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Respiratory syncytial virus infection in children with acute respiratory infections in Zambia

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SUMMARY

Epidemiological research on respiratory syncytial virus (RSV) infections in children was carried out at the Virology Laboratory, University Teaching Hospital (UTH), in Lusaka, Zambia, from January–December 1996. Specimens including 736 nasal washings and 2424 throat swabs were collected from children with acute respiratory infections (ARI) and tested for RSV by enzyme immunoassay and by virus isolation. RSV was isolated in 62 (4·1%) of 1496 throat swabs collected from March to September and was detected in 99 (16·3%) of 609 nasal washings from March to November. The average RSV isolation rate was 2·6% and the average RSV detection rate was 13·5%. The highest RSV isolation (8·1%) and detection (30·5%) rates were in June 1996. RSV antibody in the 278 serum specimens collected from Zambian children, who were hospitalized in the paediatric ward, UTH, was detected using a standard neutralization test. The antibody positive rate was 60–80% in children >4 years. It is evident that RSV is one of the main causal agents of ARI in children in Zambia.

INTRODUCTION

Acute lower respiratory tract infections are a major cause of morbidity and mortality in children in developing countries [1–3]. Viruses such as respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, and adenovirus play an important role in respiratory infections in children [2, 4]. However, there are few reports on the epidemiology of viral respiratory infections in developing countries because of the limitations in diagnostic technology.

RSV is the commonest cause of acute respiratory infections (ARI) in young children in temperate regions [5–9]. It may cause life-threatening lower respiratory tract infections [10, 11], and so it is important to monitor outbreaks of RSV infection in each region. We studied the incidence of RSV in ARI in Zambian children using cell culture techniques and an enzyme immunoassay (EIA). This is the first report documenting RSV as one of the important agents causing ARI in children in Zambia, a sub-Saharan African country.

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MATERIALS AND METHODS

Study period

An epidemiological study on RSV infection in Zambia was carried out from January to December 1996.

Patients and specimens

Patients were recruited from two urban health centres in Lusaka. Throat swab specimens of children with ARI who visited the two centres were collected for viral isolation during the study period. The specimen

collection and handling methods have already been described [12, 13]. Nasal washings were also collected for RSV antigen detection using EIA, from January to December 1996 [14]. Clinical data on each patient were collected using standard questionnaires including sex, age, clinical manifestations, clinical diagnosis and complications. Serum samples from 278 children who were hospitalized in a paediatric ward and submitted to the paediatric laboratory for various diagnostic tests were randomly selected and supplied to the Virology laboratory for RSV antibody detection.

Virus isolation and identification

Virus isolation from the throat swab specimens were performed using the previously described microplate method at the Virology Laboratory, University Teaching Hospital (UTH), Lusaka, Zambia [12, 13]. Identification of RSV isolates was performed by direct immunofluorescecence [12].

RSV antigen detection

RSV antigen in the nasal washings was detected with the EIA using TESTPACK RSV™ (Abbott Laboratories, IL, USA) [15, 16].

RSV neutralizing antibody detection

RSV antibody in the serum specimens was detected using a standard neutralization test. Briefly, Long strain virus and HEp-2 cells were used. A virus suspension containing 10 μl of 10 $TCID_{50}$ was mixed for 60 min with fourfold serial dilutions of inactivated serum in phosphate buffered saline (10 μl). The virus mixture was inoculated onto duplicate HEp-2 cell monolayers seeded in 96-well microplates. The specific cytopathic effect (CPE) of RSV was observed and antibody titre was evaluated after 3 days incubation period.

RESULTS

Patients and specimens

During the one-year study period, throat swabs were collected from 2424 children with ARI, the average number was 202 per month (range 150–240). Nasal washings were collected from 736 children with ARI from January to December 1996, the average number was 61 per month (range 39–84 per month from January to November 1996, but and 10 in December 1996). The male to female ratio was 1:1. The age distribution of children from whom throat swabs and nasal washings were collected is shown in Tables 1 and 2, respectively.

Table 1. *Number of RSV isolates and the isolation rates in children by age group. The throat swabs were collected between January 1995 and December 1996*

Age (year)	Number of samples	Number of isolates	Rate (%)
< 1	1160	38	3·3
1	713	15	2·1
2	281	5	1·8
3	170	2	1·2
4	90	2	3·3
=B 5	10	0	0
Total	2424	62	2·6

Table 2. *Number of RSV positives by EIA by age group between January and September 1996*

Age (year)	Number of samples	Number of isolates	Rate (%)
< 1	370	48	13·0
1	337	46	13·6
2	27	5	17·2
Total	731	99	13·5

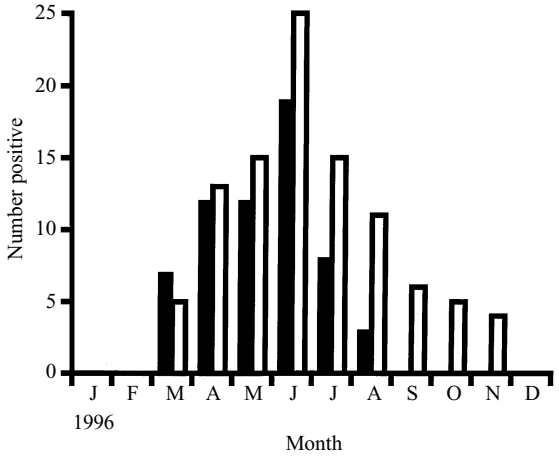

Fig. 1. Monthly distribution of RSV cases recognized by cell culture [■] and by EIA [□], January–December 1996.

RSV isolation and RSV antigen detection

RSV was isolated from 62 (4·1%) out of 1496 specimens collected from March to September. The highest RSV isolation rate was 8·1% in June 1996. RSV antigen was detected in 99 (16·3%) out of 609 nasal washings collected from March to November 1996

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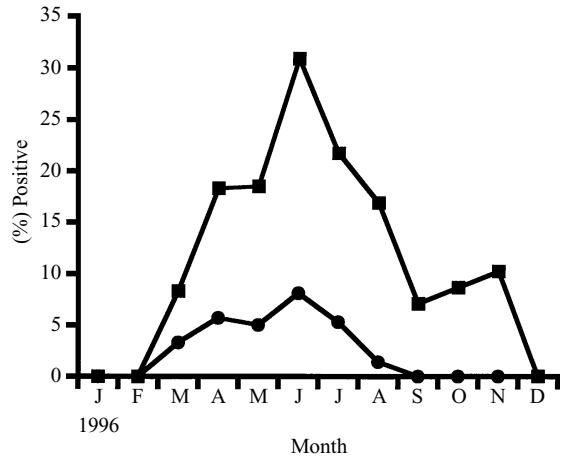

Fig. 2. Monthly RSV isolation rate (●) and antigen positive rate (■), January–December 1996.

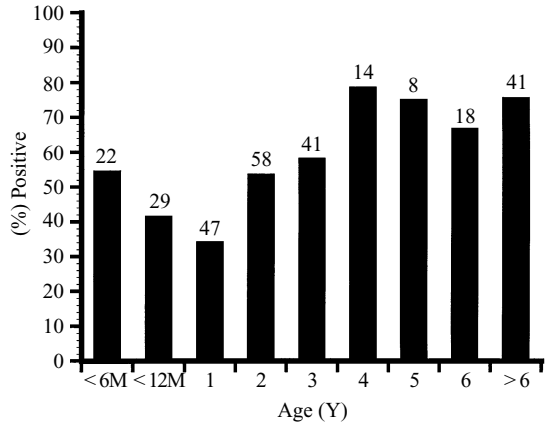

Fig. 3. RSV neutralizing antibody prevalence among Zambian children. The figures on the top of the bars indicate the number of specimens.

(Fig. 1). The RSV antigen positive rate by EIA was 13·5%, and the highest monthly rate was 30·5% in June. The monthly distribution of isolation and antigen positive rates is shown in Fig. 2. The age distribution of children, from whom RSV was isolated or was detected by EIA, is shown in Tables 1 and 2.

RSV neutralizing antibody prevalence

The RSV neutralizing antibody prevalence in Zambian children is shown in Fig. 3. The lowest RSV antibody positive rate was at 1 year of age, and it increased with age. The antibody positive rate was 60–80% among children > 4 years.

DISCUSSION

RSV is one of the main causes of ARI in children in Zambia, and seems to have a seasonal pattern. The number of RSV isolates and antigen positives identified per month increased gradually from March, peaked in June and declined thereafter. The peak months of influenza virus infections were July and August [13]. This means that in Zambia outbreaks of RSV infection among children are followed by outbreaks of influenza virus infection. The high prevalence of RSV neutralizing antibody by 4 years of age suggests that RSV is commonly transmitted among small children in Zambia.

Gbaldero and colleagues [17] and Johnson and colleagues [18] report that RSV is one of the most important viral agents, and causes bronchiolitis and asthma in Nigeria. Greenwood [19] reported that RSV was one of the most important causes of acute lower respiratory infection in the Gambia, West Africa, together with *Haemophilus influenza* type *b*. However, the reports on RSV epidemiology are very few from developing African countries and we believe that our report presents valuable information on the understanding of RSV epidemiology in Africa.

In this study, there was a difference in the rates between RSV isolation and RSV antigen detection. The difference in the rates can be explained by the difficulty associated with virus isolation. RSV is easily inactivated in a warm environment, and specimens must be inoculated onto cells as soon as possible [14]. However, there was a time lag between taking throat swabs and inoculation. Further, throat swabs are inferior to nasal washings for RSV isolation. Throat swabs were collected for RSV isolation in our study. So the RSV isolation rate by cell culture was lower than RSV antigen detection rate by EIA.

We did not analyse the clinical manifestations and outcome of RSV infections in Zambian children in this study. Further study is needed to make them clear. RSV infections in small children are severe, and life-threatening in some cases [10, 11]. The mortality rate of RSV infections in human immunodeficiency virus (HIV)-infected children is reported to be higher than that of immunocompetent children [20]. The HIV-seroprevalence in Zambian children, who were hospitalized in the paediatric ward, UTH, was reported to be 30·4% in 1995 [21]. Also, the mortality of lower respiratory tract infections was significantly higher among HIV-seropositives than among HIV-seronegatives [21]. Malnutrition in Zambian children also seems to be a co-factor for a high mortality of lower respiratory infections. So, we speculate that the mortality rate of RSV infections may be high. To clarify this speculation, further study is needed. We

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conclude that RSV is one of the major causes of ARI in Zambian children.

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