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Surveillance of respiratory viral infections by rapid immunofluorescence diagnosis, with emphasis on virus interference

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SUMMARY

During the 7-year period from September 1978 to August 1985, smear specimens of nasopharyngeal secretions from 3132 patients mainly hospitalized children, taken in different regions in Norway, were examined for respiratory viruses by the rapid immunofluorescence (IF) technique. A positive diagnosis for respiratory syncytial virus (RSV), parainfluenza virus type 1, 2 and 3 or influenza A and B virus was made for 896 patients (29%). The greatest prevalence for all these viruses was observed during the colder months with only sporadic cases during the summer months. A relative increase in parainfluenza virus activity, involving several parainfluenza virus types, was observed in every second autumn and during these periods only sporadic cases of RSV infection were diagnosed. Also both RSV and parainfluenza viruses were less frequently found during influenza virus epidemics and regional differences in RSV activity were observed. During the four autumn periods 1982–85 the monthly number of positive virus identifications by IF followed an epidemic curve, while the corresponding number of negative samples was relatively constant. The results of this study suggest interference between RSV, parainfluenza viruses and influenza virus in reaching their epidemiological peaks. It is suggested that interferon might be a mediator of this effect.

INTRODUCTION

Respiratory infections are common causes of paediatric consultations and admissions to hospital. The majority of these infections have a viral aetiology with respiratory syncytial virus (RSV) being the most important pathogen among infants and small children (Kim *et al.* 1973; Martin, Gardner & McQuillin, 1978; Ørstavik, Carlsen & Halvorsen, 1980). In children of preschool age, parainfluenza viruses are frequent causes of respiratory infections and are often associated with croup (Downham, McQuillin & Gardner, 1974; Grauballe, Johnsen & Hornsleth, 1974). Several studies (Wright *et al.* 1977; Kim *et al.* 1979; Glezen, Parades & Taber, 1980) have also emphasized the importance of influenza virus as a cause of severe respiratory tract infection in children.

In recent years the development of immunofluorescence (IF) for the detection of viral antigens in preparations of exfoliated nasopharyngeal cells has greatly improved the rapid laboratory diagnosis of viral respiratory diseases (Gardner & McQuillin, 1980). However, for those who are remote from a virus laboratory application of these techniques has been hampered by the laborious preparative

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work necessary to process the cell smears. We have developed a simplified procedure for preparing nasopharyngeal secretions using smears of undiluted aspirated material (Ånestad & Mæhle, 1981). The sensitivity and specificity of this method have been found to be comparable in our hands to the conventional method for detecting respiratory viruses by IF (Ånestad, Breivik & Thoresen, 1983). The simplicity of our method also encourages users to submit specimens during non-epidemic periods when the percentage of positives is low, and makes it suitable for epidemiological surveillance. The method was introduced in Norway near the end of August 1978 and an earlier report (Ånestad, 1985) described the epidemiological findings during the winter season 1982/3. This paper contains further epidemiological observations on the 7-year period from September 1978 to August 1985 and drawing attention to the possibility of interference between RSV, influenza virus and parainfluenza viruses outbreaks.

The possibility of mutual exclusion between these respiratory viruses has also been suggested in an earlier report (Ånestad, 1982). This assumption is based on Norwegian laboratory reports of RSV and influenza virus infection obtained during the seven winter seasons 1974/5 to 1980/1. For comparison with our IF findings, these data are presented together with the figures from the succeeding four winter seasons in the present paper.

MATERIALS AND METHODS

Patients

During the 7 years nasopharyngeal specimens from 3132 patients were examined at the Department of Virology, National Institute for Public Health. In this time the number of specimens received increased from 111 samples during the first 12 months to more than 500 samples during each of the last 3 years and were submitted from hospitals in different parts of Norway. However, during the last 2 years of the study the majority of the samples were collected from young children admitted to the departments of paediatrics at the Central Hospital of Akershus (Akershus County) and the Central Hospital of Telemark (Telemark County), both of which are in the south-eastern part of Norway. General practitioners accounted for 10–20% of the specimens and these were collected both from adults and children.

Reagents

The smears were stained by an indirect IF technique with antisera to RSV (from August 1978), influenza A virus (from January 1981), parainfluenza virus type 1, 2 and 3, and influenza B virus (from September 1981). Before use, the quality of these reagents had been thoroughly checked (Ånestad, 1985).

Epidemiological data: incidence of influenza-like disease

General practitioners report weekly the number of influenza-like illnesses to the Department of Infectious Disease Control at the National Institute of Public Health. The weekly incidence of influenza-like disease/100000 is reported for each county in the Norwegian Notification System for Infectious Diseases (MSIS).

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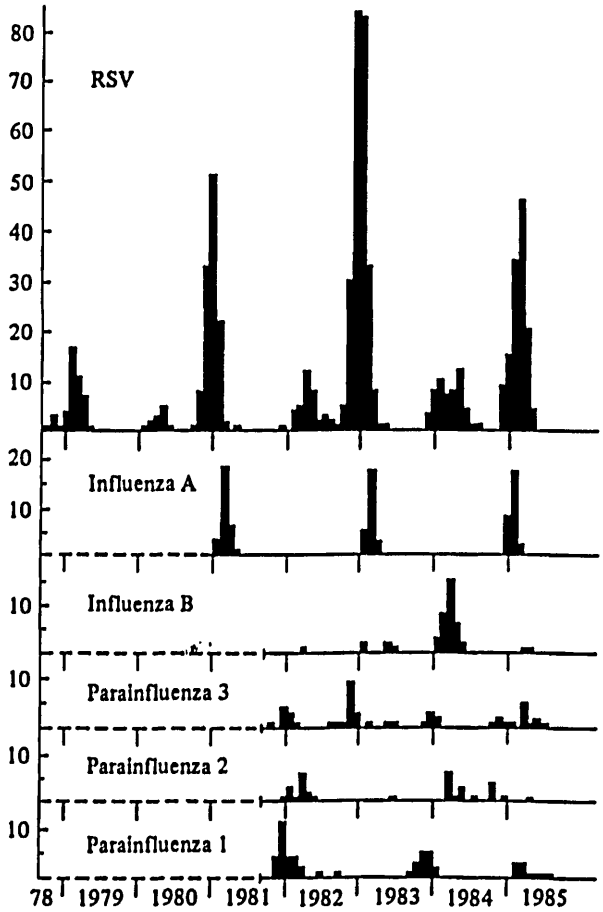

Fig. 1. Monthly virus identifications by rapid immunofluorescence diagnosis, September 1978 to August 1985.

RESULTS

A positive diagnosis for RSV, parainfluenza virus type 1, 2 and 3 or influenza A and B virus was made in 896 patients (29%) with monthly variations in positivity between 0 and 61%. The greatest activity for all these viruses was observed during the colder months with only sporadic cases during the summer months (Fig. 1).

RSV, the most frequently identified pathogen, accounted for more than half the total number of cases. In four winter seasons the RSV outbreaks started in late autumn and in each of these seasons substantial outbreaks with distinct peaks were observed. In the other three winters RSV activity was first observed near the end of the year, and in these only small RSV outbreaks were observed. Influenza A or B virus activity was observed in four of the five seasons in which tests for these viruses were included. With the exception of the winter of 1984/5 low RSV activity was observed during the influenza virus epidemics. Parainfluenza viruses were sought during four seasons and parainfluenza virus type 3 activity was observed in all of them. On the other hand, outbreaks with parainfluenza virus

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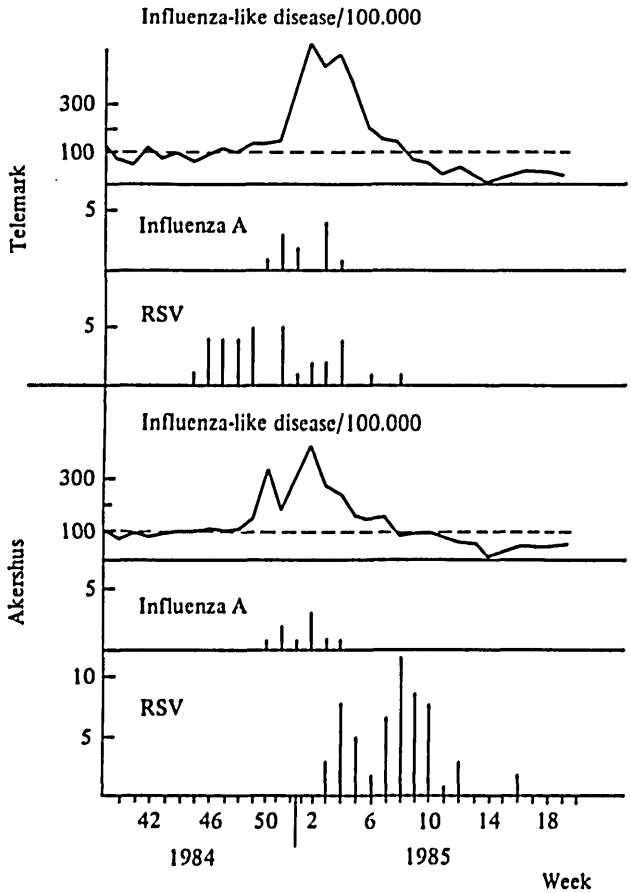

Fig. 2. Weekly incidences of influenza-like disease in Telemark and Akershus counties compared with influenza A virus and respiratory syncytial virus (RSV) identifications by rapid immunofluorescence diagnosis (vertical bars) during the winter season 1984/5.

type 1 and 2 tended to occur every second year. In 1981/2 and 1983/4 outbreaks with several parainfluenza virus types were observed in early winter, and in these periods the observed RSV activity was low. In the 1983/4 outbreak, parainfluenza virus type 2 started in early spring and continued the following autumn; in this autumn the RSV epidemic was delayed by 1–2 months compared with the 1980/1 and 1982/3 RSV epidemics. Fig. 1 also shows low parainfluenza virus activity during the influenza virus epidemic periods.

In an earlier report (Ånestad, 1985) differences in the timing of RSV outbreaks between two regions in Norway were described during the winter season 1982/3. A similar comparison was then made for both RSV and influenza virus outbreaks during the 1984/5 winter season. In Fig. 2 the weekly number of RSV and influenza virus identifications by IF are compared with the incidence of influenza-like disease/100000 in Akershus and Telemark counties during this season. Fig. 2 shows a good temporal correlation between an increased incidence of influenza-like disease (above 100/100000) and our influenza A virus identifications. In both

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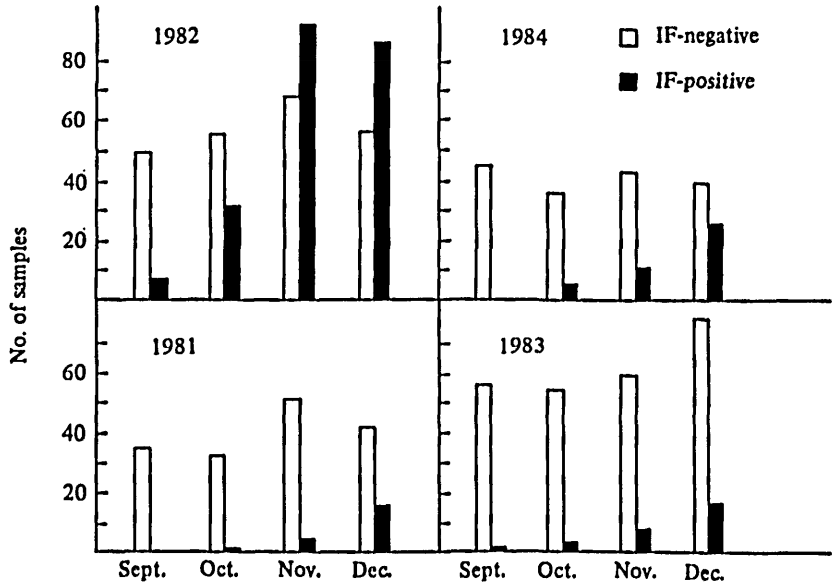

Fig. 3. Monthly number of positive virus identifications and negative samples by rapid immunofluorescence diagnosis during the autumn seasons 1981–4.

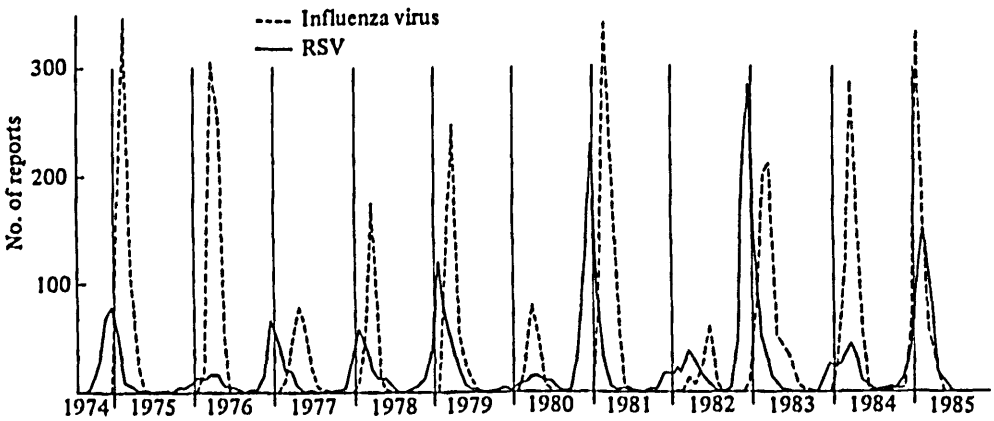

Fig. 4. Monthly number of laboratory confirmed infections with respiratory syncytial virus (RSV) and influenza A and B virus infections in Norway during an 11 years period.

counties the peaks of the influenza A virus outbreaks were almost identical, but in Telemark county the peak of the RSV outbreak may have preceded that of influenza A virus. In contrast, in Akershus county RSV activity rose as influenza activity declined. Fig. 2 also shows that RSV activity within the community had no apparent correlation with the reported incidence of influenza-like illness.

To investigate the progress of these virus epidemics, the monthly number of positive virus identifications was compared with the number of negative specimens during the last four autumns of the study period (Fig. 3). As can be seen from this figure the total number of positive virus identifications followed an epidemic curve

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while the number of negative specimens was more constant and was independent of the size of the outbreaks with these viruses.

Fig. 4 shows the monthly number of Norwegian laboratory confirmed infections with RSV and influenza virus during the 11 winter seasons 1974/5 to 1984/5. With the exception of the three consecutive winter seasons 1976/7, 1977/8 and 1978/9 the RSV outbreaks alternated between smaller and greater outbreaks every second year. Fig. 4 also shows that the winter season 1984/5 was the only one in which the peaks of the RSV and influenza virus outbreaks coincided.

DISCUSSION

Although the observation period is too short to allow any definitive conclusions the figures presented in this communication suggest some interference between RSV, influenza and parainfluenza virus activity so that when one of these epidemic respiratory viruses reached a peak the others seemed to be relatively inactive, and regional differences in RSV activity during the 1984/5 winter season (Fig. 2) could explain the apparently coincidental peak of the RSV and influenza virus outbreaks during that winter. Glezen & Denny (1973) have made similar observations in the USA. Parainfluenza virus type 3 appeared to be an exception; this virus circulated independently of the other respiratory viruses.

Small children hospitalized with respiratory virus infections probably represent only the tip of the iceberg of infections with these viruses within the population. Investigations of consecutive RSV outbreaks from Norway (Ørstavik, Carlsen & Halvorsen, 1980) and England (Martin, Gardner & McQuillin, 1978) have estimated the incidence of RSV infection severe enough to require hospitalization to be 1 in 100 and 1 in 50 in children under 1 year old respectively. On the other hand, seroepidemiological studies by Kim *et al.* (1973) have revealed that approximately one-half of the infants tested were infected by RSV during their first epidemic and almost all children had been infected after living through two RSV epidemics.

With parainfluenza virus infections, the incidence of severe respiratory tract infections is probably lower. Martin, Gardner & McQuillin (1978) estimated the risk of acquiring parainfluenza virus type 3 infection serious enough to require hospitalization to be 1 in 300 children born each year. As with RSV, infections with parainfluenza viruses are common in childhood and, by the age of 5 years, the frequency of antibodies to the different types is found to range between 60% and 90% (Glezen, Loda & Denny, 1982).

In older children and adults reinfections both with RSV and parainfluenza viruses are common. Ørstavik *et al.* (1984) have emphasized the importance of school children in transmitting these virus infections. Clinically, reinfections in this age group are usually manifested as upper respiratory tract infections indistinguishable from the common cold syndrome. The majority of these reinfections and also the majority of the primary infections will not require medical attention and consequently also escape ordinary respiratory virus surveillance systems. Figure 2 is an illustration of this, where an extensive RSV outbreak during the 1984/5 winter season apparently had no influence on the incidence of influenza-like disease.

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Virological surveilance of small children hospitalized with respiratory tract infection by rapid IF diagnosis may thus be a suitable tool for monitoring RSV and parainfluenza virus activity within the community. If the proportion of those with severe respiratory tract infection due to these viruses within the different age groups is relatively constant, the number of hospitalized children with these virus infections will to some degree reflect the relative extent of outbreaks with these viruses. However, as already mentioned, the frequency of severe respiratory tract infection associated with parainfluenza virus infection is lower than for RSV and the relative role of the parainfluenza viruses in causing respiratory tract infection within the population may be underestimated by this monitoring system.

Surveillance of children hospitalized with respiratory tract infections by rapid IF diagnosis seems to be suitable also for monitoring the occurrence of influenza virus activity in the population. This is clearly shown in Fig. 2, where good temporal correlations are seen between an elevated incidence of influenza-like disease and identification of influenza A virus in samples collected from hospitalized children.

A regular occurrence of outbreaks associated with parainfluenza virus type 3 each year and the tendency of parainfluenza virus type 1 and 2 outbreaks to occur every second year have also been observed in England (Martin, Gardner & McQuillin, 1978) and USA (Glezen *et al.* 1984). Similar alternations between smaller and greater RSV outbreaks as seen in Norway have also been observed in other Scandinavian countries (Ørstavik *et al.* 1984; Norwegian, Swedish and Finnish monthly reports of laboratory diagnosis of virus infections). However, whilst more extensive RSV outbreaks tend to occur in even-numbered years in Norway, they usually occur in odd-numbered years in Sweden and Finland. In contrast, with only a few exceptions, RSV activity is found to be very regular in Great Britain (Martin, Gardner & McQuillin, 1978; Communicable Disease Surveillance Centre and Communicable Disease (Scotland) Unit, 1980). If the suggestion that there is interference between the outbreaks of these epidemic respiratory viruses is correct, a reasonable explanation for the observed differences in RSV activity between the two areas of Europe might be that in Scandinavian countries outbreaks of parainfluenza virus type 1 and 2 may tend to occur during late autumn every second year whilst in Great Britain they may occur in alternate years. Consequently, any inhibition on the development of RSV outbreaks by parainfluenza virus activity may be relatively constant each year in Great Britain and any interference between these virus outbreaks may be obscured. Regular summer outbreaks of infection with parainfluenza virus type 3 have been observed in Great Britain (Martin, Gardner & McQuillin, 1978; Hope-Simpson, 1981). In Norway, summer outbreaks with parainfluenza viruses have never been recorded, nor have they been reported from other Scandinavian countries. This indicates real differences in the epidemiological behaviour of the parainfluenza viruses between Scandinavian countries and Great Britain. Another explanation for the observed differences in RSV epidemiology between the two areas might be that, compared with the densely populated Great Britain, interference activity due to parainfluenza and influenza viruses on RSV might be more clearly expressed in the sparsely populated Scandinavian countries. Nevertheless, the regular yearly RSV outbreaks observed in Norway during the three consecutive winter seasons 1976/

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7 to 1978/9 (Fig. 4) show that the yearly alternations between smaller and greater RSV outbreaks have not always been the rule in Norway.

Among children a variety of infectious agents not included in our immunofluorescence testing may cause respiratory tract infections serious enough to require hospitalization. However, Fig. 3 shows that these agents probably represent a more constant background activity.

Interference is regularly observed among bacteria and for many pathogenic microorganisms a struggle for existence is fought not only between the host and parasite, but also between different prokaryotic species. This dual struggle is probably of importance in evolution and interference between different viruses has repeatedly been observed *in vitro*. It therefore seems reasonable to assume that this also happens *in vivo*. If so, interferon is the most probable mediator of this effect, but other mechanisms may also be important.

Influenza and parainfluenza viruses are known to be good interferon inducers (McIntosh, 1978; Chonmaitree *et al.* 1981). In fact, it has been postulated that the raised level of circulating interferon in the acute phase of influenza virus infection may be responsible for the classical symptoms of influenza (Scott *et al.* 1981). From longitudinal family studies, Glezen (1982) estimated influenza virus attack rates to range from 14% to 50% each year. Consequently, during influenza virus epidemics there will be higher levels of interferon in the population.

In conclusion, if interference between epidemic respiratory viruses does exist, a ranking order of these viruses can probably be made with influenza virus at the top, parainfluenza viruses in the middle and RSV at the bottom.

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