Significance of respiratory virus isolations

A study in primary schoolchildren

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Horn, M. E. C., and Yealland, S. J. (1974). *Archives of Disease in Childhood*, **49**, 516. **Significance of respiratory virus isolations: a study in primary schoolchildren.** This study was undertaken in a primary school to determine how frequently viruses, *Mycoplasma pneumoniae*, and pathogenic bacteria could be isolated from children in whom there was no evidence of acute respiratory infection. The clinical status was firmly established before the virological and bacteriological results were recorded. Respiratory illness was found during 79 out of 224 interviews (35%). Specimens were taken at 112 of these interviews and children were re-examined 6 days later to ensure that no symptoms had developed during the intervening period. Viruses were isolated from 2 out of 65 (3%) of the investigations performed in asymptomatic children, compared with 11 out of 47 (23%) in those who had episodes of mild respiratory illnesses. *Haemophilus influenzae* and *Streptococcus pneumoniae* were more commonly isolated in the 47 children with symptoms than in those without. There was a close correlation between the virological findings of this study and those in children attending a nearby general practice, where the isolation rate in episodes of respiratory illness was 23% during the same period.

Evidence which supports the belief that most respiratory viruses cause the illnesses with which they are associated is that isolation rates in patients with clinical illness are higher than those in 'controls' who have no respiratory symptoms. However, it is difficult to ensure that the controls provide a true measure of the frequency of asymptomatic infection, because in most studies it has not been possible to ascertain whether they remained well during the few days after the taking of specimens. The present paper reports a study which was carried out in a primary school to find out how frequently viruses, *Mycoplasma pneumoniae*, and bacteria could be isolated from children who had no symptoms or signs of respiratory illness at the time of swabbing or within 6 days thereafter. A secondary aim was to compare the rate of isolation in these asymptomatic children with that in children who were attending school but who had mild respiratory illnesses.

This study was undertaken during a long-term survey of respiratory disease in children in a neighbouring general practice which is also being

carried out in co-operation with the Department of Microbiology at the Brompton Hospital. It has therefore been possible to compare the virological findings in this larger survey with those obtained in the schoolchildren.

Methods

Permission to carry out the study was given by the Medical Officer of Health of the London Borough of Wandsworth and by the headmistress of the primary school concerned. Written consent was sought from the parents of 40 children aged 5 to 7 years, and only 1 parent refused consent and 2 did not reply.

The school was visited on 8 occasions between January and June 1972. 4 visits were made for examination and swabbing and 4 were made 6 days after specimens had been taken to determine whether any respiratory illness had developed in the intervening period: if the child was not seen on the second visit the reason for absence was ascertained. Each child was questioned, examined, and swabbed by the same doctor throughout. Inquiry was made about recent illness and absence from school and also about the presence and duration of colds, sore throats, ear ache, and cough. The nose, throat, and ears were examined routinely, but the chest only if the child complained of cough. Rhinorrhoea, pharyngitis, acute

otitis media, and crepitations or rhonchi were considered to be evidence of illness; minimal degrees of rhinorrhoea and pharyngitis were discounted if they were not associated with symptoms of which the child complained. Symptoms and signs were recorded at each examination and a decision was made at the time as to what constituted illness. Children were then divided into two groups: those who had respiratory symptoms and signs at the time of swabbing, or who developed them within 6 days, and those without respiratory illness.

A nasal and a throat swab from each child was placed in a bottle containing transport medium (Hanks's medium with 0.2% bovine albumin) for virological studies. Viral specimens were kept chilled in an insulated bag with freeze packs and were transferred to the laboratory within 3 hours. These specimens were inoculated into human diploid embryo lung (WI-38 and HEL-35), HEp-2, and primary monkey kidney cell cultures, and onto a selective diphasic medium and supplemented PPLO agar plates for the isolation of *M. pneumoniae*.

Nasal and throat swabs were inoculated directly onto blood agar plates for bacteriology, and routine laboratory methods were used.

Results

Thirty-seven children (19 boys and 18 girls) took part in the study. Specimens were obtained for laboratory investigations at the 112 initial interviews, 59 from boys and 53 from girls. Symptoms and signs of respiratory illness were found during 79 out of the total 224 interviews: these were made up of 32 who had symptoms at the initial and follow-up

visits, 6 who only had symptoms at the time of the initial visit, and 9 who had developed symptoms by the time of the follow-up visit 6 days later. Therefore, 47 episodes of illness were investigated and the remaining 65 laboratory investigations were made in children without illness. Physical examination confirmed the children's symptoms in all but 2 instances; their estimation of the date of onset of symptoms was too vague to be useful.

The pathogenic agents isolated from children without illness and from those with minor respiratory illnesses are compared in the Table. Viruses were isolated from 2 out of the 65 (3%) specimens obtained from children who had no symptoms and signs, compared with 11 out of the 47 (23%) from those with minor respiratory illnesses. The difference is significant (χ^2 9.0, $P < 0.01$). Two viruses were grown from 13 specimens obtained from children who subsequently developed symptoms. *M. pneumoniae* was found in 2 asymptomatic children who had no recent history of illness. None of the symptom-free children from whom viruses or *M. pneumoniae* were isolated gave a history of recent illness nor had they been absent from school during the previous 2 weeks.

Pathogenic bacteria were isolated with almost equal frequency from children with and without respiratory illness with the exception of *Haemophilus influenzae* and *Streptococcus pneumoniae* which were more common in the former group. *Staphylococcus*

TABLE
Pathogenic agents isolated in schoolchildren with and without minor respiratory illnesses

Clinical assessment	No. seen	Viruses	<i>M. pneumoniae</i>	Bacteria
<i>Minor respiratory illness</i>				
Rhinorrhoea ± pharyngitis	29	Rhinovirus (1)* RSV (2) Coxsackie B2 (1)* Parainfluenza 1 (1) Poliovirus 2 (1) Adenovirus 2 (1)	— — — — — —	<i>Staph. aureus</i> N (2) <i>Staph. aureus</i> N (+ adenovirus 2) (1) <i>Staph. aureus</i> NT (1) <i>Staph. aureus</i> NT (+ Coxsackie B2) (1) — —
Rhinorrhoea + otitis media	3	—	—	<i>H. influenzae</i> N (1)
Rhinorrhoea + cough	13	Rhinovirus (1) RSV (1) — — —	— — — — —	<i>Staph. aureus</i> N (1) <i>Staph. aureus</i> NT (+ RSV) (1) <i>H. influenzae</i> N (1) <i>H. influenzae</i> N + <i>Strep. pneumoniae</i> N (1) <i>Strep. pneumoniae</i> N (1)
Rhinorrhoea + cough + otitis media	1	Rhinovirus (1)	—	—
Rhinorrhoea + cough + otitis media + bronchitis	1	Coxsackie B2 (1)	—	—
No evidence of illness	65	Rhinovirus (1) Adenovirus 2 (1) —	2 — —	<i>Staph. aureus</i> N (14) <i>Staph. aureus</i> NT (4) <i>Staph. aureus</i> NT + <i>Strep. pneumoniae</i> N (1)
Total	112	13	2	30

RSV, respiratory syncytial virus; N, nasal swab; T, throat swab.

*Isolated before onset of symptoms.

aureus was found in both groups, but group A streptococci were not cultured from the schoolchildren.

Discussion

The small number of viruses which were isolated from children who had no evidence of respiratory illness contrasts with the findings of some other workers. Holzel *et al.* (1965) reported an isolation rate of 7% in nonrespiratory illnesses in young children who were in hospital and Loda, Glezen, and Clyde (1972) obtained a figure of 8% in children under 6 years of age who had no respiratory symptoms in a group day care centre. Stott and his colleagues (1967) isolated viruses from nose and throat swabs in 26% of children admitted to hospital with diarrhoeal illnesses, but commented that some children from whom rhinoviruses were grown had mild respiratory symptoms as well as diarrhoea. In the Medical Research Council's Report (1965), viruses were isolated from 10% of healthy controls of all ages who were close contacts of individuals with acute respiratory illnesses. It seems likely that some of the contact controls may have been swabbed during the incubation period. In the present study viruses were isolated from 2 children who developed illness subsequent to swabbing. If no follow-up visit had been made in this study, the rate of virus isolation in those with no illness would have been 5% instead of 3%, but the significance would have remained unaltered ($P < 0.01$).

It is known that some agents may persist in the nose and throat in the absence of clinical illness. One adenovirus type 2, one rhinovirus, and two *M. pneumoniae* were isolated from asymptomatic children in this study. Adenoviruses have been shown by Brandt and his co-workers (1969) to be shed intermittently for prolonged periods by young children after an initial associated illness. Rhinoviruses have been isolated from 1.7% of children with nonrespiratory illnesses in an outpatient clinic (Bloom *et al.*, 1963) and from 2% of contact controls of all ages (Medical Research Council, 1965). *M. pneumoniae* has been found (Foy *et al.*, 1966) to persist in the nasopharynx for 8 to 13 weeks after infection and to have a high carrier rate in children under 12 years of age.

During the 6-month period when the schoolchildren were being sampled, 201 illnesses were investigated in children under 13 years of age in the general practice. The sampling technique, transport facilities, and laboratory facilities were the same as those in the school study and were carried out by the same workers. The percentage of virus isolations (23%) and of *M. pneumoniae* (1.4%) in ill children in the practice was almost identical to that

obtained in the schoolchildren. A wide variety of viral agents was detected in children with symptoms in both studies, as would be expected from the work of others (Medical Research Council, 1965; Glezen *et al.*, 1971; Pereira, Andrews, and Gardner, 1967). Influenza A2 virus was isolated on 10 occasions from children in the practice about the time of the first visit to the school, but none were obtained from the schoolchildren, though there was an epidemic of influenza in the community at the time. This could be explained by the fact that influenza A2 causes illness severe enough to prevent attendance at school. However, the absentee rate, according to the school records, was average for the time of the year (8%).

The isolation of a vaccine strain of poliovirus type 2 in a child of 7 years who had received polio vaccine 9 days previously is of interest. A similar isolation was made 12 days after a 10-month-old infant had received vaccine in the practice survey. Both children had evidence of illness, the former had rhinorrhoea only, but the infant also had otitis media and wheezy bronchitis. There was only this one isolation of polio virus during 5 years of investigation of acute respiratory illness among children in the practice who are routinely vaccinated. This is consistent with the findings of others (Loda *et al.*, 1972; Medical Research Council, 1965; Pereira *et al.*, 1967). Hurrell and his colleagues (1971) have, however, reported the recovery of polio viruses from the faeces, but not from the respiratory tract, in a family in which 1 child had been immunized.

The role of bacteria in minor upper respiratory illness in children has never been clearly defined. Straker, Hill, and Lovell (1939) found no evidence that bacteria caused such illnesses. By using pernasal swabs and special culture techniques, Masters *et al.* (1958) showed that there was a rise in the incidence of *Strep. pneumoniae* and *H. influenzae* in children when they had nasal discharge. Though no special methods were employed in the present study, the incidence of these two bacteria was greater in the nasal swabs of the children with minor respiratory illnesses. Group A streptococci were not recovered from the schoolchildren and were rarely obtained from children in the practice during the same period.

This study has revealed that many children considered well enough to attend school had evidence of respiratory illness and were a source from which infection could spread within the school and to the home. This supports the finding of Lambert and Stern (1972) that there was a greater prevalence of virus infection in adult members of families with children.

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The importance of virus infection in minor respiratory illnesses has been confirmed, but it has been found that viruses could rarely be isolated from children who were free of symptoms and remained so for at least 6 days.

Despite the small size of this investigation and the relatively infrequent intervals at which the school-children were sampled, useful information has been gained not only about the significance of respiratory virus isolation, but also about the incidence, age distribution, and pathogenicity of respiratory viral infections in schoolchildren. This basic method would be valuable for further studies, but several modifications are suggested. First, there should be more frequent sampling over a longer period and in varying age groups. Secondly, the absentee rate due to illness could be determined by examination of the school records. Thirdly, children who were absent due to illness at the time of visiting the school could be sampled at home. Fourthly, it would be valuable to take a second specimen at the follow-up examination in children who had developed symptoms in the intervening period; this would ensure that any agent isolated from a symptom-free child on the first occasion was also present in association with the illness which developed within 6 days. Finally, it might be possible to improve the rate of isolation of certain viruses, especially RSV and influenza—and parainfluenza—viruses, by the incorporation into the laboratory methods of immunofluorescent techniques.

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