THE BURDEN OF HUMAN CORONAVIRUS INFECTION IN CHILDREN HOSPITALISED WITH SEVERE LOWER RESPIRATORY TRACT INFECTION IN CAPE TOWN, SOUTH AFRICA (2012 – 2013)

By

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DECLARATION

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ABSTRACT

Introduction: In order to better understand the epidemiology and burden of human coronaviruses - NL63, HKU1, OC43 and 229E in South Africa, their role in the aetiology of childhood pneumonia needs to be described.

Methods: We used data collected between September 2012 – September 2013 from children aged <13 years with lower respiratory illness at Red Cross War Memorial Children's Hospital. Respiratory samples including a nasopharyngeal swab (NP) and induced sputum (IS) were taken and tested for the four strains of coronaviruses using FTD33 multiplex real-time PCR.

Results: A total of 460 respiratory samples were analysed. Of these, 258 (56.0%) were male and 19 (4.1%) HIV infected. The median age of the children was 8 (IQR 4-18) months.

Nasopharyngeal (NP) samples were obtained from 460 children while induced sputum (IS) was not available for six children due to sample loss prior to analysis, leaving 454 available for analysis. A total of 42 (9.1%, 95% CI 6.7- 12.1%) participants tested positive for HCoV in at least one of the two specimens. PCR was able to detect a total of 35 (7.7%) cases from the 454 tested IS specimens compared to 23 (5.0%) detected out of 460 NP samples.

The commonest detected HCoVs were coronavirus OC43 with 20 (4.3%) detected from either specimen followed by coronavirus NL63 or coronavirus HKU detected in 14 (3.0%) and 10 (2.2%) of positive test samples, respectively. The least common virus detected HCoV was coronavirus 229E detected in both positive test samples of one participant.

Overall HCoVs were detected in 23 (8.9%) of boys compared to 19 (9.1%) of the girls who returned a positive test; p=0.856. The overall age distribution of children with PCR detected HCoVs was similar to that of children with a negative result with median age of 10 (IQR 5-16) months and median of 8 (IQR 4- 19) months, respectively; p=0.535. Prevalence of HCoV was 11/192 (5.7%), 23/153 (15.0%) and 8/115 (7.0%) in children <6 months old, 6-18 months and over 18 months respectively; p=0.008.

Conclusion: Children aged 6 to 18 months had double the risk of other age groups.

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LIST OF ABBREVIATIONS

ALRTI	Acute lower respiratory tract infection
COVID-19	Coronavirus disease 2019
ELISA	Enzyme linked immunosorbent assay
HCoV	Human Coronavirus HKU 229E OC43 NL63
HIV	Human immunodeficiency virus
ICU	Intensive care unit
IS	Induced sputum
IQR	Inter-quartile range
LMIC	Low and middle-income country
MERS	Middle East respiratory syndrome
NP	Nasopharyngeal
PCR	Polymerase chain reaction
PERCH	Pneumonia Etiology Research for Child Health
PMTCT	Prevention of mother to child transmission
RCWMCH	Red cross war memorial children's hospital
RNA	Ribonucleic acid
RTHC	Road to health card
RSV	Respiratory syncytial virus
SARS-CoV	Severe acute respiratory syndrome – coronavirus
SSA	sub-Saharan Africa
WHO	World Health Organisation

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1 INTRODUCTION

1.1 Background

The emergence of the COVID-19 pandemic has brought into focus the role of coronaviruses as important pathogens in the aetiology of severe respiratory infection [1].Globally, respiratory tract infections are a major cause of under-five mortality [2].Specifically, a wide variety of viruses including non-severe acute respiratory syndrome (non-SARS)-related human coronaviruses (HCoVs) are responsible for 5% of all upper and lower respiratory tract infections in children below the age of five years worldwide [3]. For example, in 2010, lower respiratory tract infections caused about 5.8 million deaths in children younger than five years across the globe [4].

HCoVs 229E, NL63, HKU and OC43 are generally understood to cause mild respiratory illness in humans [6].Evidence suggests that major outbreaks of more serious diseases are caused by SARS-CoV and Middle East respiratory syndrome (MERS) CoV [3]. SARS-CoV2, a novel strain is the newest addition to the HCoV family [5]. Meanwhile, HCoVs are considered relatively harmless, but severe infection is possible in premature infants, low birthweight babies and children with chronic underlying diseases [6]. The risk of being hospitalised with HCoV is higher among infants with chronic underlying health problems than among healthy ones [7]. However, some studies have found that HCoV-NL63 infection was often linked with more severe lower respiratory tract infection [3, 8, 9].

The four major HCoV strains are often found in association with lower respiratory tract disease involving infants and children suffering from pneumonia and bronchitis [3]. Specifically, HCoVs HKU and NL63 have been associated with more serious illnesses in the case of febrile convulsion, croup, and pneumonic illness. For this reason, children below the age of five years are often at risk of being hospitalised for respiratory tract infection caused

by human coronaviruses [10]. Evidence from data collected on the individual viruses show that HCoV NL63 is more frequently associated with the development of croup compared with HCoV OC43 or HCoV 229E [11]. For example, a study by Van der Hoek et al found that at least 45% of children with HCoV NL63 infection had croup [12]. Similarly, studies by Wu et al. [13] and Han et al. [14] reported a high detection of HCoV NL63 infection in samples of children who had croup. In addition, other studies show that HCoVs such as HKU [14, 15] and NL63 [11, 16, 17, 18, 19] are frequently associated with bronchiolitis and pneumonia. A better understanding of the burden of HCoV infection as well as understanding of its contribution to viral pneumonia may inform an improvement in the management outcomes of childhood pneumonia. In addition, it has been demonstrated that more severe pneumonia is observed among infants hospitalised with respiratory disease due to detection of HCoV in respiratory samples [20]. This observation raises the possibility for inclusion of HCoV testing in routine viral panels which could have beneficial effects on describing aetiology of childhood pneumonia in future.

1.2 Aim

To describe the burden of HCoVs infection in a cohort of children hospitalised with lower respiratory tract infection to contribute to our understanding of management outcomes of childhood pneumonia.

1.3 Summary of the structure of the thesis

Chapter 1: Background

This chapter introduces the topic as well as the structure of the thesis

Chapter 2: Literature Review

This chapter reviews published literature on the burden and severity of HCoVs in children with lower respiratory tract infection.

Chapter 3: Methodology

This chapter describes the study design, methods, and procedures, including data handling and analysis as well as resolving ethical issues and study approval.

Chapter 4: Results

The study findings are reported in this chapter.

Chapter 5: Discussion

The study findings are interpreted in this chapter and are reviewed in the context of existing literature in the field.

Chapter 6: Conclusion

This chapter summarises the contribution of this study in view of existing literature and its limitations are noted. Recommendations are made for further study.

2 LITERATURE REVIEW

2.1 Introduction

South Africa has been disproportionately affected by deaths from respiratory tract infection in children below the age of five years [1]. A South African study done in 2019 at Red Cross War Memorial Children's hospital of the clinical characteristics and outcome of children admitted to paediatric intensive care with severe lower respiratory tract infection reported a mortality rate of 12.8% [20]. Another South African study during 2009-2012 describes the incidence of LRTI in children below the age of five years to be 2530-3173/100,000, while the annual incidence rate in children less than one year of age is 8446-10532/100,000 [21]. On the other hand, a national study done between 2012 and 2013 demonstrated a prevalence of 6.3% HCoV-229E in children aged 1 to 4 years hospitalised with severe respiratory tract infection [22].

Although the incidence of LRTIs such as pneumonia and bronchiolitis are similar across the globe, low and middle-income countries (LMICs) carry the biggest burden of acute respiratory infections and mortality with over 95% of deaths occurring in these settings [23]. In South Africa as in most LMICs, respiratory viruses such as HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU are rarely confirmed by laboratory diagnosis, making the burden and the contribution of these viruses to disease difficult to assess. Understanding trends of the burden of human coronaviruses is essential for the clinical management of cases and in prioritising intervention strategies to reduce childhood morbidity and mortality [24].

2.2 Methods of the literature review

This chapter seeks to review the literature around the topic of HCoV infection in young children with respect to the following:

- Epidemiology and clinical characteristics of HCoV infection in children

- Prevalence and risk factors for HCoV infection in children
- Contribution of HCoVs to viral pneumonia
- Diagnosis and treatment

A literature search was performed in PubMed (www.ncbi.nih.gov) database. A search strategy to identify the relevant literature was developed using different text and MeSH terms of the following words: 'child', 'coronavirus', 'respiratory tract', 'burden', 'incidence', 'treatment', and 'South Africa'. A total of 153 eligible studies were found, of which upon screening, 59 were used to guide the write up of this thesis. The last search was done on 25 January 2021.

2.3 Summary of the retrieved literature

2.3.1 Epidemiology and clinical characteristics of HCoV infection in children

It is well known that the four major HCoVs strains namely NL63, OC43, HKU and 229E are distributed across the globe [25]. By spreading via person-to-person transmission, HCoVs cause mild upper respiratory illness in adults. However, younger children and the elderly may sometimes suffer from more serious life-threatening conditions such as pneumonia and bronchiolitis [6]. In addition, the virus has also been shown to cause enteric and neurological illness [4]. Below, we describe the most common HCoVs of public health interest.

HCoV-229E also referred to as the prototypic strain was first isolated in 1966 from a standard tissue culture. It is proposed that HCoV-229E originated from hipposiderid bats mainly found in West Africa and camelids were adopted as possible intermediate hosts. In most healthy adults, HCoV-229E infection is frequently associated with flu-like symptoms. However, infants and the elderly are susceptible to lower respiratory tract infections [26]. Specifically, patients with impaired or weakened immune system have been reported to be vulnerable to

more serious and life-threatening HCoV-229E infection [6]. Furthermore, serological qualitative tests suggest that children with HCoV-229E infection were at the risk of developing a multisystem inflammatory syndrome known as **Kawasaki disease** [27].

After an incubation period of 2-5 days, the majority of patients infected with HCoV-229E develop illness that can last for 2-18 days. In most patients, HCoV-229E infection is commonly associated with sneezing, headache, malaise, and nasal discharge. A few patients may sometimes show signs of a fever and cough [25]. However, the clinical features of HCoV-229E are easily separable from those caused by Influenza A virus which could be attributed to co-circulation. The virus is primarily transmitted during the winter season [28].

HCoV-OC43 was isolated in 1967 and is serologically different from HCoV-229E. However, clinical features alone cannot be used when distinguishing patients infected with HCoV-229E or HCoV-OC43 [29]. Although HCoV-229E infection is one of the main causes of coryza, sore throat manifestations have been associated with HCoV-OC43 infection [25].

An association of HCoV-OC43 infection with neurologic disease such as encephalitis has been clearly demonstrated by the detection of OC43 in the neurons of mice [6]. Moreover, the virus has been associated with persistent infection within various human cell lines. For specific detection of HCoV-OC43, seven genotypes (A-G) were identified by molecular phylogenetic studies. HCoV-OC43 shows peak during winter in temperate regions [25].

HCoV-NL63 was first isolated from nasopharyngeal aspirate of a seven-month old baby in the Netherlands with many clinical symptoms including conjunctivitis, bronchiolitis, coryza and fever. As HCoV-NL63 continues to circulate in the human population, it remains the leading cause of hospitalisation for most of the respiratory infections. The virus has been shown to exist in mixed viral infection. However, it does not impact on disease severity. It is estimated that about 71% of HCoV-NL63 cases are involved in coinfection with other

respiratory viruses including parainfluenza virus, enterovirus, and rhinovirus [25]. The clinical symptoms of HCoV-NL63 infection include sore throat, cough, fever, and rhinitis [28]. In addition, HCoV-NL63 is associated with croup in young children [29].

HCoV-NL63 causes disease in all ages, with greater proportion of infection occurring in children below the age of five years. About 1-10% of patients infected with HCoV-NL63 experience cold-like symptoms annually. HCoV-NL63 is transmitted during winter in tropical and subtropical regions. However, peak incidence is recorded during spring and summer in Hong Kong [25].

HCoV-HKU was first isolated in Hong Kong from an adult patient suffering from a chronic pulmonary infection. Clinical features associated with HCoV-HKU infection include fever, chills, cough, sore throat, and nasal congestion [6]. In about 50% of cases, patients experience febrile convulsion, however, the symptoms of HCoV-HKU infection are like those caused by other viruses of the respiratory tract. HCoV-HKU infection tends to be epidemic during influenza season and patients are coinfected with another respiratory virus such as respiratory syncytial virus [25].

2.3.2 Prevalence and risk factors for human coronavirus infection in children

A significant percentage of children admitted to hospital with HCoVs had acute respiratory tract infection – 4.4% in one study [30]. HCoV-NL63 is the most identified coronavirus with an incidence rate of 2.6% [24]. In a study done in Japan, it was shown that out of 419 samples checked for HCoVs, five (1.2%) tested positive for HCoV-NL63 while others were negative [31]. Similarly, in another study conducted in Japan, HCoV-NL63 was reported in three (2.5%) out of 118 nasopharyngeal swab samples obtained from children below the age of two years hospitalised with lower respiratory infection [28]. A South African study during 2006 – 2007

showed 0.3% HCoV-229E was obtained in the nasopharyngeal swab samples of under-fives sick with bronchiolitis [32]. Another study conducted in South Africa during 2012 – 2013 detected a high prevalence (4.1%) of HCoV-229E in respiratory specimens of hospitalised patients [33].

Several factors can increase the risk of children to HCoV infection and to severe disease. Uddin et al [34] found that over-crowding in a room of children below the age of five years was associated with an increased risk of HCoV disease. More so, children from disadvantaged backgrounds were seen to be at increased risk of contracting HCoV infection compared to those from wealthier backgrounds. Other risk factors associated with HCoV disease include infant age, parental smoking, air pollution, fever, and HIV exposures [20, 34, 35, 36, 37].

The risk of being hospitalised with HCoV is higher among infants with chronic underlying health problems than among healthy ones [7]. A study done in Nepal found that the incidence of HCoV among infants with underlying pathology more than doubled those of healthy children [34]. In most published studies, older infants with isolated HCoV disease had higher incidence of the disease than neonates. This can partly be explained by the presence of maternal immunoglobulins at birth that fights against all types of HCoV [7].

2.3.3 Contribution of HCoV to viral pneumonia

Viruses remain an important cause of lower respiratory tract infection in children. Viruses recognised as the most common cause of infection include respiratory syncytial virus (RSV), adenovirus, enterovirus, parainfluenza type 3 virus and influenza virus [3]. WHO estimates suggest that viruses are responsible for about 30 to 67% of pneumonia in young children [38]. Pneumonia and bronchitis are the most common presentation of lower respiratory tract

infection in children [39]. The WHO defines pneumonia as ‘an acute episode of cough or difficulty breathing associated with an increased respiratory rate’. Severe pneumonia is described as ‘the presence of lower chest wall indrawing requiring hospital admission’ and is often characterized by hypoxia [40]. A study done in 2020 at Kilifi County Hospital in Kenya, reported that 4% of childhood pneumonia admission were associated with HCoVs 229E, NL63 and OC43 infections [39]. Similarly, a study done in 2005 in Hong Kong found that 2.4% of patients with community-acquired pneumonia were positive for HCoV HKU [40]. More so, most published studies report high incidence of pneumonia and bronchitis in children hospitalised due to HCoVs OC43, 229E and NL63 infection [16, 29, 41].

Generally, HCoVs are associated with mild respiratory diseases and are less likely to mutate to cause serious infection. However, a virulent subtype of HCoV NL63 has been reported to cause more severe infection of the lower respiratory tract in China [24]. Another study in the Netherlands reported that cases of infection with HCoV NL63 were detected among young children admitted with acute lower respiratory tract infection [27]. Currently, there is no scoring system available to predict whether coinfection with other respiratory viruses is associated with more severe pneumonia.

2.3.4 Diagnosis and treatment

Identifying the specific pathogens in children with respiratory infection has many challenges such as the difficulty in obtaining samples of good quality from the infected site as well as poor interpretation of results [42]. Nasopharyngeal specimen obtained by swab has become the most common type of specimen used in diagnosing HCoV in children due to ease of collection [24]. Many pathogens require sophisticated laboratory culture systems for growth or replication. Conventional diagnosis through cell culture or immunofluorescence has poor sensitivity. With the introduction of more sensitive nucleic acid detection tests, such as PCR, our ability to confirm cases has greatly improved (1).

Recent large multi-centre childhood pneumonia cohort studies such as Pneumonia Etiology Research for Child Health (PERCH) and Drakenstein did not include HCoV in the 33 multiplex PCR tests [1, 44], as such HCoVs would not have been detected in these studies.

Recent studies have shown that identifying a pathogen in a respiratory specimen does not necessarily indicate a causal relationship. Organisms once thought to have been pathogenic have now been found in otherwise healthy controls. Thus, the recruitment and analysis of asymptomatic controls alongside cases is invaluable in establishing a causal relationship between the pathogen and the disease.

However, there is evidence that the detection of HCoV in respiratory samples is strongly associated with severe pneumonia and need for hospitalisation [45, 46, 47, 48]. In addition, researchers have adopted various methods in searching for respiratory viruses associated with pneumonia in infants and children. Majority of studies have focused on nasopharyngeal aspirate as the most appropriate sample for diagnosis [49, 50, 51]. But choosing the best sample type for viral detection is an essential criterion for improved PCR performance. A South African birth cohort study of aetiology of childhood pneumonia found higher viral yield on induced sputum compared to nasopharyngeal swabs [1]. Similarly, in 2017 a multicentre study done in the United States using TaqMan array card found that sputum specimens had greater viral yield compared to those obtained from nasopharyngeal specimens [52]. Some studies have reported acceptable diagnostic sensitivity observed when sputum samples are used for detection of potential pathogens.

The SARS-CoV pandemic has led to discovery of effective vaccines which might be of benefit to children infected with HCoV. Currently, there is no specific antiviral treatment for clinical use against HCoVs. Therapy is primarily supportive as HCoV disease is usually self-limiting [11, 53].

2.4 Conclusion

Accounting for the burden of respiratory diseases caused by HCoVs NL63, OC43, HKU and 229E in children is difficult because of very limited available data. This paucity of data is even more pronounced in LMICs such as South Africa. There is also very little data on the role of HCoVs in pneumonia aetiology which contributes to the poor management outcomes of patients after diagnosis. Furthermore, there is very little data regarding the risk factors of HCoV infection in African populations.

3 METHODS

3.1 Introduction

The study investigates the burden of HCoV in a cohort of children hospitalised with lower respiratory tract infections. The research follows a cross-sectional design with both descriptive and analytical elements. The chapter provides details on the methods used.

3.2 Aims

This study aimed to describe the burden of HCoVs infection in children hospitalised with lower respiratory tract infection at the Red Cross War Memorial Children's Hospital (RCWMCH) over a period one year.

3.3 Objectives

The following were the study objectives:

1. To determine the proportion of infants and children admitted to Red Cross War Memorial Children's Hospital during one calendar year with acute lower respiratory tract infection (aLRTI), who are infected with HCoVs.
2. To determine the prevalence of different HCoVs in children hospitalised with aLRTI
3. To assess risk factors associated with HCoV infection.
4. To determine the outcome of children with HCoV infection hospitalised for a lower respiratory tract infection.

3.4 Study Design

This study is a sub-study of the parent study (HREC reference number 371/2011), and utilises data that was prospectively collected over a one-year period from September 7, 2012 to September 6, 2013.

3.4.1 Study Population

Infants and children below the age of 13 years who were hospitalised during the study period and presented with World Health Organization (WHO) defined severe acute lower respiratory tract infection as indicated by age-specific tachypnoea or lower chest indrawing [38]. Children could only be included into the study following written, informed parental consent.

Participants were excluded from the study if deemed too ill by the attending paediatrician to undergo induced sputum. Any child who had been hospitalised for a period of longer than 72 hours prior to recruitment were also excluded from the study to reduce the risk of including children who may have acquired the infection in hospital.

In order for the study to reflect the whole season, recruitment was limited to a maximum of four qualifying participants per day.

3.4.2 Outcomes of Interest

The primary outcome of interest is the identification of one of the HCoV's by polymerase chain reaction (PCR) on a nasopharyngeal (NP) specimen or from a sample of induced sputum (IS).

3.5 Study Procedure

After obtaining consent, a detailed clinical examination was done noting the presence of cough, apnea, duration of symptoms and antibiotic treatment before admission. Additional history taking was conducted with the primary caregiver of the child. The child vaccination status was noted as recorded on the road to health card (RTHC). A physical examination was performed on each child and oxygen saturations measured and recorded. From each child, a nasopharyngeal aspirate and induced sputum specimen were taken for molecular diagnostic testing using PCR to test for HCoV.

Each child was weighed, and the child's nutritional status was assessed using WHO weight for age Z scores [58]. The status was assessed as moderate to severely undernourished if the Z scores were lower, -2.

An ELISA test (Architect HIV Ag/Ab Combo, Abbott Diagnostics, Wiesbaden) was used to screen children with unknown status for HIV. In children younger than 18 months a positive ELISA test was confirmed with an HIV PCR test (COBAS AmpliPrep/COBAS Taqman HIV-1, Roche Molecular Diagnostics, and Pleasanton, CA). For children older than 18 months a second positive ELISA using a different test method (Enzygnost Anti-HIV1/2 Plus, Siemens/Dade Behring, and Erlangen) was sufficient to confirm HIV infection. Children under 18 months of age with a positive PCR or older children testing positive on two ELISA tests were classified as being HIV infected. Infants whose mothers were HIV positive during pregnancy but who themselves were not HIV infected were classified as HIV exposed uninfected.

The FTDResp33 multiplex real-time PCR assay was used to identify the presence of HCoV's on NP and IS samples. In addition to other respiratory pathogens, the platform assessed for nucleocapsid protein gene on the RNA to find targets specific to coronaviruses 229E, NL63, HKU and 43.

3.6 Management of study participants

All participants were managed according to the national and departmental guidelines, at the discretion of the attending paediatrician. Children were followed up until discharge and in-hospital course, duration and outcome were recorded.

3.7 Patient safety and confidentiality

As the study uses data already collected by the original study, patient care was not affected. The investigator ensured that this study was conducted in full conformity with the principles set forth in the research guideline for Good Clinical Practice and the Declaration of Helsinki in its current version, whichever affords the greater protection to the participants.

3.8 Statistics

Demographic characteristics were tabulated to provide a description of the study population. Percentages and 95% confidence intervals were used to depict proportions of categorical variables while medians with interquartile ranges were used to summarise continuous variables. The χ^2 test or Fisher's exact test were used to assess the strength of association between two categorical variables as appropriate. Continuous variables were compared using the Wilcoxon rank-sum test.

The description of HCoV was stratified by the type of coronavirus and the type of specimen used to detect the infection.

A significance level was set at a two-tailed $P<0.05$ for all analysis. Stata version 16 13 (Stata Corporation, College Station, Texas) was used to conduct all the statistical analyses.

3.9 Ethical considerations

Ethical approval was sought and obtained from the FHS Human Research Ethics Committee, University of Cape Town (HREC REF: 507/2020). As data had already been collected as part of another study, it is not clear what direct risk there may be.

4 RESULTS

4.1 Sociodemographic characteristic of study population

A total of 7792 children were admitted during the period in which the study was recruiting, including 987 that had respiratory illness. Of these, 460 (46.6%) participants were eligible and were enrolled into the study between September 2012 and September 2013. Of these, 258 (56.0%) were male and 19 (4.1%) HIV infected. The median age of the children was 8 months (IQR 4-18 months). The number of participants in crèche was 96 (20.9%) with 450 (97.8%) of the accompanying caregivers being mothers to their children. Prior use of antibiotics was reported in 173 (37.6%) children. Of those reporting prior use, the commonest antibiotics used alone were ceftriaxone and oral penicillin with 91 (52.6%) and 70 (40.5%), respectively. The remaining 12 (6.9%) had received a combination of penicillin and ceftriaxone or another antibiotic. The other baseline characteristics are shown in Table 1.

Table 1: Sociodemographic characteristic of study population (N=460)

Variable		n	%
Sex			
	Male	258	56.1
	Female	202	43.9
HIV status			
	Uninfected	349	75.9
	Exposed but negative	92	20.0
	Infected	19	4.1
Undernourished (Z score < -2)			
	Yes	45	9.8
	No	415	90.2
Creche attendance			
	Yes	96	20.9
	No	364	79.1
Presence of home-smoker			
	Yes	162	35.2
	No	298	64.8
Use of Fossil fuel at home			
	Yes	18	3.9
	No	442	96.1
Prior use of antibiotics			
	None	260	56.5
	Ceftriaxone	91	19.8
	Penicillin	70	15.2
	Other	12	2.6

	Unknown	27	5.9
Caregiver Relationship			
	Mother	450	97.8
	Father	2	0.4
	Grandmother	5	1.1
	Other	3	0.7
Breastfeeding history			
	Never breastfed	60	13.0
	Breastfed 1st four months	323	70.2
	Breastfed > four months	77	16.7

4.2 Frequency of HCoV infection strains among study population by sample

Nasopharyngeal (NP) samples were obtained for all 460 children while induced sputum (IS) was not available for six children due to sample loss prior to analysis, leaving 454 available for analysis. A total of 42 (9.1%, 95% CI 6.7- 12.1%) participants tested positive for HCoV in at least one of the two specimens. PCR was able to detect a total of 35 (7.7%) cases from the 454 tested IS specimens compared to 23 (5.0%) detected out of 460 NP samples. Figure 1.

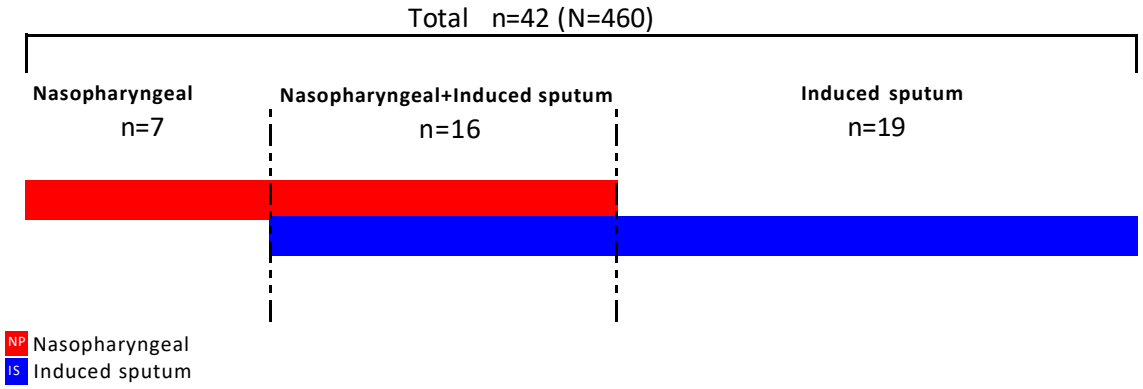

Figure 1: Number of positive specimens by type of sample analysed

The commonest detected HCoVs was coronavirus OC43 with 20 (4.3%) detected from either specimen followed by coronavirus NL63 or coronavirus HKU detected in 14 (3.0%) and 10 (2.2%) of the participants, respectively. The least common HCoV detected was coronavirus 229E detected in both samples of one individual. Both coronaviruses OC43 and NL63 were more frequently detected on IS than NP while the other two had the same frequency in both samples (Figure 2). For the one participant, coronavirus 229E was detected in both NP and IS

while coronavirus OC43 was found in both IS and NP in seven participants. Coronavirus NL63 was detected in both IS and NP in four participants, while coronavirus HKU was detected in both NP and IS in three individuals. One participant had more than one type of coronavirus in NP (coronavirus OC43 and HKU) and another one had coronavirus HKU in IS and coronavirus OC43 in NP. There were 16 participants who returned positive results and had different coronaviruses detected in both IS and NP.

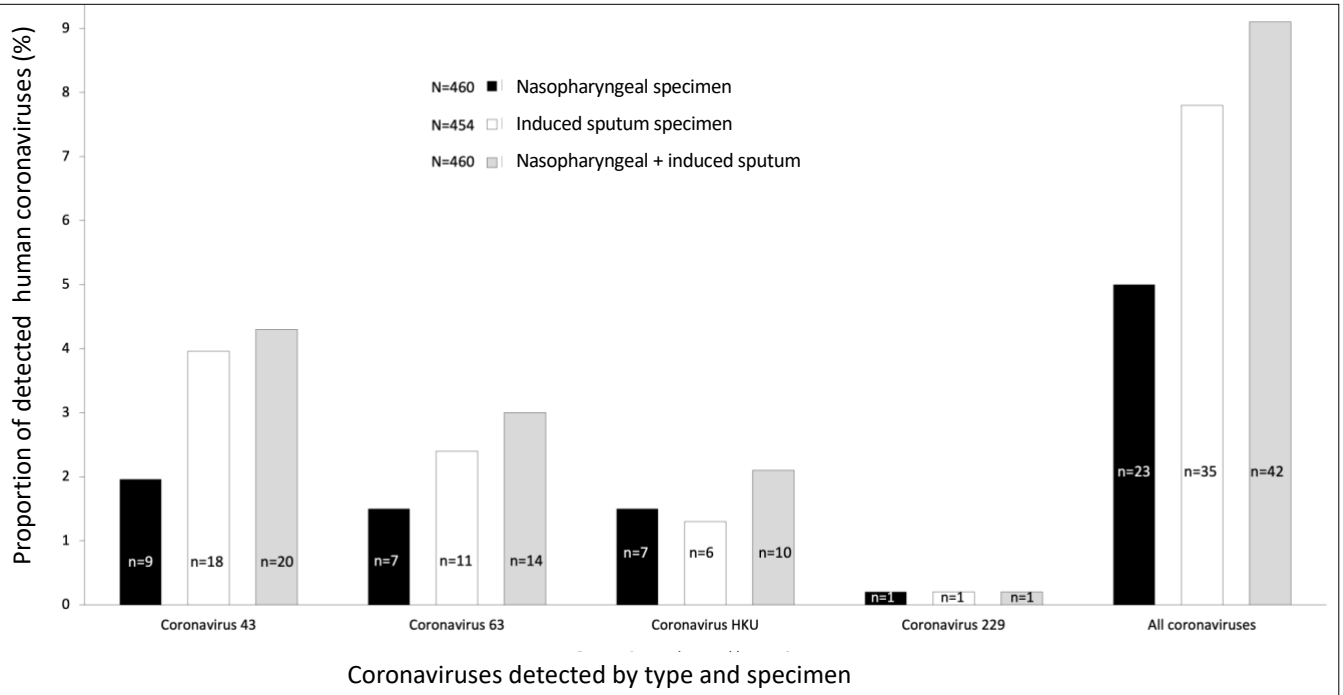

Figure 2. Proportion and number of detected human coronaviruses by type and sample used

4.3 Risk factors for HCoV infection

Overall HCoVs were detected in 23 (8.9%) of boys compared to 19 (9.1%) of the girls who returned a positive test; p=0.856. Similarly, HCoVs were detected in 30 (8.6%) of the 349 HIV unexposed uninfected children and in 12 (13.0%) HIV exposed uninfected children with none detected in HIV infected children; p=0.155. The overall age distribution of children with PCR detected HCoVs was similar to that of children with a negative result with median age of 10

(IQR 5- 16) months and median of 8 (IQR 4- 19) months, respectively; p=0.535. However, when the risk was stratified by age category, the frequency of children testing positive was 11/ 192 (5.7%), 23/153 (15.0%) and 8/115 (7.0%) in children less than six months of age, six to 18 months, and children above 18 months, respectively; p=0.008. Analysis of other potential risk factors is shown in Table 2.

Table 2: Potential risk factors for human coronavirus infection (N=460)

Assessed risk factor	n	HCoVpositive n (%)	P value
Sex			
Male	258	23 (8.9)	0.856
Female	202	19 (9.1)	
Age category			
< 6 months	192	11 (5.7)	**0.008**
6 – 18 months	153	23 (15.0)	
>18 months	115	8 (7.0)	
HIV status			
Uninfected	349	30 (8.60)	0.155
Exposed but negative	92	12 (13.0)	
Infected	19	0 (0.0)	
Undernourished (Z score < -2)			
Yes	45	3 (6.7)	0.546
No	415	39 (9.4)	
Creche attendance			
Yes	96	10 (10.4)	0.623
No	364	32 (8.8)	
Presence of home-smoker			
Yes	162	15 (9.3)	0.944
No	298	27 (9.1)	
Use of fossil fuel at home			
Yes	18	3 (16.7)	0.221
No	442	39 (8.8)	

HCoV= Polymerase chain reaction positive for human coronaviruses

4.4 Clinical presentation and outcome of HCoV infection

The commonest presentation of the study participants was cough in 456 (99.1%) with all children testing positive for HCoVs presenting with cough. Fever was found in 21 (50.0%) of HCoV positive children compared to 151 (36.1%) in PCR negative children; p=0.076. Other clinical presentations are shown in Table 3.

Table 3 Clinical presentation of participants by coronavirus status

Variables		No (n/%)	Yes (n/%)	P value
Cough				
	Negative	4 (0.96	414 (99.04)	
	Positive	0 (0.00)	42 (100.00)	0.524
Fever				
	Negative	151(36.12)	267 (63.88)	0.076
	Positive	21 (50.00)	21 (50.00)	
Apnoea				
	Negative	390 (95.59)	18 (4.41)	0.916
	Positive	40 (95.24)	2 (4.76)	
Cyanosis				
	Negative	394 (94.71)	22 (5.29)	0.884
	Positive	40 (95.24)	2 (4.76)	

There were no deaths reported in the study. The median length of hospital stay was 2 days (IQR 1 – 4) days in the group testing negative for coronaviruses compared to a median of 1.5 days (IQR 1-3) days in the group testing positive, p=0.557. Only 14 children required a high dependency care or critical care admission with one (2.4%) and 13 (3.1%) in the coronavirus positive and negative groups, respectively; p=0.629.

The median oxygen saturation of children who tested positive for coronaviruses was 97% (IQR 95 – 98%) compared to 96% (IQR 95- 98%) in children who did not test positive for coronavirus; p=0.492. 7 (16.7%) out of the 42 children who tested positive for HCoV required oxygen supplementation, compared to 107 (25.5%) in the group without detected HCoVs; p=0.201.

5 DISCUSSION

5.1 Introduction

This study shows that human coronaviruses are common in children admitted with respiratory illness. The prevalence of HCoVs was significantly high in children between the ages of six and 18 months. In general, children with HCoVs did not present differently from children who tested negative for coronaviruses. Similarly, the clinical outcome did not differ between the two groups. Of interest, the induced sputum specimen was able to detect more cases of coronaviruses than the traditionally used nasopharyngeal specimen.

Meanwhile, a large proportion of children had received antibiotics prior to admission, which probably reflects severity of disease that necessitated antibiotics before referral to RCH as per Integrated Management of childhood Illness (IMCI) protocols.

5.2 Prevalence

Several studies have described the detection of human coronaviruses in respiratory samples with most of them using nasopharyngeal specimen collected from patients with upper respiratory tract infection [25, 49, 50]. In this study, HCoVs NL63, OC43, 229E and HKU were tested from both nasopharyngeal (NP) swab specimen and induced sputum (IS) in infants and children with lower respiratory tract infection using a multiplex real-time PCR. Although the original study was not specifically designed to detect coronaviruses, however, our data showed that coronoviruses were common in this group of children with a prevalence of almost 10%. The study group consisted of hospitalised patients and therefore would not have picked up children infected with coronavirus who are ill and can stay at home, or who are asymptomatic.

Using IS in addition to NP doubled the detection rate by identifying cases that otherwise would have been missed by the NP specimen alone. The Drakenstein child health study in South

Africa reflect that induced sputum specimens provided better viral yield for identification of potential pathogens than nasopharyngeal swabs [1]. Our study is in keeping with this findings as it has shown that more positive cases were detected on IS compared to NP. In the light of COVID-19 precautions, the use of IS must always be done under proper ventilation with PPE.

5.3 Clinical presentation and outcome

The presence of fever was higher in children testing positive for HCoV in the study although this finding was not statistically significant. This is also the experience with COVID-19 where the risk of disease in children is associated with fever. In a study done in South Korea, Chang et al found that the presence of fever was a significant parameter for predicting risk of COVID-19 disease [55]. A possible explanation for this may be the interraction between cytokines and chemokines during inflammatory response to infection and injury [56].

The study identified children between the ages of 6 and 18 months as showing a significantly higher risk of coronavirus infection that was double that of other age groups. This highlights the possible effects of waning maternal antibodies which occurs in children older than 6 months [20]. Other possible reasons include social interaction, creche attendance and effect of carriers in older children and adults [59].

Compared to RSV and other respiratory viruses in which children younger than 6 months got severe infection and vaccines have been targeted for maternal vaccination to protect them [37]. This finding is consistent with the literature in which several studies have found that older children who got coronavirus were less likely to get severe respiratory illness requiring ICU and high care [7, 22]. But this is not the case with older children where HCoV infection is seen to occur frequently later in life than expected. This might explain the reason why the outcome seem to be slightly better in children with HCoV infection.

The risk of HCoV infection was not associated with HIV status. This contrasts with other pathogens in published studies in South Africa in which the risk of respiratory pathogens such as RSV was shown to be associated with HIV exposure among children [20, 37]. It is not fully clear why this association with coronavirus as shown in our reported results is missing. The small sample size in which the analysis was based could be the reason why it was not detected. However, HIV remains a major problem in sub-Saharan Africa (SSA) with more children having in utero exposure to HIV even if uninfected on account of successful implementation of the prevention of mother to child transmission (PMTCT) strategy [21]. A number of studies show this group of children as a growing concern for high risk for infectious diseases [20, 21].

In our study children that come from households using fossil fuel had a higher risk to have coronavirus detected in their respiratory tract, however this finding did not reach statistical significance and does not identify fossil fuel as a risk in children. This contrast with what is observed in other contexts where solid fuel generally pose a risk to children with respiratory tract infection [57]. The study found similar frequencies of HCoV infection in children who live with a smoker in their homes and those without a household smoker. In addition, HCoV may be underrepresented in this study as it included only hospitalised children, not those in the community with milder coronavirus disease.

6 GENERAL CONCLUSIONS

This study investigated the burden of HCoVs in a cohort of children hospitalised with lower respiratory tract infections. Our findings highlight important new information regarding the role of human coronaviruses in the aetiology of childhood pneumonia, but also provide explanation on the risk factor for HCoV associated pneumonia in children. The study findings further reflect on the low risk of severe disease as children are disproportionately spared even in the same household with those having the disease. It is even more important in the context of COVID-19 which poses a less risk to children. However, it is important to explore in greater detail the genomic and clinical characteristics of human coronaviruses circulating in the paediatric population as this may assist in tracking potential virulent HCoV strains capable of causing more severe illness in children.

From the review of published literature, nasopharyngeal secretions from children are widely accepted as a means of diagnosing respiratory pathogens including human coronaviruses. Although other findings exist in support of induced sputum over nasopharyngeal swabs as a better method for screening respiratory pathogens, there is no agreement regarding the choice of sample type for testing in children with lower respiratory tract infection. Moreover, should we even be testing for coronaviruses routinely if they do not seem to cause any severe disease? Perhaps occasional studies/audits may be useful for coronavirus testing as they can easily undergo mutations and are capable of causing the re-emergence of SARS. More so, would identifying viral aetiologies contribute to antibiotic stewardship and avoidance of unnecessary antibiotic use?

6.1 Limitations

As the study is from a secondary data analysis, it may have been limited by the small sample size. Although the study was sufficiently powered, it had low precision and could not

demonstrate statistically significant associations. The prospective design of the study may have also limited the supposed variables that were known to predict certain risk factors such as HIV and fossil fuel. Additionally, only respiratory disease caused by HCoV was included in the study; it is not possible from the data available for this study to comment on other disease associated with HCoV.

6.2 Recommendations

6.2.1 Recommendations for future study

This study is restricted to the burden of human coronaviruses in children presenting mainly with respiratory illness. There is a need to assess the broader burden of HCoV infection in children presenting with other illnesses such as febrile convulsions / encephalopathy / croup / and Kawasaki disease in order to define the true HCoV burden.

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APPENDICES

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27 August 2020

HREC REF: 507/2020

A/Prof R Muloiwa
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Student: ALYABD002@myuct.ac.za

Dear A/Prof Muloiwa

PROJECT TITLE: CO-INFECTION RATES BETWEEN NON-SEVERE ACUTE RESPIRATORY SYNDROME (NON-SARS)-RELATED HUMAN CORONAVIRUSES (HCOVS) AND OTHER RESPIRATORY VIRUSES AMONG HOSPITALISED CHILDREN WITH SEVERE LOWER RESPIRATORY TRACT INFECTION IN CAPE TOWN, SOUTH AFRICA (2012-2013)-MPHOIL CANDIDATE-DR ABDULMUMUNI ALIYU.

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020.

Approval is granted for one year until the 30 August 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: - Dr Abdulmumuni Aliyu will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

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