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The viral etiology of acute respiratory infections in children in Uganda

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The role of viruses in respiratory diseases of young children in Uganda was studied. A viral etiology was established in 36% of the infections investigated. The most important pathogens were found to be respiratory syncytial virus and parainfluenza viruses, which were responsible for 26% of infections investigated. They caused both upper and lower respiratory tract diseases. There was little or no seasonal variation in the etiology of these infections. Adenoviruses were found to be less important and were etiologically related to only 4% of respiratory disease cases. Influenza viruses and enteroviruses were also found to be associated with respiratory infections. However, they were less frequent and their role was insignificant. The role of multiple virus infections was also insignificant.

Considerable knowledge on the viral etiology of acute respiratory infections has been accumulated (*1, 2, 3*). However, most of these data originate from studies carried out in countries with a temperate climate, whereas the problem has been studied to a lesser extent in the tropics. In many developing countries little is known on the subject, although acute diseases of the respiratory tract, particularly in children, are an important public health problem (*4*) and longitudinal studies of rural Ugandan children have shown that kwashiorkor may develop following the anorexia that often accompanies infections, including those of the respiratory tract (*17*). In addition, a knowledge of the etiology of such infections is a prerequisite for any steps leading to their control. The problem is therefore of great concern to WHO, and it was decided to begin to study this question in Uganda, where a WHO Team for Special Studies in Virology has been operating since 1969.

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MATERIAL AND METHODS

Study area

The study was carried out from April 1972 to October 1973, in close collaboration with the Department of Paediatrics, Mulago Hospital, Kampala, and with the child welfare clinics of the Medical Research Council, in an area surrounding the cities of Kampala and Entebbe. The altitude of the region is about 1300 m and the temperature is moderate, usually not exceeding 28°C or falling below 9°C. The rainy seasons (April–June and October–December) are distinct from the dry seasons, although the relative humidity is high throughout the year. The three-month mean temperatures, relative humidity, and rainfall of the area, as established during the study period, are shown in Table 1.

Study population

The study population consisted of inpatients and outpatients aged 0–3 years with acute upper or acute lower respiratory tract disease classified as upper respiratory infection (URI), bronchitis, bronchiolitis, or bronchopneumonia. The clinical diagnosis was established after physical examination and auscultation of the patients. X-rays were taken only in exceptional cases. The control group consisted of children of the same age and from the same area suffering from kwashiorkor but with no signs of respiratory disease. Both sexes were represented approximately equally in both groups.

Table 1. Three-month mean temperatures, relative humidity, and rainfall in the Kampala–Entebbe region, April 1972–September 1973

	April–June 1972	July–September	October–December	January–March 1973	April–June	July–September
Temperature (°C)	20.1	19.1	20.0	20.8	20.7	19.0
Relative humidity (%)	92.0	93.0	96.0	94.0	91.0	93.0
Rainfall (mm)	122.6	86.1	220.7	61.0	166.4	72.7

Material collected

Throat and nasal swabs for the isolation of viruses and mycoplasmas, and acute and convalescent serum samples, were collected from the patients for serological investigation.

The throat and nasal swabs for virus isolation were pooled immediately after collection in 3 ml of transport medium (medium 199 + 0.5% albumin + a mixture of antibiotics), whereas the throat swabs for mycoplasma isolation were transported in 3 ml of PPLO broth (Difco) supplemented with penicillin. Both kinds of swab were kept under refrigeration before being further processed in the laboratory—usually within 4–6 hours.

Laboratory techniques

Isolation experiments were carried out in HeLa, Hep-2, and primary vervet monkey kidney tissue cultures, as well as in mycoplasma artificial broth and agar media (*5*). Three passages (10 days each) were performed before the specimen was considered negative. Identification of recovered strains was performed by standard haemadsorption inhibition or neutralization test procedures (*5*). Isolated *Mycoplasma* species were identified by the growth inhibition test (*5*). Some of the identification experiments were carried out by the WHO Collaborating Centre for Animal Mycoplasmas, Aarhus, Denmark. Acute and convalescent sera from patients were stored at —20°C and tested simultaneously against the following complement-fixing (CF) antigens: influenza A and B; parainfluenza types 1, 2, and 3; respiratory syncytial (RS) virus; adenovirus (group); herpesvirus hominis; and *Mycoplasma pneumoniae*. The complement fixation test was performed in microtitre plates with standard methods (*5*). Sera were inactivated for 30 min at 56°C prior to testing; 2–8 units of antigen and 2 units of complement with overnight fixation were used. Sera from which enteroviruses were recovered were also tested for an increase in neutralizing antibody against the strain isolated. Four-fold or higher antibody rises between acute and convalescent serum samples were considered as positive, regardless of whether or not the virus had been isolated from the patient.

RESULTS

Rates of virus and mycoplasma recovery from patients with various forms of respiratory infection, and from the control group (kwashiorkor patients), are presented in Table 2. Of 662 throat and nasal swabs taken from respiratory-disease patients, 18% yielded viruses and 20%, mycoplasmas. The corresponding proportions, for the 222 throat and nasal swabs taken from kwashiorkor patients, were 9% and 18%. Although the mycoplasma recovery rates of these two groups of patients were similar, virus recovery rates in children with kwashiorkor were significantly lower than those for children with respiratory infections. There was no significant difference in the number of viruses and mycoplasmas recovered

Table 2. Viral and mycoplasma recovery rates in children 0–3 years of age with respiratory infections, by clinical diagnosis, and in the control group (kwashiorkor)

Diagnosis	No. tested	Children positive for: virus		mycoplasma	
		No.	%	No.	%
URI	186	31	17	38	20
Bronchitis	180	36	20	46	26
Bronchiolitis	20	2	10	2	10
Bronchopneumonia	276	47	17	48	17
Total	662	116	18	134	20
Control group (kwashiorkor)	222	19	9	41	18

Table 3. Pattern of viruses recovered from children 0–3 years of age with various forms of respiratory disease, and from the control group (kwashiorkor)

Diagnosis	No. of viruses recovered (100%)	Virus types (%)																											
		RS virus	Parainfluenza virus			adenovirus									echovirus							coxsackievirus							polio-virus		herpesvirus hominis
			1	2	3	1	2	3	5	6	7	11	14	21	1	7	9	11	19	20	31	A9	B1	B2	B3	B4	B5	B6	1	3	
URI	31	—	—	—	10	16	20	—	—	—	—	—	—	—	—	—	—	—	3	3	—	3	—	13	10	—	—	3	13	3	3
Bronchitis	36	—	8	—	10	8	10	3	—	3	3	3	—	3	—	6	—	—	—	—	3	3	6	—	6	3	—	—	6	10	6
Bronchiolitis	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	100
Broncho-pneumonia	47	2	2	2	4	9	19	—	—	—	4	—	2	—	2	—	2	7	—	—	—	2	—	7	7	4	—	—	4	2	19
Total	116	1	4	1	8	10	16	1	—	1	2	1	1	1	1	2	1	2	1	1	1	2	2	6	7	2	—	1	7	5	12
Control group (kwashiorkor)	19	—	—	—	—	11	11	—	5	11	—	—	—	—	5	—	—	—	—	5	—	—	—	—	—	—	5	—	16	—	31

from male and female patients, nor was there any significant difference in virus and mycoplasma recoveries in patients with URI, bronchitis, or bronchopneumonia. The number of patients with bronchiolitis was too small to be evaluated.

The pattern of virus types recovered from patients with respiratory infections and from the control group is presented in Table 3. In total, 116 virus strains were recovered from patients with respiratory infections. Of these, adenoviruses were the most frequently isolated (33%) followed by coxsackieviruses (20%), parainfluenza viruses (13%), polioviruses and herpesvirus hominis (12%), echoviruses (9%), and RS virus (1%). From the patients with kwashiorkor, 19 virus strains were recovered in all. Adenoviruses were isolated with approximately the same frequency (38%) from patients with kwashiorkor as from those with respiratory diseases. However, there were differences in the types recovered. Adenoviruses types 1, 2, and 6 were common isolates in both groups, whereas types 3, 7, 11, 14, and 21 were isolated only from the respiratory disease group. The recovery rates of polioviruses (16%) and echoviruses (10%) were also comparable in the two groups. However, a wider variety of virus types was seen in respiratory disease patients. Herpesvirus hominis was more frequently isolated in kwashiorkor children, whereas coxsackieviruses were far less frequently isolated in this group. In addition, no parainfluenza or RS virus was recovered from these patients. There were no significant differences in the virus types recovered in URI, bronchitis, and bronchopneumonia patients, nor was there a significant seasonal prevalence of virus types in these infections. In approximately 5% of cases, 2–3 different viruses—mainly combinations of adenoviruses and echoviruses or coxsackievirus groups—were recovered simultaneously from the same patient.

Unlike viruses, mycoplasma species isolated from patients with various respiratory diseases and from the control group were comparable, with the exception of *M. pneumoniae*, which was isolated only from a child with kwashiorkor, without any overt respiratory disease (Table 4).

The results of serological investigation, regardless of virus isolation, from patients with various forms of respiratory disease and from the control group are presented in Table 5. Altogether 405 patients with respiratory disease were investigated, the viral etiology being established in 36% of cases. RS virus was found to be the most important pathogen, since it was responsible for 17% of all these infections. Next

Table 4. Pattern of mycoplasmas recovered from children 0–3 years of age with various forms of respiratory diseases, and from the control group (kwashiorkor)

Diagnosis	No. of mycoplasmas recovered (100%)	Mycoplasma species (%)			
		M. salivarium	*M. orale*	*M. hominis*	*M. pneumoniae*
URI	38	71	21	8	–
Bronchitis	46	67	24	9	–
Bronchiolitis	2	100	–	–	–
Bronchopneumonia	48	58	27	15	–
Total	134	66	24	10	–
Control group (kwashiorkor)	41	64	27	7	2

came parainfluenza viruses (9%) and adenoviruses (4%). In the parainfluenza group, type 3 was the most common (5%), followed by type 1 (3%) and type 2 (1%). The role of other virus groups, such as influenza virus, coxsackievirus, and echovirus, was much less important. The same applies to cases of respiratory infection in which two types of virus were involved simultaneously. There were combinations of RS virus and adenovirus, RS virus and parainfluenza viru stype 3, RS virus and coxsackievirus type B3, and RS virus and coxsackievirus type B4. No significant difference in the etiology of infections was seen with respect to sex. Regarding the various clinical categories of respiratory disease, the most frequent pathogens of the lower respiratory tract were found to be RS virus and parainfluenza viruses. The former was responsible for 13% of bronchitis, 25% of bronchiolitis, and 20% of bronchopneumonia cases; the latter, for 16% of bronchitis and 7% of bronchopneumonia cases. These two categories of virus were also found to be important pathogens of upper respiratory tract infections, in more than 23% of which they were etiologically involved. Adenoviruses were less important in these infection, being etiologically involved in 7% of URI, 3% of bronchitis, and 4% of bronchopneumonia cases. The role of other virus groups, namely influenzavirus, coxsackievirus, and echovirus, or a combination of virus types, was of minor importance. A 16-fold rise of CF antibody to RS virus occurred in a kwashiorkor patient without any sign of respiratory infection.

Table 6 shows the etiology of respiratory infections observed during the study period. There was little seasonal variation in the prevalence of infections due to most of the tested viruses, especially RS virus, which was responsible for 15–22% of infections in each of the seasons under study. The prevalence of parainfluenza virus infections ranged from 7% to 11% except for the period October–December 1972, during which 19% of these infections were detected. They were due mainly to parainfluenza virus type 3, which alone was responsible for 14% of them. In other seasons, the prevalence did not exceed 5%. Adenovirus infections also showed little seasonal variation except for the period April–June 1972, when no such infections were observed. Respiratory infections due to coxsackieviruses and echoviruses were found to be less frequent and evenly distributed throughout the study period. They were, however, not detected between January and March 1973—the only season when few cases of influenza A and B were detected.

DISCUSSION

The results of the study confirmed the important role of certain respiratory viruses in the etiology of acute respiratory disease in infants and young children in Uganda. More than one-third of the cases of this disease were of viral origin. The most important of the viruses involved appeared to be RS virus and parainfluenza viruses, which were involved in 26% of all respiratory infections investigated. Even this high proportion may be an underestimation when it is taken into consideration that not all RS virus infections may be detected by complement fixation tests, especially in infants and young children. In previous studies carried out in the tropics, RS virus and parainfluenza virus infections were shown to be associated mainly with lower respiratory tract disease, and they occurred either sporadically or in

Table 5. Etiology of respiratory infections in children 0–3 years of age, by clinical diagnosis, and in the control group (kwashiorkor) for comparison

Diagnosis	Tested	Positive (%)	Percentage with 4-fold or greater antibody rise to:																		
			influenza-virus:		parainfluenza virus types:			RS virus	Adenovirus group	coxsackie-virus types:				echo-virus types:				Combinations of viruses			
																		adeno+RS viruses	coxsackie-virus types:		parainfluenza virus type 3+RS
			A	B	1	2	3			A9	B1	B2	B3	7	11	20	31		B3+RS	B4+RS	
URI	127	37	2	<1	2	<1	4	17	7	<1	–	<1	–	<1	–	<1	–	<1	–	–	<1
Bronchitis	121	36	–	–	5	–	11	13	3	–	2	–	2	–	–	–	<1	–	–	–	–
Bronchiolitis	20	25	–	–	–	–	–	25	–	–	–	–	–	–	–	–	–	–	–	–	–
Broncho-pneumonia	137	36	–	–	2	2	3	20	4	1	–	–	2	–	1	–	–	–	<1	<1	–
Total	405	36	<1	<1	3	1	5	17	4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Control group (kwashiorkor)	58	2	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 6. Etiology of respiratory infections in children 0–3 years of age, by seasons

Period	Tested	Positive (%)	Percentage with 4-fold or greater antibody rise to:																		
			influenza-virus:		parainfluenza virus types:			RS virus	Adenovirus group	coxsackie-virus types:				echo-virus types:				Combinations of viruses			
																		adeno+RS viruses	coxsackie-virus types:		parainfluenza virus type 3+RS
			A	B	1	2	3			A9	B1	B2	B3	7	11	20	31		B3+RS	B4+RS	
April–June 1972	96	30	–	–	2	1	4	16	–	1	1	–	1	1	–	–	1	–	1	1	–
July–Sept. 1972	87	38	–	–	2	1	4	22	7	–	–	–	1	–	–	–	–	1	–	–	–
Oct.–Dec. 1972	58	48	–	–	3	2	14	16	7	2	–	–	–	–	2	2	–	–	–	–	–
Jan.–March 1973	46	39	4	2	4	–	4	20	3	–	–	–	–	–	–	–	–	–	–	–	2
April–June 1973	64	30	–	–	2	0	3	15	3	–	–	2	3	–	2	–	–	–	–	–	–
July–Sept. 1973	54	35	–	–	4	2	5	15	7	–	2	–	–	–	–	–	–	–	–	–	–

small epidemic outbreaks during the cool months of the year or in the rainy season (*6*, *7*, *8*, *9*). In this study, both upper and lower respiratory tract infections due to these viruses were detected. No marked seasonal distribution was observed, a rather endemic pattern of prevalence being seen throughout the year. The factors influencing such prevalence are not known, and further long-term studies are needed to elucidate the problem. However, a similar pattern of prevalence was observed also in another tropical country (*10*).

Although the number of RS virus infections detected serologically was high, the rate at which the virus was isolated from specimens collected from patients was very low. This may have been due to the adverse effect of transport, to the low sensitivity of the Hep-2 and HeLa cell lines used for isolation purposes, or to the fact that specimens were collected at a late stage in the disease, since children were often brought to the clinic several days after the onset of the infection. This may also explain the low recovery rate of parainfluenza viruses from patients compared with the high number of positive results of serological tests. These difficulties clearly demonstrate the need for other techniques, such as immunofluorescence, which not only give an answer rapidly but overcome problems related to temperature, transport, cell variability, and the arrival of specimens late in the course of the illness (*11*).

Adenoviruses were the viruses most frequently recovered in this study; however, they seemed to be less important than RS virus and parainfluenza viruses in the acute respiratory infections investigated. Most of the adenovirus strains isolated were of types 1 and 2, which are known to be commonly isolated from normal children in temperate climates (*12*). These strains were isolated with approximately the same frequency from children with respiratory disease and from the control group of children. Nevertheless, they were responsible for 7% of upper respiratory tract infections and the role of higher types (especially type 7) in lower respiratory tract disease should not be overlooked.

In addition to adenoviruses, a wide variety of enteroviruses were recovered from throat swab specimens. However, their role in acute respiratory infections, compared with that of respiratory viruses, was insignificant.

Infections in which two different types of virus were involved have been reported in previous studies (*6*, *9*, *13*). In this study, we also observed such cases, although they were few in number. Furthermore, it is not entirely clear whether these were true dual infections or whether sequential infection occurred. It is of interest that, in all such cases, one of the infecting agents was RS virus.

The role of herpesvirus hominis in respiratory diseases has not yet been clearly defined (*1*, *14*, *15*). In our study, numerous strains of that virus were isolated from both respiratory disease patients and controls. However, no antibody rises in any of the patients or controls were detected.

The recovery of a relatively large number of poliovirus strains is not surprising, since poliomyelitis is endemic in the study area and immunization against the disease was carried out simultaneously with the study.

Unlike viruses, *M. pneumoniae* played a negligible role in the respiratory diseases of childhood investigated, since neither the organism nor a rise in antibody to it was detected. These findings correspond to those reported in temperate climates (*16*). On the other hand, *M. pneumoniae* was isolated from the respiratory tract of a symptomless child with kwashiorkor. However, no serum was available from this case to prove the infection. Other human mycoplasma species were recovered with approximately the same frequency from both groups of children, and their distribution, also, is in agreement with previous findings (*16*).

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RÉSUMÉ

L'ÉTIOLOGIE VIRALE D'INFECTIONS RESPIRATOIRES AIGUËS CHEZ LES ENFANTS EN OUGANDA

Le rôle des virus dans les maladies respiratoires chez les nourrissons et les jeunes enfants en Ouganda a été recherché. L'étiologie virale a été confirmée dans 36% des infections étudiées. Il s'est révélé que les agents pathogènes les plus importants étaient les virus respiratoires syncytiaux (RS) et les virus parainfluenza, qui étaient responsables de 26% de la totalité des infections respiratoires étudiées. Ils provoquaient des affections des voies respiratoires supérieures et profondes. La fréquence de ces infections ne connaissait que des variations saisonnières minimes, sinon nulles, mais la prévalence avait plutôt une allure endémique. Il a été trouvé que les adénovirus sont peu importants et n'ont de relation étiologique qu'avec 4% des cas de maladies respiratoires. En outre, des virus grippaux de même que des entérovirus ont été trouvés associés avec des infections respiratoires, mais plus rarement, et leur rôle était insignifiant. Les infections virales mixtes se sont également révélées peu importantes.

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