Surveillance of respiratory viral infections by rapid immunofluorescence diagnosis, with emphasis on the epidemiological development of respiratory syncytial virus infections

By G. ÅNESTAD

Department of Virology, National Institute of Public Health, Geitmyrsveien 75, 0462 Oslo 4, Norway

SUMMARY

Surveillance of certain respiratory viral infections by applying immunofluorescence (IF) examinations to samples of nasopharyngeal secretions has been evaluated using a simplified procedure for the preparation of cell smears. Samples from 711 children living in different parts of Norway were examined during the winter 1982/83 and a positive diagnosis was made for 290 children (41 %). Temporal epidemic peaks were observed for respiratory syncytial virus (RSV), parainfluenza virus type 3 and influenza virus. On the other hand, the monthly number of negative samples was almost constant throughout the period. Differences in timing of RSV outbreaks were observed between two regions in Norway. Compared to rapid IF diagnosis, RSV notifications obtained by serological examinations were delayed by several weeks. Rapid virus diagnosis by IF examinations with our simplified procedure for preparation of nasopharyngeal samples seems to be suitable for the epidemiological surveillance of respiratory viral infections, both for its simplicity of preparation of the samples and for its accuracy in defining the time of the actual virus infection. Nevertheless, the method is not without pitfalls; a close cooperation between those who take the specimens and the laboratory is essential, and the IF examinations should be performed by an experienced microscopist.

INTRODUCTION

Rapid diagnosis of respiratory viral infections by immunofluorescence (IF) examinations of exfoliated nasopharyngeal cells may be of great help to the clinicians both for the management of the patient in question and for the control of nosocomial viral spreading. In addition, this method may form the basis for epidemiological surveillance of these virus infections. We have developed a simplified procedure for preparation of nasopharyngeal secretions using prepared smears of undiluted aspirated material (Ånestad & Mæhle, 1981). The sensitivity and specificity of this method in our hands has been found to be comparable to the conventional procedure in detecting respiratory viruses by IF examinations (Ånestad, Breivik & Thoresen, 1983). In the present communication we report

further experience with this method during the winter season 1982/83 in Norway, with special reference to the usefulness of the method for epidemiological surveillance.

MATERIALS AND METHODS

Patients

During the period September 1982 to April 1983 nasopharyngeal smears were submitted from 642 children in 14 hospitals located in different parts of Norway. General practitioners submitted samples from 69 patients.

Collection and preparation of nasopharyngeal secretions

The collection and preparation of samples have been described earlier (Ånestad & Mæhle, 1981). In brief, nasopharyngeal secretions were aspirated with a mucus collector and the undiluted aspirated material was squeezed out from the plastic outlet immediately after collection, deposited on 2–3 slides and air dried. The slides were sent to the virus laboratory by regular mail delivery.

To obtain consistent preparations with a sufficient number of cells on the slides, comments on the quality of the smears were included in the reports. This was particularly important for inexperienced users.

Antisera

Bovine antisera to respiratory syncytial virus (RSV), parainfluenza virus type 3 and influenza A virus were obtained from Wellcome Reagents. These antisera had been standardized and quality checked by members of the European Group for Rapid Virus Diagnosis (Ørstavik et al. 1984). Antisera to parainfluenza virus types 1 and 2 and influenza B virus were prepared in guinea-pigs by intranasal instillation of high-titred infectious virus. The immunization was repeated once, and blood was drawn two weeks after the last immunization when the animals were killed.

The guinea-pig antisera were thoroughly checked for cross-reactivity to other respiratory viruses. End-point titres were determined on virus-infected cell cultures for each antiserum. Antisera without cross-reactivity and IF titre of 1000 or higher were accepted and used at a working dilution of at least 1 in 50.

Our experience has been similar to that described by Riggs (1979) and Gardner (1984) that post-infection antisera are usually free of antibodies reacting with cellular components and no absorption of the antisera with cells grown in culture was found to be necessary. When tested on clinical samples the guinea-pig antisera exhibited some non-specific staining with the mucus present in the smears, but at a working dilution of at least 1 in 50 this non-specific staining did not cause serious problems in interpreting the IF examinations.

Immunofluorescence staining and microscopy

At the virus laboratory the slides were fixed in acetone for 10 min. Circular areas $\frac{1}{2}$–2 cm in diameter were marked with nail polish in places on the slides where the smears were of moderate thickness. Areas with clumps of material were omitted. The smears were stained according to an indirect IF technique with anti-bovine and anti-guinea-pig conjugates (Wellcome Reagents) labelled with fluorescein
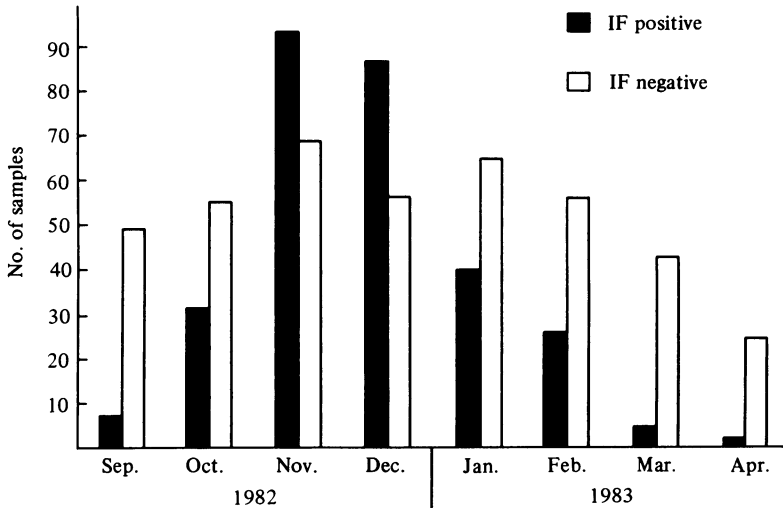
Fig. 1. Monthly number of virus identifications by immunofluorescence (IF) examinations and negative samples during the winter season 1982/83.

isothiocyanate (FITC). To prevent precipitation of FITC crystals in the mucus, the conjugates were centrifuged at 3000 *g* for 10 min before use. The IF examinations were performed with a Zeiss fluorescence microscope with incident light and mercury lamp illumination. The smears were screened at low magnification (160 ×) and findings were confirmed at 400 × .

As described previously (Ånestad & Mæhle, 1981; Ånestad, Breivik & Thoresen, 1983), the exfoliated cells were embedded in mucus either separately or as small clusters. In our hands the homogeneous background fluorescence of the mucus did not interfere with the intracellular staining of the virus-infected cells. Smear samples with at least two exfoliated cells with characteristic IF staining as described by Gardner & McQuillin (1980) were recorded as positive.

Serological examinations for respiratory syncytial virus

During the investigation period, serum samples from 774 patients were examined for antibodies to RSV by the complement fixation test (CFT). The Randall strain of RSV grown in HEp-2 cells was used as antigen in this test. The optimum antigen dilution was determined by a chessboard titration with a pool of sera obtained from small children with a recent RSV infection. A titre rise of at least fourfold or a titre of 80 or greater was considered diagnostically significant. These tests were part of our routine diagnostic service for respiratory virus infections, and the majority of these serum samples were obtained from patients from whom nasopharyngeal samples were not received.

RESULTS

A positive diagnosis for one of the viruses listed above was made for 290 children (41 %). A peak in the number of virus identifications was observed near the end of November, whilst the monthly number of negative samples was almost constant
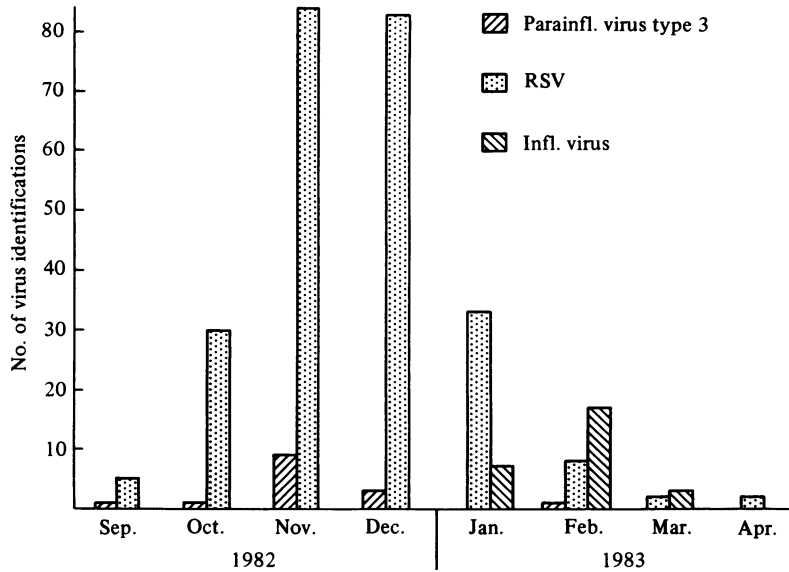
Fig. 2. Monthly virus identifications of respiratory syncytial virus (RSV), parainfluenza
(parainfl.) virus type 3 and influenza (infl.) A and B virus.

throughout the investigation period (Fig. 1). RSV was identified in samples from
247 children, parainfluenza virus type 3 from 15 and parainfluenza virus type 1
in one sample. No parainfluenza virus type 2 was detected. Twenty-five influenza
A and two influenza B virus infections were diagnosed.

Distinct peaks in the number of identifications of parainfluenza virus type 3 and
RSV were observed in the middle and near the end of November respectively, while
the influenza virus epidemic peaked in the middle of February (Fig. 2). No
influenza virus activity was observed during the most active phase of the RSV
epidemic.

The majority of the samples were submitted from hospitals and general
practitioners in the North-western part of Middle Norway and in the South-eastern
part of Southern Norway. The weekly incidence of RSV infection in these regions
is shown in Fig. 3. Both the onset and the peak occurrence of the RSV infections
were observed 3–4 weeks earlier in the sparsely populated North-western part of
the country compared with the more densely populated South-eastern region.
However, the epidemiological pattern of the RSV epidemic in the two regions was
almost identical. Similar differences in regional occurrence were not found for the
other viruses.

RSV infection was diagnosed by serological tests in 47 patients. The monthly
distribution of the RSV infections notified by virus identification and by serology
is shown in Fig. 4. Compared with the rapid IF diagnosis, the epidemic curve
obtained by serological examinations was delayed by approximately one month.

The simplified method for rapid IF diagnosis was introduced at the beginning
of the winter season 1978/79, and our results for RSV identification obtained
during the present winter season are compared with the results from the previous
four winter seasons in Fig. 5. In three seasons (1978/79, 1980/81 and 1982/83) the
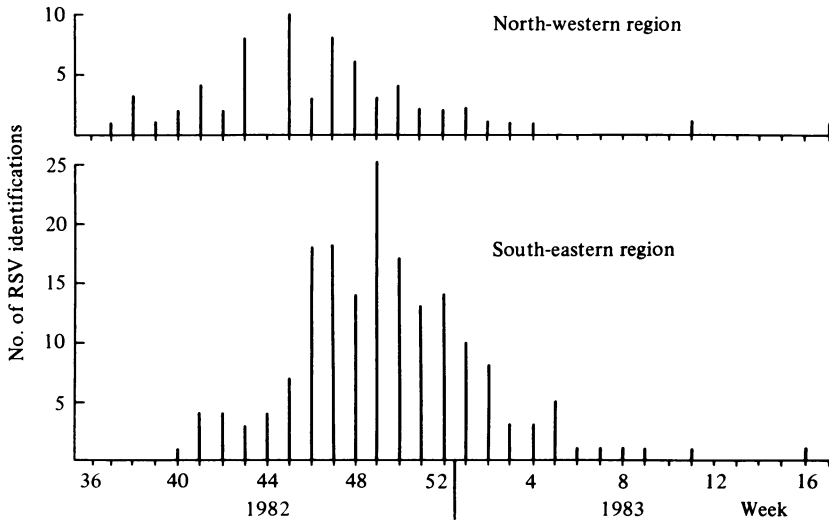
Fig. 3. Weekly identifications of respiratory syncytial virus (RSV) in samples submitted from the South-eastern and the North-western regions in Norway.
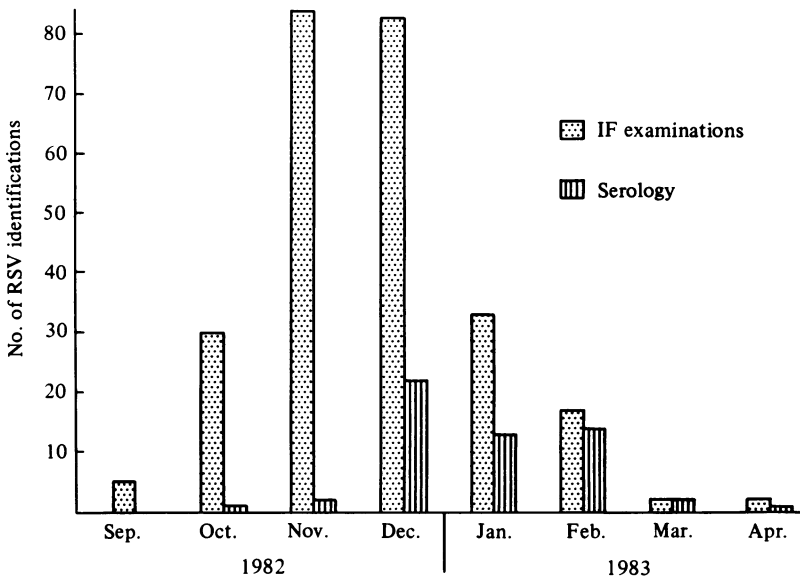
Fig. 4. Monthly distribution of respiratory syncytial virus (RSV) infections notified by virus identifications by immunofluorescence (IF) examination and by serology (CFT).

RSV epidemics started in early autumn and in these years extensive outbreaks were observed. On the other hand, in 1979/80 and 1981/82 the first cases of RSV infection were not recorded until nearly the end of the year, and in both these winters relatively few cases of RSV infection were recorded. However, in all five winter seasons the RSV outbreaks peaked 2–4 months after the first virus identifications.
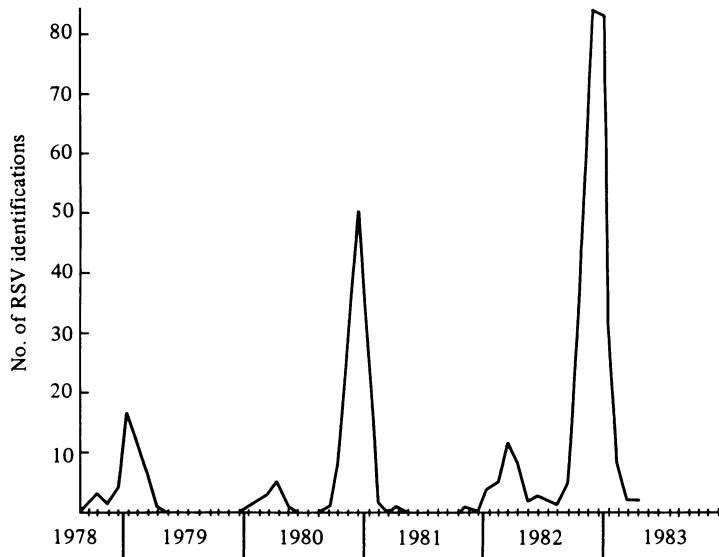
Fig. 5. Monthly respiratory syncytial virus (RSV) identifications: August 1978 to April 1983.

DISCUSSION

The use of standardized high-quality reagents is crucial in all IF work, and where such reagents were used the presence of some mucus in the cell smears did not cause too much confusing non-specific staining. It should, however, be underlined that the IF examinations should be performed by a thoroughly experienced microscopist. Bell *et al.* (1983) successfully used monoclonal antibodies for rapid IF detection of RSV in clinical samples. Such antisera with a low background activity may reduce the amount of non-specific staining and thus facilitate the microscopical examinations of the cell smears.

A close cooperation with those who take the original specimens is essential and, as mentioned before (Ånestad, Breivik & Thoresen, 1983), it is essential to aspirate adequate amounts of material. If not, the number of exfoliated cells will be too small to allow a proper diagnosis.

With these precautions in mind, our simplified method for preparation of nasopharyngeal secretions seems to be suitable for surveillance of certain respiratory virus infections for the following reasons. First, the preparation of cell smears can easily be performed by persons with little or no laboratory experience. This makes the method available also to small hospitals and general practitioners without access to well-equipped laboratory facilities. Secondly, the simplicity of the method encourages the users to continue submitting samples in non-epidemic periods when the percentage of positive samples is low. This makes it possible to recognize the start of an epidemic.

Fig. 1 clearly shows that the virus identifications during the investigation period followed an epidemic curve. On the other hand, the monthly figures of negative samples were almost constant throughout the period. This suggests that there are

infectious agents not identified by our IF methods or other causative factors which are endemic in Norway and cause respiratory tract infection throughout the year.

Development of epidemics of respiratory viral infections can be different in countries even in close geographical relationship to each other. For RSV, such differences have repeatedly been observed between Scandinavian countries (Ørstavik *et al.* 1984; Norwegian, Swedish and Finnish monthly reports on laboratory diagnosis of virus infections). Similar differences have also been observed between England and Scotland (Communicable Disease Surveillance Centre and Communicable Disease (Scotland) Unit, 1980). Fig. 3 clearly shows that the development of an epidemic of RSV may also have a different timing in different regions within the same country.

In this study, RSV notifications based on serological examinations were delayed by several weeks compared with the figures obtained by the rapid IF diagnosis. Therefore, epidemiological information is always retrospective unless based on virus identification.

Our observations that an early start of RSV infections is usually followed by more extensive outbreaks are in accord with similar observations from other Scandinavian countries (Norwegian, Swedish and Finnish monthly reports on laboratory diagnosis of virus infections). Armed with these data we have been able to predict the extent of the RSV outbreaks for the coming months for the last three winter seasons in Norway (the Norwegian Notification System for Infectious Diseases (MSIS)). Such predictions may have practical value, for instance in the planning of preventive measures.

We have observed repeatedly that the epidemic prevalence of influenza does not coincide with that of RSV (Ånestad, 1982). Also during the present winter season the first influenza virus identifications were made well after the culmination of the RSV epidemic. The observation of such phenomena is only possible by applying diagnostic methods which correctly reflect the true epidemiological situation. For this purpose rapid diagnosis of respiratory viral infections by IF seems to be very suitable.

REFERENCES

ÅNESTAD, G. (1982). Interference between outbreaks of respiratory syncytial virus and influenza virus infection. *Lancet* i, 502.

ÅNESTAD, G., BREIVIK, N. & THORESEN, T. (1983). Rapid diagnosis of respiratory syncytial virus and influenza A virus infections by immunofluorescence: experience with a simplified procedure for the preparation of cell smears from nasopharyngeal secretions. *Acta Pathologica et Microbiologica Scandinavica* B **91**, 267–271.

ÅNESTAD, G. & MÆHLE, O. R. (1981). Rapid diagnosis of respiratory syncytial (RS) virus infection by immunofluorescence: a simplified procedure for the preparation of nasopharyngeal suction specimens. *Acta Pathologica et Microbiologica Scandinavica* B **89**, 285–287.

BELL, D. M., WALSH, E. E., HRUSKA, J. F., SCHNABEL, K. C. & HALL, C. B. (1983). Rapid detection of respiratory syncytial virus with a monoclonal antibody. *Journal of Clinical Microbiology* **17**, 1099–1101.

COMMUNICABLE DISEASE SURVEILLANCE CENTRE AND COMMUNICABLE DISEASE (SCOTLAND) UNIT (1980). Respiratory syncytial virus activity in the United Kingdom, 1979. *Journal of Infection* **2**, 93–97.

GARDNER, P. S. (1984). Viral diagnosis by immunofluorescence. In *Recent Advances in Virus Diagnosis* (ed. M. S. McNulty & J. B. McFerran), pp. 1–14. Boston: Martinus Nijhoff.

GARDNER, P. S. & McQUILLIN, J. (1980). *Rapid Virus Diagnosis. Application of Immunofluorescence*, 2nd ed. London: Butterworth.

ØRSTAVIK, I., GRANDIEN, M., HALONEN, P., ARSTILA, P., MORDHORST, C. H., HORNSLETH, A., POPOW-KRAUPP, T., McQUILLIN, J., GARDNER, P. S., ALMEIDA, J., BRICOUT, F. & MARQUES, A. (1984). Viral diagnosis using the rapid immunofluorescence technique and epidemiological implications of acute respiratory infections among children in different European countries. *Bulletin of the World Health Organisation* **62**, 307–313.

RIGGS, J. L. (1979). Immunofluorescence staining. In *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections* (ed. E. H. Lennette & N. J. Schmidt), pp. 141–151. Washington, D.C.: American Public Health Association.