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S.A. Madhi · N. Ramasamy · K. Petersen · A. Madhi
K.P. Klugman

Severe Lower Respiratory Tract Infections Associated with Human Parainfluenza Viruses 1–3 in Children Infected and Noninfected with HIV Type 1

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Abstract The aim of this study was to compare the clinical course of severe lower respiratory tract infections associated with human parainfluenza virus types 1–3 (HPIV 1–3) in hospitalised children infected with the human immunodeficiency virus type 1 (HIV-1) versus that in hospitalised children not infected with HIV-1. Children were enrolled prospectively as part of a broader study that evaluated the aetiology of lower respiratory tract infections in HIV-1-infected and -noninfected children from March 1997 through March 1999. HPIV types 1–3 were isolated from nasopharyngeal aspirate samples that were analysed using immunofluorescein monoclonal antibody assays. Thirty percent (24 of 80) of the children from whom HPIV was isolated were infected with HIV-1. Sixty-six percent (47of 62) and 22% (14 of 62) of the HPIV isolates that were typed were subtypes 3 and 1, respectively. The clinical presentation of severe lower respiratory tract infection was similar in both HIV-1-infected and -noninfected children, except that the former were less likely to have wheezing (4.2% vs. 28.6%, *P*=0.01). Furthermore, the duration of hospitalisation was longer in HIV-1-infected children than in HIV-1-noninfected children (median 11.5 days [range 1–15 days] vs. median 7.5 days [range 1–22 days]; *P*=0.02), and mortality was higher (5 of 24 [20.8%] infected children vs. 0 of 56 non-infected children; *P*=0.001). Importantly, four of five (80%) of the HIV-1-infected children who died had other concurrent illnesses or predisposing factors for severe HPIV-associated disease. HPIV-associated lower respiratory tract infection causes greater morbidity and mortality in HIV-1-infected children than in HIV-1-noninfected children; however, this may be due to other concurrent illnesses in HIV-1-infected children.

S.A. Madhi (✉) · N. Ramasamy · K. Petersen · A. Madhi
K.P. Klugman
Medical Research Council Pneumococcal Diseases Research Unit,
National Health Laboratory Service,
University of the Witwatersrand, SAIMR–Room 11,
PO Box 1038, Bertsham 2013, Republic of South Africa
e-mail: shabirm@mail.saimr.wits.ac.za
Tel.: +27-11-489-8786, Fax: +27-11-489-8692

S.A. Madhi
Paediatric Infectious Diseases Research Unit,
Wits Health Consortium, University of the Witwatersrand,
SAIMR–Room 11, PO Box 1038, Bertsham 2013,
Republic of South Africa

K.P. Klugman
Department of International Health, Emory University,
Rollins School of Public Health, Room 764, 1518 Clifton Road,
Atlanta, GA 30322, USA

Introduction

Impaired humoral and cell-mediated immunity in children infected with human immunodeficiency virus type 1 (HIV-1) increases their risk for developing severe lower respiratory tract infection (LRTI) following respiratory virus infections [1, 2]. Within the Paramyxoviridae family of viruses, respiratory syncytial virus (RSV) and human parainfluenza virus (HPIV) are the two major viruses associated with LRTI. Mortality among children hospitalised with LRTI caused by respiratory syncytial virus (RSV) was fourfold higher in HIV-1-infected children than in HIV-1-noninfected children (7.6% vs. 1.7%) [3], while a mortality rate of 28% has been described among HIV-1-infected children hospitalised with measles virus infection [4]. There is, however, limited data on HPIV infection in HIV-1-infected children [1, 5, 6, 7, 8], even though it is recognised as a common cause of LRTI among otherwise healthy children [9].

The risk of hospitalisation with HPIV-associated severe LRTI in HIV-1-infected children aged 2–24 months has been estimated to be 8.5-fold (95% confidence interval [CI], 5.0–10.5) greater than that in HIV-noninfected children (estimated incidence rates, 893/100,000 vs. 108/100,000 children aged 2–24 months, respectively) [5]. Among HIV-1-infected children hospitalised for severe LRTI, isolation rates of HPIV 1–3, RSV and influenza A/B have been similar, reported as 4.2, 5.3 and 5.4%, respectively [5]. This is in contrast to isolation rates observed in HIV-1-noninfected children from the same community, among whom RSV and influenza A/B

were isolated more frequently than HPIV 1–3 (18.1, 11.8 and 7.9%, respectively) [5]. Some of the children reported in the above-mentioned study [5] were included in this report. Aside from the latter children, there have been reports of only six other HIV-1-infected children with HPIV-associated LRTI [1, 6, 7, 8], three of whom died and all of whom had other concurrent opportunistic infections [1, 6, 7, 8].

This paucity of reports of HPIV among HIV-1-infected children is somewhat surprising, considering that HPIV has been documented as an important cause of morbidity among other immunosuppressed individuals [10]. The importance of HPIV as a cause of acute respiratory tract infection is emphasised by a study that found 27% of acute respiratory tract infections detected over a 3-year period in immunocompromised adults, either with leukaemia or bone marrow transplants, to be associated with common respiratory viruses; among these acute respiratory tract infections, 15% were caused by HPIV [10]. Children with primary combined immunodeficiencies have also been described to have severe and fatal LRTI events associated with HPIV infection [9, 11]. Mortality following HPIV-associated LRTI among HIV-1-noninfected immunocompromised individuals ranges between 15 and 30% [12].

The aim of this study was to compare the clinical presentation and course of severe LRTI associated with HPIV in hospitalised children with and without HIV-1 infection.

Subjects and Methods

Study Site

Children were enrolled prospectively into a broader study in which the aetiology of severe LRTI was evaluated between March 1997 and March 1999 at Chris Hani-Baragwanath Hospital (Soweto, South Africa), where approximately 5% of all children born between 1997 and 1999 were estimated to be infected with HIV-1 [3, 5]. While only children aged between 2 and 60 months were enrolled between March 1997 and February 1998, all children less than 5 years of age were enrolled during the subsequent 12 months. HIV-1-infected children did not receive any antiretroviral treatment, nor were they treated with ribavirin or any other antiviral agent during their hospitalisation.

Inclusion Criteria

Children hospitalised for LRTI who fulfilled the World Health Organisation (WHO) clinical criteria for severe pneumonia (i.e. tachypnea adjusted for age and lower chest wall indrawing and/or intercostal recession in malnourished children) [13] or who had an oxygen saturation of <90% as measured by pulse oximetry and in whom HPIV 1–3 was isolated within 24 h of admission were included in the study. Other than the above-mentioned clinical criteria for severe pneumonia, no specific clinical scoring system was used in determining the degree of respiratory distress or wheezing.

Hospitalised children who were participating in a randomised trial that began in 1998 in which a nonavalent pneumococcal conjugate vaccine was evaluated were excluded from this study, since immunisation with the vaccine may have potentially altered the clinical presentation and course of disease in these children.

The study was conducted according to the current research laws of the Republic of South Africa, and ethical clearance was obtained from the Committee for Research on Human Subjects of the University of the Witwatersrand.

Identification of Respiratory Viruses

HPIV was identified using a direct pooled-immunofluorescent test for respiratory viruses that included monoclonal antibodies for RSV, HPIV 1–3, influenza A/B virus and adenovirus. The test was performed on nasopharyngeal aspirate samples obtained within 24 h of hospital admission. Only those specimens that tested positive in the pooled immunofluorescent test but negative for RSV upon subsequent testing using a specific mouse anti-RSV monoclonal fluorescent antibody test (Chemicon International, USA) were further evaluated for HPIV 1–3 virus by shell-vial culture after 48 h of incubation. Testing for HPIV 1–3 was also performed using an immunofluorescein monoclonal antibody assay (Chemicon International). Further details of the method used to collect samples have been described previously [5]. While typing for individual HPIV types 1, 2 and 3 was performed during the first half of the study period, only a pooled test that included immunofluorescein monoclonal antibodies for HPIV 1, 2 and 3 was performed between March 1998 and October 1998, due to resource constraints. Typing for individual HPIV types 1, 2 and 3 was reinstituted for the last 5 months of the study.

Other Tests

To confirm the HIV-1 status of the children, the polymerase chain reaction (PCR) (either qualitative DNA PCR or quantitative RNA PCR; Amplicor HIV-1 Monitor Test, version 1.5; Roche Diagnostics, USA) was performed in children who were less than 18 months of age and had reactive HIV ELISA tests; these tests have been described previously [3]. Methods used for measuring leucocyte counts, C-reactive protein and oxygen saturation in room air and for reading of chest radiographs have been described as well [3].

Chest radiographs were categorised as being normal (no visible lung parenchymal infiltrate), consistent with bronchopneumonia (an interstitial or patchy nonconfluent lung parenchymal infiltrate) or consistent with alveolar consolidation (consolidation of confluent lung air space).

Clinical Information

Clinical signs and symptoms were recorded prospectively. Some information, e.g. history of fever and axillary temperatures, was recorded only from March 1998 onwards. Children were also screened for other underlying diseases that may have predisposed them to severe HPIV disease, including prematurity (<36 weeks of gestational age and <6 months chronological age at the time of illness); chronic lung diseases; congenital heart disease and underlying conditions other than HIV infection that may have predisposed to immunosuppression [14].

Statistical Analysis

Analysis was performed using Epi Info software (version 6.04 c, Centers for Disease Control and Prevention, Atlanta, USA; and version 6.04 b, World Health Organisation, Geneva, Switzerland). Normally distributed continuous variables of equal variances were analysed using an unpaired Student's *t*-test, while nonparametric results were analysed using the Kruskal-Wallis H test. Categorical variables were analysed using the Mantel Haenszel chi-squared test or the Fisher exact test when a cell had an expected value of less than five observations. An α value of 0.05 was considered statistically significant.

Table 1 Demographic features of HIV-1-infected and -noninfected children hospitalised with lower respiratory tract infection associated with human parainfluenza virus types 1–3

Demographic feature	HIV-1-infected children (*n*=24)	HIV-1-noninfected children (*n*=56)	*P* value
Median age in months (range)	8 (2–27)	7.5 (1–53)	0.82
Male:female ratio	1.2:1	1.2:1	0.96
No. (%) with underlying illness	7 (29.2)	12 (21.4)	0.96
Chronic lung disease[a]	2 (8.3)	4 (7.1)	0.99
Congenital heart disease[b]	1 (4.2)	4 (7.1)	0.91
Prematurity[c]	3 (12.5)	3 (5.3)	0.35
Other[d]	1 (4.2)	1 (4.2)	0.51
No. (%) who received oral antibiotics	13 (54.2)	11 (19.6)	0.002
No. (%) exposed to cigarette smoke	12/23 (52.2)	22 (39.2)	0.42

[a] Includes one child each with lymphocytic interstitial pneumonitis and pulmonary tuberculosis with chronic lung disease in the HIV-1-infected group and one child each with chronic lung disease, cystic bronchopulmonary dysplasia, diaphragmatic hernia and pulmonary tuberculosis in the HIV-1-noninfected group

[b] Includes one child with ventricular septal defect in the HIV-1-infected group and two such children in the HIV-1-noninfected group. In addition, there was one child with complex congenital heart disease and one with pulmonary atresia with a Blalock-Taussig shunt in the HIV-1-noninfected group

[c] Includes only children who were born at <36 weeks of gestational age and who were <6 months of age at the time of hospitalisation

[d] Includes one HIV-1-infected child with renal cell carcinoma and one HIV-1-noninfected child with biliary atresia

Results

Demographic Features

The prevalence of HIV-1 infection among children identified as having HPIV-associated severe LRTI was 24 of 80 (30%). No child was hospitalised on more than one occasion with HPIV-confirmed LRTI during the study period. The demographic features of HIV-1-infected and -noninfected children were similar, with a slight male dominance in both groups. The disease burden was greatest in children less than 1 year of age (Table 1). Furthermore, underlying conditions that may have predisposed to severe HPIV-associated LRTI were also similar in HIV-1-infected and -noninfected children. HIV-1-infected children were more likely to have been receiving antibiotics at the time of their hospitalisation.

Subtyping of Human Parainfluenza Virus Isolates

Six of 24 (25%) HPIV isolates from HIV-1-infected children and 12 of 56 (21.4%) HPIV isolates from HIV-1-noninfected children were not subtyped. There were no differences between HIV-1-infected and -noninfected children in the types of HPIV isolated among the 62 isolates that were subtyped. HPIV isolates were identified as type 1 in 14 of 62 (22.6%) cases (4 HIV-1-infected and 10 HIV-1-noninfected) and as type 3 in 47 of 62 (75.8%) cases (14 HIV-1-infected and 33 HIV-1-noninfected). There was only a single isolate of HPIV 2 that was identified in an HIV-1-noninfected child. Furthermore, 4 of 56 (7.1%) HIV-1-noninfected children and none of the HIV-1-infected children were identified as having concurrent influenza A/B or adenovirus infections (P=0.31). Viruses isolated concurrently from the HIV-1-noninfected children included two isolates of influenza A virus and two isolates of influenza B virus.

Clinical Presentation

There were no differences between HIV-1-infected children and HIV-1-noninfected children in the clinical presentation of fever, loss of appetite or vomiting. Furthermore, the clinical signs present upon hospital admission and associated with LRTI were similar in HIV-1-infected and -noninfected children, except that HIV-1-infected children were less likely to have wheezing (4.2% vs. 28.6%; Table 2).

Laboratory values and radiologic features of chest radiographs were also similar in HIV-1-infected and -noninfected children: there were no clinical differences in leucocyte counts, C-reactive protein levels, number of children with bacteraemia, or radiologic features on chest radiograph (Table 3). *Staphylococcus aureus* was the most common bacterial isolate cultured from blood or pleural fluid, recovered from three of six (50%) children with bacterial coinfection (Table 3).

In both groups, most changes on chest radiographs were consistent with either bronchopneumonic changes or normal chest radiographs. Other features on chest radiographs that occurred with similar frequency in both HIV-1-infected and -noninfected children included atelectasis (2 of 61; 3.3%), hilar lymphadenopathy (14 of 61; 23%) and hyperinflation (24 of 61; 39.3%).

Clinical Course of Disease

The overall duration of hospitalisation was longer in HIV-1-infected children than in HIV-1-noninfected children (median 11.5 days [range, 1–15] vs. 7.5 days [range, 1–22], respectively; P=0.02]. There was a similar trend observed for duration of hospitalisation when only those children with HPIV 3 were evaluated (median 6.5 days [range, 1–15] vs. 4.0 days [range 1–20], respectively; P=0.07). No such difference in the duration of

Table 2 Clinical signs and symptoms observed in HIV-1-infected and -noninfected children hospitalised with severe lower respiratory tract infection due to human parainfluenza virus types 1–3

Clinical sign	HIV-1-infected group (*n*=24)	HIV-1-noninfected group (*n*=56)	*P* value
Mean temperature in °C (±SD)[a]	37.2 (±0.8)	37.2 (±0.8)	0.68
Mean percent O_2 saturation (±SD)[b]	86.6 (±4.4)	88.4 (±4.3)	0.91
No. (%) with cyanosis	4 (16.7)	7 (12.5)	0.72
No. (%) with clubbing	4 (16.7)	3 (5.4)	0.18
No. (%) with crackles	18 (75.0)	42 (75.0)	0.99
No. (%) with wheezing	1 (4.2)	16 (28.6)	0.01
No. (%) with bronchial breathing	4 (17.4)	5 (8.9)	0.43
No. (%) with stridor	2 (8.3)	1 (1.8)	0.21

[a] Calculated on the basis of 16 and 40 observations in HIV-1-infected and -noninfected children, respectively
[b] Measured in room air by pulse oximetry. Calculated on the basis of 16 and 40 observations in HIV-1-infected and -noninfected children, respectively

Table 3 Results of clinical investigations in HIV-1-infected and -noninfected children hospitalised for severe lower respiratory tract infection due to human parainfluenza virus types 1–3

Clinical investigation	HIV-1-infected group (*n*=24)	HIV-1-noninfected group (*n*=56)	*P* value
No. (%) with bacterial coinfection[a]	3/22 (13.6)	3/51 (5.2)	0.35
Median leucocytes/ml (range)	12 (4–21.4)	14.2 (6.6–65.2)	0.03
Median CRP in mg/l (range)[b]	8 (3–200)	22.5 (3–200)	0.07
Chest radiograph[c]			
No. (%) with alveolar consolidation	6 (33.3)	13 (30.2)	0.81
No. (%) with patchy infiltrate	11 (61.1)	24 (55.8)	0.49
No. (%) with no parenchymal infiltrate	1 (5.6)	6 (14)	0.66

CRP, C-reactive protein
[a] *Haemophilus influenzae* type b, *Staphylococcus aureus* and *Escherichia coli*, respectively, were cultured from blood in each of the three HIV-1-infected children; in the HIV-1-noninfected children, *Streptococcus pneumoniae* was cultured in one child and *Staphylococcus aureus* in two children (from blood in 1 case, and from pleural fluid in 1 case)
[b] C-reactive protein was measured in 23 HIV-1-infected and 75 HIV-1-noninfected children
[c] Involves 18 observations in HIV-1-infected children and 43 observations in HIV-1-noninfected children. There was also one child each with pleural effusion and with pulmonary cavitation in the HIV-1-noninfected children, both of whom had *Staphylococcus aureus* coinfection

Table 4 Characteristics of the five HIV-1-infected children with lower respiratory tract infection due to human parainfluenza virus (HPIV) who died

Patient no.	Age (months)	Gender	Nutritional status	HPIV subtype	CD category[a]	Leucocyte count (cells/ml)	CRP (mg/dl)	Finding on chest radiograph	Blood culture result	Concurrent illness	Duration of illness (days)	Other
1	4	male	normal	3	N	12.0	7	alveolar consolidation	no growth	gastroenteritis	5	
2	9	female	wasted	1	C	6.4	23	not done	*S. aureus*	septicaemia	1	[b]
3	9	female	wasted	3	C	4.0	135	alveolar consolidation	no growth	septicaemia	15	[c]
4	3	male	normal	3	A	8.1	8	bronchopneumonia	not done	none	1	[d]
5	5	female	wasted	3	C	not done	3	bronchopneumonia	*S. epidermidis*	none	8	[e]

CRP, C-reactive protein
[a] Centers for Disease Control and Prevention, HIV-1 clinical categorisation [15]
[b] Community-acquired methicillin-resistant *Staphylococcus aureus* was isolated from blood, and the child had a plasma RNA level of >750,000 copies/ml
[c] Child deteriorated following suspected nosocomial pneumonia on day 13 of hospitalisation, when the repeat leucocyte count was 1.2 cells/cm^3, but repeat blood cultures were negative
[d] Child had a plasma HIV-1 RNA level of >750 000 copies/ml
[e] Child was born prematurely at <36 weeks of gestational age

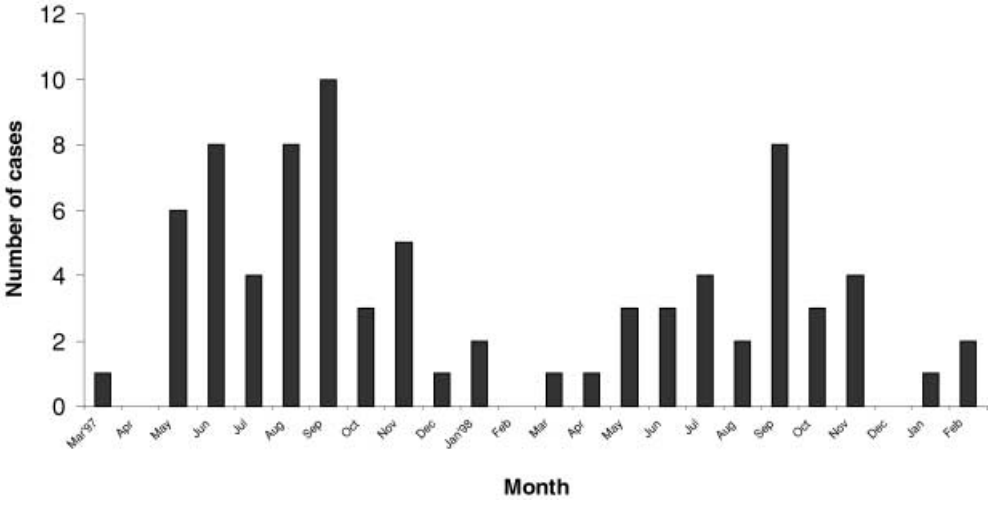

Fig. 1 Monthly pattern of human parainfluenza virus types 1, 2 and 3 isolated from HIV-1-infected and -noninfected children hospitalised with severe lower respiratory tract infection from March 1997 to February 1999

hospitalisation between HIV-1-infected and-noninfected children was observed with HPIV-1-associated LRTI (median 6.0 days [range, 1–9] vs. 5.0 days [range, 2–21], respectively; P=0.72). Furthermore, overall mortality was higher among HIV-1-infected than -noninfected children (5 of 24 [21%] vs. 0 of 56, respectively; P=0.001). The HPIV 3 specific mortality rate was also increased among HIV-1-infected compared with -noninfected children (4 of 14 [29%] vs. 0 of 33, respectively; P=0.005]. The HPIV 1 specific mortality rate was 25% (1 of 4 children). The details of the five HIV-1-infected children who died are shown in Table 4. Importantly, four of these five children had other concurrent illnesses or predisposing factors for severe HPIV-associated disease.

Seasonality of Human Parainfluenza Virus Infection

There was no difference between HIV-1-infected and -noninfected children in the seasonality of occurrence of HPIV types 1–3. HPIV types 1–3 were isolated perennially, although more cases of infection occurred in the cooler months (April–September) than in the warmer months (October–March) (Fig. 1).

Discussion

As observed with LRTI caused by other members of the Paramyxoviridae family of viruses, LRTI caused by HPIV 1–3 was associated with a poorer outcome in HIV-1-infected children than in HIV-1-noninfected children. This increased morbidity and mortality among HIV-1-infected children may, however, be related to the presence of other co-morbidities such as septicaemia, as described previously [1, 6, 7, 8]. The mortality rate observed in HIV-1-infected children was intermediate between that observed for LRTI due to other viruses belonging to the Paramyxoviridae family of viruses, namely RSV and measles, and was higher than that observed for LRTI due to influenza virus (8%) [3, 4, 16]. Variation in the above-mentioned mortality associated with HPIV and other respiratory viruses in HIV-1-infected children may, however, be due to differences in the natural histories of these viruses as well as to differences in the host immune responses. The relative role of the host immune response in the pathogenesis of illness caused by respiratory viruses may also play a role [1].

The mortality rate observed among HIV-1-infected children in this study was similar to that described for HPIV-associated LRTI (15–30%) among other groups of immunocompromised but HIV-1-noninfected individuals [12]. Whether prolonged shedding of HPIV among HIV-1-infected children, as has been reported in other case reports among HIV-1-infected children infected by HPIV [1, 6], may have contributed to the increased morbidity was not evaluated in this study.

The small number of HIV-1-infected children reported in this and other studies evaluating LRTI caused by RSV, influenza A/B or measles virus is a further limitation in evaluating for significance regarding the relative mortality rates in children with these viral LRTIs [3, 4, 16]. Furthermore, the lack of testing for pneumonia due to *Pneumocystis carinii* or cytomegalovirus, which, in a separate study from the same centre, was described in 44% of HIV-1-infected children who died [17], limits a firm conclusion from being made regarding morbidity and mortality related to HPIV-associated LRTI among HIV-1-infected children in particular. Although children were not investigated for *Pneumocystis carinii* pneumonia (PCP) in this study, data from this area and other studies in African children indicate that the median age of diagnosis of PCP in children is 3–4 months [17, 18, 19]. This reduces the possibility that all of the increased morbidity observed in HIV-1-infected children in this study can be attributed solely to concurrent PCP infection, since the median age of HIV-1-infected children in this study was greater than that observed for African children with PCP [17, 18, 19].

Importantly, the rate of bacterial coinfection in HIV-1-infected children in this study was not greater than that in HIV-1-noninfected children. This finding is in contrast to

that observed among HIV-1-infected children with LRTI due to RSV or influenza virus, in whom the rate of bacterial coinfection was greater than that found in HIV-1-noninfected children [3, 15]. It is unlikely that the lack of increased rate of bacterial coinfection among HIV-1-infected children in this study is due solely to more of these children being on antibiotics at the time of their presentation, since other indirect markers of concurrent bacterial pneumonia, including alveolar consolidation on chest radiograph, might still have been expected to occur with increased frequency if there were an increased risk of bacterial coinfection.

The lower incidence of wheezing among HIV-1-infected children observed in this study has been described previously for RSV-associated LRTI [3, 20] and may be related to altered immune responses following HPIV infection in HIV-1-infected children [1]. Furthermore, the potential perturbations of the cell-mediated immune response that is known to occur among HIV-1-infected individuals [2] may have contributed to the increased morbidity and mortality observed among these children.

Unfortunately, the lack of subtyping of HPIV isolates during at least 6 months of the study period limits our ability to delineate whether there is any subtype-specific seasonality to HPIV-associated LRTI in this area, as has been documented in the USA for HPIV subtypes 1–3 [21]. The data from the first year, when all HPIV isolates were subtyped, indicated that HPIV 3 was present perennially; however, there was a peak period during the cooler months of the year (data not shown). The number of LRTI cases due to HPIV 1was too small for any conclusions to be made regarding the seasonality of subtype 1. Perennial isolation of HPIV 3, which included a peak period during the warmer months of the year, has also been reported during more recent years (1996–1998) in New York, USA, although seasonality was more clearly defined during previous years (1992–1995) [12]. As the present study was limited to children with LRTI, it is not surprising that HPIV 2 was isolated from only one child, since this subtype is an uncommon cause of pneumonia and bronchiolitis and more commonly causes croup or bronchitis in hospitalised children [9, 21].

An additional limitation of this study is that, although shell-vial culture followed by immunofluorescence testing has been shown to be sensitive (80–100%) in diagnosing HPIV 1–3 infection [22, 23], it is possible that some children may have tested negative due to shedding of low viral titres and were consequently excluded from this analysis.

In conclusion, HPIV 1–3 is associated with greater morbidity and mortality among HIV-1-infected children than among HIV-1-noninfected children, but further studies are required to define the extent to which coinfection with *Pneumocystis carinii* and cytomegalovirus impacts this morbidity and mortality. The data gained from such studies would enable physicians to better quantify the need for interventions against respiratory viruses in HIV-1-infected children.

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