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Original Article

Human coronavirus NL63 infections in infants hospitalised with acute respiratory tract infections in South Africa

Heidi Smuts

Division Medical Virology/NHLS, Department Clinical Laboratory Sciences, Faculty Health Sciences, University of Cape Town, Cape Town, South Africa.

Correspondence: H. Smuts, Division Medical Virology/NHLS, Department Clinical Laboratory Sciences, Faculty Health Sciences, University of Cape Town, Anzio Road, Observatory, 7925 Cape Town, South Africa. Email: heidi.smuts@uct.ac.za

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Background Human coronavirus NL63 (HCoV-NL63) is a novel respiratory virus which is associated with respiratory tract infections in children.

Objective To determine the role of HCoV-NL63 in infants and young children hospitalised with acute respiratory tract infections (ARI) in Cape Town, South Africa.

Methods Respiratory specimens were collected from 1055 infants and young children hospitalised with ARI in 2003–2004. Samples were screened by RT-PCR to detect HCoV-NL63 and human metapneumovirus (hMPV). Standard shell vial culture and immunofluoresence was used to detect the common respiratory viruses including RSV, influenza A and B viruses, parainfluenza viruses 1, 2, 3, adenovirus and CMV.

Results A respiratory virus was found in 401/1055 (38·0%) samples. HCoV-NL63 was detected in 9/1055 (0·85%) with peak activity during autumn (67%). Most patients had a diagnosis of pneumonia or lower respiratory tract infection (6/9; 67%).

Conclusions This is the first report of HCoV-NL63 infections in hospitalised children in Africa. During the 2-year period HCoV-NL63 played a minor role in ARI in children.

Keywords Human coronavirus NL63, infants, respiratory tract infection, South Africa.

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Introduction

A number of respiratory viruses including influenza viruses, respiratory syncytial virus (RSV), parainfluenza viruses, adenovirus and the recently described human metapneumovirus (hMPV) play an important role in acute respiratory tract infections (ARI) in children. Infections with these viruses may often lead to hospitalisation. However, in a substantial portion of respiratory infections the aetiological agent is not known. There has been renewed interest in human coronaviruses (HCoV) as a cause of some of these infections.

Coronaviruses are large enveloped single-stranded RNA viruses that can infect both humans and a variety of domestic animals causing respiratory and enteric illness. Until recently human coronavirus (HCoV) 229E and OC43, identified in the 1960s,[1] were the only known coronaviruses to infect humans. Although primarily responsible for mild infections including the common cold[2] reports of more severe upper and lower respiratory tract infections associated with HCoV-229E and HCoV-OC43 have been documented.[3,4] The identification of a coronavirus, SARS-CoV, as the causative agent of severe acute respiratory syndrome in 2003[5] has resulted in an increased interest in this group of viruses. Subsequently two new human coronaviruses, HCoV-NL63[6,7] and HCoV-HKU1,[8] have been described. Both infect young children, the elderly and immunocompromised and can lead to severe respiratory tract infections requiring hospitalisation. The prevalence and clinical importance of HCoV-NL63 in the South African hospital setting is not known.

Methods

In this retrospective study 1055 nasopharyngeal, tracheal aspirate and bronchoalveoloar lavage samples were taken from children (age 13 days to 5 years) hospitalised with

respiratory tract infections in 2003 and 2004 in the Red Cross War Memorial Children's Hospital in Cape Town. Croup as a specific diagnosis was not reported for any of these children. Samples had previously been screened by using an indirect immunofluorecence assay (Light Diagnostics, Chemicon International, Temecula, CA, USA) for the common respiratory viruses including RSV, influenza viruses A and B, parainfluenza viruses 1, 2, 3, adenovirus and CMV. hMPV was also tested for using reverse-transcription-PCR (RT-PCR). In the South African setting, where the prevalence of HIV is high, all infant respiratory samples are routinely screened for CMV as in our setting this virus is a major cause of pneumonia in HIV-infected children.

For the detection of HCoV-NL63, RNA was extracted from 200 μl of sample using the Seek Viral RNA kit according to the manufacturer's instructions (Talent, Trieste, Italy). Ten microlitres RNA was reverse transcribed into cDNA using random primers (Roche Diagnostics GmbH, Penzberg, Germany) and the iScript kit (Bio-Rad, Hercules, CA, USA). PCR amplification of a region of the 1b gene of HCoV-NL63 was used for screening and positive samples were confirmed by amplification of a portion of the 1a gene. Briefly 10 μl of cDNA was added to a 50 μl of PCR mix containing 2 IU Supertherm polymerase (JMR Holdings, Kent, UK), 1·5 mm $MgCl_2$, 200 μm each dNTP and 0·2 μm primers. PCR was performed with two sets of outer primers, one set to the 1b gene and one targeting the 1a gene from the study of van der Hoek *et al.*[6] To improve sensitivity a nested PCR was performed using 2·5 μl outer product and inner primers described by Smuts *et al.*[9] The 1b and 1a PCR products were 169 and 520 bp respectively. The 1a amplicons were sequenced directly and the nucleotide sequences were deposited into GenBank (EU477762–EU477770).

Results

A respiratory virus was detected in 401/1055 (38·0%) samples collected over the 2-year period from 2003 to 2004. The detection rate was higher in 2004, 248/559 (44·4%), compared with 2003, 153/496 (30·8%). CMV was most frequently found (158/1055; 15·0%) followed by adenovirus (n = 65; 6·2%), RSV (42; 4·0%), parainfluenza 3 (n = 32; 3·0%), hMPV (n = 28; 2·6%), influenza virus A (n = 8; 0·76%), parainfluenza virus 1 (n = 6; 0·57%), parainfluenza virus 2 (n = 4; 0·38%) and influenza virus B (n = 1; 0·09%). In 48 (4·5%) samples a known respiratory virus was grown in shell vial culture but could not be further identified. Of CMV-positive respiratory samples 44·9% (71/158) were from HIV-infected children, 24·0% (38/158) from HIV-negative children and for the remainder the HIV status was unknown. In both HIV-positive and HIV-negative groups the rates of co-infection with another known respiratory virus were similar, 12/71 (16·9%) and 7/38 (18·4%) respectively.

HCoV-NL63 was detected in 4/496 (0·81%) and 5/559 (0·89%) samples from 2003 and 2004 respectively. All HCoV-NL63-infected children, with the exception of one aged 30 months, were under 2 years (Table 1). The majority, 6/9 (66·7%), were <6 months. The HIV status of only those children from 2004 was known and 4/5 HCoV-NL63-infected children from this year were HIV-positive. In two instances a co-pathogen was identified, hMPV and adenovirus. In 2003 3/4 positive samples were collected in March (autumn) while in 2004 HCoV-NL63-positive samples were found in March, May, August and September (Table 1). A diagnosis of pneumonia or lower respiratory tract infection was made in six (67%) children. Two HCoV-NL63-positive infants aged 50 and 71 days respectively, required admission to the intensive care unit; both were HIV-positive.

Table 1. Clinical data of HCoV NL63-infected children

Patient no.	Sex	Age, months	Date of sample	Type sample	HIV status	Diagnosis	Co-pathogen
ZA649-03	M	30	18/03/03	NPA	NK	NK	None
ZA641-03	F	NK	19/03/03	TA	NK	Gastoenteritis/vomiting	None
ZA691-03	M	3	25/03/03	NPA	NK	Pneumonia	hMPV
ZA3343-03	M	5	23/12/03	NPA	NK	LRTI	None
ZA877-04	M	4	24/03/04	NPA	+	NK	None
ZA992-04	F	2	4/05/04	TA	+	Pneumonia	None
ZA1507-04	F	3	13/05/04	NPA	+	LRTI	None
ZA2660-04	M	19	18/08/04	NPA	–	LRTI, adenoidectomy	Adenovirus
ZA2934-04	M	2	9/09/04	BAL	+	Pneumonia	None

NK, not known; TA, tracheal aspirate; NPA, nasopharyngeal aspirate; BAL, bronchoalveolar lavage; LTRI, lower respiratory tract infection; hMPV, human metapneumovirus.

Discussion

To determine the role HCoV-NL63 plays in respiratory illness in infants, respiratory samples that had previously been screened for RSV, influenza viruses A and B, parainfluenza viruses 1, 2, 3, adenovirus, hMPV and CMV were also tested by RT-PCR for HCoV-NL63. Due to limited resources, screening for other viruses such as enteroviruses, rhinoviruses and the other HCoVs was not undertaken. This may be considered a limitation of the study as there is accumulating evidence that these viruses, in particular rhinoviruses, may play a more significant role in lower respiratory tract infections than previously recognised.[10] The role and clinical significance of the recently identified human bocavirus[11] and polyomaviruses[12,13] in respiratory disease is still under investigation. Ideally comprehensive screening of respiratory samples for most if not all respiratory viruses and relevant respiratory bacteria should be undertaken in order to obtain greater insight into the epidemiological significance of these pathogens in respiratory disease in the local setting. Further this would be beneficial to the clinical management of patients, including the administration of appropriate antiviral drugs and antibiotics.

CMV was the most prevalent virus detected in the study samples. CMV-pneumonia is an important life-threatening complication in HIV-infected infants in South Africa. A post-mortem study of HIV-infected children in Kwazulu-Natal, South Africa showed frequent (52%) CMV detection in lung tissue compared with uninfected controls (4%).[14] The significance of CMV detection in respiratory samples from uninfected children is not known but probably represents viral shedding from either a recently acquired primary infection or reactivation.

This study is the first to report the presence of HCoV-NL63 in children hospitalised with respiratory illness in Africa. HCoV-NL63 was found to circulate in infants and young children in both 2003 and 2004 with similar low prevalence rates of 0·8%. This finding is lower than that reported in previous studies where detection of HCoV-NL63 ranged from 1% to 7·3%[6,7,15–20] although higher prevalences of 8·8% and 9·3% have also been reported.[21,22] Most HCoV-NL63-positive children (75%) were under 6 months, indicating that this younger age group may be more susceptible to severe infections requiring hospitalisation; a finding supported by other studies where 38/76 (50%), 4/5 (80%) and 11/12 (92%) of HCoV-NL63 infections occurred in this age group respectively.[7,19,21] Further, immunosuppression may also contribute to increased susceptibility to severe HCoV-NL63 infection. In this study 4/5 HCoV-NL63-infected whose HIV status was known, were HIV infected. All four were <4 months of age and two required admission to ICU. It is not known whether maternal antibodies, if present, would provide protection or reduce the severity of infection. This protection is likely to be most effective in infants under 2 months. In this study very few infected infants were under this age, indicating a possible protective advantage. This observation is supported by the findings of other studies.[7,15–17] In a very recent study by Dijkman *et al.*, HCoV-NL63 specific maternal antibodies were found in all newborns studied. These antibodies disappeared within 3 months providing further evidence of their possible protective role in early life.

In a recently published study[9] of ambulatory children presenting with acute wheezing at the outpatient's department of the same hospital in 2004, 3·6% (3/83) showed evidence of HCoV-NL63 infection. This is significantly different ($P = 0{\cdot}0186$) from the prevalence in the hospitalised group. This indicates that the virus is circulating in the community, probably causing mild symptoms which may trigger a wheezing episode requiring medical attention. In contrast the lower rate of HCoV-NL63 infection identified in the hospitalised children suggests that the virus rarely causes severe lower respiratory tract infections. However, all HCoV-infected children in the ambulatory group were over 12 months of age supporting the possibility that younger children are more susceptible to severe infections requiring hospitalisation.

HCoV-NL63 infections appear to be seasonal; 67% of infections occurred during autumn. No HCoV-NL63 infection was detected during the winter months in either 2003 and 2004, a finding also noted in the ambulatory population[9] This pattern differs from that previously reported where HCoV-NL63 infection was predominantly found during the winter season.[6,15–19,21,22] Detection during early spring and summer indicates that HCoV-NL63 may circulate at low levels throughout the year.

In this study the clinical symptoms of HCoV-NL63-infected infants were similar to those previously reported with lower respiratory tract infections including pneumonia predominating in this group.[6,7,15,17,20,21] In this study only specimens from hospitalised children were examined resulting in a bias towards children with more severe respiratory illness. Mild HCoV-NL63 symptoms have also been reported[9,16,23] indicating that HCoV-NL63 infections are probably more severe in the very young and immunocompromised. The role of HCoV-NL63 in enteric disease has not been established but reports of gastroenteritis associated with coronavirus infection have been documented with frequencies ranging from 6% to 33%.[15,17,20,22] In this study one child had a history of gastroenteritis and vomiting. A significant association with HCoV-NL63 infection and croup has been made[23] but in this study it could not be determined if any of the samples were from children with croup.

In conclusion these findings suggest that although HCoV-NL63 is circulating in the community it plays a minor role in severe respiratory tract infections in young children who require hospitalisation.

Ethical approval

Ethical approval (018/2004) was granted by the Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, South Africa.

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