Respiratory Syncytial Virus Infection in Infants Hospitalized with Respiratory Illness in Northern Jordan

by M. M. M. Meqdam,* M. O. Rawashdeh,** H. Masaadeh,* A. A. Shurman** and N. Abuharfeil*

*Department of Applied Biology, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan
**Department of Paediatrics, Princess Rahma Hospital, Irbid, Jordan

Summary
During the winter seasons of 1993 and 1994, a total of 256 nasopharyngeal aspirates (NPA) from infants aged less than 1 year old admitted to the pediatric ward of Princess Rahma Hospital, northern Jordan, with bronchiolitis and/or pneumonia, were tested for the presence of respiratory syncytial virus (RSV) using direct immunofluorescence assay (DFA) and cell culture (CC). Of the 256 specimens, 129 (50 per cent) were found positive by both DFA and CC, whereas 24 specimens (9 per cent) and 16 specimens (6 per cent) were found positive by DFA and CC, respectively. In an evaluation of the collected NPA specimens detected by DFA, a sensitivity of 89 per cent and a specificity of 78 per cent were demonstrated. These data suggest that virus isolation in CC is still important for the diagnosis of RSV, although DFA is a valuable, rapid diagnostic assay.

Introduction
Respiratory syncytial virus (RSV) is one of the common causes of upper and lower respiratory tract infections in children. The virus is the major cause of bronchiolitis and pneumonia in infants and children less than 2 years old worldwide. Although the mortality rate for hospitalized cases is less than 5 per cent, it is higher in patients with congenital heart disease, pulmonary disease, leukaemia, and in those who are immunocompromised. As RSV causes a severe disease in infants and young children, its treatment and prevention are important public health concerns.

Although the treatment and prevention of RSV infection are limited, an antiviral drug (ribavirin) has been used successfully for some patients, but a safe preventive vaccine is not available. The prevention of transmission of RSV infection in hospital has been directed towards strict compliance with contact isolation procedures.

The diagnosis of RSV is useful for initiating antiviral therapy, eliminating the use of antibiotics and isolating patients to diminish nosocomial spread.

RSV is a very labile virus and stringent adherence to transport guidelines and immediate inoculation into cell culture is important for recovery of the virus. The virus grows slowly in cell culture and frequently more than 7 days is needed to detect the cytopathic effect (CPE). A change of medium at 5–7 days is helpful in accelerating CPE. CPE is usually seen as fused, irregular and refractile sheet of cells with many nuclei. As cell culture needs relatively long monitoring times, a rapid test for detecting infection with RSV is needed.

Direct immunofluorescence assay (DFA) is a rapid and sensitive method for detecting RSV antigens in cells of nasopharyngeal aspirates (NPA). Limitations of DFA for RSV detection are the necessity of a skilled microscopist and prompt specimen processing.

The aim of this study was to determine the incidence of RSV causing respiratory illness in hospitalized infants and to compare the results of virus isolation in CC with those obtained by DFA using monoclonal antibodies.

Materials and Methods
Patients
This study involved 256 infants aged less than 1 year old hospitalized at the Princess Rahma Hospital, northern Jordan with a presumptive diagnosis of bronchiolitis and/or pneumonia.

Specimen collection
NPA were collected by using a sterile infant suction catheter (Kendall, 10Fr—50cm, with two side eyes, Thailand) connected to a vacuum pump. The catheter was introduced through each nostril yielding about 1 ml of aspirate which was transported to the laboratory on wet ice, and processed immediately. One portion was suspended in 1 ml of Hanks solution including 200 µl penicillin/ml, 0.2 mg streptomycin per millilitre, and 10 µg amphotericin B/ml, for inoculation on cell culture; the other portion was used for DFA.

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TABLE 1  
**Age and sex distribution of infants positive for RSV by both DFA and CC**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>No. of infants (M, F)*</th>
<th>No. of positive infants (M, F)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–&lt;3</td>
<td>82 (47, 35)</td>
<td>32 (24, 20)</td>
</tr>
<tr>
<td>3–&lt;6</td>
<td>59 (35, 24)</td>
<td>23 (14, 11)</td>
</tr>
<tr>
<td>6–&lt;9</td>
<td>39 (21, 18)</td>
<td>15 (11, 13)</td>
</tr>
<tr>
<td>9–12</td>
<td>76 (45, 31)</td>
<td>30 (21, 15)</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td>129</td>
</tr>
</tbody>
</table>

*F, female; M, male.

Virus isolation

Half ml of the NPA suspension was inoculated into a 25-cm² surface flask containing HEp-2 cells, and covered with 1 ml of growth medium [Minimal Essential Medium, MEM, 10 per cent fetal bovine serum (FBS), 10 μl penicillin/ml, 0.1 mg streptomycin/ml, 0.29 mg L-glutamine/ml, sodium carbonate]. After adsorption for 1 h at 37°C, 5 per cent CO₂, the supernatant was replaced by 5 ml of maintenance medium (MEM, 2 per cent FBS, 100 μl penicillin/ml, 0.1 mg streptomycin/ml, 0.29 mg L-glutamine/ml, sodium carbonate). The flask was incubated at 37°C, 5 per cent CO₂ and examined by an inverted microscope (Kyowa, Tokyo, Japan) daily for 2 weeks for detection of CPE. Flasks showing CPE were washed twice with 5 ml phosphate-buffered saline (PBS) and scraped off with a rubber policeman. After vigorous pipetting, a single cell suspension was formed and 15 μl of this suspension was pipetted onto each of two-well slide. The slides were processed as in immunofluorescence below. Flasks showing no CPE after 2 weeks of incubation were tested by DFA, if the NPA were positive for RSV by DFA on arrival.

Immunofluorescence

The other portion of NPA was suspended in about 0.5 ml PBS to reduce the viscosity, depending on quantity and aspect, mixed and centrifuged at 3000 rpm for 10 min using Hettich (EBA 8S, West Germany) centrifuge. The cell pellet was washed twice in PBS for 10 min. The sediment was next resuspended in 200 μl PBS. Fifteen microlitres of the cell suspension was spotted per well of a 10-well slide (5 mm diameter). The slide was air dried, and fixed with ice-cold acetone at 4°C for 10 min. Ten microlitres of monoclonal fluorescein isothiocyanate-labelled human anti-RSV IgG (AMICO Laboratories Inc., Nashville, Tennessee, USA) was added per well. The slide was incubated at 37°C for 30 min in a humid chamber and then washed three times with PBS. After mounting in phosphate-buffered glycerol (80–10 per cent, v/v), the slide was read using a fluorescence microscope (BX 300, West Germany) at x400 magnification. Specimens yielding less than an average of 10 columnar epithelial cells per well were rejected. RSV-infected and uninfected HEp-2 cells were stained as positive and negative controls, respectively, in each trial. The presence of one positive columnar epithelial cell was required to consider a specimen positive for RSV antigen.

Results

A total of 256 specimens were tested for RSV by DFA and CC. Table 1 shows the age and sex distribution of the positive cases for RSV by both DFA and CC. Males are slightly outnumbered by the females. The comparison of DFA with CC for isolation of RSV is shown in Table 2. Both methods identified 129 positive and 87 negative specimens. In comparison of DFA with CC, DFA has a sensitivity of 89 per cent and a specificity of 78 per cent. A total of 24 specimens were positive by DFA and negative by CC, whereas 16 specimens were positive by CC and negative by DFA.

Discussion

This is the first study from Jordan to examine the role of RSV infection as the etiological agent of bronchiolitis and pneumonia in infants aged less than 1 year old. The samples were collected during the cold winter months as bronchiolitis and pneumonia is most prevalent during this period in northern Jordan.

Although RSV is a common cause of bronchiolitis and pneumonia in infants, other viruses have been associated with these conditions. Therefore, rapid laboratory diagnosis is important for patient care and

### Table 2

**Comparison of DFA with CC for isolation of RSV on 256 NPA-specimens**

<table>
<thead>
<tr>
<th>RSV DFA result</th>
<th>RSV culture result</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>% Positive predictive value</th>
<th>% Negative predictive value</th>
<th>% Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>+</td>
<td>129</td>
<td>24</td>
<td>89 (129/145)</td>
<td>84 (129/153)</td>
<td>84 (216/256)</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>16</td>
<td>87</td>
<td>78 (87/111)</td>
<td>84 (87/103)</td>
<td>84 (216/256)</td>
</tr>
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</table>

*Values in parentheses are the actual numbers used for per cent determinations.*
control of nosocomial infection. Treatment with antiviral agents such as ribavirin can be instituted and the use of antibiotics avoided.

RSV in NPA specimens may be diagnosed by immunofluorescence, ELISA, and direct immunofluorescence. As fluorescence microscope is available in our laboratory, the use of DFA for rapid diagnosis of RSV is convenient, cost effective, and offers early therapeutic intervention and enhances understanding of the transmission of RSV among hospitalized patients. The use of monoclonal antibodies has a greater RSV detection rate than polyclonal antibodies.

In this study, a sensitivity of 89 per cent and a specificity of 78 per cent compared with CC isolation of RSV is similar to other results reported previously. However, others have reported a higher sensitivity and specificity ranging between 89 and 95 per cent. Of 145 RSV culture-positive specimens, 16 (11 per cent) were DFA negative. From previous studies, 9–19 per cent of the RSV culture-positive specimens were negative. These findings emphasize the importance of conventional CC for identifying RSV antigens missed by a rapid diagnostic technique such as DFA. Furthermore, false-positive results may occur in DFA when non-specific fluorescence caused by excess mucus or fluorescing neutrophils are read as positive by inexperienced technologists. It is noteworthy that routine-culture of DFA-negative specimens provided obvious therapeutic and epidemiological advantages in addition to isolation of viruses other than RSV.

The use of DFA requires experience and a smear containing columnar epithelial cells. HEP-2 cells were selected for RSV isolation because of their maximal recovery of RSV. The cells were inoculated with the specimens before they became confluent, thus increasing the chances of observing the RSV syncytia.

In this study, of 153 RSV-positive specimens by DFA, 24 (16 per cent) were culture-negative. Some of our specimens that were received in the late afternoon were refrigerated and cultured the following morning. This delay may diminish the recovery of RSV in culture, but not affect antigen detection by DFA, as RSV antigen is not destroyed by prolonged storage. In addition, a false-positive result may occur in the late stage of the disease; DFA detects RSV antigen in respiratory columnar epithelial cells although the virus is not recoverable in culture. Other viruses may hinder RSV isolation and furthermore HEP-2 cells have been demonstrated to quickly overgrow, thus reducing the sensitivity to detect RSV.

In conclusion, our findings support the previous data suggesting that, the use of a single test for RSV detection is inappropriate. There is a need for a complementary use of culture and nonculture methods for detection both viable and non-viable virus. This will be mostly beneficial for the immediate initiation of antiviral treatment.

References

18. Shakkinen HK, Halonen PE, Arestila PP, Salmi AA. Detection of respiratory syncytial virus, parainfluenza type


