology students did not differ. We cannot explain this but we also found no significant differences between the two groups for CF or/and HI antibody levels against parainfluenza, RS, influenza C viruses and *Mycoplasma pneumoniae* (data not shown). There was one influenza B epidemic in 1975–6 which led to seroconversion in 20–40% in samples of children of different ages in and around Tübingen (Ley, 1981), and a second smaller one in 1978–9. The incidence of infection for the entire surveillance period, as demonstrated by HIT, CFT or both, was 3·50 per 100 person-years for the student nurses and 2·29 for the medical technology students. Protection was closely correlated with HI-antibody titres. Twenty (79·9) of the 26 cases occurred in students with titres of < 8; 5 (19·2%) in students with titres of 8, and only 1 (3·8%) in a student with a titre of 16. The comparatively low protective titres may be accounted for by the low antibody avidity of the B/Wellington/1/75-like antigen.

Reinfections of adults with human coronavirus occur frequently (Monto & Lim, 1974; Gerna *et al.* 1979; Reed, 1984). In our study HI-antibody titres to human coronavirus OC43 in the student nurses were consistently higher than in the

medical students, a finding which cannot be explained by the few detected titre rises (student nurses 8; medical technology students 4). It may well be that we also measured cross-reacting antibodies due to infection with related strains (McIntosh *et al.* 1974; Larson, Reed & Tyrrell, 1980) and that the short-lived and low antibody response due to cross-reactive antibodies did not show up as fourfold titre increases in our test system.

It has been speculated (Reed, 1984) that antigenic variants within the two main groups of human coronavirus (Macnaughton, Madge & Reed, 1981) are at least partially responsible for the reinfections observed in adults. Although we cannot exclude a role of such antigenic variants in reinfection, it should be pointed out that we found a good correlation between preinfectious titres to the OC43 strain, which was isolated almost 10 years before the start of our study (McIntosh *et al.* 1967), and protection against serologically confirmed infection (Table 7).

As was to be expected from older literature, the population immunity to OC43-like viruses in the FRG, as reflected by HI titres, was high (Henigst, 1974; Sarateanu & Ehrengut, 1976); the incidence of serologically demonstrated infections per 100 person-years, however, was only 1·8 in student nurses and 0·9 in medical technology students. These incidences are much lower than those reported in a family study conducted in Tecumseh, Michigan (Monto & Lim, 1974).

Based on a 4-year surveillance period between 1966 and 1969 the incidence for 20- to 29-year-old persons was 9·8% per year, as determined by HIT. The total titre increases to OC43 were raised to 15·1% by the additional use of CFT. In the Tecumseh study, however, two larger epidemics, occurring within the 4-year instigation period accounted for most of the infections, and the absence of such major epidemics in our study may explain the different findings.

We would like to express our gratitude to all participants in the study for their cooperation; to Dr W. Duckert, Director of the Department of Paediatrics at the Municipal Hospital in Reutlingen, as well as Ms Gisela Fürst and Elsbeth Nonnenmacher, nursing instructors, for their assistance; to Messrs. Eichenseher and Juranek, Dr Grund-Krehl, and Ms Black, Department of Medical Documentation and Data Processing, and to Mss M. Kiedaisch and L. Jansen for their secretarial help.

Part of the study was supported by a grant of the German Green Cross.

REFERENCES

Bashe, W. J., Stegmiller, H., Leonids, D. & Greenwald, P. (1964). Failure of polyvalent vaccine to provide clinical protection against Asian influenza. *New England Journal of Medicine* **270**, 870–874.

Cox, D. R. (1953). Some simple approximate tests for Poisson variates. *Biometrika* **40**, 354–360.

Delem, A. & Jovanovic, D. (1978). Correlation between rate of infection and preexisting titre of serum antibody as determined by single radial hemolysis during an epidemic of influenza A/Victoria/3/75. *Journal of Infectious Diseases* **137**, 194–196.

Dowdle, W. A., Kendal, A. P. & Noble, G. R. (1979). Influenza viruses. In *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections* (ed. E. H. Lennette and N. J. Schmidt), 5th ed., pp. 585–609. Washington: American Public Health Association.

FOY, H. M., ALLAN, I., BLUMHAGEN, J. M., COONEY, M. K., HALL, C. & FOX, J. P. (1981). A/USSR and B/Hong Kong vaccine: field experiences during an A/Brazil and an influenza B epidemic. *Journal of American Medical Assocation* **245**, 1736–1740.

FRANK, A. L., PUCK, J., HUGHES, B. J. & CATE, TH. R. (1980). Microneutralization test for influenza A and B and parainfluenza 1 and 2 viruses that uses continuous cell lines and fresh serum enhancement. *Journal of Clinical Microbiology* **12**, 426–432.

GERNA, G., CEREDA, P. M., REVELLO, M. G., GERNA, M. & COSTA, J. (1979). A rapid microneutralization test for antibody determination and serodiagnosis of human coronavirus OC43 infections. *Microbiology* **2**, 331–344.

GERTH, H.-J. & MOK-HSU, Y. CH. (1981). Reactogenicity and serological response to polyvalent aqueous and $Al(OH)_3$ adsorbed tween ether split product influenza vaccine in young adults 1979. *Infection* **9**, 85–90.

GERTH, H.-J., GRÜNER, CH., MÜLLER, R. & DIETZ, K. (1987). Common respiratory and gastrointestinal illness in paediatric student nurses and medical technology students. *Epidemiology and Infection* **98**, 33–45.

HALL, C. B., GEIMAN, J. M., DOUGLAS JR., R. G. & MEAGHER, M. P. (1978). Control of nosocomial respiratory syncytial viral infection. *Pediatrics* **62**, 728–732.

HAWKES, R. A. (1979). General principles underlying laboratory diagnosis of viral infections. In *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections* (ed. E. H. Lennette and N. J. Schmidt), 5th ed., pp. 3–48. Washington: American Public Health Association.

HENIGST, W. (1974). Vorkommen von Antikörpern gegen Coronavirus (OC43) in der gesunden Bevölkerung und bei Respirationstraktkranken. *Zentralblatt Bakteriologie und Hygiene I, Abt. Orig. A* **229**, 150–158.

HOBSON, D., CURRY, R. L., BEARE, A. S. & WARD-GARDNER, A. (1972). The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *Journal of Hygiene* **70**, 767–777.

HOPPS, H. E. & PARKMAN, P. D. (1979). Mumps virus. In *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections* (ed. E. H. Lennette and N. J. Schmidt), 5th ed., pp. 663–653. Washington: American Public Health Association.

KASEL, J. A. (1979). Adenoviruses. In *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections* (ed. E. H. Lennette and N. J. Schmidt), 5th ed., pp. 229–255. Washington: American Public Health Association.

KNOCKE, K.-W. & KAMOLZ, R. (1979). Ergebnisse der virologischen Diagnostik aus 33 kooperierenden medizinisch-virologischen Untersuchungsstellen der Bundesrepublik Deutschland. **21**. Mitteilung. Jahresbericht.

KSIAZEK, T. G., OLSON, J. G., IRVING, G. S., SETTLE, C. S., WHITE, R. & PETRUSSO, R. (1980). An influenza outbreak due to A/USSR/77 like (H_1N_1) virus aboard a US navy ship. *The American Journal of Epidemiology* **112**, 487–494.

LARSON, H. E., REED, S. E. & TYRRELL, D. A. J. (1980). Isolation of rhinoviruses and coronaviruses from 38 colds in adults. *Journal of Medical Virology* **5**, 221–229.

LAYDE, P. M., ENGELBERG, A. L., DOBBS, H. I., CURTIS, A. C., CRAVEN, R. B., GRAITCER, P. L., SEDMAK, G. V., ERICKSON, J. D. & NOBLE, G. R. (1980). Outbreak of influenza A/USSR/77 at Marquette University. *Journal of Infectious Diseases* **142**, 347–352.

LEY, K. M. A. (1981). Serologisch-epidemiologische Untersuchungen mit Hilfe der Hämagglutinationshemmungsreaktion. Influenza-B-Viren im Raume Tübingen 1975 und 1976. Inaugural Dissertation. Med. Fakultät (Theoretische Medizin) Eberhard-Karls-Universität, Tübingen.

MCINTOSH, K., CHAO, R., KRAUSE, H. E., WASIL, R., MOCEGA, H. E. & MUFSON, M. A. (1974). Coronavirus infection in acute lower respiratory tract disease of infants. *Journal of Infectious Diseases* **130**, 502–507.

MCINTOSH, K., DEES, J. H., BECKER, W. B., KAPIKIAN, A. Z. & CHANOCK R. M. (1967). Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proceedings of the National Academy of Sciences of the United States of America* **57**, 933–940.

MACNAUGHTON, M. R., MADGE, M. H. & REED, S. E. (1981). Two antigenic groups of human coronaviruses detected by using enzyme-linked immunosorbent assay. *Infection and Immunity* **33**, 734–737.

MAYNARD, J. E., DULL, H. B., HANSON, M. L., FELTZ, E. T., BERGER, R. & HAMMES, L. (1968). Evaluation of monovalent and polyvalent influenza vaccines during an epidemic of type A2 and B influenza. *The American Journal of Epidemiology* **87**, 148–157.

MEIKLEJOHN, G. (1983). Viral respiratory disease at Lowry Air Force Base in Denver 1952–1982. *Journal of Infectious Diseases* **148**, 775–784.

MONTO, A. S. & LIM, S. K. (1974). The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection. *Journal of Infectious Diseases* **129**, 271–277.

PÖHN, H.-PH. (1977). Epidemiologie der A/Hongkong (H3N2)-Influenza in der Bundesrepublik Deutschland einschliesslich Berlin (West). Bundesgesundheitsblatt **20**, 308–309.

PÖHN, H.-PH. (1981). Zur Influenza-Situation in der Bundesrepublik Deutschland. *Fortschritte der Medizin* **99**, 1325–1326.

PYHÄLÄ, R. & AHO, K. (1975). Serum H1 antibody and protection against influenza: a follow-up survey at community level of three epidemics caused by different H3N2 variants. *International Journal of Epidemiology* **4**, 127–129.

REED, S. E. (1984). The behaviour of recent isolates of human respiratory coronavirus in vitro and in volunteers: Evidence of heterogeneity among 229E-related strains. *Journal of Medical Virology* **13**, 179–192.

SARATEANU, D. E. & EHRENGUT, W. (1976). Seasonal serological pattern of coronavirus infections in Hamburg. *Immunität und Infektion* **4**, 141–143.

TYRRELL, D. A. J. (1980). Influenza vaccines. *Philosophical Transactions of the Royal Society of London* B **288**, 449–460.

UMBACH, K. TH. (1980). Influenza A – Seroimmunität 1968–1976. Inaugural Dissertation. Med. Fakultät (Theoretische Medizin) Eberhard-Karls-Universität, Tuebingen.

WILLERS, H. (1981). Influenza in Niedersachsen 1978–1981. In *Schutzimpfung gegen Influenza* (ed. F. Deinhardt and W. Hennessen). Gegenwärtiger Stand, Zukunftsaussichten. Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten in Zusammenarbeit mit Internationales Grünes Kreuz, Genf, und Deutsches Grünes Kreuz, Marburg, S. 34–36.

WILLERS, H. & HÖPKEN, W. (1979). Epidemiology of influenza in Lower Saxony during the period 1968–1978 with particular emphasis on subtypes A (H3N2) and A (H1N1) in winter 1977–78. *Medical Microbiology and Immunology* **167**, 21–27.