Role of Human Metapneumovirus, Human Coronavirus NL63 and Human Bocavirus in Infants and Young Children With Acute Wheezing

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The role of the novel respiratory viruses, human metapneumovirus (hMPV), human coronavirus NL63 (HCoV NL63) and human bocavirus (HBoV), in wheezing illness in children has not been well studied, especially in Africa. The aim of this study was to investigate the prevalence of hMPV, HCoV NL63 and HBoV in South African children with acute wheezing. A prospective study of consecutive children presenting with acute wheezing to a pediatric hospital from May 2004 to November 2005 was undertaken. A nasal swab was taken for reverse transcription-polymerase chain reaction (RT-PCR) and PCR for hMPV, HCoV NL63 and HBoV; when positive, the genes were sequenced. Shell vial culture for RSV, influenza A and B viruses, adenovirus and parainfluenza viruses 1, 2, 3 was performed on every 5th sample. Two hundred and forty two nasal swabs were collected from 238 children (median age 12.4 months). A novel respiratory virus was found in 44/242 (18.2%). hMPV, HBoV, and HCoV NL63 was found in 20 (8.3%), 18 (7.4%), and 6 (2.4%) of samples, respectively. Fifteen of 59 (25%) samples were positive for other respiratory viruses. Viral co-infections, occurred in 6/242 (2.5%). Phylogenetic analysis showed co-circulation of hMPV and HCoV NL63 A and B lineages, although only HBoV genotype st2 was found. Viruses are an important cause of wheezing in preschool children; hMPV, HCoV NL63, and HBoV are less common than the usual respiratory pathogens. *J. Med. Virol.* ***80:906–912, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: novel respiratory viruses; wheezing; pediatric

INTRODUCTION

Viral infections are a common precipitant of acute wheezing and asthma exacerbations in children. Many respiratory viruses have been associated with acute wheezing, including respiratory syncytial virus (RSV), rhinovirus, influenza viruses, parainfluenza viruses, and enteroviruses [Johnston et al., 1995; Taussig et al., 2003; Wilson, 2003; Jartti et al., 2004; Tan, 2004; Kusel et al., 2006]. Recently human metapneumovirus (hMPV) [van den Hoogen et al., 2001], human coronavirus NL63 (HCoV NL63) [Fouchier et al., 2004; van der Hoek et al., 2004] and human bocavirus (HBoV) [Allander et al., 2005] have been described. The importance of these respiratory viruses as a trigger of acute wheezing episodes has not been well studied in African children.

Jartti et al. [2002] first showed that hMPV may cause acute wheezing in young children. Subsequently other investigators have confirmed a significant association between hMPV infection and wheezing in young children, particularly in winter [Schildgen et al., 2005; Williams et al., 2005; Chung et al., 2007]. There is relatively little information on the importance of HCoV NL63 and HBoV as a trigger of acute wheezing and no information on the prevalence of these in African children. Studies from developed countries have reported that children infected with HCoV NL63 and HBoV may present with symptoms of wheezing [Arden et al., 2005; Esper et al., 2005; Moes et al., 2005; Foulongne et al., 2006; Ma et al., 2006; Allander et al., 2007].

The aim of this study was to investigate the prevalence of these novel respiratory viruses in young South African children with acute wheezing.

METHODS

A prospective study of children aged 2 months to 6 years sequentially presenting to the ambulatory section of Red Cross Children's Hospital, South Africa from May 2004 to November 2005 (two winter seasons) with acute wheezing was undertaken. Children were eligible if they had a history of cough or difficulty breathing within the prior 5 days and wheezing on auscultation or hyperinflation of the chest. Clinical and sociodemographic information were recorded.

Written, informed consent was obtained from a parent or guardian. The study was approved by the Ethics Committee of the Faculty of Health Sciences, University of Cape Town, South Africa.

A nasopharyngeal swab was obtained, placed in viral transport medium and transported to the Virology laboratory on the same day. After a clarification step (2,000 rpm for 7 min) the medium was stored at $-20°C$.

A general shell vial culture using a pool of monoclonal antibodies detecting RSV, influenza A and B viruses, adenovirus and parainfluenza viruses 1, 2, and 3 was performed on every 5th sample by an indirect immunofluorescence assay (Light Diagnostics, Chemicon International, Temecula, CA). Further specific virus identification on pool-positive samples was not undertaken.

RNA was extracted from 200 μl of the respiratory sample using the Talent Seek Viral RNA kit (Talent Sri, Trieste, Italy) according to the manufacturer's instructions. The purified RNA sample was converted into cDNA using random primers (Roche Diagnostics, Penzberg, Germany) and the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). A nested PCR reaction was performed targeting the fusion (F) and nucleocapsid (N) genes of hMPV and the 1b (protease) and 1a (RNA polymerase) genes of HCoV NL63 (Table I).

In the extraction procedure described above DNA was also co-incidentally isolated and could be used in the detection of the DNA virus, HBoV. A semi-nested PCR reaction was performed targeting the NP-1 (nonstructural) and VP1/2 (capsid) genes of HBoV (Table I).

The PCR products of the hMPV N gene, HCoV NL63 1a gene and the VP1/2 gene of HBoV were purified with a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced on a ABI 310 sequencer with a fluorescent dye terminator kit (Applied Biosystem, Foster City, CA).

The nucleotide sequences were aligned using CLUSTALG software (version 1.4) [Thompson et al., 1997]. A neighbor-joining phylogenetic tree was constructed using the Treecon software program (version 1.3b) with 500 bootstrap resamplings (van de Peer, University of Konstanz 1994–2001).

For statistical analysis continuous variables were expressed as median and inter quartile ranges and compared using Kruskal−Wallis Test. Categorical characteristics were analyzed using Fisher's Exact test.

RESULTS

Two hundred and thirty eight children were enrolled and 242 nasal swab samples were taken. The median (25th–75th percentile) age of children was 12.4 (6–25) months; 124 (52%) were under 12 months, while 174 (73%) were <24 months.

A novel respiratory virus was found in 44/242 (18.2%) nasal samples of which 36 (14.9%) were single infections (Table II). hMPV, HBoV, and HCoV NL63 were detected in 20 (8.2%), 18 (7.4%), and 6 (2.5%) samples respectively. Of 59 samples tested for other common respiratory viruses, 15 (25.4%) were positive; 4 of these were dual infections with one of the novel viruses. Dual viral infection was uncommon, occurring in 6/242 (2.5%)

TABLE I. PCR Primers Used for Amplification

Gene	Forward	Reverse	References
hMPV			
F outer	5′ CTT TGG ACT TAA TGA CAG ATG	5′ GTC TTC CTG TGC CTA ACT TTG	Peret et al. [2002]
F inner	5′ CTG AAC TAG CCA GAG CTG T	5′ CAT TGA TTC CTG CTG CTG TGT C	Smuts et al. [2004]
N outer	5′ GAG AAG AGC TGG GTA GAA G	5′ CAA ACA AAC TTT CTG CT	Peret et al. [2002]
N inner	5′ CAG AGG CC (CT) TCA GCA CCA	5′ CTG CCT GTA GAG GAT GAG C	This study
HCoV NL63			
1b outer	5′ GTG ATG CAT ATG CTA ATT TG	5′ CTC TTG CAG GTA TAA TCC TA	van der Hoek et al. [2004]
1b inner	5′ TTG GTA AAC AAA AGA TAA CT	5′ TCA ATG CTA TAA ACA GTC AT	Arden et al. [2005]
1a outer	5′ CTT TTG ATA ACG TCA CT ATG	5′ CTC ATT ACA TAA AAC ATC AAA CGG	Arden et al. [2005]
1a inner	5′ GGT CAC TAT GTA GTT TAT GAT G	5′ GGA TTT TTC ATA ACC ACT TAC	Arden et al. [2005]
HBoV			
NP-1 outer	5′ TAA CTG CTC CAG CAA GTC CTC CA	5′ GGA AGC TCT GTG TTG ACT GAA T	Smuts and Hardie [2006]
NP-1 inner	5′ CTC ACC TGC GAG CTC TGT AAG TA	5′ GGA AGC TCT GTG TTG ACT GAA T	Smuts and Hardie [2006]
VP1/2 outer	5′ GCA CTT CTG TAT CAG ATG CCT T	5′ CGT GGT ATG TAG GCG TGT AG	Smuts and Hardie [2006]
VP1/2 inner	5′ CTT AGA ACT GGT GAG AGC ACT G	5′ CGT GGT ATG TAG GCG TGT AG	Smuts and Hardie [2006]

TABLE II. Clinical and Demographic Details of Children With Wheezing

	Virus positive patients n = 53					Virus negative patients n = 185	ρ
	hMPV (n = 18)	HCoV NL63 (n = 5)	HBoV (n = 13)	Other (n = 11)	Dual (n = 6)		
Age, months (median; IQR)	7.5 (6.5; 8.1)	22.1 (13.9; 24.2)	6.6 (4.3; 16.3)	12.4 (5.9; 18.1)	11.5 (9.0; 17.6)	14.6 (6.9; 31.1)	0.0205
Male (%)	12 (67)	2 (40)	8 (62)	7 (64)	2 (33)	112 (61)	NS
Prematurity (%)	3 (17)	0	1 (8)	1 (9)	0	23 (12)	NS
Clinical symptoms (%)							
Cough	15 (83)	5 (100)	8 (62)	10 (90)	6 (100)	168 (91)	NS
Wheeze	17 (94)	4 (80)	9 (69)	10 (90)	4 (66)	161 (87)	NS
Breathing difficulty	8 (44)	3 (60)	7 (54)	2 (18)	5 (83)	94 (51)	NS
Rhinorrhea	10 (55)	4 (80)	8 (62)	11 (100)	3 (50)	125 (68)	NS
Night waking	14 (78)	4 (80)	5 (38)	6 (69)	5 (83)	106 (57)	NS
Fever >37.5°C	9 (50)	4 (80)	2 (15)	5 (45)	5 (83)	74 (40)	0.045
Diarrhoea	4 (22)	2 (40)	2 (15)	3 (27)	0	21 (11)	NS
Vomiting	4 (22)	2 (40)	3 (23)	3 (27)	0	67 (36)	NS
Duration of symptoms, days (Median; IQR)	3 (1; 4)	5 (4; 6)	5 (3; 14)	4 (2; 8)	5 (3; 5)	3 (2; 7)	NS
No. previous episodes (%)							
0	8 (44)	2 (40)	4 (31)	2 (18)	3 (50)	35 (19)	NS
1	3 (17)	1 (20)	2 (15)	3 (27)	1 (17)	25 (14)	NS
2	2 (11)	1 (20)	0	2 (18)	0	23 (12)	NS
3	0	0	3 (23)	0	1 (17)	29 (16)	NS
≥5	5 (27)	1 (20)	1 (8)	4 (36)	1 (17)	64 (35)	NS
Hospital admission							
Ward/Overnight	4	1	3	5	3	67 (28)	NS
ICU	0	0	0	0	0	6 (3)	NS

IQR, interquartile range; NS, not significant; hMPV, human metapneumovirus; HCoV NL63, human coronavirus NL63; HBoV, human bocavirus; ICU, intensive care unit.
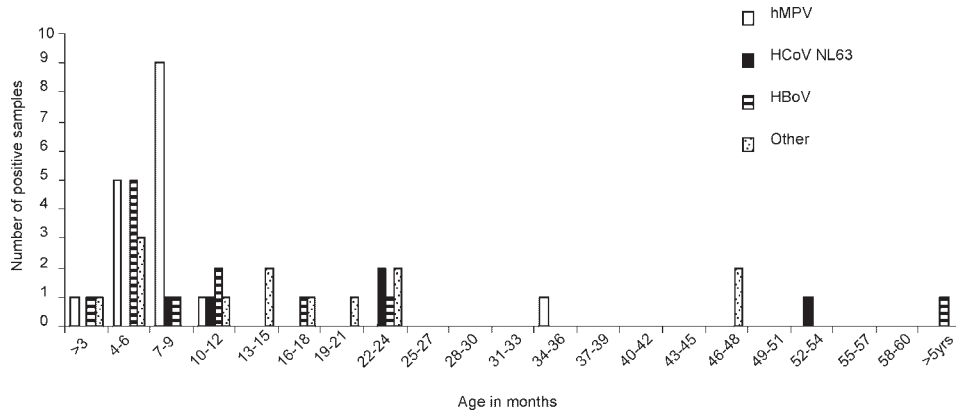
Fig. 1. Age distribution of wheezing children infected with hMPV, HBoV, HCoV NL63, or other common respiratory viruses.

cases. However, 5/6 HBoV cases had associated viral co-infection, 3 with other respiratory viruses and 2 with hMPV. The novel respiratory viruses occurred predominantly in children under 2 years of age (38/44; 86.4%) (Fig. 1), as did other respiratory viruses (14/15; 93.3%). Moreover, infections with hMPV or HBoV were found predominantly in infants in whom 16/18 (88.8%) hMPV and 9/13 (69.2%) HBoV cases occurred. HCoV NL63 occurred in children of all ages.

Novel respiratory viruses were detected mainly in the autumn or winter seasons (28/44, 63.6%) when 9/20 (45%) hMPV, 3/6 (50%) HCoV NL63, and 16/18 (88.8%) HBoV isolates were identified (Fig. 2A–D).

Most children presented with cough (83%), wheezing (83%), or rhinorrhea (68%). Fever (39%) and gastrointestinal symptoms (23%) were less common (Table II). Symptoms were similar in children with and without viral infection.

Most children had mild illness; only 11 children required hospitalization, none to intensive care unit. In 17/44 (38.6%) children this was the first wheezing episode; 15 (34.1%) had a history of 2 or more prior wheezing episodes.

Phylogenetic analysis showed that children were infected with hMPV from group A and B lineages and both sublineages, A1, A2, B1, and B2 (Fig. 3A). Further, two samples, ZA169-05 and ZA185-05, formed a new subgrouping within lineage A, A3, with high bootstrap values. No apparent lineage was more prevalent during the winter or summer months. During 2004 only hMPV lineage B was detected, while in 2005 isolates from the A lineage were more frequently detected (7/11). Limited sequence data is available for HCoV NL63 with ZA85-05 most closely related to the prototype strains, HCoV NL63 and HCoV NL, of lineage A and ZA08-04 grouped with lineage B (Fig. 3B). All HBoV positive samples clustered with HBoV strain st2 (Fig. 3C).

DISCUSSION

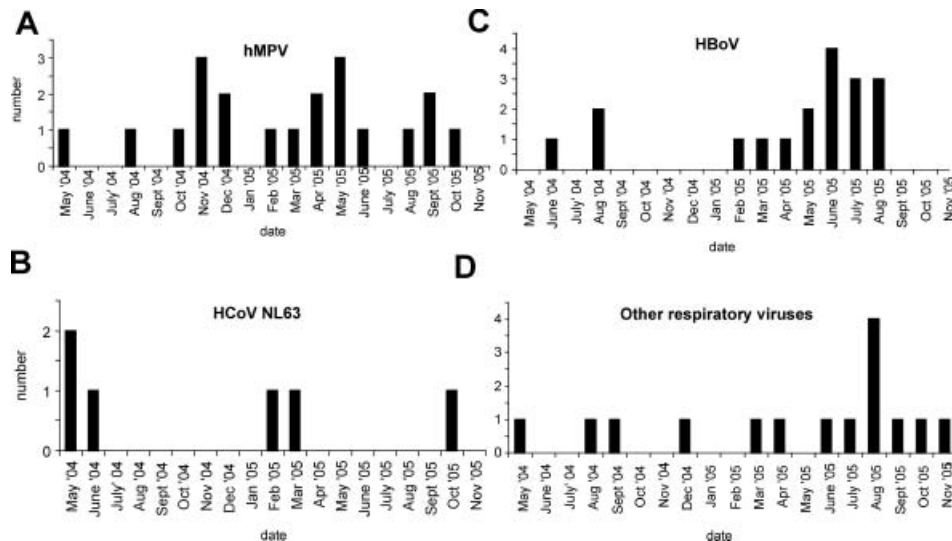A novel respiratory virus was detected in 18% of young children with acute wheezing. hMPV was the most

Fig. 2. Seasonal distribution of (**A**) hMPV, (**B**) HCoV NL63, (**C**) HBoV, (**D**) other viruses in children with acute wheezing over a 19-month period, including two winter seasons.
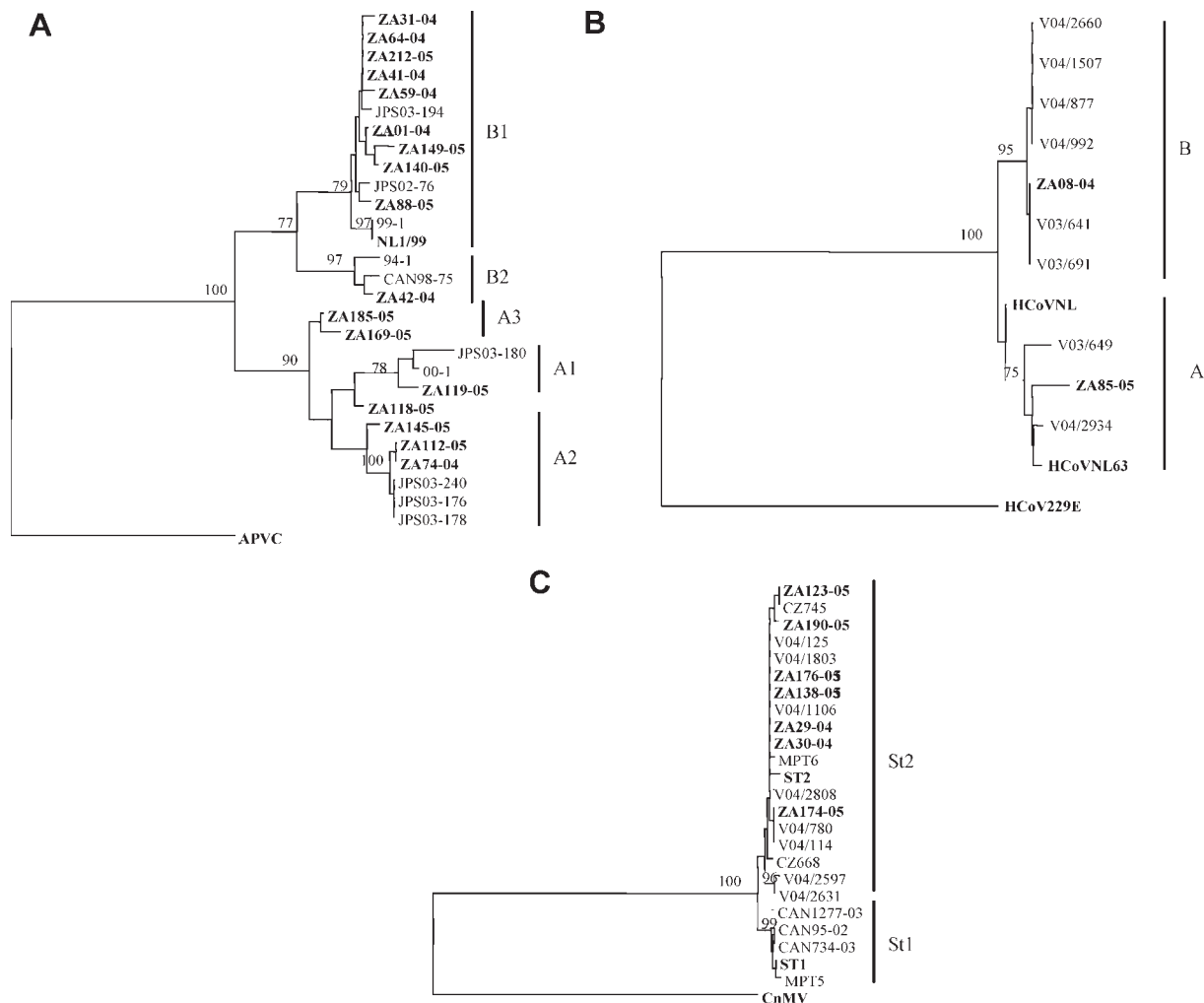
A

B

C

Fig. 3. Phylogenetic analysis of the (**A**) N gene sequences of hMPV; (**B**) 1a gene sequences of HCoV NL63 and (**C**) VP1/2 gene sequences of HBoV obtained from South African children with acute wheezing. A neighbor-joining phylogenetic tree was constructed with 500 bootstrap resamplings. The trees were rooted with sequences obtained from GenBank: N gene of avian pneumovirus subgroup C (APVC accession number AY579780); 1a gene of coronavirus HCoV 229E (HCoV 229E accession number AF304460) and VP1/2 gene of canine minute virus (CnMV accession number AB158475). Lineage and sublineages are indicated. The nucleotide sequences from this study have been deposited in GenBank (EU189107-EU189131).

common novel virus followed by HBoV and HCoV NL63. For comparison, in the subset of samples that were also tested for the other common respiratory viruses, 25% were positive. The viral detection rate was lower than reported in other studies where viruses have been found in up to 90% of cases [Jartti et al., 2004; Williams et al., 2005; Allander et al., 2007; Chung et al., 2007; Ong et al., 2007]. A number of factors may account for this including patient selection, methodology of viral detection, storage of samples, use of a subset for detection of common viruses and lack of testing for additional respiratory viruses such as rhinoviruses, CMV, other coronaviruses or picornaviruses. Due to resource limitations, only a subset of specimens could be tested for a limited number of respiratory viruses. Further children with relatively mild illness were studied, which may also account for lower rates of viral identification as infection rates have been reported to be higher in wheezing

children requiring hospitalization compared to an ambulatory population [Rakes et al., 1999; Thumerelle et al., 2003; Heymann et al., 2005]. Nevertheless, our study indicates that respiratory viruses, including the novel viruses, are an important trigger of acute wheezing in young children. Furthermore, this is the first report of the incidence of novel viruses in African children with wheezing.

The prevalence of hMPV is similar to that reported in other studies. In a Finnish study 8% of children with wheezing exacerbations had detectable hMPV [Jartti et al., 2002]. A second study reported hMPV in 9% of wheezing children compared to 1% in a control group without wheezing [Williams et al., 2005]. Recurrent wheezing was a common diagnosis in almost half of hMPV-infected children compared to 24% in RSV-infected children [Garcia-Garcia et al., 2006a,b]. A recent study from Korea found 8% of children under

6 years hospitalized with acute wheezing had evidence of hMPV infection [Chung et al., 2007].

There is accumulating evidence that HBoV can trigger wheezing. Recent studies report HBoV infections in 5–6% of children hospitalized with acute wheezing, similar to the prevalence in our study [Allander et al., 2007; Chung et al., 2007]. Wheezing has been reported as a clinical feature of HBoV infection in 14–72% children [Kesebir et al., 2006; Ma et al., 2006; Weissbrich et al., 2006; Qu et al., 2007]. In addition, dual viral infection, as occurred in 5 of 6 HBoV-infected children in our study, has previously been reported as a common feature of HBoV infection with co-infection rates ranging from 33% to 80% [Choi et al., 2006; Smuts and Hardie, 2006; Weissbrich et al., 2006; Sloots et al., 2006a; Allander et al., 2007].

There are few studies on the role of the newly described coronaviruses in children with acute wheezing although other coronaviruses, HCoV 229E and HCoV OC43, may play a minor role in asthma exacerbations [Thumerelle et al., 2003; Jartti et al., 2004]. In our study HCoV NL63 was infrequently detected (2%), a similar rate to that reported from Korea where 1% of young children hospitalized with acute wheezing were HCoV NL63 infected [Chung et al., 2007]. HCoV NL63 infection has been more strongly associated with a clinical presentation of croup [Moes et al., 2005; van der Hoek et al., 2005; Choi et al., 2006; Han et al., 2007].

The winter predominance of common respiratory and novel viruses is consistent with other studies [Jartti et al., 2002; Arnold et al., 2006; Kesebir et al., 2006; Weissbrich et al., 2006; Sloots et al., 2006b]. hMPV has a winter circulation pattern similar to RSV but there are reports of year round detection as found in this study [Esper et al., 2004; Williams et al., 2004]. HBoV infections were predominately (72%) found in the winter months, a finding confirmed by other studies [Smuts and Hardie, 2006; Weissbrich et al., 2006; Sloots et al., 2006a]. The lack of seasonality for HCoV NL63 may be due to the small number of isolates or may indicate true lack of a seasonal trend.

Phylogenetic analysis showed co-circulation of both A and B lineages of hMPV and HCoV NL63. Neither lineage was associated with a different spectrum of symptoms or age prevalence. There was a cyclical nature to the appearance of the various hMPV lineages. In 2004 only B1 and B2 sublineages were detected while from early 2005 lineage A became more prevalent. In Cape Town the hMPV lineages circulating in young children have been monitored over a 5-year period. In 2001 only A1 was detected; in 2002 and 2003 A1 and B2 were found; 2004 the predominant sublineage was B1 and to a lesser extent B2 while in 2005 sublineage A1, A2, A3, and B1 were detected [Smuts et al., 2004 and unpublished data]. A study undertaken in Johannesburg, South Africa from 2000 to 2002 showed similar but slightly different circulation patterns [Ludewick et al., 2005]. This complex pattern of co-circulation may be related to pre-existing immunity in the general population to one or more sublineages allowing the emergence

of a different sublineage with a changed antigenic profile. These changes in the dominant virus lineage have also been reported in respiratory viruses like RSV [Peret et al., 2000; Venter et al., 2001]. The identification of a novel sublineage of lineage A in this study, has also been observed by Huck et al. [2006] who reported an increased divergence of lineage A. HBoV is a highly conserved virus and thus sequence analysis of the more variable capsid gene allowed for the phylogenetic differentiation of strains. In this study all HBoV isolates were closely related to st2.

REFERENCES

Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci 102:12891–12896.

Allander T, Jartti T, Gupta S, Niesters GM, Lehtinen P, Osterback R, Vuorinen T, Waris M, Bjerkner A, Tiveljung-Lindell A, van den Hoogen BG, Hyypia T, Ruuskanen O. 2007. Human bocavirus and acute wheezing in children. Clin Infect Dis 44:904–910.

Arden KE, Nissen MD, Sloots TP, Mackay IM. 2005. New human coronavirus, HCoV NL63, associated with severe lower tract disease in Australia. J Med Virol 75:455–462.

Arnold JC, Singh KK, Spector SA, Sawyer MH. 2006. Human bocavirus: Prevalence and clinical spectrum at a children's hospital. Clin Infect Dis 43:283–288.

Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, Lee JH, Song EK, Kim SH, Park JY, Sung JY. 2006. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. Clin Inf Dis 43:585–592.

Chung J-Y, Han TH, Kim SW, Kim CK, Hwang E-S. 2007. Detection of viruses identified recently in children with acute wheezing. J Med Virol 79:1238–1243.

Esper F, Martinello RA, Boucher D, Weibel C, Ferguson D, Landry MC, Kohn JS. 2004. A 1-year experience with human metapneumovirus in children aged <5 years. J Infect Dis 189:1388–1396.

Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. 2005. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. J Infect Dis 191:492–498.

Fouchier RA, Hartwig NG, Besteboer TM, Niemeyer B, de Jong JC, Simon JH, Osterhaus AD. 2004. A previously undescribed coronavirus associated with respiratory disease in humans. Proc Natl Acad Sci USA 101:6212–6216.

Foulongne V, Rodiere M, Segondy M. 2006. Human bocavirus in children. Emerg Infect Dis 12:1251–1253.

Garcia-Garcia ML, Calvo C, Martin F, Perez-Brena P, Acosta B, Casas I. 2006a. Human metapneumovirus infections in hospitalised infants in Spain. Arch Dis Child 91:290–295.

Garcia-Garcia ML, Calvo C, Perez-Brena P, De Cea JM, Acosta B, Casas I. 2006b. Prevalence and clinical characteristics of human metapneumovirus infections in hospitalised infants in Spain. Ped Pulmon 41:863–871.

Han TH, Chung JY, Kim SW, Hwang ES. 2007. Human coronavirus—NL63 infections in Korean children, 2004–2006. J Clin Virol 38:27–31.

Heymann PW, Platts-Mills TA, Johnston SL. 2005. Role of viral infections, atopy, antiviral immunity in the etiology of wheezing exacerbation among children and young adults. Pediatr Infect Dis J 24:S217–S222.

Huck B, Scharf G, Neumann-Haefelin D, Puppe W, Weigl J, Falcone V. 2006. Novel human metapneumovirus sublineage. Emerg Infect Dis 1:147–150.

Jartti T, van den Hoogen B, Garofalo RP, Osterhaus ADME, Ruuskanan O. 2002. Metapneumovirus and acute wheezing in children. Lancet 360:1393–1394.

Jartti T, Lehtinen P, Vourinen T, Osterback R, van den Hoogen B, Osterhaus ADME, Ruuskanen O. 2004. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 10:1095–1101.

Johnston SL, Pattemore PK, Sanderson G, Smith G, Lampe S, Josephs L, Symington P, O'Toole S, Myint SH, Tyrell DA. 1995. Community study of the role of viral infections in exacerbations in 9–11 years old children. Br Med J 310:1225–1229.

Kesebir D, Vanquez M, Weibel C, Shapiro E, Ferguson D, Landry ML, Kahn JS. 2006. Human bocavirus infection in young children in the United States: Molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. J Infect Dis 194:1276–1282.

Kusel MMH, de Klerk N, Holt PG, Kebadze T, Johnston SL, Sly PD. 2006. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life. A birth cohort study. The Ped Infect Dis J 25:680–686.

Ludewick HP, Abed Y, van Niekerk N, Boivin G, Klugman P, Madhi SA. 2005. Human metapneumovirus genetic variability, South Africa. Emerg Infect Dis 11:1074–1078.

Ma X, Endo R, Ishiguro N, Ebihara T, Ishiko H, Ariga T, Kikuta H. 2006. Detection of human bocavirus in Japanese children with lower respiratory tract infections. J Clin Micro 44:1132–1134.

Moes E, Vijgen L, Keyaerts E, Zlateva K, Li S, Maes P, Pyrc K, Berkhout B, van der Hoek L, van Ranst M. 2005. A novel pancoronavirus RT-PCR assay: Frequent detection of human coronavirus NL63 in children hospitalised with respiratory tract infections in Belgium. BMC Infect Dis 5:6–16.

Ong BH, Goa Q, Phoon MC, Chow VTK, Tan WC, van Bever HP. 2007. Identification of human metapneunovirus and *Chlamydophilia pneumoniae* in children with asthma and wheeze in Singapore. Singapore Med J 48:291–293.

Peret TCT, Hall CB, Hammond GW, Peidra AP, Storch GA, Sullender WA, Tsou C, Anderson LJ. 2000. Circulation patterns of group A and B human respiratory syncytial virus genotypes in 5 communities in North America. J Infect Dis 181:1891–1896.

Peret TCT, Bolvin G, Li Y, Cowillard H, Humphrey C, Osterhaus ADME, Erdman DD, Anderson LJ. 2002. Characterization of human metapneumovirus isolated the patients in North America. J Infect Dis 185:1660–1663.

Qu XW, Duan ZJ, Qi ZY, Xie ZP, Gao HC, Liu WP, Huang CP, Peng FW, Zheng LS, Hou YD. 2007. Human bocavirus infection, People's Republic of China. Emerg Infect Dis 13:165–168.

Rakes GP, Arruda R, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, Platts-Mills TA, Heymann PW. 1999. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care: IgE and eosinophil analyses. Am J Resp Crit Care Med 159:785–790.

Schildgen O, Wilkesmann A, Simon A. 2005. Wheezing in patients with human metapneumovirus infection. J Allergy Clin Immunol 117:223.

Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. 2006a. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. J Clin Virol 35:99–102.

Sloots TP, Mackay IM, Bialasiewicz S, Jacob KC, McQueen E, Harnett GB, Siebert DJ, Masters BI, Young PR, Nissen MD. 2006b. Human metapneumovirus, Australia, 2001–2004. Emerg Infect Dis 12:1263–1266.

Smuts H, Hardie D. 2006. Human bocavirus in hospitalised children, South Africa. Emerg Infect Dis 12:1457–1458.

Smuts HE, Kannemeyer J, Smit L, Smith T. 2004. Human metapneumovirus infection in children hospitalised with respiratory tract disease. SAMJ 94:359–361.

Tan CW. 2004. Viruses in asthma exacerbations. Curr Opin Pulm Med 11:21–26.

Taussig LM, Wright AL, Holberg CJ. 2003. Tuscon children's respiratory study: 1980 to present. J Allergy Clin Immunol 111:661–675.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins PG. 1997. The ClustalX windows interface: Flexible strategies fro multiple sequence alignment aided by quality analysis tools. Nucl Acid Res 24:4876–4882.

Thumerelle C, Deschildre A, Bounquillon C, Santos C, Sardet A, Scalbert M, Delbecque I, Debray P, Dewilde A, Turuk D, Leclerc F. 2003. Role of viruses and atypical bacteria in exacerbations of asthma in hospitalized children: A prospective study in the Nord-Pas de Calais region (France). Pediatr Pulmonol 35:75–82.

van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 7:719–724.

van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJM, Wolthers KC, Wertheim-van Dillen PM, Kaandorp J, Spaargaren J, Berkhout B. 2004. Identification of a new human coronavirus. Nat Med 10:368–673.

van der Hoek L, Sure K, Ihorst G, Stang A, Pyrc K, Jebbick MF, Peterson G, Foster J, Berkhout B, Uberla K. 2005. Croup is associated with the novel coronavirus NL63. PLOS Med 2:764–770.

Venter M, Madhi SA, Tiemessen CT, Schoub BD. 2001. Genetic diversity and molecular epidemiology of respiratory syncytial virus over four consecutive seasons in South Africa: Identification of new subgroup A and B genotypes. J Gen Virol 82:2117–2124.

Weissbrich B, Neske F, Schubert J, Tollman F, Blath K, Blessing K, Kreth W. 2006. Frequent detection of bocavirus DNA in German children with respiratory tract infections. BMC Infect Dis 6:109–6115.

Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, Wright PF, Crowe JE. 2004. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. New Eng J Med 350:443–450.

Williams JV, Tollefson SJ, Heymann PW, Carper HT, Patrie J, Crowe JE. 2005. Human metapneumovirus infection in children hospitalised for wheezing. J Allergy Clin Immunol 115:1311–1312.

Wilson NM. 2003. Virus infections, wheeze and asthma. Paed Resp Rev 4:184–192.