

## **Viruses associated with acute respiratory infections in Royal Air Force personnel**

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### SUMMARY

All respiratory illnesses which were reported to the medical officers between September 1966 and December 1967 on a Royal Air Force station of 350 men were studied virologically.

Three periods of increased respiratory infections were observed: two occurred in the autumn, one in each year, and the third in the winter during January and February. The autumnal outbreaks were associated mainly with rhinovirus infections, and high isolation rates (82.1, 65.9 %) were achieved at these times. Few of the illnesses during the winter outbreak could be diagnosed in the laboratory, and no evidence was found of infection with 'coronaviruses'.

Despite the entrance of 30 fresh recruits direct from civilian life every 5 weeks, the respiratory infections encountered on the station were very similar to those in the local population and were not predominantly infections with adenoviruses, Coevirus, and *Mycoplasma pneumoniae*, as previously reported from larger military recruit centres.

### INTRODUCTION

Many workers have reported studies of the acute respiratory infections experienced by new entrants to the armed forces, most of them at large initial training establishments. The value of many such investigations, however, has been limited by the small proportion of cases in which a diagnosis could be confirmed in the laboratory.

In 1967 it became possible for us to investigate the respiratory illnesses in a Royal Air Force station which received small intakes of officer cadets straight from civilian life to undergo their initial aircrew training. The purpose of the study was to compare the importance of the viruses isolated from respiratory illnesses in this small unit with those reported from larger recruit centres. Furthermore, it was hoped that a combination of serology, a range of tissue cultures, and the use of organ cultures would allow a diagnosis to be made with sufficient frequency that the major causes of respiratory infections could be defined.

## MATERIALS AND METHODS

*Population studied*

All Royal Air Force personnel with acute respiratory illness who reported to the Station medical officers at R.A.F. South Cerney between September 1966 and December 1967 were included in this study. The total complement of the station was approximately 350 officers and men and its main function was the initial training of officer cadets for aircrew duties. Cadets were admitted direct from civilian life to a course lasting 15 weeks. Each course consisted of approximately 33 cadets and every 5 weeks one course graduated and another course began its training so that three courses (*ca.* 100 cadets) were present on the station at any given time.

*Specimens*

A nose swab, two throat swabs, and a specimen of blood were taken from each patient at the initial interview and a second specimen of blood was drawn between 2 and 3 weeks later, if the patient had not been posted by this time.

*Virology*

One throat swab was transported dry to the laboratory and inoculated on a blood agar plate. The nose swab and second throat swab in transport medium were held and sent to the laboratory at 4° C. All such specimens were examined in tissue cultures of monkey kidney, the Bristol line of HeLa cells, human embryo diploid fibroblast (WI-38), and human embryo kidney (HEK), and newborn mice were inoculated—all as previously described (Higgins, Ellis & Boston, 1963; Higgins, Boston & Ellis, 1964). Viruses isolated were identified by haemadsorption inhibition, haemagglutination inhibition, or neutralization test except for rhinoviruses which were confirmed by their ability to grow in rolled cultures at 33° C. at pH 7 but not in stationary cultures with alkaline medium at 37° C. or by their instability in acid medium.

Specimens which failed to yield an agent by these methods were inoculated into organ cultures of human embryonic ciliated epithelium (Hoorn, 1966), the fluids from which were tested in tissue cultures and newborn mice as described for the original specimens. No fewer than four passes in organ culture, the last of which, at least, was by the modified method of Tyrrell & Blamire (1967), were carried out on all specimens which failed to yield a virus. The fluid from the final pass was spun at 60,000g for 30 min., the pellet resuspended in a few drops of distilled water and applied to a formvar or formvar-carbon-coated grid and stained with 2% phosphotungstic acid at pH 6.9. The grids were then examined in a Hitachi HS 7S, a Phillips 100, or a Phillips 200 electron microscope.

*Serology*

Paired sera were tested by the method of Bradstreet & Taylor (1962) for fourfold or greater rises in complement-fixing antibodies to influenza A, B and C viruses, psittacosis, Q fever, adenoviruses, respiratory syncytial virus, Sendai virus, and *Mycoplasma pneumoniae*. Sera from cases which failed to yield an agent were also tested for antibodies against the 229E (Hamre & Procknow, 1966) strain of

'coronavirus' (Almeida *et al.* 1968) by the same method and against the OC-43 strain of 'coronavirus' (McIntosh, Becker & Chanock, 1967) in a similar fashion but using  $1\frac{3}{4}$  units of complement.

The 229E antigen was an infected WI-38 tissue culture fluid and the OC-43 antigen an infected mouse brain suspension. All other antigens were supplied by the Standards Laboratory, Colindale.

## RESULTS

Three periods of increased respiratory illness were observed during the period studied (Fig. 1). Between September and December 1966, 39 cases of acute respiratory infection were seen; 35 were seen between January and March 1967; and 44 between September and December 1967, an incidence of 7.96, 8.33 and 7.49/1000 persons/week respectively. From April to August 1967 only 11 patients with respiratory infections consulted the medical officers, an incidence of 1.43/1000/week.

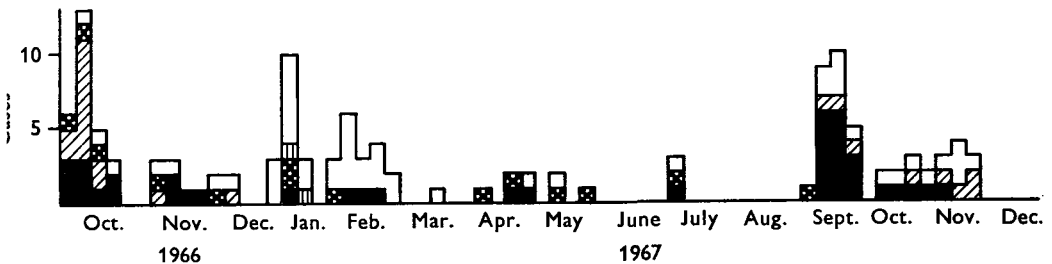


Fig. 1. Virological diagnosis of cases of acute respiratory infections seen between September 1966 and December 1967. ▨, Rhinovirus M; ■, Rhinovirus H; ▩, other pathogens; □, diagnostic serology; □, illness without isolation of pathogens or diagnostic serology.

## Isolations

A total of 77 agents (Table 1) were isolated from 76 (59%) of the 129 illnesses. Of the infecting agents, 62 (80.5%) were rhinoviruses with H-types isolated twice as frequently as M-types. A quarter of the H-type rhinoviruses were isolated only in organ culture and of the 30 isolations in tissue culture 22 were detected in HEK cultures but only 17 in WI-38. Similarly, seven of the 22 M-type rhinoviruses were isolated only in organ culture and a further six only in monkey kidney cultures. Eight strains were detected in both monkey kidney and WI-38 and one in monkey kidney, WI-38 and HEK. The strains isolated only in monkey kidney were readily isolated from the specimens but proved increasingly difficult to pass or adapt to other tissue cultures so that typing of these strains was not possible. Typing was attempted with 11 other rhinoviruses, one of which was not identifiable with the 22 sera available. Of the ten strains which were typed, three belonged to type 4, two to each of types 1B and 29, and one to each of types 1A, 15 and 30.

No structures resembling avian infectious bronchitis virus or the parainfluenza group of viruses were seen on electron microscopy of the concentrated fourth or later passage organ culture fluids of the specimens which failed to yield an agent detectable in the tissue culture systems employed.

*Distribution of isolations*

The most striking feature of this survey is the comparison between the high isolation rates associated with the two autumnal outbreaks of respiratory illness and the quiescent period during the summer, 82.1, 65.9 and 72.7 % respectively, and the failure to detect an infecting agent for the majority of illnesses in the early part of 1967, when the isolation rate was only 20 % (Fig. 1).

Table 1. 77 Isolations from 76 of 129 respiratory illnesses between September 1966 and December 1967

No. of strains	Agent
40	Rhinovirus H-type
22	Rhinovirus M-type
4	Herpes simplex virus
2	Poliovirus type 1
1	Parainfluenza virus type 1
1	Coxsackie virus type A10
1	Coxsackie virus type B3
1	Adenovirus type 5
1	$\beta$ -haemolytic streptococci Group A*
4	$\beta$ -haemolytic streptococci Group G
77	

\* Rhinovirus H-type also isolated.

*Serology*

Paired sera were examined from 49 of the 76 patients who yielded an agent, but no rises in antibody were demonstrated. This was because the agents isolated were mainly rhinoviruses and other viruses, antigens to which were not used in the complement fixation test. A rise in antibody titre to respiratory syncytial virus antigen from 1 in 16 to 1 in 64 in one pair of sera and against both adenovirus and influenza C virus antigen from 1 in 8 to 1 in 32 in another were the only positive findings among the 42 paired sera from patients from whom no virus was grown. No rising titre to either 'coronavirus' was detectable and no serum contained antibody at 1 in 8 to 229E but five pairs of sera had unchanging titres between 1 in 8 and 1 in 32 to OC-43 antigen.

*Association of virus with clinical illness*

The frequency with which each clinical type of respiratory illness was seen and the agents isolated from these cases is shown in Table 2. Sore throats accounted for half the respiratory infections seen; colds and tracheitis were each responsible for approximately a quarter of the cases. One case of laryngitis was the only other infective respiratory condition diagnosed during the period. Rhinoviruses were isolated from almost two-thirds of patients suffering from colds or tracheitis but from little more than one-third of those with sore throats. All isolations of enteroviruses and herpes simplex virus and of four of the five streptococci were associated with sore throats.

The severity of the illness may be measured by whether the patient was suffi-

ciently ill to warrant admission to sick quarters or could be treated as an out-patient, and also by the length of time the patient was off duty. This information was available for 77 of the cases seen during the first year and its relationship to the agents isolated is summarized in Table 3. The number of infections with viruses other than rhinoviruses and those with streptococci are too small to comment on with the exception that all four streptococcal infections were considered to need in-patient treatment and that the average time off duty for all patients with 'other virus' infections was of the same order as that for in-patients from whom no agent or a rhinovirus was isolated. There is a close similarity between the proportion of patients admitted and those treated as out-patients, the range and mean duration of time off duty for rhinovirus infections, and for those where no agent was isolated.

Table 2. *Laboratory diagnosis in relation to clinical illness*

Diagnosis	Cold	Pharyngitis	Tonsillitis	Tracheitis	Laryngitis
Agents isolated	1 Parainfluenza virus type 1	8 Rhinoviruses type H	8 Rhinoviruses type H	13 Rhinoviruses type H	Nil
	11 Rhinoviruses type H	8 Rhinoviruses type M	3 Herpes simplex viruses	6 Rhinoviruses type M	—
	8 Rhinoviruses type M	1 Herpes simplex virus	1 Coxsackie virus type A 10	1 Adenovirus type 5	—
	1 Streptococcus group A†	2 Poliovirus type 1	3 Streptococcus group G	—	—
	—	1 Coxsackie virus type B3	—	—	—
	—	1 Streptococcus group G	—	—	—
Fourfold rise in antibody	Nil	1 Respiratory syncytial virus	Nil	Nil	Nil
	—	1 Influenza C virus*	—	—	—
	—	1 Adenovirus*	—	—	—
Negative	12	11	16	11	1
Total	32	34	31	31	1

\* Same pair of sera. † Rhinovirus H-type also isolated.

Table 3. *Severity of illness in relation to laboratory diagnosis*

Isolate	Rhinoviruses		Other viruses		Streptococci		No agent	
	Yes	No	Yes	No	Yes	No	Yes	No
Admitted to sick quarters								
Number of patients	20	12	6	2	4	0	23	10
Days off duty: Range	2-5	1-2	2-3	2-3	2-4	—	2-6	1-2
Total	51	23	15	5	11	—	59	19
Days off duty: Mean	2.55	1.92	2.50	2.50	2.75	—	2.57	1.90

## DISCUSSION

The view that a comprehensive range of tissue cultures and the use of organ cultures would improve the results in the study of acute respiratory infections is justified. The limitations of the use of only HEK or WI-38 for the isolation of rhinoviruses (Higgins, 1966*a*) are confirmed, as are the advantages of employing organ cultures of ciliated epithelium when attempting to detect these viruses (Tyrrell & Bynoe, 1966; Higgins, 1966*b*).

The agents most commonly isolated were not adenoviruses, Coxsackie virus type A 21, and *Mycoplasma pneumoniae*, as previously reported from larger military establishments (Johnson, Bloom, Mufson & Chanock, 1962; Chanock, Fox & James, 1967; Oei & van der Veen, 1967; Mantyljarvi *et al.* 1967; Mogabgab, 1968; van der Veen, Oei & Abarbanel, 1969) and the preponderance of rhinovirus infections more closely resembles that found in the local population (Higgins, 1967) or among university students (e.g. Gwaltney & Jordan, 1966; Hamre, Connelly & Procknow, 1966; Phillips, Melnick & Grim, 1968) and families (e.g. Hendley, Gwaltney & Jordan, 1969; Fawzy *et al.* 1967). These findings would indicate that a unit of 350 men with intakes of 30 fresh individuals at regular intervals is too small for the introduction of many new viruses or possesses too few susceptibles to support an outbreak. Although the serotyping of rhinoviruses was very limited, at least seven different serotypes were detected among the 11 strains tested showing that no period of increased respiratory illnesses was attributable to an outbreak with one particular serotype.

The failure to determine the cause of the majority of the respiratory infections in the early part of 1967 by the use of tissue culture had led us to believe that the illnesses were likely to be the result of infection with 'coronaviruses'. This may be so although we failed to demonstrate the presence of typical virus particles in fluid from the organ cultures infected with these specimens or to show a rise in antibody to 229E or OC-43 in paired sera from these cases. This work was begun before Tyrrell & Blamire (1967) reported the importance of the modified method of organ culture for the isolation of these viruses; and although the later organ culture passages were by this method the earlier ones were performed as originally described by Hoorn (1966) and this could have prevented the growth of these viruses. Furthermore, strains of 229E virus have been isolated in diploid fibroblast of human embryonic intestine (Kapikian *et al.* 1969) but the authors failed to grow the strain in organ cultures of ciliated epithelium and this may apply to other 'coronaviruses'. Not all 'coronaviruses' so far isolated are serologically related to either 229E or OC-43 (McIntosh *et al.* 1969) so that negative serological findings do not exclude the possibility that these infections were caused by 'coronaviruses'. However, the close similarity in severity and duration of the illnesses from which no agent was isolated and those where a rhinovirus infection was demonstrated suggests that the causal organism was more likely to be a fastidious rhinovirus which cannot be detected in tissue cultures similar to HS (Hoorn & Tyrrell, 1966) and ST (Tyrrell, Bynoe & Hoorn, 1968) for the illness associated with 'coronavirus' infections has been shown to be shorter than those following infection with rhinoviruses (Bradburne, Bynoe & Tyrrell, 1967).

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