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# Viral diagnoses using the rapid immunofluorescence technique and epidemiological implications of acute respiratory infections among children in different European countries

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*From November 1978 to October 1981, a total of 7716 specimens of nasopharyngeal secretions were examined by the rapid immunofluorescence technique to determine the frequency of infections caused by the respiratory syncytial virus (RSV), influenza virus A, and parainfluenza viruses 1 and 3. The tests were carried out in six different virus laboratories located in Newcastle upon Tyne (England), Copenhagen, Oslo, Stockholm, Turku (Finland), and Vienna; laboratories in Lisbon and Paris participated in the study for shorter periods. The specimens were collected from infants and children less than 6 years of age who had been admitted to hospital with an acute respiratory infection. Standardized techniques and quality controlled reagents were used. At least one of the above viruses was detected in 1927 (25%) of the specimens: RSV in 1475, influenza virus A in 123, parainfluenza virus 1 in 110, and parainfluenza virus 3 in 237 specimens. Respiratory syncytial virus dominated in all centres, but in some Scandinavian centres distinct outbreaks due to this virus occurred only once or twice during the 3 years' study period. Three outbreaks of RSV were observed in Newcastle, but here an unprecedented delay of the first winter's epidemic occurred. The delay was associated with prolonged school closures in the area, and with a very early outbreak of influenza. Parainfluenza virus 3, which was predominantly a summer virus in Newcastle, was most frequently encountered during the colder months of the year in the other centres.*

Acute respiratory infections account for a large number of admissions to hospitals and considerable morbidity among infants and children (*1*). The majority of these infections are of viral etiology (*2*) which may be determined by applying the immunofluorescence antibody technique on cells collected from the respiratory tract secretions of the patient; this technique provides a rapid and reliable diagnosis within hours of obtaining the specimen (*3*). One of the aims of the European Group for Rapid Virus Diagnosis, founded in 1975, is to make available standardized reagents for rapid diagnostic techniques, including the immunofluorescence antibody test (*4*).

In 1978, the European Group organized a three-year comparative study of respiratory virus infections in children in six different European countries. The aims of the study were to promote the use of the immunofluorescence antibody technique as a rapid but sensitive and accurate routine method for diagnosing respiratory virus infections in European virus laboratories, to introduce techniques that can be used for handling specimens at a distance from the virus laboratory, and to study the epidemiology of some respiratory virus infections in children by standard techniques in different parts of Europe.

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A preliminary report from the first 7 months of the study was published in 1980 (*5*).

The present report summarizes the virological findings at the end of a three-year study programme.

## MATERIALS AND METHODS

### *Laboratories*

The study was started in six virus laboratories located in Copenhagen, Newcastle upon Tyne (England), Oslo, Stockholm, Turku (Finland), and Vienna. Two other laboratories (in Lisbon and Paris) joined the study at a later stage. All the participants were either experienced in the immunofluorescence antibody technique or had attended the course on the use of this test for rapid diagnosis in Oslo in March 1978, which was organized by the European Group for Rapid Virus Diagnosis in conjunction with the World Health Organization.

### *Patients and specimens*

Nasopharyngeal secretions were collected from children less than six years of age who were admitted to hospital because of an acute respiratory infection. The specimens were taken on ice to the virus laboratory, and the cells from the secretions were washed and prepared on microscope slides as described by Gardner & McQuillin (*3*). In a few centres, some of the specimens were prepared in the hospital and the slides were sent to the virus laboratory.

The population covered by the different virus laboratories varied from only a section of a city to an entire city and surrounding areas.

### *Methods and reagents*

The specimens were examined by the indirect immunofluorescence test for the following four viruses: respiratory syncytial virus (RSV), influenza virus A, and parainfluenza viruses 1 and 3. Calf immune sera against RSV and influenza virus A were obtained from Wellcome Reagents; rabbit immune serum against parainfluenza virus 1 from the Stockholm laboratory; and rabbit immune serum against parainfluenza virus 3 from the Newcastle laboratory, which owing to a shortage of supply was replaced by a calf anti-parainfluenza 3 immune serum (Wellcome) during the study. Fluorescein isothiocyanate conjugated anti-bovine and anti-rabbit globulin were obtained from Wellcome Reagents.

All the reagents were standardized and quality checked by the Newcastle and Stockholm laboratories, and latterly by the Central Public Health Laboratory, Colindale, London, according to a protocol approved by the European Group as outlined by Gardner & McQuillin (*3*).

Table 1. Total number of nasopharyngeal specimens examined and number of virus-positive specimens for each centre

| Centre | Total no. of specimens | No. of virus-positive specimens | | | | |
|---|---|---|---|---|---|---|
| | | Respiratory syncytial virus | Influenza virus A | Parainfluenza virus 1 | Parainfluenza virus 3 | Total |
| Newcastle | 3331 | 648 (19.5)[a] | 48 (1.4) | 65 (2) | 136 (4.1) | 897 (26.9) |
| Copenhagen | 665 | 127 (19.1) | 14 (2.1) | 9 (1.4) | 8 (1.2) | 158 (23.7) |
| Oslo | 1444 | 260 (18) | 25 (1.7) | 9 (0.6) | 46 (3.2) | 340 (23.5) |
| Stockholm | 706 | 120 (17) | 9 (1.3) | 21 (3) | 28 (4) | 178 (25.2) |
| Turku | 1309 | 239 (18.9) | 27 (2.1) | 4 (0.3) | 17 (0.8) | 287 (21.9) |
| Vienna | 261 | 63 (24.1) | – | 2 (0.8) | 2 (0.8) | 67 (25.7) |
| Total | 7716 | 1457 (18.9) | 123 (1.6) | 110 (1.4) | 237 (3.1) | 1927 (25) |

[a] Figures in parentheses are percentages.

## RESULTS

A total of 7716 specimens were examined in the six original laboratories during the study period from November 1978 to October 1981 (Table 1). A virus was detected in 1927 (25%) of these specimens, the percentage of virus-positive specimens in the different laboratories varying from 21.9% to 26.9%.

Most of the specimens were obtained during the winter months from December to February (37%), the percentages for spring, summer, and autumn being 24, 14, and 25, respectively; 35% of the winter specimens were positive for at least one of the four viruses mentioned above, compared with 19% for specimens obtained in each of the other seasons. The seasonal distribution of positive specimens was less prominent at Newcastle than at the other centres, probably because of local epidemiological factors affecting RSV and parainfluenza virus 3 (see below).

The Paris laboratory participated in the study from October 1980 to October 1981 and examined 625 specimens, of which 88 (14%) were found to be virus positive. The Lisbon laboratory examined specimens during only two winter periods, and a virus was detected in 34 (16.2%) out of 210 specimens examined. As the findings of these two laboratories are incomplete, they cannot be included in the detailed discussions of the epidemiological findings. However, winter outbreaks of RSV were observed in both these centres, and influenza virus A was detected during the winter in Paris. Only three parainfluenza virus strains were diagnosed, too few to detect any epidemiological pattern.

### *Respiratory syncytial virus*

RSV was by far the most frequently encountered

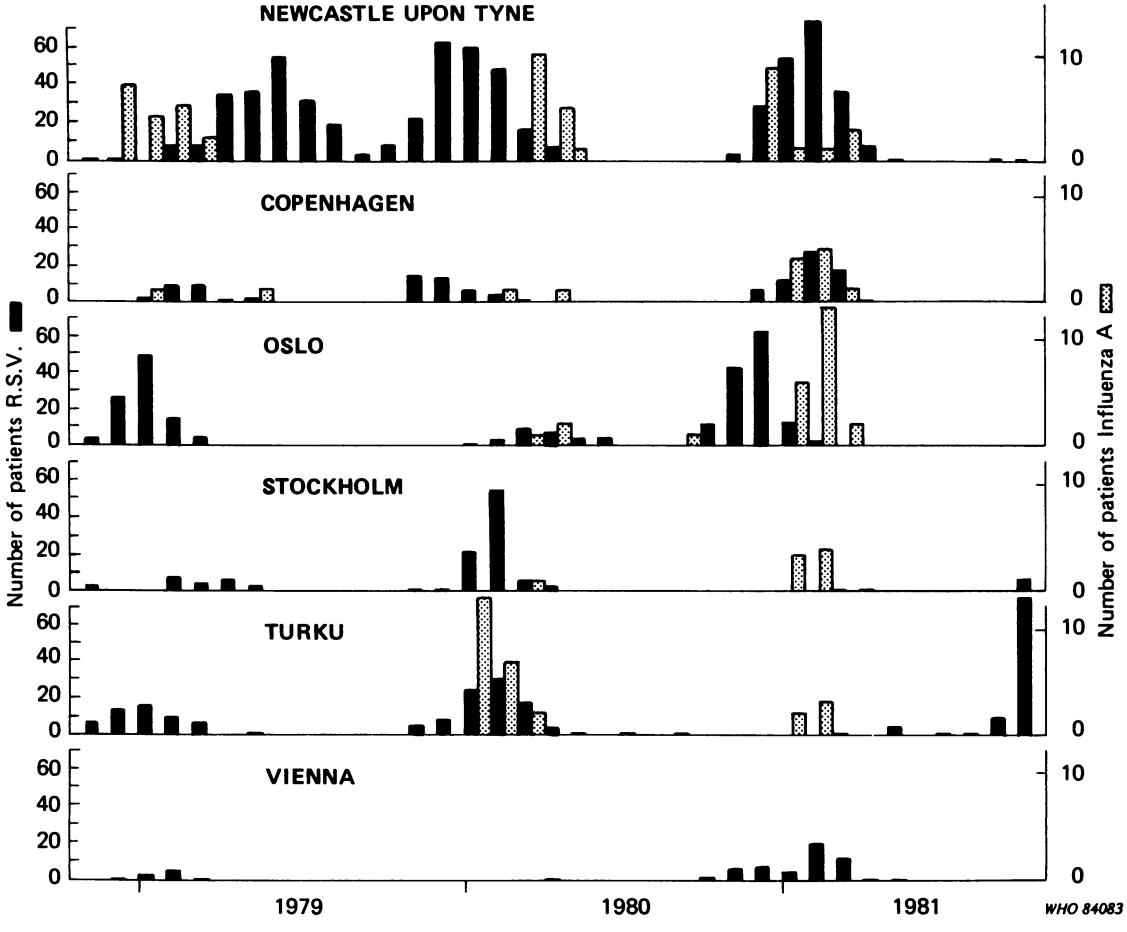


Fig. 1. Number of patients with respiratory syncytial virus (RSV) and influenza virus A infections every month during 1979–81 in the six centres. (*Note*: different scales for the two viruses).

virus in all centres (Table 1). With one exception, RSV was detected almost exclusively during the colder months (Fig. 1). The exception was Newcastle where, during the winter of 1978–79, the expected winter epidemic did not take place but an outbreak started in April and peaked in June 1979.

Extensive outbreaks occurred in Oslo during the first and the third winters of the study period with only a few cases in the second winter, whereas in the Stockholm laboratory the opposite situation was observed. Turku also saw very few cases of RSV infection among hospitalized children during the winter of 1980–81, whereas an outbreak was evidently under way in October 1981.

*Influenza virus A*

Altogether 123 (1.6%) of the specimens were positive for this virus. Every winter/spring, a number of such cases occurred in Newcastle whereas in Copenhagen, Oslo, and Stockholm these cases only occurred in the winter of 1981 (Fig. 1). In Turku a distinct outbreak occurred in the winter of 1980. The only influenza A infection which did not occur during the winter was a child who had recently arrived in Oslo from a subtropical area and fell ill in September 1980.

*Parainfluenza virus 1*

This virus was the least frequently encountered in the study and was detected in only 110 (1.4%) of the specimens. However, the frequency varied considerably between the different laboratories (Table 1). The virus was most often detected during late autumn/winter in Newcastle, Copenhagen, and Stockholm, whereas only sporadic cases of parainfluenza virus 1 infections were diagnosed in the other centres (Fig. 2).

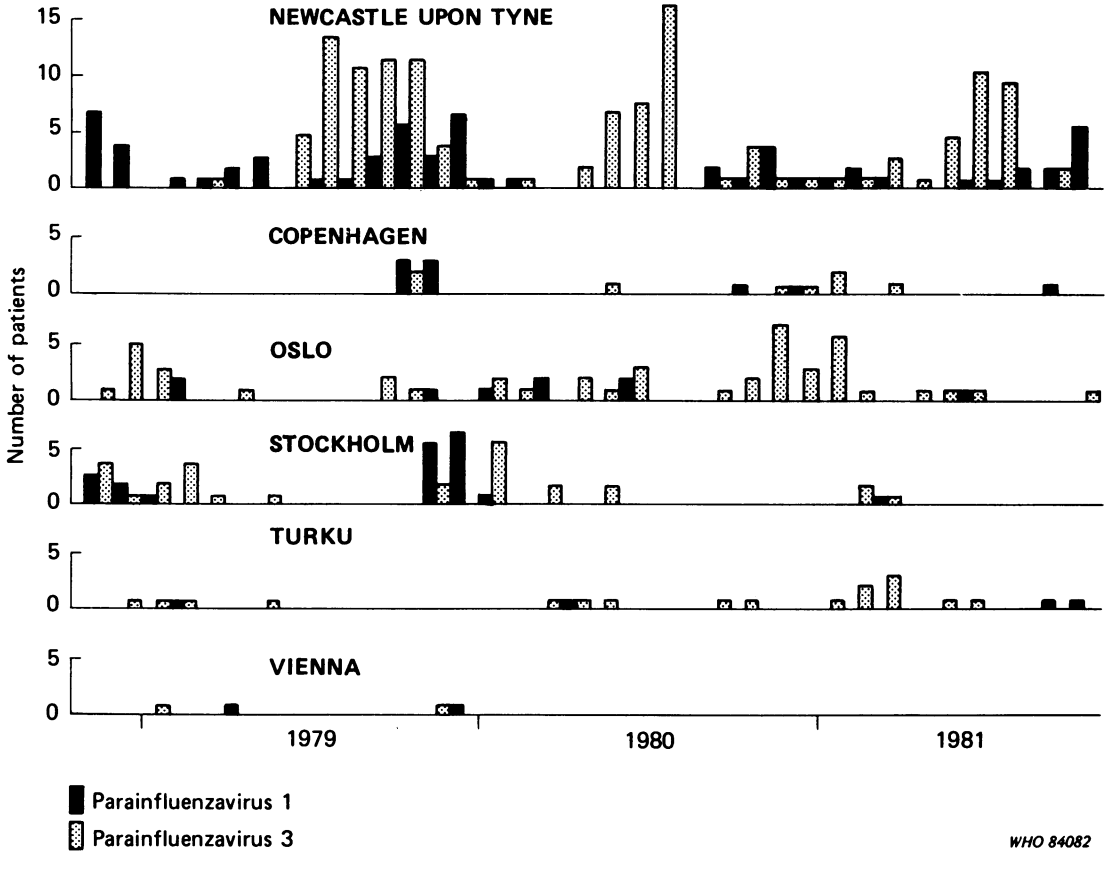


Fig. 2. Number of patients with parainfluenzavirus 1 and parainfluenzavirus 3 infections every month during 1979–81 in the six centres.

*Parainfluenza virus 3*

Next to RSV, parainfluenza virus 3 was the second most often detected, but, as with parainfluenza virus 1, the frequency varied considerably between the different centres (Table 1). In Newcastle the majority of cases occurred in the summer or early autumn, but this kind of seasonal prevalence was not observed in the other laboratories where parainfluenza virus 3 was most frequently encountered during the colder months (Fig. 2).

## DISCUSSION

Longitudinal studies on respiratory virus infections in hospitalized children have been the subject of many reports during the last several years (*2*, *6–9*). Most previous studies have been confined to a single centre, and the present study was undertaken in an attempt to compare the situation in different countries. Whereas a cell culture technique may be difficult to standardize, the immunofluorescence test used in the present study was standardized by using uniform reagents that had been quality controlled, and by careful instructions to the participants during the course on this test in March 1978.

The importance of respiratory syncytial virus as a cause of serious acute respiratory illness in this age group was demonstrated in all centres. Three outbreaks of RSV infection occurred in Newcastle during the three years of the study period, but a different epidemic pattern was found in the other centres. The observation that a distinct outbreak in one winter was followed by only a few cases the following winter, which was what happened in Oslo, Stockholm, and Turku, may have been due to different population densities in these localities, compared with Newcastle where distinct, yearly outbreaks had been observed even prior to the present study (*10*). However, the regular occurrence of outbreaks in alternate years cannot be predicted in the three other centres because in Oslo distinct outbreaks of RSV infections had been observed in five of the seven winters preceding the present study (*11*).

The delay in the expected 1978–79 winter epidemic of RSV infection in Newcastle cannot be fully explained, but the following two events may have contributed.

(1) The closing down of schools in the colder areas of Scotland and the north of England during January and February because of lack of heating (caused by a strike affecting oil transport), which may have delayed the transmission of RSV among schoolchildren. It is known that schoolchildren are important transmitters of RSV in families as well as to smaller children and infants who are more likely to develop serious symptoms (*12–15*).

(2) The presence of influenza virus A infections, several of which had already been diagnosed in Newcastle in December 1978 (Fig. 1). It is reported that in Melbourne, Australia, a delay in the yearly RSV epidemic was associated with an early outbreak of influenza virus infection (E. Uren, personal communication, 1982).

Recently it has also been suggested that influenza virus infections within a community may affect the outcome of RSV outbreaks (*16*, *17*). In the present study, an influenza outbreak followed the RSV outbreaks in Newcastle during the winter of 1979–80 and in Oslo in 1980–81, suggesting that the influenza virus may have interfered with RSV transmission. On the other hand, outbreaks of RSV infection occurred in Newcastle in 1980–81 and in Turku in 1979–80, at the same time as when influenza virus A was prevalent in the community (Fig. 1). A study of the influenza and RSV epidemics in Newcastle over a 6-year period showed that epidemics with both viruses occurred together every year, which was explicable on the grounds that RSV infects a younger age group than influenza (*10*).

The total number of influenza virus A strains detected was small and the virus occurred only during a few months of the study period. During some of these months, however, the specimens positive for influenza virus A amounted to more than 20% of the total number of specimens received at the centre. Influenza virus A was almost absent from the Scandinavian centres during the first year of the study when influenza virus B predominated in these areas.

Children may often be admitted to hospital with symptoms not connected with the respiratory tract (such as febrile convulsions), which are due to influenza virus infections (*18*, *19*); the figures obtained in the study may therefore have underestimated the importance of influenza virus A as a cause of serious illness in this age group.

In the Newcastle, Copenhagen, and Stockholm laboratories, parainfluenza virus 1 was mainly detected in the late autumn, as has been reported elsewhere (*2*, *20*, *21*). Only a few, sporadic cases of infections with this virus were diagnosed in the other centres, which may have been due to the fact that in these areas children with croup were usually treated in the hospitals' ear, nose, and throat departments where samples for virological studies were not taken.

In the case of parainfluenza virus 3 infections, the following difference in the epidemiology was observed. While this virus predominated as a cause of summer illness in Newcastle and other regions in the United Kingdom (*22*), it was in other countries most often detected during the colder months of the year.

We cannot explain this different seasonal prevalence, but it has recently been suggested that parainfluenza virus 3 may be heterogeneous, one subtype possessing and the other lacking a genetic mechanism for "cold-season" prevalence (*23*). Reports from the USA have indicated summer prevalence in some areas and autumn prevalence in others (*21*).

Rapid diagnosis of respiratory virus infections by the immunofluorescence test helps clinicians in their management of the individual patient, e.g., by making possible a more rational and economical therapy, a shorter stay in the hospital for the patient, and less use of antibiotics (*24*). The diagnoses may also help in the control of hospital cross-infections (*25, 26*). This test is less expensive than other, conventional methods of virus isolation (in cell culture or in chick embryo) and identification and has been recommended as the method of choice by a WHO Scientific Group (*27*).

The use of a standardized immunofluorescence technique in the present study has made it possible to compare the epidemiology of four different respiratory viruses among hospitalized children in six different countries. The study confirmed that respiratory syncytial virus and influenza virus A were present in all centres at approximately the same time during the winter epidemics, unless there was some exceptional reason such as the late occurrence of a RSV epidemic one year in Newcastle. Local differences in clinical diagnosis and age composition among the patients studied may explain the differences between centres in the prevalence of parainfluenza virus 1 and, to a lesser extent, influenza virus A because of their association with croup. However, striking differences in the epidemiology of RSV and of parainfluenza virus 3 infections were revealed between centres. For the parainfluenza viruses in particular, further studies are necessary to elucidate the epidemiological differences.

## RÉSUMÉ


### DIAGNOSTICS VIRAUX PAR LA TECHNIQUE DE L'IMMUNOFLUORESCENCE RAPIDE ET INCIDENCES ÉPIDÉMIOLOGIQUES DES INFECTIONS RESPIRATOIRES AIGUËS CHEZ DES ENFANTS DE DIFFÉRENTS PAYS D'EUROPE

De novembre 1978 à octobre 1981, 7716 spécimens de sécrétions nasopharyngées ont été examinés par la technique de l'immunofluorescence rapide afin de déterminer la fréquence des infections provoquées par le virus respiratoire syncytial (VRS), le virus grippal A ou les virus paragrippaux 1 et 3. Les épreuves ont eu lieu dans six laboratoires de virologie différents situés à Newcastle upon Tyne (Angleterre), Copenhague, Oslo, Stockholm, Turku (Finlande), et Vienne; des laboratoires de Lisbonne et de Paris ont participé à l'étude pendant des périodes de plus courte durée. Les spécimens ont été recueillis auprès de nourrissons et d'enfants de moins de 6 ans hospitalisés pour des infections respiratoires aiguës. On s'est servi de technique normalisées et de réactifs dont la qualité avait été contrôlée. Au moins un des quatre virus précités a été décelé dans 1927 (25%) des spécimens: VRS (1475 spécimens), virus grippal A (123), virus paragrippal 1 (110) et virus paragrippal 3 (237). Le virus respiratoire syncytial a dominé dans tous les centres mais, dans certains centres scandinaves, des poussées épidémiques distinctes dues à ce virus ne se sont produites qu'une ou deux fois pendant les trois années de l'étude. Trois poussées de VRS ont été observées à Newcastle où la première épidémie hivernale s'est produite avec un retard sans précédent. Ce retard a été associé à des fermetures prolongées d'établissements scolaires dans la région, et à une poussée très précoce de grippe. Le virus paragrippal 3, essentiellement estival à Newcastle, a été le plus souvent observé pendant les mois froids dans les autres centres.


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