J. Hyg., Camb. (1969), **67**, 255
Printed in Great Britain

Patterns of infections with adenovirus types 4, 7 and 21 in military recruits during a 9-year survey

By J. VAN DER VEEN, KIEM GIOK OEI and M. F. W. ABARBANEL

Department of Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands

(*Received* 13 *September* 1968)

Numerous studies have shown the importance of adenoviruses as a cause of acute respiratory disease in military recruits. However, there are few reports on surveys of respiratory infections in military populations conducted for a period of years in order to obtain reliable and detailed data on the epidemiological pattern of adenovirus infection (Bloom *et al.* 1964; Rosenbaum *et al.* 1965). Such data are required to provide a well-founded base for the control of adenovirus illness. The present study was established for this purpose.

This paper summarizes the results of a survey of respiratory infection in military recruits over a 9-year period. It was designed primarily to acquire information on the behaviour of adenovirus infections. From 1963 to 1967 additional studies were made to assess the part played by other respiratory pathogens. Results of studies undertaken during brief periods before 1963 and designed to appraise the incidence of infection with adenovirus type 21, *Mycoplasma pneumoniae* and Coxsackie A-21 virus were described previously (van der Veen & Dijkman, 1962; van der Veen & van Nunen, 1963; Oei & van der Veen, 1967).

MATERIALS AND METHODS

Study population

The investigation was conducted from February 1958 through January 1967 in the military training centre at Ossendrecht, The Netherlands. A recruit is defined as any newly enlisted soldier, without previous military service, receiving basic training. The recruits were predominantly between the ages of 19 and 21 and came from all areas of The Netherlands. At intervals of 2 months, groups of recruits entered the camp to receive an 8-week course of basic training. The size of the groups varied from about 750 to 1350 men between 1958 and 1961 and from about 1600 to 2600 men between 1961 and 1967. During the period of basic training no other men were introduced into the camp.

The recruits were formed into companies of slightly more than 200 men, all of which followed a similar training schedule. The companies were trained separately and were housed in separate barracks. Each barrack was partitioned into rooms, $23 \times 26 \times 9{\cdot}5$ ft. housing up to 20 men, who slept in bunks of the double-decker type. Recruits from the same company ate together. Opportunities for contact

between men from different companies were limited by these circumstances to occasional associations at the common mess hall and to attendance at the theatre during off-duty hours. Following $2\frac{1}{2}$ weeks service, recruits received a $1\frac{1}{2}$-day leave every week. There was an interval of 4–5 days between two successive courses of basic training. During this interval the barracks were not occupied.

After completing the course of basic training, a portion of the recruits varying from one-third between 1958 and 1961 to three-quarters between 1961 and 1967 were shipped to duty at other posts. The remaining recruits stayed for another 8 weeks for further training. These advanced recruits were formed into separate companies. After completing the course of continued training they were transferred elsewhere. Opportunities existed for contact between fresh and advanced recruits at the mess hall and theatre. The permanent personnel who operated the centre and instructed the recruits varied during the study period between 200 and 750 men. The total strength of the camp varied between 1500 and 3500 men. The investigation was limited to recruits in basic training.

Collection of specimens

Specimens were collected from patients who were admitted to the sick quarters with symptoms of acute respiratory disease associated with rectal temperature greater than 38·0° C. (100·4° F.). Acute phase blood specimens and throat swabs for virus isolation were taken on the first or second day after admission. In addition throat swabs for isolation of streptococci were obtained shortly after admission over a 3-year period from 1962 to 1965. Convalescent blood specimens were collected 10–14 days after the first sample.

Furthermore, paired sera were collected from randomly selected recruits from each company of each group. The recruits were bled within 2 days after arrival in the camp and again at the end of the eighth week of basic training. Of the recruits of two groups who received training in February and March 1958 and in April and May 1959, 6 % and 4 % respectively were bled. Of the recruits of the remaining groups the percentage of men bled varied between 8 and 14. In all, paired sera were obtained from 8765 randomly selected men.

Sera from blood samples were frozen and stored at − 20° C. until tested. During the first two years of the study throat swabs were mailed to the laboratory. The swabs were then transferred to a tube containing 2 ml. of Hanks's balanced solution, and were squeezed against the glass. Subsequently, fluids from the swabs were frozen and preserved at − 20° C. After 1960, throat swabs for virus isolation were placed in tubes containing 2 ml. of GLY medium (0·5 % gelatin, 0·5 % lactalbumin hydrolysate, and 0·1 % yeast extract in Hanks's balanced salt solution). Fluids from the swabs were kept at 2–4° C. until arrival in the laboratory. They were then stored at − 50° C. Before inoculation, fluids from the swabs were thawed and centrifuged at 3000 rev/min. for 10 min.; the supernatants were used.

Isolation tests

From February 1958 to October 1962 throat swabs from patients who were serologically positive for adenovirus were tested routinely for virus. The specimens

were inoculated into cultures of HeLa cells. After October 1962 throat swabs were inoculated routinely into cultures of HeLa cells, rhesus monkey kidney cells and human diploid cells, irrespective of the results of serological tests. Specimens from patients serologically positive for adenovirus were also inoculated into cultures of human thyroid cells. In addition to virus isolation tests, throat swabs collected between October 1962 and October 1965 were tested for the presence of haemolytic streptococci. Cultures of human diploid cells consisted of WI-38, 'Gabi' or N-3 cells. The WI-38 cell strain was purchased from Flow Laboratories Inc., Rockville, Md. The 'Gabi' cell strain was kindly supplied by Dr R. Gispen, Utrecht. The N-3 cell strain, derived from human embryonic lung, was established in our laboratory. HeLa cells were maintained in medium consisting of 5% horse serum and 0·5% lactalbumin hydrolysate in Hanks solution. For maintenance of the other cell cultures, medium 199 (Difco) supplied with 2·5% chicken serum was used. An inoculum of 0·2 ml. of the virus isolation specimen was added to each of duplicate tubes of each kind of cell culture. During the first years of the study inoculated cultures were subjected to stationary incubation. After October 1962 a drum rotating 12 times/hr. was used for incubating inoculated cultures. Passages were made by inoculation of 0·2 ml. of fluid into fresh tubes of cell cultures. The total incubation period was 21 days. Following one or two passages, all inoculated cell cultures were tested for haemadsorption with guinea-pig erythrocytes at 4° and 37° C. Viruses isolated were typed by neutralization or haemadsorption-inhibition tests with rabbit antisera against prototype strains.

Throat swabs for the isolation of streptococci were transferred to tubes containing Pike's enrichment medium. After incubation at 37° C. for 4–18 hr., subcultures on blood agar plates were made. The plates were incubated aerobically and anaerobically at 37° C. for 24 hr. and subsequently were examined for colonies of haemolytic streptococci. Representative colonies were tested for sensitivity to bacitracin. Sensitive strains were considered to be of group A.

Serological tests

Tests with sera from recruits for neutralizing antibody titre against adenoviruses were carried out according to procedures described previously (van der Veen, Abarbanel & Oei, 1968). The viruses used were the prototype strains of adenovirus types 4, 7 and 21.

The following strains of viruses were used as antigens for complement-fixation (CF) tests: prototype strain of adenovirus type 4; influenza A-1/Netherlands/49 and influenza A-2/Netherlands/60; influenza B (Lee); influenza C (Taylor); parainfluenza 1 (61–1264); parainfluenza 2 (Greer); parainfluenza 3 (24,249); Sendai; RS (Long); Coxsackie A-21 (58–5178); and *Mycoplasma pneumoniae* (Mac). The Coxsackie A-21 virus and the strains of parainfluenza 1 and 3 viruses were isolated in this laboratory. CF antigens were prepared from chorioallantoic membranes of infected chick embryos for influenza A and B viruses, from allantoic fluid of infected chick embryos for influenza C and Sendai viruses, and from infected cell cultures for the other viruses. CF antigen of *M. pneumoniae* was prepared according to the technique described by Chanock *et al.* (1962). With the exception of influenza

viruses, virus suspensions to be used as CF antigens were inactivated at 56° C. for 30 min.

Between 1958 and 1962, CF tests were performed in transparent plastic sheets with 0·125 ml. unit volumes and overnight fixation at 4° C. according to a minor modification of the Kolmer technique (van der Veen, 1955). After October 1962, CF tests were done in microtitre plates according to the technique described by Sever (1962). The lowest initial serum dilution tested was 1 in 5. Titres were expressed as the highest initial serum dilution causing complete or nearly complete (more than 3+) fixation of complement. Paired sera were always titrated simultaneously. A fourfold or greater rise in antibody between paired sera was considered significant.

RESULTS

Incidence of adenovirus illness and infection

During the 9-year study period, 89,200 recruits entering service at bi-monthly intervals received an 8-week course of basic training (section 1 of Fig. 1). The morbidity of patients admitted with febrile respiratory illness is shown in section 2 of Fig. 1. The expected influence of season is clearly seen, rates for the winter and spring being, in general, much higher than those for the other seasons.

Paired sera were obtained from 2776 of 4408 patients admitted to the sick quarters and were tested for increase in CF antibody titre against adenovirus. In all, 938 (34 %) of the 2776 patients tested showed serological evidence of infection with adenovirus. The incidence of adenovirus illness was estimated by applying the rate of serologically diagnosed cases to the total respiratory illness admission rate for each group of recruits (section 3 of Fig. 1). Outbreaks of adenovirus illness occurred in the winter and spring, and in some years in the late fall, whereas the incidence was very low or nil during the summer and early fall.

In addition to the admission rate for adenovirus illness, an estimate of the total incidence of adenovirus infection was made. Paired sera spanning the 8-week training interval were collected from roughly 10 % of all recruits. In all, 8765 recruits were randomly selected. The sera were tested for CF antibody titre against adenovirus. It is assumed that a rise in CF antibody against adenovirus may be detected by the eighth day after infection. Thus, the 8-week interval allowed for the detection of antibody which might have developed if infection occurred during the seventh week of basic training or earlier. The percentages of positive serum pairs are shown in section 4 of Fig. 1. These percentages give an estimate of the total incidence of adenovirus infection in the various groups. The seasonal distribution of adenovirus infection was similar to that of adenovirus illness. The incidence was high during the cold season. This contrasted with the summer, when infections were virtually absent. During epidemic periods about 20–60 % of the recruits were infected with adenovirus at some time during training.

Prevalence of different adenovirus types

The recovery of adenovirus from throat swab specimens from patients with acute respiratory disease is summarized in section 5 of Fig. 1. In all, adenoviruses

were isolated from 633 patients. A remarkable finding was the successive appearance of different serotypes. Type 7 was prevalent from 1958 to 1960 and was replaced in 1960 by type 21. Three years later type 21 in turn was replaced by type 4. Besides strains of these types, one strain of type 1, five strains of type 2 and eight strains of type 3 were recovered during the study period.

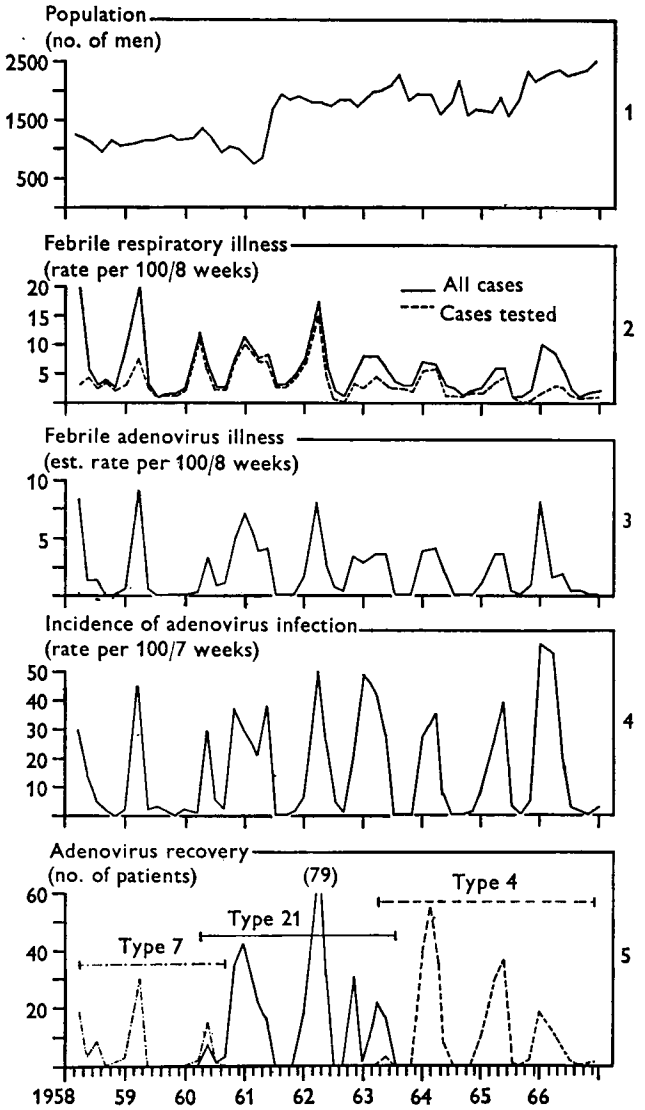

Fig. 1. Adenovirus infection among military recruits at Ossendrecht, 1958–67.

Serological studies were carried out to measure the population's prior experience with adenoviruses and to investigate whether changes in immunity of the general population occurred as estimated by frequency of neutralizing antibody. Sera collected from randomly selected recruits at the time of entry into service were

tested for neutralizing antibody against adenovirus types 4, 7 and 21. The sera were tested at a dilution of 1 in 4 and 1 in 16. Sera from nine groups of recruits who entered service in different years between 1958 and 1967 were used. The percentages of sera containing antibody to the various adenovirus types are shown in Table 1. The values give an estimate of the frequency of antibody among young adults of the general population.

No substantial changes in frequency of antibody at a serum dilution of 1 in 4 were found for any of the three types. This applied also to a comparison of the frequency of antibody at a dilution of 1 in 16. The data suggest that the immunity of incoming recruits remained fairly constant during the 9-year study period.

Table 1. *Prevalence of neutralizing antibody against adenovirus types in sera of incoming recruits in different years*

Time of collection of sera	No. of men studied	Percentage with antibody*		
		Type 4	Type 7	Type 21
June 1958	108	50 (24)	58 (37)	54 (34)
June 1959	116	63 (22)	57 (32)	54 (33)
June 1960	98	60 (23)	51 (29)	53 (34)
June 1961	117	66 (28)	62 (30)	44 (28)
June 1962	107	64 (26)	56 (31)	50 (31)
June 1963	107	65 (32)	51 (29)	49 (32)
June 1964	114	64 (26)	52 (31)	50 (33)
June 1965	116	68 (31)	52 (29)	56 (40)
June 1966	101	51 (31)	56 (29)	49 (34)

* Titres of 1/4 or more; in parenthesis titres of 1/16 or more.

Proportion of infections associated with illness

An attempt was made to estimate the proportion of adenovirus infections which was associated with clinical illness. Estimates were calculated for infections with type 7, type 21 and type 4. To allow comparison with the total incidence of infection, only cases occurring during the first 7 weeks of basic training were included and increase in CF antibody was used as the sole criterion for infection. The study was further confined to outbreaks in which a reasonably representative proportion (60 % or more) of the patients with acute respiratory illness was studied.

As seen in Table 2, the estimated ratio of the total amount of infection to the amount of clinical infection showed only minor fluctuations among different outbreaks. Thus, it would appear that the serotype had little influence upon the severity of infection. Likewise, there was no evidence that the infections occurring in the winter were more severe than those occurring in the fall or spring.

Adenovirus illness by week of training

For each group of recruits record was made of the number of patients admitted with adenovirus illness by week of training. Because of the small number of admissions in a group, the distribution of cases by week shows considerable chance fluctuation. To overcome this difficulty, the numbers of admissions in different

groups were combined to give the composite distribution of cases by week. The composite distribution was calculated for each of the three successive periods in which, respectively, adenoviruses type 7, type 21 and type 4 were prevalent. In Fig. 2 the cumulative percentages of cases by week are compared for the three serotypes.

Table. 2 *Ratio of total amount of adenovirus infection to amount of adenovirus illness*

Prevalent adenovirus type	Period	Est. rate/100/7 weeks: All adenovirus infections	Est. rate/100/7 weeks: Adenovirus illness	Est. ratio of total:clinical
Type 7	Apr.–May 1958	14	1·0	14:1
Type 21	Oct.–Nov. 1960	37	3·3	11:1
	Dec.–Jan. 1961	30	3·7	8:1
	Feb.–Mar. 1961	21	1·7	12:1
	Apr.–May 1961	38	3·3	12:1
	Feb.–Mar. 1962	50	7·1	7:1
	Apr.–May 1962	25	2·0	12:1
Type 4	Dec.–Jan. 1964	26	2·4	11:1
	Feb.–Mar. 1964	35	3·1	11:1

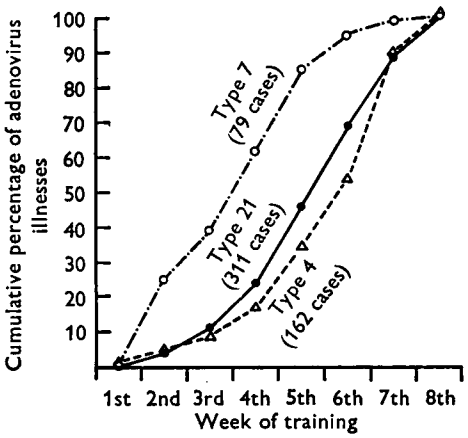

Fig. 2. Adenovirus illness by week of training.

Cases of illness due to type 7 occurred at a relatively early time during the training period. Over 50% of these illnesses were observed by the fourth week of training. There was a delay of about 2 weeks in the cumulative percentages of cases due to type 21 and type 4 when compared to corresponding percentages of type 7 illnesses. Less than one fourth of the cases due to type 21 and type 4 fell within the first month of training and 50% of the cases were observed by the sixth week.

Comparison of CF test and virus isolation

A comparison was made of the sensitivity of the CF technique and virus isolation tests in detecting adenovirus infection. Data relative to patients with acute respiratory illness from whom throat swabs were tested for the presence of virus,

irrespective of the results of the CF test, were employed. The comparative study was confined to patients admitted during outbreaks of adenovirus infection. In all, 1061 patients were included. The patients were divided into three groups according to the prevalent serotype (type 7, type 21 or type 4). Table 3 shows that in each group virus isolation tests were less sensitive in detecting infection than was the CF technique. In all, adenovirus was isolated from 72 % of the cases diagnosed by CF test and from only 8 % of the serologically negative cases.

Table 3. *Comparison of CF test and virus isolation test for detecting adenovirus infection*

Prevalent adenovirus type	CF antibody rise to adenovirus	No. of patients studied	Adenovirus isolated	
			No.	Percentage
Type 7	Positive	67	42	63
	Negative	18	2	11
Type 21	Positive	390	285	81
	Negative	98	18	18
Type 4	Positive	287	210	73
	Negative	201	5	2
Totals	Positive	744	537	72
	Negative	317	25	8

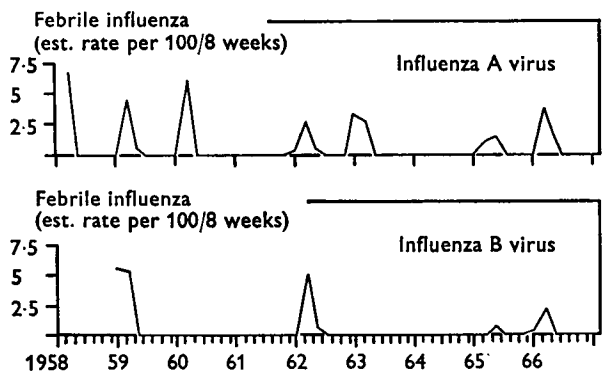

Fig. 3. Influenza among military recruits at Ossendrecht, 1958–67.

Other respiratory agents

Paired sera from the patients and randomly selected recruits who were studied for evidence of adenovirus infection were also tested for increase in CF antibody against influenza A and B viruses. As seen in Fig. 3, outbreaks of influenza A occurred at 1- or 2-year intervals, those of influenza B at 3- or 4-year intervals. Subclinical infections with influenza virus as evidenced by antibody rises in randomly selected recruits were found exclusively during outbreaks. The total incidence of infection with influenza A virus varied between 9 % and 44 % among different outbreaks of influenza A. The total incidence of infections with influenza B virus during outbreaks of influenza B ranged from 7 % to 36 %.

Between October 1962 and October 1966, paired sera from 570 patients

serologically negative for adenovirus and influenza A and B viruses were tested for CF antibody titre against various other respiratory agents. In addition, virus isolation tests were done routinely in these cases. Adenovirus was isolated from 23 of the 570 patients. Table 4 shows that infection with other respiratory pathogens as evidenced by virus isolation or antibody rise or both was detected in 87 of the remaining 547 patients.

Table 4. *Evidence of infection with various agents in patients with acute respiratory disease negative for adenovirus and influenza virus*

	Year of study				
	1962–3	1963–4	1964–5	1965–6	Totals
No. studied	189	176	87	95	547
No. positive					
Influenza C	15	2	3	1	21
Parainfluenza	3	2	5	4	14
RS	1	0	0	1	2
Coxsackie A-21	6	7	0	3	16
Echo-28	0	3	0	0	3
Picorna unidentified	4	13	2	3	22
M. pneumoniae	3	4	2	0	9

Influenza C virus was relatively more active in the spring and summer of 1963; during this period 15 of the 21 patients positive for influenza C were admitted. All but three of the CF antibody rises for Coxsackie A-21 virus occurred throughout a 12-month period between April 1963 and April 1964. It is dubious whether these rises were due to infection with Coxsackie A-21 virus, since no increase in neutralizing antibody to the virus was found and no virus was recovered in these cases. In a previous study we reported that Coxsackie A-21 virus was active in the military population at Ossendrecht in the fall of 1961 (Oei & van der Veen, 1967). CF antibody rises for parainfluenza virus and *M. pneumoniae* were observed throughout the 4-year period. Cases positive for *M. pneumoniae* were also tested for increase in amount of fluorescent stainable antibody against *M. pneumoniae;* antibody rises were found in all instances. As in the findings of the present study, *M. pneumoniae* was found to be a minor cause of acute respiratory disease in recruits at Ossendrecht during a separate study conducted in 1961 and 1962 (van der Veen & van Nunen, 1963).

Haemolytic streptococci of group A were recovered from 10 (3%) of 286 patients with serological evidence of adenovirus infection and from 40 (10%) of 397 patients who were serologically negative for adenovirus. The data do not allow a reliable appraisal of the role of haemolytic streptococci in producing acute respiratory disease, since the mere presence of *Streptococcus pyogenes* in the throat cannot be regarded as diagnostic of streptococcal illness (Williams, 1958). The difference in incidence of recoveries of streptococci between patients with adenovirus illness and those with illness not due to adenovirus was 7%. If it is assumed that the excess of recoveries in the latter group or part of it was aetiologically associated with illness, and if, subsequently, the excess rate is applied to the total

number of patients studied, then it may be estimated that the proportion of total respiratory illness due to group A haemolytic streptococci was 4% or less.

The percentage distribution of aetiological diagnoses in recruits with acute respiratory illness is graphically presented in Fig. 4. Adenovirus represented the major cause of illness for which a cause was found. The next most common diagnostic category was influenza. Other agents were of minor importance. A number of patients showed serological evidence of simultaneous infection with adenovirus

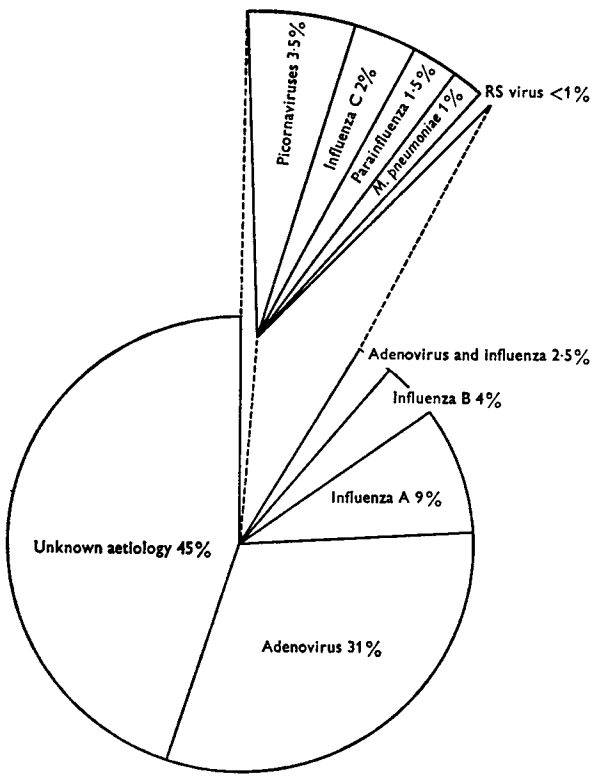

Fig. 4. Distribution of aetiological diagnoses in recruits with acute respiratory illness.

and influenza virus. Probably, the proportion of double infections was underrated, since no serological tests for parainfluenza, RS and Coxsackie A-21 viruses and *M. pneumoniae* were done in patients with adenovirus infection and influenza. In as many as 45% of the cases, no aetiological diagnosis could be made. Although insensitivity of tests for detecting infection or loss of infectivity of agents during collection and processing of specimens may be a cause of failure in establishing diagnoses, it seems likely that at least a part of these illnesses were produced by presently unrecognized agents or families of agents. In a previous study we reported that an attempt to uncover agents in acute respiratory disease of unknown aetiology by application of interference tests was unsuccessful (Custers & van der Veen, 1967).

DISCUSSION

Perhaps the most interesting finding in this survey was the successive appearance of different serotypes. In a previous study we reported the prevalence of adenovirus type 14 in the winter of 1955 (van der Veen & Kok, 1957). The present data show that type 7 was prevalent from 1958 to 1960, type 21 from 1960 to 1963 and type 4 from 1963 up to the end of the study period in 1967. Studies conducted among Finnish military recruits between 1964 and 1966 suggested also that different adenovirus types may be prevalent in successive seasons (Mäntyjärvi, 1966). This pattern of adenovirus infection is substantially different from that reported for military populations in the United States (cf. review by Huebner, Rowe & Chanock, 1958). Types 4 and 7 were found to be the most prominent causes of adenovirus illness in American recruits, whereas type 3 played a less important role. No periodic variation in prevalence of these types was observed.

We thought that the successive appearance of different serotypes might be related to variation in the proportion of immunologically susceptible persons among the incoming recruit population. However, the data did not support this concept. The immunity of the population against types 4, 7 and 21 remained fairly constant during the 9-year study period. Possibly, the prevalence of type 7 during the first years of the study and the subsequent appearance of type 21 originated from the behaviour of adenovirus in the general population. Previous studies on children in hospital with acute respiratory disease in the Netherlands showed that type 7 was the predominant cause of adenovirus illness from 1958 to 1960 whereas type 21 was predominant in 1961 (Kapsenberg, 1962; van der Veen & van Zaane, 1963). Because of the relatively high frequency of contact between recruits and their families, there was ample occasion for the introduction of viruses into the training centre from the civilian population.

The important role of adenovirus in causing respiratory illness in recruits at Ossendrecht is evident. About 20–60 of every 100 recruits who received training in the winter or spring were infected at some time during the period of training, and about 7–14 of every 100 infections were severe enough to require admission to the sick quarters. Adenovirus accounted for about 30–70 % of all cases of acute respiratory illness occurring during the colder months of the year. Since reasons for reporting sick and criteria for admission of patients to sick quarters or to the hospital may vary in different training centres, in different years for the same centre and in different companies of the same population, the results of surveys of adenovirus illness in different military populations are difficult to compare. Studies designed to permit an estimate of the total incidence of adenovirus infection, irrespective of the presence and severity of illness, are better suited for comparison of incidence rates. Surveys of the total incidence of adenovirus infections in military recruits are scarce and are confined to relatively small numbers of recruits and brief periods of time. Studies conducted on United States Army and Navy recruits during an 8-week training interval in winter showed that about 50–80 % of the men became infected with adenovirus as measured by CF tests (Hilleman *et al.* 1955; Grayston *et al.* 1959; McNamara, Pierce, Crawford & Miller, 1962; Forsyth, Bloom, Johnson

& Chanock, 1964). In a training centre in Finland, unreported adenovirus infection as evidenced by a rise in CF antibody was found in 24 % of 159 recruits studied between February and April 1964 (Mäntyjärvi, 1966).

The seasonal pattern of adenovirus infection among recruits at Ossendrecht was characterized by the occurrence of extensive epidemics in the late fall, winter and spring and a very low incidence or absence of illness in the summer and early fall. A similar pattern was observed in military recruits at Fort Dix, N. J. (Hilleman *et al.* 1957), and at training centres in Finland (Mäntyjärvi, 1966), and in advanced recruits at Camp Lejeune, N.C. (Bloom *et al.* 1964). In contrast, studies at the Great Lakes Naval Training Centre showed that in this locality adenovirus illness occurred throughout the year, with the highest incidence usually occurring in the winter and early spring (Woolridge *et al.* 1956; Rosenbaum *et al.* 1965). Furthermore, during a comparative study of two companies at this centre who received training in the summer months and the winter months respectively no difference in the total amount of serologically detectable adenovirus infection was found (McNamara *et al.* 1962).

Several hypothetical explanations—such as crowding, variation in virus dosage, influence of the indoor relative humidity upon virus spread and variation in host defence mechanisms—might be advanced for the seasonal occurrence of epidemics of adenovirus infection. The present data offer no clue as to the nature of this phenomenon. However, the data do show that, at least in this population, the as yet undetermined seasonal factor influenced the incidence rate of subclinical as well as clinical adenovirus infection. During the warmer months of the year the activity of adenovirus appeared to be negligible.

SUMMARY

Investigations to define the epidemiological pattern of adenovirus infection in military recruits were carried out in a training centre at Ossendrecht, The Netherlands, during a 9-year period. Extensive outbreaks of adenovirus illness occurred in the winter and spring, and in some years in the late fall. The seasonal variation in total incidence of adenovirus infection as estimated by frequency of antibody rises in randomly selected recruits correlated well with that of adenovirus illness. In the cold season 20–60 of every 100 men were infected, whereas no or very little activity of adenovirus was found during the summer.

Type 7 was prevalent during the first 2 years of the study period, type 21 during the next 3 years and type 4 during the last 4 years. The proportion of adenovirus infection associated with clinical illness, the distribution of adenovirus illness by week of training and the immunity of incoming recruits against adenovirus infection as estimated by frequency of neutralizing antibody were compared for type 7, type 21 and type 4.

Adenovirus was responsible for about one third of all cases of acute respiratory illness. The next most common diagnostic category was influenza. Other respiratory agents (parainfluenza, RS and Coxsackie A-21 viruses, *Mycoplasma pneumoniae* and haemolytic streptococci) were of less importance.

This work was supported in part by the United States Department of the Army through its European Research Office.

We are indebted to the following persons who contributed to this study: C. P. A. van Boven, R. Brouwer, J. H. E. Custers, J. H. Dijkman, A. Prins and A. R. van der Werff, medical officers; Mr A. P. K. M. I. van Nieuwstadt, Miss M. C. J. van Nunen, Dr G. C. J. van der Ploeg and Dr H. J. A. Sonderkamp, members of the staff of our laboratory; and Misses C. H. Bronswijk, G. J. Hof, A. D. E. M. Leeuwenberg, W. T. C. J. Smulders and C. M. G. Stevens, and Mrs A. M. van Snik, technicians.

Major-General Dr H. J. van der Giessen and Brigadier General W. P. Blokpoel, former Directors-General of Medical Services of the Royal Netherlands Army, and Colonel Dr B. J. W. Beunders, former Head of the Division of Preventive Medicine, granted facilities for the conduct of this study.

REFERENCES

Bloom, H. H., Forsyth, B. R., Johnson, K. M., Mufson, M. A., Turner, H. C., Davidson, M. A. & Chanock, R. M. (1964). Patterns of adenovirus infections in marine corps personnel. I. A 42-month survey in recruit and nonrecruit populations. *Am. J. Hyg.* **80**, 328.

Chanock, R. M., James, W. D., Fox, H. H., Turner, H. C., Mufson, M. A., & Hayflick, L. (1962). Growth of Eaton PPLO in broth and preparation of complement fixing antigen. *Proc. Soc. exp. Biol. Med.* **110**, 884.

Custers, J. H. E. & van der Veen, J. (1967). Failure to detect interfering agents in febrile respiratory illnesses. *Antonie van Leeuwenhoek* **33**, 213.

Forsyth, B. R., Bloom, H. H., Johnson, K. M. & Chanock, R. M. (1964). Patterns of adenovirus infections in marine corps personnel. II. Longitudinal study of successive advanced recruit training companies. *Am. J. Hyg.* **80**, 343.

Grayston, J. Th., Woolridge, R. L., Loosli, C. G., Gundelfinger, B. F., Johnston, P. B. & Pierce, W. E. (1959). Adenovirus infections in naval recruits. *J. infect. Dis.* **104**, 61.

Hilleman, M. R., Werner, J. H., Dascomb, H. E., Butler, R. L. & Stewart, M. T. (1955). Epidemiology of RI (RI-67) group respiratory virus infections in recruit populations. *Am. J. Hyg.* **62**, 29.

Hilleman, M. R., Gauld, R. L., Butler, R. L., Stallones, R. A., Hedberg, C. L., Warfield, M. S. & Anderson, S. A. (1957). Appraisal of occurrence of adenovirus-caused respiratory illness in military populations. *Am. J. Hyg.* **66**, 29.

Huebner, R. J., Rowe, W. P. & Chanock, R. M. (1958). Newly recognized respiratory tract viruses. *A. Rev. Microbiol.* **12**, 49.

Kapsenberg, J. G. (1962). Een epidemie bij kinderen veroorzaakt door adenovirus type 7. II. Etiologisch onderzoek. *Ned. Tijdschr. Geneesk.* **106**, 108.

Mäntyjärvi, R. (1966). Adenovirus infections in servicemen in Finland. *Annls. Med. exp. Biol. Fenn.* **44**, Suppl. 4.

McNamara, M. J., Pierce, W. E., Crawford, Y. E. & Miller, L. F. (1962). Patterns of adenovirus infection in the respiratory diseases of naval recruits. A longitudinal study of two companies of naval recruits. *Am. Rev. resp. Dis.* **86**, 485.

Oei, Kiem Giok & van der Veen, J. (1967). Epidemiological study of Coxsackie A-21 virus infections in military recruits. *Am. J. Epidem.* **85**, 93.

Rosenbaum, M. J., Edwards, E. A., Frank, P. F., Pierce, W. E., Crawford, Y. E. & Miller, L. F. (1965). Epidemiology and prevention of acute respiratory disease in naval recruits. I. Ten years' experience with microbial agents isolated from naval recruits with acute respiratory disease. *Am. J. publ. Hlth.* **55**, 38.

Sever, J. L. (1962). Application of a microtechnique to viral serological investigations. *J. Immun.* **88**, 320.

van der Veen, J. (1955). A rapid and simple method for conducting large series of complement fixation tests and antistreptolysin 'O' titrations. *J. Lab. clin. Med.* **45**, 323.

VAN DER VEEN, J., ABARBANEL, M. F. W. & OEI, KIEM GIOK (1968). Vaccination with live type 4 adenovirus: evaluation of antibody response and protective efficacy. *J. Hyg., Camb.* **66**, 499.

VAN DER VEEN, J. & KOK, G. (1957). Isolation and typing of adenoviruses recovered from military recruits with acute respiratory disease in The Netherlands. *Am. J. Hyg.* **65**, 119.

VAN DER VEEN, J. & DIJKMAN, J. H. (1962). Association of type 21 adenovirus with acute respiratory illness in military recruits. *Am. J. Hyg.* **76**, 149.

VAN DER VEEN, J. & VAN NUNEN, M. C. J. (1963). Role of *Mycoplasma pneumoniae* in acute respiratory disease in a military population. *Am. J. Hyg.* **78**, 293.

VAN DER VEEN, J. & VAN ZAANE, D. J. (1963). Infectie met adenovirus type 21 bij kinderen met acute aandoeningen van de onderste luchtwegen en longen. *Ned. Tijdschr. Geneesk.* **107**, 808.

WILLIAMS, R. E. O. (1958). Laboratory diagnosis of streptococcal infections. *Bull. Wld Hlth Org.* **19**, 153.

WOOLRIDGE, R. L., GRAYSTON, J. Th., WHITESIDE, J. E., LOOSLI, C. G., FRIEDMAN, M. & PIERCE, W. E. (1956). Studies on acute respiratory illness in naval recruits, with emphasis on the adenoviruses (APC–RI). *J. infect. Dis.* **99**, 182.