J. Hyg., Camb. (1962), **60**, 51
Printed in Great Britain

Respiratory viruses in a residential nursery

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(*Received* 31 *August* 1961)

INTRODUCTION

From October 1957 to May 1958, a longitudinal prospective survey of infantile morbidity was undertaken in a residential nursery, near Sheffield. The objects of this survey were twofold, to investigate the roles of the adenoviruses and of the newer respiratory viruses in the aetiology of any illnesses observed.

Since 1953, when the first strains of these viruses later known (Enders, Bell, Dingle, Francis, Hilleman, Huebner & Payne, 1956) as adenoviruses were recognized (Rowe, Huebner, Gilmore, Parrott & Ward, 1953; Hilleman & Werner, 1954), their place in the family of respiratory viruses has gradually become clearer. Antibody surveys (Jordan, Badger, Curtiss, Dingle, Ginsberg & Gold, 1956; Jordan, Badger & Dingle, 1958) have shown that infection with Types 1–7 of these viruses occurs in a large proportion of the population during childhood and early adult life. Infection with adenoviruses of Types 3, 4 and 7 is very frequently accompanied by acute respiratory disease and epidemics associated with such infection are well known amongst recruit populations (Hilleman, Werner, Adair & Dreisbach, 1955; Van der Veen & Kok, 1957; Tyrrell, Balducci & Zaiman, 1956). In the case of adenoviruses of Types 1, 2 and 5, although mild (Roden, Pereira & Chaproniere, 1956) and severe (Chany, Lépine, Lelong, Le-Tan-Vinh, Satgé & Virat, 1958; Deinhardt, May, Calhoun, & Sullivan, 1958) illnesses are known to occur in association with infection, most of the recoveries of virus have been made from tonsil or adenoid tissue, where the virus apparently lies dormant (Rowe, Huebner, Hartley, Ward & Parrott, 1955; Zaiman, Balducci & Tyrrell, 1955). Antibody studies show, however, that infection with these types of adenovirus is very common during the first five years of life and it has been assumed that the majority of these infections result in very mild or inapparent illness. In the present study, interest was centred on these latent adenoviruses.

In recent years, a number of new viruses have been recovered from cases of acute respiratory illness. A recent and comprehensive review of this field has been made by Andrewes (1959).

Many mild respiratory illnesses were likely to be observed among the infants in the Sheffield nursery during the period of study and it was therefore expected that one, at least, of these newer respiratory viruses would be recovered. In the event, outbreaks were observed of illnesses attributable to Parainfluenza 3 and to Influenza A viruses.

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METHODS

Clinical methods

The population at risk

The residential nursery in which the survey was made was the main nursery in Sheffield for children aged less than five years coming into the care of the City Children's Department. The staff was resident and there were few visitors. Admissions to and discharges from the nursery were irregular and infrequent. Forty-six children (aged from one month to 4 years) were admitted to the nursery over the 8 months of the survey. They were observed for a total period of 144 child-months in a population which fluctuated from 11 to 22, the mean population being 18; throughout this period, the proportions of children of different ages remained the same. The sex ratio of the children was approximately unity (24 boys to 22 girls), but their total experience in the nursery (86·2 child-months (boys) and 57·8 child-months (girls) differed significantly ($p < 0{\cdot}05$).*

Seven children were observed over the whole duration of the study; six infants were studied for one week or less. Between these extremes, the children were observed for a variety of periods, 27 being under observation for at least 2 months.

Methods of observation and the assessment of illness

Each child was seen twice weekly by the same observer and physically examined whenever unwell. In addition, the attendants were questioned about each child's health during the three or four days between visits. With this routine, errors in defining the duration and severity of illnesses were minimized, althought it was observed that significantly more illnesses were reported as commencing on visiting days than on non-visiting days. Any abnormal signs or symptoms were recorded on a proforma closely resembling that used by Dingle, Badger, Feller, Hodges, Jordan & Rammelkamp (1953). When the children were ill enough to be confined to bed, twice daily temperatures were taken by the nursery nurse in charge, but not otherwise.

In the present study, 'illness' was defined as the presence of a single symptom abnormal for an individual, the same definition as that used by Dingle *et al.* (1953). To be recorded on the proforma, such symptoms must usually have been present for 24 hr. or more. It is known that the emphasis placed by the investigator on any one symptom may distort the reporting of illness; for example, Lidwell & Sommerville (1951) focused attention on the 'runny nose type of cold', which was stressed as the criterion of illness. In the nursery, when inquiring after the children's health, it was mentioned, but not stressed, that the 'usual winter coughs and colds' were being investigated.

Illnesses were considered to end on the day before that on which the child's health was considered to be normal. Following Simpson (1958), a period of 24 hr. freedom from symptoms was considered sufficient to separate two episodes of illness. For analysis of numbers present in the nursery during individual weeks

* Throughout this study, the χ^2 test, using Yates' correction for small numbers where appropriate, was used as a test of significance.

or months, a child was considered as present for any week of which he spent four or more days in the nursery, and for any month of which he spent two or more weeks in the nursery. A child's age during any particular illness was taken as that child's age (to the nearest month) on the first day of that illness.

Bearing in mind the probable errors in both directions, it is possible that a reasonably accurate estimate was made of the number of episodes of illness that occurred in the nursery during the period of observation.

Laboratory methods

The collection of specimens

Two throat swabs were taken weekly from each child. One was taken into 4 ml. 50% skimmed milk/50% physiological saline and was stored, within 30–40 min. of collection, at −26° C. This throat swab was later tested for adenoviruses and, in the influenza epidemic, for influenza virus also. The second throat swab was taken into 4 ml. medium 199 (Morgan, Morton & Parker, 1950) with 0·5% bovine plasma albumin added; the throat swab fluid was stored at −70° C. This specimen was tested for the newer respiratory viruses. Specimens of serum were obtained from the children whenever possible. These were stored, without preservative, at −26° C.

Tissue cultures

(*a*) *Rhesus monkey-kidney tissue cultures.* Rhesus monkeys were obtained from Shamrock Farms, Brighton, and held in isolation for 4 or 5 weeks before being killed by deep nembutal anaesthesia and concurrent exsanguination. Tissue cultures were prepared by a modification of the method described by Melnick (1955). A cell concentration at the time of distribution of about 50,000–75,000 cells per tissue culture tube was used.

(*b*) *H.Li.*1. *tissue cultures.* Cultures of the H.Li.1. strain of human embryonic liver cell tissue culture (Westwood, Macpherson & Titmuss, 1957), a cell line very similar to HeLa cells in its sensitivity to adenoviruses (Flewett & Hoult, 1958), were obtained from Dr T. H. Flewett and the cell line was established in bottle cultures. Successive subcultures were made from these by treatment with versene and subsequent distribution of cells at an initial concentration of about 75,000 cells per tissue culture tube.

(*c*) *HeLa tissue cultures.* These were obtained from the Public Health Laboratory, Salisbury, through the courtesy of Dr P. J. Wormald. These were trypsinized and distributed at about 50,000 cells per tissue culture tube.

Media

(*a*) *Rhesus monkey-kidney tissue cultures.* Rhesus monkey-kidney tissue cultures were grown to confluence in 5% calf serum and 0·5% lactalbumin hydrolysate in Hanks' balanced salt solution containing 0·03% sodium bicarbonate and 100 units penicillin and 100 μg. streptomycin/ml. They were maintained in a variety of media, only two of which are here relevant. The first of these media was 0·5% calf serum and 0·5% lactalbumin hydrolysate in Hanks' balanced salt

solution with 0·105% added sodium bicarbonate and added antibiotics. The second was medium 199 with 0·92% sodium bicarbonate added.

(*b*) *H.Li.*1 *tissue cultures.* H.Li.1. tissue cultures were grown almost to confluence in 7% calf serum and 7% human serum in medium 199 with 0·92% added sodium bicarbonate. They were then washed twice in Hanks' saline and maintained in 5% calf serum and 0·5% lactalbumin hydrolysate in Earle's balanced salt solution.

(*c*) *HeLa tissue cultures.* HeLa tissue cultures were grown to confluence in Gey's balanced salt solution with 0·5% lactalbumin hydrolysate, 5% calf serum and 0·09% sodium bicarbonate with added antibiotics. They were maintained in 0·25% lactalbumin hydrolysate and 5% calf serum in Gey's balanced salt solution with 0·112% sodium bicarbonate and added antibiotics.

Viruses

Adenoviruses. Strains of adenoviruses Types 2 and 5 were used which had been recovered from throat swabs collected during the study. A strain of adenovirus Type 1 was obtained from Dr H. G. Pereira, National Institute for Medical Research, London, N.W. 7.

Parainfluenza 3. The Moss strain of Parainfluenza 3 virus, recovered from an ill child in the nursery during this study, was used.

Influenza A. The Iksha strain of Influenza A_2 was used. This strain was provided through the courtesy of Dr F. E. Buckland, M.R.C. Common Cold Research Unit, Salisbury.

Sera

Adenoviruses. Adenovirus immune sera were used which had been prepared in rabbits by Dr D. A. J. Tyrrell.

Parainfluenza 3. An immune rabbit serum prepared against the prototype Parainfluenza 3 strain was kindly provided by Dr R. M. Chanock.

Influenza A. A rabbit serum immune to Influenza A/Singapore/1/57 was used which had been prepared by Dr S. K. R. Clarke.

Serological methods

Haemagglutination-inhibition

The antigens used were:

Influenza A—an allantoic fluid pool of the Iksha strain of Influenza A_2.

Parainfluenza 3—the supernatant fluids from rhesus monkey-kidney tissue cultures infected with the Moss strain of Parainfluenza 3.

Method. Plastic plates were used for haemagglutination-inhibition tests, the method being that recommended by the World Health Organization (1953). Non-specific inhibitor was removed from sera by overnight treatment with crude cholera filtrate* and eight partial haemagglutinating units of antigen were used.

* A commercially available filtrate of a culture of *Vibrio cholerae* (Phillips–Roxane) was used.

Antigen and sera were allowed to react for one hour at room temperature before 0·5 % fowl erythrocytes were added. Dilutions were made in normal physiological saline buffered to pH 7·1 with phosphate buffer.

Complement-fixation

The antigens used were:

Adenovirus and Influenza A—prepared by the Standards Laboratory (Dr Bradstreet), Central Public Health Laboratory, Colindale, London, N.W. 7., and obtained from the Public Health Laboratory, Salisbury, through the kindness of Dr P. J. Wormald.

Parainfluenza 3—prepared from rhesus monkey-kidney tissue cultures, infected with the Moss strain of Parainfluenza 3, by three cycles of alternate freezing (−70° C.) and thawing (35° C.).

Method. Overnight fixation, using a modified Fulton and Dumbell technique (Balducci, Zaiman & Tyrrell, 1956) was employed.

Neutralization tests

The viruses used in these tests were the strains of adenovirus which have been described. Twofold dilutions of the sera under investigation were held with approximately 100 TCD_{50} of virus for 2 hr. at 37° C. before inoculation into HeLa tissue cultures. Serum and virus dilutions were made in Gey's balanced salt solution.

RESULTS

The incidence of respiratory illness in the nursery

During the 8 months of observation, there were 94 episodes of illness in the nursery. Ninety-one of these were respiratory illnesses, the remaining three being gastro-intestinal episodes. An average of 8·3 illnesses per year was experienced by each child, there being no significant difference between the incidence in boys and in girls.

The incidence of illness by months is given in Fig. 1 and shows the high incidence in October 1957, falling to reach a low level by the end of April 1958. The incidence of respiratory illness by weeks is given in Fig. 2 and also in Fig. 3, where the data are presented in a way which allows for the fluctuations which occurred in the nursery population.

Four major outbreaks of respiratory illness were observed. These were in October, November and December 1957 and in February 1958. The aetiology of the October outbreak is known (Influenza A) and also that of the outbreak which was observed in February (Parainfluenza 3). It is of interest that 44 of the 91 observed episodes of respiratory illness were experienced in one or other of these two outbreaks.

The outbreak of influenza

On the first visit to the nursery, on 8 October 1957, five children were found to have fallen ill on that day or within the previous two or three days. By 23 October, all but two of the 22 children at risk (aged from 6 months to 4 years, mean age

$2\frac{1}{2}$ years) had succumbed to infection. Twenty-four illnesses were recorded, as four of the children had second attacks of illness, beginning within a week or so of recovery from their initial episode.

Fifteen of these 24 illnesses were, clinically, influenza. They presented with a sudden onset of moderately high fever (99°–100° F.) and considerable malaise, together with a reddened pharynx and a dry cough. In about one third of cases,

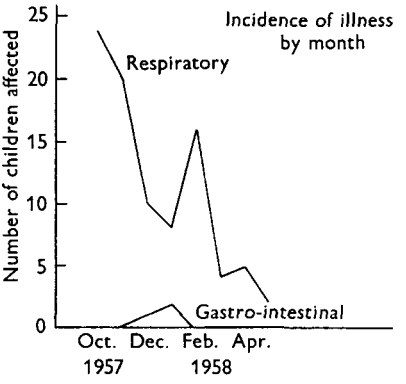

Fig. 1

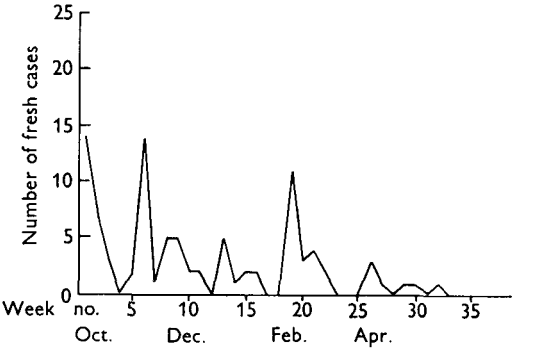

Fig. 2

Fig. 1. The incidence of illness in the nursery, by months.

Fig. 2. The incidence of respiratory illness in the nursery, by weeks (*a*).

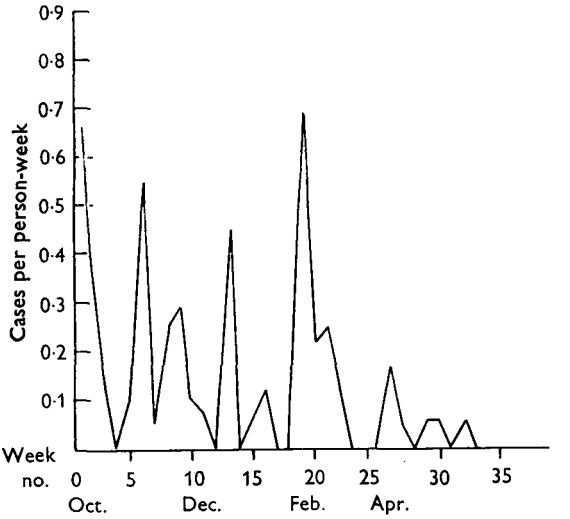

Fig. 3. The incidence of respiratory illness in the nursery, by weeks (*b*).

there was conjunctival suffusion and there was mild bronchitis in the same proportion of children. The remaining nine cases of illness appeared as respiratory illnesses of other kinds, for example, mild colds. It is of interest that all the infants had mild colds. The frequencies with which the various signs and symptoms appeared in the 24 illnesses are given in Table 1. The cumulative frequencies and daily prevalence of selected signs and symptoms are given in Fig. 4.

Virological investigations

Fourteen throat swabs were collected from ill children during the outbreak and were inoculated, by the intra-amniotic route, into 10-day old embryonated eggs. One strain of Influenza A_2 was recovered. This was isolated from a throat swab taken from a $2\frac{1}{2}$ year old girl on the fourth day of a typical attack of influenza. No sera were available from this child.

Table 1. *Symptoms and signs observed during two outbreaks of respiratory illness in a residential nursery*

Influenza A		20
	100 %	cases
Common features		
Pharynx injected	85	17
Pyrexia (> 99° F.)	80	16
Pyrexia (> 100° F.)	60	12
Nasal discharge	80	16
Rhinitis	80	16
Less common features		
Conjunctival suffusion	30	6
Sweating	30	6
Bronchitis	30	6
Pharyngeal exudate	15	3
Infrequent features		
Vomiting	10	2
Diarrhoea	10	2
Shivering	10	2
Splenomegaly	10	2
Epistaxis	5	1
Cervical lymph node enlargement	5	1
Nasal obstruction	5	1
Duration of fever (average		
99° F. or more	3·3 days	
100° F. or more	2·2 days	
Maximum temperature (average)		
	101·1° F.	

Parainfluenza 3		16
	100 %	cases
Common features		
Nasal discharge	95	15
Pharynx injected	70	12
Rhinitis	65	11
Cough	60	10
Malaise	50	8
Less common features		
Cervical lymph node enlargement	30	6
Bronchitis	30	6
Pyrexia (> 99° F.)	30	6
Pyrexia (> 100° F.)	25	5
Infrequent features		
Diarrhoea	5	1
Duration of fever (average)		
99° F. or more	2·6 days	
100° F. or more	2·0 days	
Maximum temperature (average)		
	100° F.	

No specimens of sera were available from any of the children during the acute phase of their illnesses, but convalescent sera, taken from one to nine months later, were available from six children. These sera were tested for haemagglutination-inhibiting and complement-fixing antibodies against the Iksha strain of Influenza A_2 and against Influenza A soluble antigen respectively. The results of these tests are given in Table 2. They show that high titres of both types of antibody were present in one out of the six sera, that haemagglutination-inhibiting antibody only was present in two sera and that complement-fixing antibody only was present in one serum. In two sera, neither type of antibody was present. As there had been no outbreak of influenza-like illness in the nursery between the October outbreak and the collection of the sera and as antibodies to the A_2 type of influenza were unknown in this age group before the 1957 pandemic (Jensen,

1957; Clarke, Heath, Sutton & Stuart-Harris, 1958), it is probable that the high titres recorded relate to the outbreak of influenza described. The recovery of influenza virus at that time supports this probability.

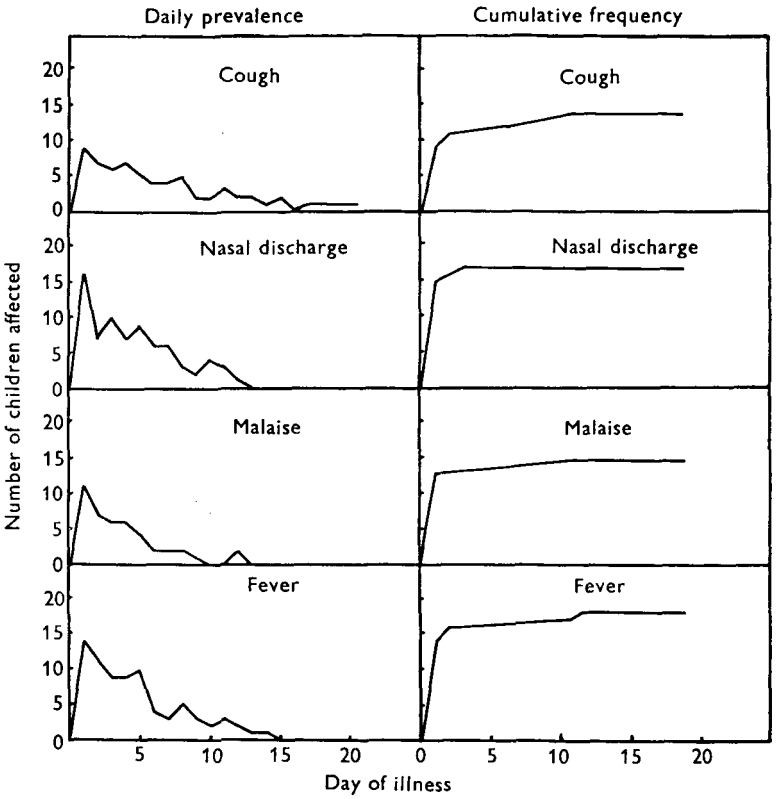

Fig. 4. The daily prevalence and cumulative frequency of signs and symptoms during the outbreak of Influenza A.

The outbreak of Parainfluenza 3 *infection*

The outbreak of respiratory illness

The index case in this outbreak occurred on 13 February 1958, after the nursery had been free from any infection for $2\frac{1}{2}$ weeks. On that day, Mark J., a coloured infant aged 11 months, was noticed to be irritable. The next day, a purulent nasal discharge appeared and his pharynx was seen to be reddened, without any exudate. After 2 weeks in this state, a cough developed and scattered coarse rhonchi were heard. Mark was not completely well again until 15 March.

At the beginning of the outbreak, there were 16 children in the nursery and nearly all were at risk throughout its whole duration. Seven of these children had also been observed during the influenza epidemic. A further six children were admitted after the outbreak began, but before all the children had recovered, making a total of 22 children at risk. Their ages ranged from 4 months to 3 years, the mean age being 2 years.

Following the index case, all 16 children who were in the nursery at that time fell ill with similar signs and symptoms to those of Mark J. The only children who escaped clinical infection were those six who entered the nursery after the initial spread of illness. The percentage frequencies of signs and symptoms are given in Table 1 and the daily incidences and cumulative frequencies of selected signs and

Table 2. *Investigations on sera collected from children resident in the nursery*

		Antibodies against							
		Parainfluenza 3		Influenza A_2		Adenovirus type			
						1	2	5	—
Name of child	Date on which serum was collected	HAI*	CF†	HAI	CF	NT‡	NT	NT	CF
Mark J.	18. ii. 58	10¶	< 2	< 5	24	32	*12*§	12	48
	2. vii. 58	120	8	—‖	—	64	*8*	8	*6*
Anita N.	5. iii. 58	10	—	—	—	*24*	< 24	> *96*	—
	2. vii. 58	120	—	320	6	*48*	< 4	> *64*	24
Violet C.	2. vii. 58	240	—	< 5	6	< 4	*48*	24	*24*
Neil C.	18. xi. 57	80	4	—	—	—	—	—	—
	2. vii. 58	240	6	240	32	32	*48*	38	*48*
Susan M.	18 ii. 58	40	< 4	< 5	< 8	64	*12*	32	*24*
	5. iii. 58	40	4	—	—	—	—	—	—
Neil Ch.	4. ii. 58	60	4	—	—	< 4	4	*12*	*48*
Samuel A.	2. vii. 58	240	5	—	—	< 4	12	> 64	6
Carole A.	2. vii. 58	240	6	—	—	< 4	< 4	12	48
Claire M.	4. ii. 58	15	< 4	—	—	—	—	—	—
	14. v. 58	120	—	—	—	—	—	—	—
Philip N.	2. vii. 58	—	—	120	4	4	> 64	24	24

* HAI, Haemagglutination–inhibiting antibody.

† CF, Complement-fixing antibody.

‡ NT, Neutralizing antibody.

§ Serum titres are italicized in cases where adenovirus of this type was recovered from child.

‖ No test (insufficient or anti-complementary serum or child not present in nursery during outbreak of infection due to test virus).

¶ Titres of sera are expressed as the reciprocals of the end-point dilution.

symptoms are given in Fig. 5. These show that there appeared to be a biphasic character to the illnesses. In the first phase, there was a sudden onset of nasal discharge and malaise (including listlessness and irritability) which improved, often within a week or so. Relapse then occurred with fever, cough and, in many cases, cervical lymphadenopathy and bronchitis. This latter phase persisted for 2 or 3 weeks before recovery was complete.

The relationship of laboratory studies to clinical events

During the 4 months from 1 February to 30 May 1958, a period which covered the outbreak, 129 throat swabs from the children were tested in rhesus monkey-kidney tissue cultures. These tissue cultures were maintained in serum-free or in low (0·5 %) serum media, suitable for the growth of parainfluenza viruses. A haemagglutinating agent was detected in tissue cultures inoculated with 16 out of the 129 throat swabs. These 16 throat swabs had been taken at the time of the

outbreak from 12 out of the 22 children who had been at risk and only one throat swab was from a child who had not been ill at the time of the outbreak.

Two strains of this haemagglutinating virus were established in subcultures of the positive tissue cultures and were shown (Sutton, Clarke & Tyrrell, 1959) to be serologically identical with the Parainfluenza 3 virus of Chanock, Parrott, Cook, Andrews, Bell, Reichelderfer,Kapikian, Mastrota & Huebner (1958). For technical reasons, (loss of infectivity on storage and contamination with simian viruses), it was not found possible to confirm the identity of the remaining strains of virus.

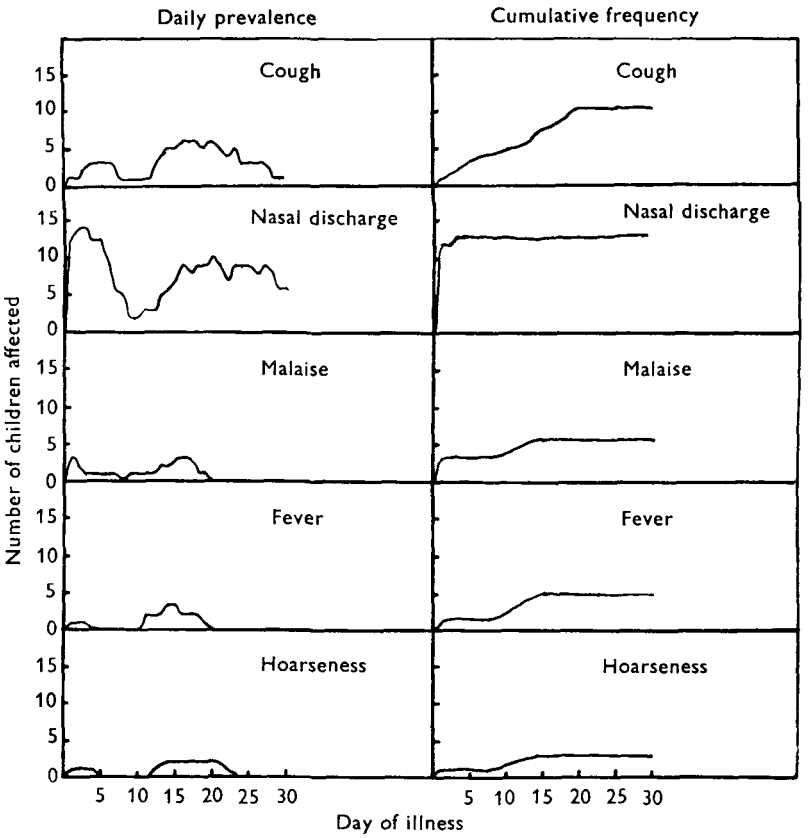

Fig. 5. The daily prevalence and cumulative frequency of signs and symptoms during the outbreak of illness associated with Parainfluenza 3 infection.

Paired sera from five children were tested against one of the recovered strains of Parainfluenza 3 virus and the results of these tests are given in Table 2. Three of these pairs showed a fourfold or greater rise in haemagglutination-inhibiting antibody and one also showed a fourfold rise in complement-fixing antibody. Three out of the four single convalescent sera showed suggestively high titres of haemagglutination-inhibiting antibody. These rises in antibody, coupled with the recovery of virus at the time of illness, suggest that in these cases illness was associated with infection by Parainfluenza 3 virus. The following case report is

of one such child in whom infection with this virus was shown to have occurred during the outbreak.

Case report. Claire M., a white infant aged 13 months, developed a mucoid nasal discharge on 14 February 1958; this persisted for 10 or 11 days and she appeared well by 26 February. On 28 February she was again ill with a purulent nasal discharge and a cough. Her cervical lymph nodes became enlarged. By 20 March full recovery had taken place. A haemagglutinating virus was recovered from throat swabs taken on 4, 11 and 18 March. The virus recovered from 4 March throat swab was identified as Parainfluenza 3 virus. Paired sera showed an eight-fold rise in haemagglutination-inhibiting antibody.

The clinical results were tabulated together and include cases which were shown to be infected with Parainfluenza 3 virus and others. As the nursery was a virtually closed community, it is likely that all the children were infected with the same virus, but this is not in any way proved.

The recovery of adenoviruses throughout the study

During the 8 months of the study, each child's throat was swabbed, on an average, every 7·6 days, and from one to 32 (average 11·5) throat swabs were taken from each child for adenovirus investigation. Five hundred and thirty-seven throat swabs were thus collected from 46 children, who were observed in the nursery for 144 child-months.

From each throat swab, 0·5 ml. of fluid was inoculated into each of three H.Li.1 tissue culture tubes. After an overnight period of adsorption, the tissue culture media were changed. As inocula containing small amounts of adenovirus may not produce cytopathic changes in tissue cultures for several weeks (Kjellén, 1956; Evans, 1958), the inoculated tissue cultures were observed for 14 days or more. The cells and tissue culture fluids of the groups of tubes in which one or more had not exhibited a cytopathic change were then harvested and passaged for a further 14 days in H.Li.1 tissue cultures before being discarded as negative. The cells and culture fluids of those culture tubes which showed the characteristic changes of adenovirus infection were harvested and typed by neutralization against specific rabbit antisera.

Twenty-four recoveries of adenovirus were made from throat swabs taken from 15 children. Four of these adenoviruses were Type 1, 12 were Type 2 and eight were adenovirus Type 5. The temporal distribution of these recoveries is given in Fig. 6; it can be seen that there was a noticeable amount of periodicity. This periodicity can also be seen in Fig. 7, which shows the recoveries of virus from five children observed for 30 weeks or more. In four children (Michael R., Susan M., Neil C. and Anita N.), recurrent isolations of the same type of adenovirus were made at intervals of 3–12 weeks. In two children (Michael R. and Anita N.), a change was observed in the type of adenovirus recovered.

In the absence of paired sera, it is not possible to state whether infection occurred at the time that the viruses were recovered. Sera were available from nine of the children and their neutralizing and complement-fixing antibodies

against adenovirus were estimated. The results are given in Table 2 and show that neutralizing antibodies were present to the types of adenovirus recovered and that complement-fixing antibodies were also present.

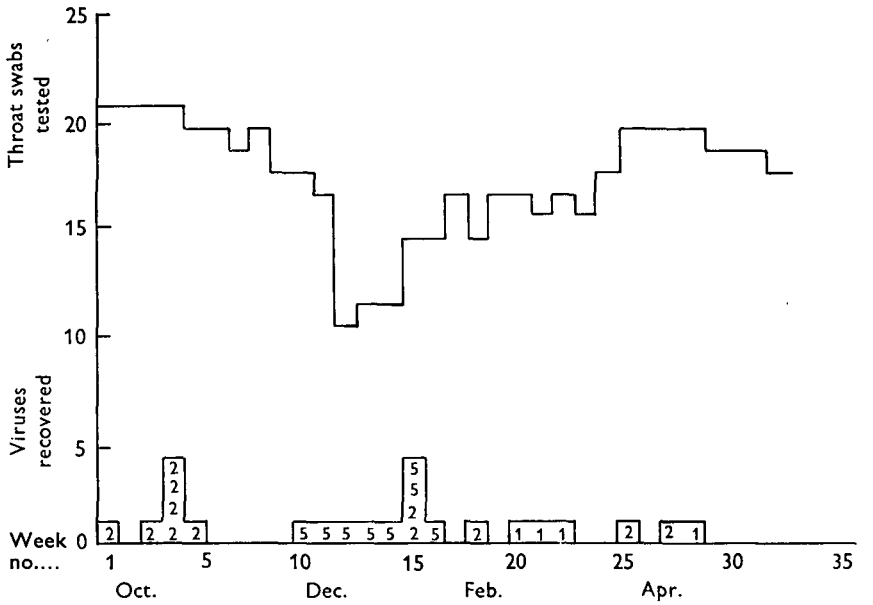

Fig. 6. The recovery of adenoviruses in the nursery. 1, adenovirus type 1; 2, adenovirus Type 2; 5, adenovirus Type 5.

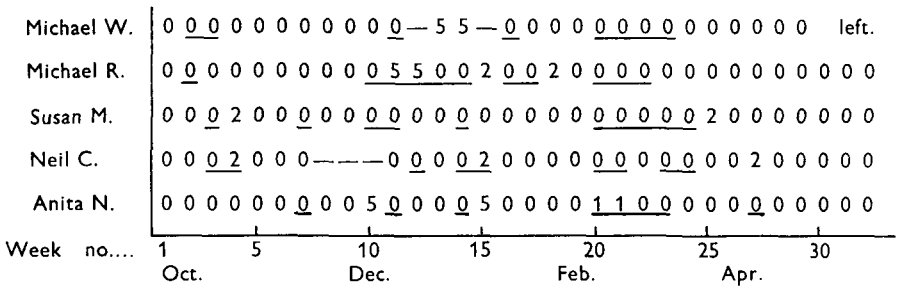

Fig. 7. Adenovirus isolations from five children and their relation to respiratory illness. 1, adenovirus Type 1; 2, adenovirus Type 2; 5, adenovirus Type 5; 0, no adenovirus recovered; —, specimen not tested. Underlining denotes periods when children were ill.

The relation of adenovirus recoveries to respiratory illness

The relation of adenovirus recoveries (as adenoviruses recovered per throat swab tested) to respiratory illness in the nursery (as illnesses per person per week) is given in Fig. 8. In two of the outbreaks of illness, October and February, the viral aetiology was known; in these and in the remainder of the illnesses observed, where the aetiology remained unknown, there was no clear relation between the

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incidence of illness and that of adenovirus recoveries. In Table 3 the recoveries of adenoviruses and their relation to respiratory illnesses are compared in a different way. In the three types of adenovirus recovered (Types 1, 2 and 5), only one

Table 3. *The recoveries of adenovirus and their relation to respiratory illness*

Type of adenovirus recovered	State of child when throat swab was taken: Ill	State of child when throat swab was taken: Healthy	Total no. of throat swabs
1	1	3	4
2	3 (6)	9 (18)	12
5	2	6	8
Total no. of adenoviruses of Types 1, 2, 5	6	18	24
Throat swabs from which adenovirus of Types 1, 2 or 5 was recovered	6	18	24
Throat swabs from which no adenovirus was recovered	169	344	513
Total no. of throat swabs	175	362	537

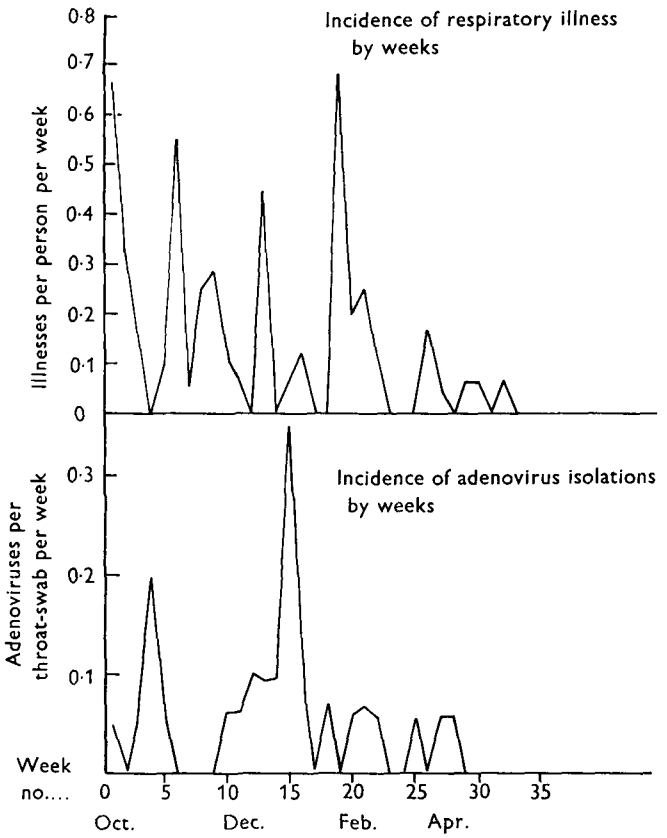

Fig. 8. The incidence of respiratory illness and of adenovirus isolations in the nursery, by weeks.

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quarter were from children who were ill at the time that their throats were swabbed. Eighteen recoveries of adenovirus were made from 362 throat swabs taken from healthy children; six recoveries of adenovirus were made from 174 throat swabs taken from ill children. The excess of isolations from healthy children over those from ill children is significant ($p < 0{\cdot}02$).

Table 4. *Adenoviruses recovered from children observed in the nursery for long and for short periods*

	Children observed for a period of	
	30 weeks or more	Less than 30 weeks
No. of children who yielded adenoviruses	7	7
No. of children who did not yield adenoviruses	1	31

$p < 0{\cdot}01$.

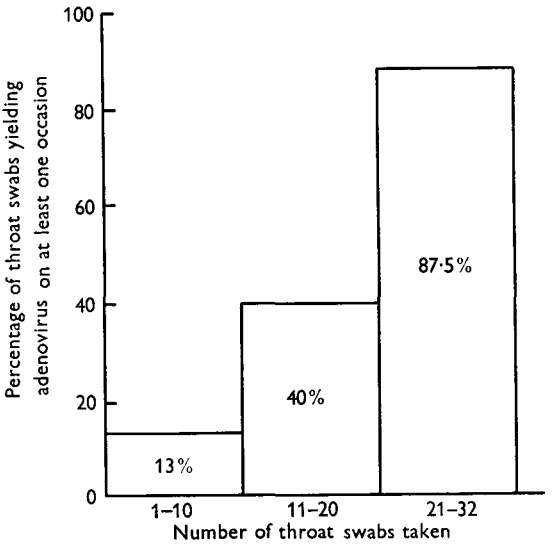

Fig. 9. The percentage of throat swabs yielding adenovirus on at least one occasion related to the number of throat swabs taken from individual children.

The children from whom adenoviruses were recovered

The 24 adenoviruses isolated were recovered from 15 children; thus one third of the 46 children under observation yielded all the adenoviruses. Possible differences were investigated between this group from whom viruses were recovered and their companions from whom none could be isolated. No significant difference could be found in the numbers of illnesses experienced by the two groups, when the data were adjusted to allow for the varying time spent in the nursery by those children who excreted virus and those who did not. There was no significant difference in the age composition of the two groups. There was, however, a significant ($p < 0{\cdot}01$)

https://doi.org/10.1017/S0022172400039309 Published online by Cambridge University Press

difference between the number of children observed for 30 weeks or more from whom adenoviruses were recovered and those who were observed for less than that period from whom adenoviruses were recovered (Table 4). The proportion of children from whom adenoviruses were recovered on at least one occasion rose from 13 % of those from whom one to ten swabs were taken to 87·5 % in those who were swabbed from 20 to 32 times (Fig. 9). These figures suggest that, in the nursery, adenoviruses could be recovered from the throats of most infants if the duration of observation and number of throat swabs taken were sufficient.

DISCUSSION

In recent years, a number of outbreaks of respiratory illness associated with Parainfluenza 3 infection have been reported (Chanock *et al.* 1958; Lelong, Vialette, Cotlenko, Chany & Nodot, 1959; Labzoffsky, Cooper, Morrissey, & Lesiak, 1959; Sutton *et al.* 1959; Bukrinskaya & Paktoris, 1960) and many descriptions of influenzal outbreaks appeared following the 1957 pandemic (Woodall, Rowson & McDonald, 1958; Fry, 1957; Watson, 1960). Similarly, many workers have described the recovery of adenoviruses of Types 1, 2 and 5 from cases of respiratory illness (Roden *et al.*, 1956; Katz, Jordan, Badger & Dingle, 1957; Bell, Ward, Huebner, Rowe, Suskind & Paffenbarger, 1956) and from tonsils and adenoids taken from healthy children (Rowe *et al.* 1955; Zaiman *et al.* 1955; Andrews & McDonald, 1957; Huebner, Rowe, Ward, Parrott & Bell, 1954; Tyrrell, 1958; Evans, 1958). In no published investigation, however, have studies on these viruses been made in the same population, *pari passu* with concurrent clinical observations.

In the Sheffield nursery, two outbreaks of respiratory illness occurred which could be attributed to specific virus infections; the children in the nursery during these episodes were substantially the same and the populations at risk to the two viruses were therefore comparable. Analysis of the symptomatology showed slight differences between the clinical picture following infection with Influenza A and that following infection with Parainfluenza 3 virus. Infection with Parainfluenza 3 virus produced a milder infection than that with Influenza A (as judged by incidence and duration of pyrexia), but the children were ill for a longer period when infected by the former virus.* Nevertheless, although the symptomatology of the two outbreaks appears to differ, this only became apparent on retrospective analysis and, during the episodes, it would not have been possible to make confident diagnosis in individual cases on clinical appearances alone. It seems probable that, even in comparable populations, host factors play a very large part in determining the illness resulting from any specific infection.

When the recoveries of adenoviruses from the children's throats were compared with those of Parainfluenza 3 and influenza A viruses, differences in the patterns of recovery became evident. Whereas the recoveries of Influenza A and Parainfluenza 3 viruses were closely related to episodes of respiratory illness among the children, no such relation applied to the adenovirus recoveries. The recoveries

* It is possible that this longer period resulted from super-infection with a different, unidentified, virus or from reinfection with the same virus.

of adenoviruses of Types 1, 2 and 5 in the nursery were independent of any illness in the children, although there was a definite periodicity in the types of virus isolated. It appeared that, in the Sheffield nursery, adenoviruses could be detected in most children's throats if enough throat swabs were taken over a long enough period. The pattern of excretion suggested silent epidemics sweeping through the nursery rather than the fortuitous shedding by several children of the same type of adenovirus; antibody studies did not, however, confirm this. There was no evidence of reactivation of latent adenovirus infection by superinfection with some other microbial agent, such as is well known in the case of herpes simplex virus; in fact, the recoveries of adenovirus were significantly associated with health in the children who yielded virus.

The general pattern of recoveries of adenovirus of Types 1, 2 and 5 in the Sheffield nursery was reminiscent of that found in the normal nasopharyngeal bacterial flora (Straker, Hill & Lovell, 1939). This, coupled with the lack of relation to respiratory or other illness in the nursery, suggests that these viruses may be components of the normal viral flora of the nasopharynx, at least in infants.

SUMMARY

A longitudinal survey of respiratory illness in a Sheffield residential nursery is described. Over 8 months, outbreaks of respiratory illness occurred, two of which were associated with infection by Influenza A and Parainfluenza 3 viruses. The symptomatology is described of the illnesses resulting from these infections. The incidence of adenovirus recoveries over the same period was also investigated and suggests that adenoviruses of Types 1, 2 and 5 may be components of the normal viral flora of the nasopharynx in infants.

I am indebted to Professor C. H. Stuart-Harris for much help and advice during the course of this investigation. I also wish to thank Miss M. O'Malley (Sheffield) and Mr A. S. Greasby, F.I.M.L.T. (Salisbury) for technical assistance; Dr F. E. Buckland, Dr R. M. Chanock, Dr S. K. R. Clarke, Dr T. H. Flewett, Dr H. G. Pereira, Dr D. A. J. Tyrrell and Dr P. J. Wormald for the provision of tissue cultures, viruses and sera; and the Childrens' Department of the City of Sheffield for permission to study the children in the Moss nursery.

The investigation was carried out during the tenure of a Medical Research Council Fellowship in Clinical Research.

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