EPIDEMIOLOGY AND PULMONARY SEQUELAE IN CHILDREN WITH HUMAN METAPNEUMOVIRUS ASSOCIATED LOWER RESPIRATORY TRACT INFECTION

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DECLARATION

I, Lesego Matlhogonolo Ramocha, student number 0703905g, declare that this Thesis is my own work and that I contributed adequately towards research findings published in the articles stated below which are included in my Thesis.

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Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of her Thesis.

Article 1: Title: Epidemiology of Human Metapneumovirus-associated Lower Respiratory Tract Infections in African Children: Systematic Review and Meta-analysis

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DEDICATION

This thesis is dedicated to my beloved parents Goitsemang Ramocha and Martin Ramocha. I am forever thankful for the love and support you have given me throughout the years. To my brother Patrick Ramocha, thank you for being my number one fan. I also dedicate this thesis to friends and church members who have been with me throughout this entire journey.

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THESIS ABSTRACT

Background: Human Metapneumovirus (hMPV) has been associated with upper and lower respiratory tract infections (LRTI).

Aims: 1) Conduct a systematic review on epidemiology of hMPV-associated LRTI in African children; 2) Determine the incidence and clinical course of hMPV-associated hospitalisation during the first five years of life in South African children; 3) Investigate the effect of maternal HIV-1 status on transplacental transfer of anti-hMPV neutralisation antibodies from mother to babies; 4) Describe the pulmonary sequelae of children hospitalised with hMPV associated LRTI during the first two years of life.

Methods: We conducted a systematic review and meta-analysis to evaluate the epidemiology of hMPV associated LRTI in African children under five years of age. We used the search terms "("Human metapneumovirus" AND "Africa") OR ("hMPV" AND "Africa")" up to September 17, 2020. Incidence estimation and clinical course of hMPV-associated hospitalisation in Soweto, South Africa, 2015-2017, was nested within a larger study in which children under five years of age hospitalised at a large public hospital in Soweto, South Africa, with symptoms consistent with LRTI, were tested for presence of a panel of common respiratory pathogens, including hMPV. The hMPV neutralizing antibodies study was a case-control study of the level of transplacental transfer of hMPV neutralising antibody in 53 mother-infant dyads in which the mother was living with HIV-1 matched to 53 mother-infant dyads in which the mother was HIV-1 negative. To describe the hMPV pulmonary sequelae we conducted a case-control study that drew data from infant pulmonary function tests in 98 cases and 496 controls.

Results: The prevalence of hMPV-associated LRTI among hospitalised cases was 4.7% [95% confidence interval (CI): 3.9–5.6, I^2=95.0]. In the hMPV-associated LRTI hospitalisation study, hospitalisation was higher in children less than six months compared with other age groups. In the third study, transplacental transfer of hMPV neutralizing antibodies was significantly lower in women living with HIV-1 compared with women without HIV-1; with cord blood to mother ratio being 0.71 median (interquartile range [IQR], 0.50-1.0) vs 1.4 [IQR 0.71- 2.0] (genotype A) and 0.71 median [IQR 0.50-1.4] vs 1.4 [IQR 0.71-2.0] (genotype B). hMPV LRTI lead to abnormal lung function in the first two years of life. In the first year of life hMPV-associated LRTI was associated with higher respiratory rate (27.91 [IQR 25.88-31.78] than controls (27.17 [IQR 24.44-30.72], p=0.05). Furthermore, the

inspiratory time (Ti) and lung clearance index (LCI) were higher in controls (1.00 seconds [IQR 0.89-1.10] than in cases (0.93 seconds [IQR 0.86-1.04], p=0.03) and 7.27 [IQR 6.79-8.03] than in cases 6.81 [IQR 6.43-7.59] p=0.0001) respectively. Lung function at two years also showed higher respiratory rate in cases (25.19 [IQR 24.41-28.01] than controls (24.35 [IQR 22.21-26.89], p=0.05).

Conclusion: Infants are at increased risk of hMPV-associated LRTI hospitalisation and subsequent pulmonary sequelae. Transplacental transfer of the maternal hMPV neutralizing antibody was lower in women living with HIV, however, it was not associated with any difference in risk of the infants being hospitalised for hMPV- associated lower respiratory tract infections.

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ABBREVIATIONS

%:	Percentage
°C:	Celcius
9-valent PCV:	9-valent pneumococcal conjugate vaccine
AFV:	Area of flow-volume loop
AIDS:	Acquired immunodeficiency syndrome
aLRTI:	Acute Lower respiratory tract infection
AMSTAR:	Assessment of multiple systematic reviews
Ar:	Argon
ART:	Antiretroviral therapy
ASSA 2008:	Actuarial society of South Africa
ATS/ERS:	The American Thoracic Society and European Respiratory Society
BoSS:	Babies of Soweto Study
bpm:	Beats per minute
BTPS:	Body Temperature and Pressure Saturated
C:	Capacitance
CAP:	Community-acquired pneumonia
CFR:	Case fatality ratio
CHBAH:	Chris Hani Baragwanath Academic hospital
CI:	Confidence interval

CLWH:	Children living with HIV
cm:	Centimetre
cmH2O/l/s:	Centimeters of Water Column
CMR:	Child to mother ratio
CO2:	Carbon dioxide
CRF:	Case report forms
Ct:	Cut-off threshold
DSR:	Dynamic spatial reconstructor
ECMO:	Extracorporeal membrane oxygenation
EEL:	Total end expiratory level
ELISA:	Enzyme-linked immunosorbent assay
FOT:	Forced oscillation technique
FRC:	Functional residual capacity
Fres:	Frequency
GBS:	Group B streptococcus
He:	Helium
HEU:	HIV exposed uninfected
Hib:	Haemophilus influenzae type b
HIV:	Human immunodeficiency virus
hMPV:	Human metapneumovirus
hPa:	Hectopascal
HREC (Med):	Human research ethics committee (Medical)
HUU:	HIV unexposed uninfected

Hz:	Hertz
I2:	Heterogeneity
IC50:	Inhibitory concentration 50
IgG:	Immunoglobulin G
ILI:	Influenza like illness
IMDM:	Iscove's Modified Dulbecco's Medium
Inf A:	Influenza A
iPFT:	Infant pulmonary function test
IQR:	Interquartile range
IS481:	Transposable b pertussis
ISAAC:	International Study of Asthma and Allergies in Childhood
Kg:	Kilograms
kPa:	Amplitude pressure
l/s:	Litres per second
LCI:	Lung clearance index
LLC-MK2:	Rhesus Monkey Kidney Epithelial Cells
LMIC:	Low- and middle-income country
LRTI:	Lower respiratory tract infection
mAbs:	Monoclonal antibodies
MBW:	Multiple inert gas washout technique
ml:	Millilitres
mmHg:	Millimeters of mercury
Mmol:	Millimoles
MR:	Mortality rate

MTEF: Mean tidal expiratory flow

MTIF: Mean tidal inspiratory flow

MV: Minute ventilation

NL: Netherlands

NO: Nitric oxide

NOS: Newcastle-Ottawa scale

NPA: Nasopharyngeal aspirates

NPS: Nasopharyngeal swabs

O2: Oxygen

OR: Odds ratio

PCP: Pneumocystis pneumonia

PERCH: Pneumonia aetiology research for child health

PMTCT: Prevention of mother-to-child transmission

PRISMA: Preferred Reporting Items for Systematic and Meta-Analyses

PSG: Penicillin, streptomycin and glutamine

PTEF: Peak tidal expiratory flow

PTIF: Peak tidal inspiratory flow

R: Resistance

RMPRU: Respiratory and Meningeal Pathogens Research Unit

RR: Relative risk

Rrs: Resistance

RSV: Respiratory syncytial virus

RTHC: Road to Health Chart

RT-PCR: Reverse transcription polymerase chain reaction

RT-qPCR: real-time quantitative polymerase chain reaction

RV: Residual volume

SARI: Severe acute respiratory infection

SD: Standard deviation.

SF6: Sulphur hexafluoride

SOP: Standard operating procedure

TBFVL: Tidal breathing flow volume loop

TE/TTot: Ratio of expiratory time to total respiratory time

tE: Total expiratory time

TE: Expiratory time

TEF10: Flow at 10% of VT remaining

TEF25: Flow at 25% of VT remaining

TEF50: Flow at 50% of VT remaining

TEF75: Flow at 75% of VT remaining

TI/TE: Ratio of inspiratory time to expiratory time

TI/TTot: Ratio of inspiratory time to total respiratory time

TI: Inspiratory time

TIF50: Inspiratory flow at 50% of VI

TLC: Total lung capacity

TMP-SMX: Trimethoprim-sulfamethoxazole

TPEF/T: Ratio of time to peak tidal expiratory flow to expiratory time

TPEF: Time to peak tidal expiratory flow

TPIF: Time to peak tidal inspiratory flow

tPTEF: Time to peak tidal expiratory flow

TTot (sec): Total breath time in seconds

UR: Uncertainty range

URTI: Upper respiratory tract infection

VE: Expired tidal volume

VE: Expiratory volume

VI: Inspiratory volume

VPEF: Volume at peak tidal expiratory flow

VPIF: Volume at peak tidal inspiratory flow

VPTEF: Volume at peak tidal expiratory flow

VT: Tidal volume

WHO: World health organisation

Xrs: Reactance

X^2 test: Chi square

Zrs: Impedance

Zrs: Impedance

PREFACE

The format structure of this thesis is the dived block format, which is used within the Faculty of Health Sciences in the University of the Witwatersrand. It consists of a full introductory chapter, followed by the methods chapter. The next chapter is the results. The manuscripts that have been derived (published or under review) from this thesis are used as the basis for each individual results chapter. The results chapter has a short introductory section, brief methods section, full results section, full discussion and references section. After the results chapter follows the integrated discussion and conclusion chapter at the end.

Chapter 1

A literature review that focuses on key components of the thesis which are: lower respiratory infections, HIV exposure, maternal antibodies and pulmonary function in children less than five years with hMPV.

Chapter 2

A comprehensive methods chapter of the four components contributing to this thesis.

Chapter 3

Results of the systematic review paper. The systematic review has been peer-reviewed and published in The Pediatric Infectious Diseases Journal. The systematic review evaluated the epidemiology of hMPV-associated LRTI, including severe acute respiratory infection (SARI), hospitalization and clinically diagnosed severe pneumonia in African children under five years.

Chapter 4

Reports on results of the 2nd objective, which evaluated the epidemiology of Human metapneumovirus-associated hospitalisation in HEU and HUU children less than five years in South Africa.

Chapter 5

Reports on the results of objective three which evaluated the effect of maternal HIV-1 on transplacental transfer of anti-hMPV neutralizing antibodies from mothers to their babies. The study also evaluated the association of transplacental-acquired anti-hMPV neutralizing antibody and risk for hMPV associated lower respiratory tract infection hospitalization in the first six months of life, using a nested case control study design.

Chapter 6

In this chapter, we report novel data on the clinical and pulmonary function sequelae in children following hMPV-associated LRTI hospitalisation during infancy. Pulmonary sequelae were evaluated using questionnaires and pulmonary function techniques, including, tidal breathing flow volume loop (TBFVL), and multiple inert gas washout (MBW).

Chapter 7

This chapter comprises an integrated discussion and conclusion of all the studies contributing to this thesis.

STUDENT CONTRIBUTIONS

Protocol and ethics: The study protocol was prepared by Lesego Ramocha (LR), who also attained the ethical clearances for all the studies.

Paper 1: A systematic review and meta-analysis paper.
Project title*:* Epidemiology of Human Metapneumovirus-associated Lower Respiratory Tract Infections in African Children.
Role of the student: LR was responsible for literature search, screening of study titles, data extraction and data analysis. LR was also responsible for submission of the paper to journals, revision and publication. LR is the corresponding author for this paper.

Paper 2: A retrospective analysis of hMPV incidence.
Project title: Human metapneumovirus-associated hospitalization in HIV-1 exposed uninfected and HIV-1 uninfected children less than 5 years in South Africa.
Role of the student: LR was responsible for accessing the databases of the parent study, retrieval of the data and accessing medical files of patients where data was missing. LR cleaned, analysed and interpreted the data. LR wrote the manuscript, was also responsible for submission of the paper to journals and revision. Manuscript is still under review.

Paper 3: This a case case-control study that analysed archived serum from a previous birth cohort study.
Project title: Fetal transfer of human metapneumovirus neutralizing antibodies is reduced from mothers living with HIV-1.
Role of the student: LR was responsible for locating suitable participants from the database and retrieving their data. LR located stored serum for cases and controls. LR performed neutralisation assays, analysed results and wrote the manuscript. LR was also responsible for submission of the paper to journals, revision and publication

Chapter 1: Introduction

1.1 Lower respiratory tract infections in children

Children under the age of five years contributed to 5.3 million deaths globally in 2018, a large proportion of these occurring in LMICs and sub-Saharan Africa (1). The World Health Organisation (WHO) reported that the under-five mortality rate (U5MR) in the African region was eight times higher than in the WHO European Region (76 vs 9 per 1000 live births). The majority of all under-five deaths occur in the sub-Saharan region where 1 in 13 children died before they reached their fifth birthday (2). Lower respiratory tract infections (LRTI), especially pneumonia, are one of the main causes of childhood mortality outside of the neonatal period worldwide, contributing to the 0.921 million [95% uncertainty range (UR) 0.812 −1.117] under-five deaths globally (3). In sub-Saharan Africa, the leading causes of mortality in children under five years was pneumonia (0.490 million [UR 0.417–0.631]).

In South Africa the U5MR has declined substantially over the past decade. In 2015 the U5MR was estimated at 37 - 40 deaths per 1 000 live births compared to 2003 where it was 78.1 per 1000 live births (4). Mortality as a consequence of acute LRTI had been largely reduced due to the implementation of immunisation strategies that target important causes of LRTI, such as *Bordetella pertussis, Haemophilius influenzae type b and Streptococcus pneumoniae* (5).

The burden of LRTI is substantially higher in low-income countries (LIC) compared to high-income countries (HIC). A systematic review of children younger than five years from 2010 reported that hospitalisation incidence from severe LRTI in LIC was twice as high (19.7/1000 children (95% confidence interval (CI) 17.1–22.7)) as in HIC (9.9/1000 children; 95% CI 7.4–13.3) (6). Africa had the highest hospitalisation incidence for severe and very severe LRTI in children across all the WHO regions (Table 1.1). The global case fatality ratio (CFR) for severe LRTI was 2.1% (95% CI 1.4–3.1); and it was approximately four fold higher in LIC (2.3%; 95% CI 1.6–3.4) compared with HIC (0.6%; 95% CI 0.4–0.8) (6). Furthermore, Africa had the highest mortality rates of all WHO regions.

Table 1.1: Global LRTI hospitalisation incidence and CFR of children less than 5 years by WHO region (per 1000 children per year) (7)

	Africa	**Europe**	**America**	**Eastern Mediterranean**	**South-East Asia**	**Western Pacific**
Severe ALRI Incidence of admissions	22.6 (15.3-33.4)	7.3 (4.6-11.6)	19.8 (14.6-27)	12.1 (8.6-17.2)	17.8 (12.2-26.1)	17.3 (13.4-22.3)
Case fatality	3.9**%** (2.7-5.5)	0.4**%** (0.3-0.5)	1.3**%** (0.8-1.9)	7.6**%** (4.1-13.9)	2.1**%** (1.1-4)	2.3**%** (1.7-3.2)
Very severe ALRI Incidence of admissions	15 (10.4-21.6)	Data not available	3 (1.9-4.7)	Data not available	3.7 (1.7-8)	6.3 (3.5-11.2)

Abbreviations: LRTI, lower respiratory tract infection; ALRI, acute lower respiratory tract infection. Data taken from publication by Nair et al, 2010, Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: a systematic analysis.

In South Africa, Cohen et al. reported that children less than five years had an overall LRTI hospitalisation incidence ranging from 2530 to 3173 per 100 000 population with the highest incidence being in infants (8 446–10 532/100 000). The CFR for <5 year olds hospitalised with LRTI was 2% (150/8512) (7).

1.2 Risk factors for severe lower respiratory tract infection

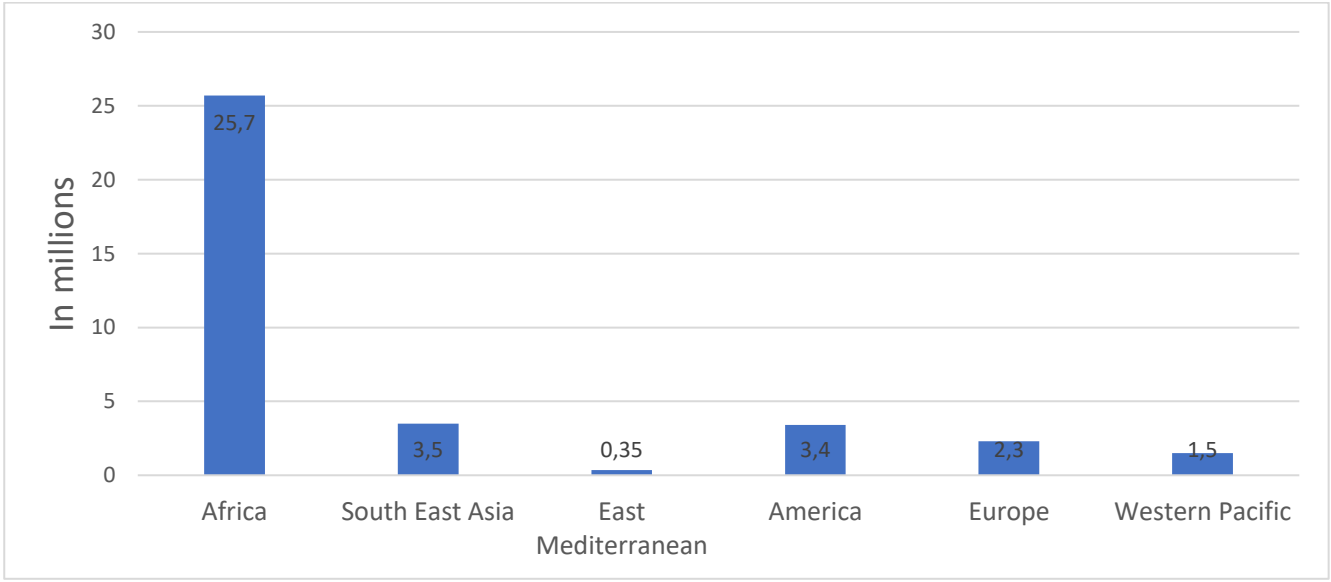
The risk factors for the development of LRTI overlap with causes of increased mortality due to LRTI. Sonego et al. conducted a systematic review that explored the risk factors for death from LRTI in children under five years living in LIC. The authors reported that being an infant younger than two months, having a diagnosis of *Pneumocystis jirovecii*, and the presence of chronic underlying diseases or HIV/AIDS, were strongly associated with mortality among children with severe pneumonia as per WHO definition (8). For social-economic and environmental factors the following were all associated with higher mortality: young maternal age, low maternal education, low socio-economic status, smoke exposure and indoor air pollution. Receipt of childhood vaccines (relative risk: 0.46, 95%CI: 0.36–0.58) and antenatal care (RR 0.50, 95%CI: 0.31–0.81) were associated with lower risk of death (8). Other studies indicated that overcrowding, prematurity, history of LRTI in the family, male gender, poor

living conditions, malnutrition and faulty breast feeding and weaning practices are risk factors for mortality from LRTI (9, 10).

1.3 Lower respiratory tract infections and HIV exposure in Sub-Saharan Africa

Africa has the highest prevalence of HIV-1 in the world and sub-Saharan Africa accounts for 71% of the global burden of HIV infection (11-13). In 2017 there were 25.7 million people living with HIV in Africa and two thirds of new HIV infections globally occurred in Africa (14) (Table 1.2). In 2018, there were an estimated 14.8 million (95%CI: 11.1-18.3 million) children aged 0-14 years globally who were HIV-1 exposed, but uninfected (HEU). 13.2 million of these children resided in sub-Saharan Africa (15).

Figure 1.1: Number of people living with HIV classified according to WHO regions



Published report from Global Health Observatory data repository, WHO 2017

South Africa has 7 200 000 [95% CI 6 600 000-7 900 000] people living with HIV of which 61% are receiving ART (14). The maternal HIV-1 prevalence in South Africa is estimated to be 30% (16); however, the implementation of prevention of mother-to-child transmission (PMTCT) has successfully reduced the vertical transmission rates of HIV-1 to children in South Africa to <1% (17). Nonetheless, there remains a substantial and growing population

of HIV-1 exposed uninfected (HEU) children, who exhibit reportedly higher rates of morbidity and mortality compared with HIV-1 unexposed uninfected (HUU) children (18, 19).

HEU children are at increased risk of premature births, low birth weight and developing severe LRTI (20-24). A Zimbabwean study reported an overall mortality in 2-6 month old infants to be three times higher in HEU (9.2%) than in HUU (2.9%) children; and the main cause of death was acute respiratory infection (21). Furthermore, a study in Botswana reported that HEU children with LRTI were at an increased risk of pneumonia treatment failure (47%) compared with HUU children (24%). (25). In South Africa, HEU infants under six months have been reported to be at increased risk of LRTI hospitalisation (26).

1.4 LRTI aetiology

LRTI in children is caused predominantly by viral and bacterial organisms; the Pneumonia Etiology Research for Child Health (PERCH) multi-country case-control study in children less than five years hospitalised with severe and very severe pneumonia reported that viruses contributed to 61.4% (95% credible interval [CrI] 57.3-65.6) and bacteria 27.3% (23.3-31.6) of aetiological agents in cases (27).

1.4.1 Viruses

The aetiology of viral LRTI in children includes respiratory syncytial virus (RSV), influenza and parainfluenza viruses, rhinovirus, adenovirus, and hMPV (7, 28, 29) . In the PERCH study, the most commonly detected virus in African and Asian children less than five years of age with severe or very-severe LRTI was RSV, with a prevalence of 26.5%, followed by rhinovirus (23.0%), and bocavirus (13.1%); hMPV was detected in 6.8% (27). Globally, the most common viral cause of LRTI in children is RSV (30, 31). A systematic review reported that RSV-LRTI was associated with 3.2 million (2.7–3.8) hospitalisations and 59 600 (48 000–74 500) deaths in children under five years in 2015 (32). In South Africa, Cohen et al. reported the prevalence of viruses in 6517 out of 8393 (78%) children with LRTI, the most common virus being Rhinovirus (37%), followed by RSV (26%), influenza (7%) and hMPV (5%) (7).

1.4.1.1 Human metapneumovirus

Since 2001 hMPV has been well-established as a cause of upper and lower respiratory tract infection in children younger than five years (33, 34). It is one of the leading causes of paediatric viral LRTI (27).

1.4.1.1.1 hMPV taxonomy

hMPV belongs to the Paramyxoviridae family in the Pneumovaridae, subfamily. The Pneumovaridae, are further divided into pneumoviruses and metapneumoviruses (including hMPV). Metapneumoviruses were solely associated with upper respiratory tract infections in birds (35) and not mammals, until they were isolated in nasopharyngeal aspirate samples of 28 children under five years with LRTI in the Netherlands in 2001 (34).

Just like other Paramyxoviridae viruses, hMPV has intrinsic glycoproteins, namely the fusion glycoprotein (F), the attachment glycoprotein (G) and a small hydrophobic protein (SH) (36). During virus entry into the cell cytoplasm, the F protein facilitates fusion of the viral and cellular membranes.

hMPV is an enveloped, single stranded, negative strand RNA virus (37). The two major hMPV lineages are A and B and they have four sublineages (A1, A2, B1 and B2) (34, 38-40). Some studies have reported a dominance of genotype A, while other studies have reported no dominance during hMPV circulation (41-43). Ludewick et al. showed that although both genotypes co-circulated over a three-year study period, genotype A dominated in 60.9% and genotype B in 39.1% of 92 hMPV positive samples. In China and Japan genotypes A2 were the most common circulating hMPV genotypes (42, 44). The detection of lineage subtype A1 has become rare (45, 46). hMPV often presents in seasonal epidemics during winter to spring in temperate climates, with some studies also reporting perennial circulation (34, 47, 48).

There is conflicting data on whether hMPV associated acute respiratory illness (ARI) severity differs between Genotype A (causing more severe disease) and Genotype B (43, 49-56).

Repeated hMPV infection has been reported globally, especially in children less than five years. In a quest to identify the duration of hMPV antibody protection, Van den Hoogen et al. used the animal model at different time points after inoculation with NL/1/00 (representing hMPV sublineage A1) and NL/1/99/ (hMPV sublineage B1) by plaque-

reduction virus-neutralization assay. Initial infection with hMPV strains induced high neutralisation titres that cross neutralised well between the genotypes. After 74 weeks the antibodies levels had declined to undetectable levels and hMPV re-infections were detected (57).

1.4.1.1.2 Detection of hMPV

Van den Hoogen et al. identified hMPV from nasopharyngeal aspirates of children less than five years with LRTI, using reverse transcriptase PCR (RT PCR) which was designed after facing challenges to isolate the virus using standard cell culture (34). Since then, RT PCR assays have become the gold standard for hMPV detection in most laboratories (58). hMPV is usually restricted to the respiratory tract (54). The sensitivity, specificity and reproducibility of different types of PCR assays in detecting hMPV have been explored across studies (59-62). When real-time RT-PCR was compared to conventional RT-PCR, it showed its increased sensitivity by detecting an additional 9.6% of hMPV positive isolates compared to conventional RT-PCR (63).

hMPV can also be detected using cell culture, although it is challenging because of its selective growth, mildly cytopathic nature and lack of specific diagnostic reagents, as well as being time consuming (63). hMPV can be cultured in few cell lines and its replication requires trypsin, which is necessary for cleavage of F protein.

hMPV replicates readily in Vero cells, LLC-MK2 cells and HEP-2 cells (64, 65). Deffrasnes et al. showed that hMPV replicates better in LLC-MK2 compared to Vero cells, while HEP-2 cells showed the least hMPV replication. Our collaborator in the Netherlands generated a subclone of Vero-WHO 118 by selecting for its equal permissiveness to all genetic lineages of hMPV and it is used for neutralisation assays (57, 66-68).

1.4.1.1.3 hMPV epidemiology

The aetiology of LRTI includes bacterial and respiratory virus infections, including hMPV. It has been almost two decades since hMPV was first identified as a cause of upper and lower respiratory tract infection (34). Studies have found hMPV infections in children with LRTI globally (55, 69-71). The contribution of hMPV to LRTI morbidity and mortality had not been

systematically reviewed in African countries at the time of this thesis. A systematic review and meta-analysis to define the epidemiology of hMPV-associated LRTI, including severe acute respiratory infection (SARI) hospitalisation or clinically diagnosed severe pneumonia, in children under five years of age, in African countries was undertaken as part of the research contributing to this thesis (See Appendix 1 for the full systematic review) and is reported in Chapter 3.

Infection with hMPV has been associated with hospitalisation, all stages of severity of respiratory disease, and death in high-risk groups such as children less than five years of age, immunocompromised, and elderly individuals (26, 33, 72, 73). HIV-exposed children are at increased risk [IRR 1.4; 95% confidence interval (CI): 1.1–2.0] of being hospitalized for hMPV-associated severe LRTI (26).

1.4.2 Bacteria and fungi

Streptococcus pneumoniae is the commonest bacterial pathogen in the pathogenesis of community-acquired pneumonia (CAP). Madhi et al. reported that bacteraemia was significantly more common among children living with HIV (CLWH) compared with HIV-uninfected children aged 2-60 months who required hospitalisation for CAP (74). The isolation of *S. pneumoniae* was more common in CLWH: 41/548 (7.5%) vs HUU: 17/617 (2.6%), followed by *Haemophilus influenzae* type b: (CLWH: 19/548 (3.5%) vs HUU: 11/617 (1.8%)), *Staphylococcus aureus* (CLWH 10/548 (1.8%) vs HUU 4/617 (0.7%)), *Escherichia coli* (CLWH*:* 7/548 (1.3%) vs HUU: 1/617 (0.2%)) and Salmonella species (CLWH: 3/548 (0.5%) vs HUU: 3/617 (0.5%)). Overall, CLWH had a longer duration of hospitalisation compared with HUU children (6.76 days; (SD ± 5 5) vs 5.94 days (SD 5.86); P< 0.00001). The case fatality rate (CFR) was higher in CLWH (72/548, 13.1%) compared with HUU (13/617, 2.1%; P< 0.00001).

The systematic review contributing to this thesis reported that the bacterial coinfection most frequently associated with hMPV was *S. pneumoniae* (72, 73, 75). All studies on bacterial coinfection were done in South Africa. Groome et al. reported *S. pneumoniae* coinfections identified by lytA quantitative real-time PCR detection using whole blood specimens in 7.3% of children with hMPV-associated SARI hospitalisation (72). *S. pneumoniae* was further identified in blood culture by Madhi et al. in 4/189 (2.1%) of infants with hMPV (76). In a randomized controlled trial comparing an experimental 9-valent pneumococcal conjugate

vaccine (PCV) or placebo (77), hospitalisation for hMPV-associated LRTI was 45% (95% CI: 19%–62%, P = 0.002) and 53% (95% CI: 3%–77%; P = 0.035) lower in PCV-9 compared with placebo recipients in HIV-uninfected children and CLWH (who were not treated with antiretrovirals), respectively. In the same study, 25% (2/8) of CLWH not on antiretroviral therapy hospitalised for hMPV-associated LRTI had concurrent *Pneumocystis jiroveci* pneumonia (PCP) (77).

1.4.2.1 Prevention and treatment of hMPV LRTI

There is currently no preventative vaccine or specific treatment for hMPV infection. The management of hMPV LRTI is supportive. Monoclonal antibodies have been tested in animal models but none have been licenced due to limited effectiveness and safety concerns (78, 79).

Studies done in animal models have shown that neutralising monoclonal antibodies (mAbs) that target the hMPV viral coat proteins produces immunity in animals (79). In hMPV, the prime target of mAbs has been reported to be the F protein as it is highly conserved and has a high titre neutralising antibody response. The G and SH proteins were shown to have a negligible contribution in inducing hMPV-neutralising antibodies (80). The effectiveness of surface glycoproteins mAbs varies across different studies with different mAbs (78, 79).

Ulbrandt et al. isolated three mAbs (MAb 338, MAb234 and MAb 628) that neutralised all hMPV subgroups in vitro and in vivo using the half maximal inhibitory concentration (IC50) of < 5µg/ml (79). The mAbs MAb 234 and MAb 338 had the most potent neutralising capability and they showed higher affinity, binding to the F protein from hMPV group A and B sequences. The MAb 338 and MAb 234 exhibited similar characteristics to Palivizumab, a monoclonal antibody that is used as prophylaxis in infants at risk of developing severe RSV disease. In this study, humanisation of MAb 338 and MAb 234 demonstrated potential to prevent hMPV LRTI. A similar study by Schuster et al. demonstrated that the human mAb 54G10 neutralised all four subgroups of hMPV in vitro and binds to hMPV F protein with high efficiency (78).

There are currently few studies that show conduct of hMPV phase 1 clinical trials. Shaw et al conducted a randomized, placebo-controlled study in healthy adults aged 18–49 using mRNA-1653 which is a mRNA-based investigational combination vaccine against hMPV

and PIV3. The study evaluated four vaccine dose levels which were given intramuscularly. The results showed that the mRNA-1653 vaccine was tolerated by participants at all dose levels and neutralizing antibodies against hMPV and PIV3 were seen at baseline in all participants. A single dose of mRNA-1653 boosted serum neutralization titers against both hMPV and PIV3, however, a second dose did not show any change in results (81). A similar study in 2018, developed a live attenuated hMPV vaccine (rHMPV-Pa) which was administered intranasally and evaluated it in 15 adults, 15 hMPV -seropositive children, and 15 hMPV -seronegative children. The study showed that neutralizing antibody response was not detected in adults and hMPV -seropositive children. However, neutralization response was detected in only 3 hMPV -seronegative children. The study was then stopped due to low infectivity rate and over attenuation of rHMPV-Pa (82) .However, there are concerns about using the humanized mAbs due to development of side effects associated with cardiac/respiratory hospitalisations (83, 84).

1.5 Lower respiratory tract infections and maternal antibody transfer and the protection of the newborn.

Due to the absence of preventive and treatment options for hMPV LRTI, further research into mechanisms for natural protection from disease is important. These include the role of transplacental transfer of maternal antibodies in lowering the risk of severe hMPV LRTI in early infancy. Currently, no studies, to my knowledge, have evaluated the association of transplacental acquired hMPV neutralizing antibody and the risk for hMPV LRTI hospitalisation in young children.

Transplacental acquisition of maternal IgG antibodies has been associated with protecting young infants against infectious diseases (85, 86). In humans, transplacental transfer of maternal antibody into the foetal systemic circulation stops at or soon after birth, although the coating of mucosal surfaces by antibodies (IgA) in breast milk can confer some protection (87). Maternally-derived antibody levels in blood wane by approximately six months of age. The transfer of the immunoglobulins is dependent on factors such as gestational age of the foetus, the level of maternal IgG (mostly IgG1) and placenta integrity (88).

A reduction in transplacental transfer of maternal antibodies from mother to new-borns has been observed in HEU infants compared with HUU infants in some, but not in all studies (89-91). This reduction was linked to factors including placental inflammation, which compromises the placental integrity (92, 93). Reduction in transplacental transfer of antibodies could be partly responsible for the increased susceptibility of HEU children to infectious diseases during early infancy. Furthermore, lower maternal IgG antibody levels in women living with HIV compared with HIV-uninfected women, could also contribute to reduced antibody levels acquired by HEU new-borns. Reductions in antibody levels in women living with HIV-1 may be due to a reduced ability to respond to an antigen, or loss of memory recall to the antigens. In South Africa, HEU children at birth reportedly have lower maternally derived antibodies against measles, *H. influenzae* type b, *S. pneumoniae, B. pertussis*, tetanus toxoid, and hepatitis B surface antigen compared with HUU infants (94, 95). Similarly a study from Brazil reported differences in levels of transplacental acquired antibodies to different viruses (Herpes Simplex, Varicella-Zoster, and measles virus), as well as tetanus toxoid in HEU compared with HUU new-borns. (96). Women living with HIV had higher IgG levels to Varicella-Zoster virus compared with the HIV uninfected women, albeit no differences in other antigens. The transplacental transfer of antibodies to Varicella-Zoster virus*, tetanus toxoid, measles, streptolysin 0*, and *S. pneumoniae* was reduced in new-borns of women living with HIV-1 compared with HUU infants.

In contrast to the above results, a study in Malawi showed that the different antibody levels in pregnant HIV infected and uninfected women did not influence the antibody levels in their new-borns (97). In Botswana, a study on the transfer of maternal antibodies by breast-feeding reported that there was no difference in the immunologic profile of breast milk (IgA) of women living with and without HIV, however, the mortality rates of CLWH at two years was four times higher compared with HEU children (98).

1.5.1 hMPV and maternal antibody transfer

Serological evidence of hMPV infection has been found in children less than five years old (34, 51, 99). Wolf et al. used serological analysis in healthy Israeli children during their first two years of life to study exposure to hMPV (100). They reported that at the age of two months, 80% of the children had anti-hMPV IgG antibodies. By the age of seven and 13 months the IgG levels had declined by 40% and 30% respectively. New hMPV infections

were detected in 13% at seven months, 23% at 13 months and 55% at 24 months of age. These results suggested that waning antibody levels may be associated with repeat hMPV infections in children.

A reduction in transplacental transfer of maternal IgG antibodies of a related respiratory pathogen, RSV, has been observed in HEU compared with HUU children (89, 101).

There is, however, currently no data on transplacental transfer of anti-hMPV antibodies from mother to child and evaluation of whether differences exist between HEU and HUU children. A reduction in transplacental transfer of antibodies could increase the susceptibility of HEU children to hMPV-associated LRTI hospitalisation during early infancy (26).

1.6 Pulmonary function sequelae

1.6.1 Factors affecting pulmonary function

Genetic and environmental factors, as well as viral infections are all associated with pulmonary function. Environmental risk factors that have been associated with impaired lung function during infancy are smoking, household heating, certain building materials such as asbestos, and mode of cooking, such as wood burning fires. These can all affect the structure of the smaller airways, causing chronic airway obstruction and a decline in pulmonary function (102, 103).

1.6.1.1 Pulmonary function sequelae after LRTI

LRTIs are associated with clinical long-term pulmonary sequelae. Gray et al. examined the lung function in six week old infants and one-year-old children, with or without previous LRTI. Children who had experienced LRTI had significant increased respiratory rate, decreased tidal volume and increased lung clearance index compared with those without previous LRTI (104). These results suggest that lower respiratory tract illnesses acquired within the first year of life impair lung function. It has also been demonstrated that infants exposed to intra-uterine tobacco smoke had significantly lower tidal volumes and higher lung clearance index compared with children whose mothers never smoked. In addition children born to women living with HIV had higher tidal volumes compared with children born to HIV negative

mothers (1.7 mL [0.06 to 3.3] p=0.04) (105). These findings suggest that other environmental factors contribute to a decline in lung function.

A systematic review on long-term sequelae of pneumonia in children under five years, reported that the risk of at least one major sequela was higher in hospitalised children (13.6% 95% confidence interval (95% CI) 6.2–21.1%) compared with non-hospitalised children (5.5% 95% CI 2.8–8.3%) (106). Furthermore, adenovirus pneumonia had the highest risk (54.8% 95% CI 39.2–70.5%) for major sequelae compared to other pathogens studied. Studies investigating the risk of impaired lung function post-RSV LRTI using pulmonary function tests have shown evidence of small airway obstruction in most cases (107-109).

1.6.1.2 Pulmonary function sequelae after hMPV LRTI

The impact of hMPV on lung function has not yet fully elucidated. Coverstone et al. reported that hMPV LRTI in early life predisposed to an increased risk of subsequent wheezing events later in life, however, the authors did not find an increased risk of asthma following hMPV LRTI in early life (hMPV 5/38 vs controls 6/30, p 0.44) (110). The number of participants with asthma in both groups were low. In another study the authors showed that maternal asthma increased the risk for more severe disease in children less than two years old admitted for severe hMPV LRTI (OR 4.72; 95% CI, 1.39–16.01) (111) (56). Broughton et al. showed that children born prematurely who develop hMPV LRTI were more likely to have high airway resistance at one year follow up [154]. The number of hMPV cases (four cases) was low, although the results were similar to children with RSV from the same study. The lung function results of this study were further confounded by prematurity, as the lungs at that stage are not fully developed.

1.6.1.3 Animal studies of hMPV lung function sequelae

Although there is no animal model that precisely resembles the anatomy of a human being and how it responds to diseases, animal models such as rodents and non-human primates have been used to study the physiological effect of various pathogens. The use of pulmonary function techniques in rodents have been shown to provide important and reliable information on the alteration of pulmonary function during respiratory pathology [155, 156].

A study conducted on an animal model by Bao et al. showed evidence that alveolar epithelial cells infected with hMPV secrete increased levels of cytokines and chemokines which trigger the pulmonary inflammatory cascade that leads to significant obstructive disease of the airways (112). A similar response of long-term pulmonary inflammation was also seen in mice after being infected with hMPV; the mice developed significant obstructive airways disease (113, 114). The results of these studies strongly suggest that hMPV infection induces lung function abnormalities in rodents.

1.6.1.4 General introduction to pulmonary function testing in children

Lung growth and development continues after birth throughout childhood (128). Paediatric lung function tests can be used to assess and monitor the impact of various respiratory infections in children. Spirometry is the most commonly used technique for the determination of pulmonary function in adults and children over six years of age, however, due to the complexity of proper performance of spirometry, it is not suitable for preschool children. In infancy and the preschool age group, the ideal PFT are ones that require minimal patient cooperation. This includes the use of forced oscillation techniques (FOT), tidal breath flow-volume loop (TBFVL) measurements and multiple breath washout technique (MBW) (129). Infant PFT can confirm airway obstruction and other abnormal physiological patterns, including measurement of lung volume and its components (total lung capacity (TLC), residual volume (RV), and functional residual capacity (FRC)), which is vital in the assessment of lung function and used in the diagnosis of lung function abnormalities (123, 125, 129).

1.6.2 Specific infant pulmonary function testing

1.6.2.1 Tidal breath flow-volume loops

TBFVL measures multiple air flow and volume indices, including inspiratory and expiratory time, time to peak tidal expiratory flow (tPTEF), volume at peak tidal expiratory flow (VPTEF) and expired tidal volume (VE). It can be used to predict pathology, severity of the disease and monitor disease progression in children (115, 116).

1.6.2.2 Multiple breath wash-out technique

MBW is used to evaluate the distribution of ventilation and perfusion in the lungs, through the LCI and to measure FRC (117). The LCI is a sensitive marker to assess ventilation distribution inhomogeneity in the lungs and is able to identify pathology caused by inflammation or obstructive airways disease (118-120). The FRC measures the volume of air in the lungs after passive expiration and is used as a measurement of air-trapping, which signifies small airway disease (121, 122). In previous studies, the inert gases, nitric oxide (NO), Helium (He), Argon (Ar) and Sulphur hexafluoride (SF_6) have been used for monitoring lung function outcomes (123-126). Recently LCI has gained momentum in epidemiological studies and has been shown to be a sensitive marker of lung function abnormalities (127, 128).

1.7 Justification and objectives

The contribution of hMPV to LRTI morbidity and mortality has not been systematically reviewed in children in African countries. There are still sparse data on the epidemiology of hMPV especially in African children. This is particularly true for settings with a high prevalence of *in utero* HIV-1 exposure, such as Sub-Saharan Africa. We therefore conducted a systematic review and meta-analysis to evaluate the epidemiology of hMPV-associated LRTI in African children under 5 years of age, as well as a study describing hMPV epidemiology in Soweto, South Africa, to estimate the incidence and clinical course of hMPV-associated LRTI.

Furthermore, there is little information on the role of HIV status on maternal hMPV antibody transfer to their infants. We conducted a study on hMPV antibody transfer from mothers with and without HIV infection to their children. There is currently no available information on transplacental transfer of anti-hMPV neutralizing antibodies from mother to child or comparisons of the level of antibodies in children from mothers with vs without HIV infection. Therefore, in this thesis we determined the effect of HIV status on hMPV antibody status in women and their infants. In our study we used neutralization assay to determine the level of anti-hMPV antibodies, which provides a more robust estimate of putative protection when

compared to other methods like ELISA, which usually measure both protective and non-protective antibodies.

Thirdly, there is a dearth of information on the pulmonary sequelae of hMPV-associated LRTI hospitalisation. We performed a lung function study on the pulmonary sequelae of hMPV-associated LRTI infections in one and two year old children. The use of lung function techniques in children who have recovered from LRTI can help to effectively monitor pulmonary changes. This approach is ideal for LMICs, where there is a general paucity of data regarding childhood respiratory morbidity and mortality. To our knowledge, there is no longitudinal study that has assessed the impact of hMPV on clinical and measured lung function, or which has assessed lung function at two time points after an hMPV infection. Importantly, we used robust measurements of the infant PFT to assess the pulmonary sequelae following hMPV Infection in the first two years of life, as well as a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire for the clinical aspects relating to presence of asthma, wheezing and atopic features in the past year.

All of these aspects of our study would contribute to the knowledge of hMPV epidemiology, prevention, and sequelae, and therefore stimulate further research into this subject, with regards to treatment and vaccination for hMPV, especially in LMICs.

1.8 Study objectives

1. To conduct a systematic review and meta-analysis of the epidemiology of hMPV-associated LRTI in African children less than five years of age.
2. To determine the incidence and clinical course of hMPV-associated acute LRTI in South African children less than five years of age.
3. To determine the influence of maternal HIV-1 status on the transplacental transfer of hMPV neutralising antibodies.
4. To describe the pulmonary sequelae of children hospitalised with hMPV-associated LRTI during the first two years of life.

Chapter Two: Methods

2.1 Epidemiology of Human metapneumovirus-associated lower respiratory tract infections in African children: Systematic review and meta-analysis

2.1.1 Search strategy

The systematic review was conducted according to the Preferred Reporting Items for Systematic and Meta-Analyses (PRISMA) guidelines. We searched Science Direct, PubMed, Cochrane Central, Scopus and the WHO regional database using the terms “("Human metapneumovirus" AND "Africa") OR ("hMPV" and "Africa")” and included all identified publications through to 17 September 2020 (Table 2.1). ClinicalTrials.gov was reviewed for identification of any unpublished trials. There were no limitations to the language or year of publication. The protocol was registered under PROSPERO CRD42018114131.

2.1.2 Inclusion and exclusion criteria

This review included studies involving children less than five years of age with LRTI hospitalisation, SARI or clinically diagnosed severe pneumonia in the community. Eligible study designs were randomised and non-randomised control studies, prospective and retrospective cohort studies, cross sectional and case-control studies. We only included studies conducted over at least a full calendar year. Excluded from the analyses were systematic reviews, case reports and series, narrative reviews, editorials and animal studies. In instances of multiple publications of the same study, the most comprehensive publication was retained. The Human Research Ethics Committee of the University of the Witwatersrand waived this systematic review (Reference number: W-CBP-190218-2).

2.1.3 Data extraction and management

Two reviewers (LR and EM) independently screened the titles and abstracts of the studies and assessed their eligibility. Full text of eligible studies was obtained and disagreement on eligibility was resolved between the two reviewers. Data were extracted using standardised data extraction forms, including information on hMPV incidence and prevalence, outcomes, study design, study duration, sample size, location, coinfection with other respiratory infections, method of hMPV detection, participant characteristics and study quality. The two reviewers independently extracted data from 10/74 (13.5%) of eligible studies. After

achieving good concordance (>80%), the remainder was extracted by one reviewer (LR). In case of lack of clarity during extraction, the second reviewer (EM) was consulted to discuss and resolve issues.

Table 2.1: hMPV systematic review search strategy

	Science direct search: 17-09-2020	Search Details	Number of results
#1	hMPV	(hMPV AND Africa) OR (Human metapneumovirus AND Africa)	790

	PubMed search: 17-09-2020	Search Details	Number of results
#1	hMPV	(hMPV [All Fields] AND ("Africa"[MeSH Terms] OR "Africa"[All Fields])) OR (hMPV [All Fields] AND "Africa"[MeSH Terms]) OR (((human metapneumovirus[All Fields] AND ("Africa"[MeSH Terms] OR "Africa"[All Fields]))) OR (human metapneumovirus[All Fields] AND "Africa"[MeSH Terms])))	69

	The Cochrane Library search: 17-09-2020	Search Details	Number of results

#1	hMPV	("Human metapneumovirus" AND "Africa") OR ("hMPV " and "Africa")	5

	WHO regional database: 17-09-2020	Search Details	Number of results
#1	hMPV	(human metapneumovirus AND Africa) OR (hMPV AND Africa)	34

	Scopus: 17-09-2020	Search Details	Number of results
#1	hMPV	(human metapneumovirus AND Africa) OR (hMPV AND Africa)	576

Table 2.2: Characteristics of studies included in the systematic review

Author (year)	Date form completed	Report title (title of paper)	Country	Publication type	Study funding source	Type of study	Participants
PERCH group, 2019	2020/09/23	Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study.	Bangladesh, The Gambia, Kenya, Mali, South Africa, Thailand, and Zambia.	Journal article	Bill & Melinda Gates Foundation	Case-control	Cases were children aged 1–59 months admitted to hospital with severe pneumonia. Controls were age-group-matched children randomly selected from communities surrounding study sites.
El Basha, 2019	2020/09/22	Prematurity, a significant predictor for worse outcome in viral bronchiolitis: a comparative study in infancy.	Egypt	Journal article	This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.	Cross-sectional study	Infants in the first year of life with clinical diagnosis of bronchiolitis
Jallow, 2019	2020/09/22	Epidemiological, clinical and genotypic features of human Metapneumovirus inpatients with influenza-like illness in Senegal, 2012 to 2016.	Senegal	Journal article	This study was supported by the US Department of Human Health services by grant number IDSEP140020–01-	Cross-sectional study	Outpatients presenting to healthcare sentinel sites were screened for Influenza-like Illness (ILI) surveillance.

					00 via the International division of Pasteur Institutes. These funds enabled the setup of the sentinel surveillance network for influenza in Senegal.		
Oketch, 2019	2020/09/22	Human metapneumovirus prevalence and patterns of subgroup persistence identified through surveillance of paediatric pneumonia hospital admissions in coastal Kenya, 2007–2016.	Kenya	Journal article	This study was supported by the Welcome Trust, United Kingdom (grants102975, 100542, 084633, and 077092). The funder had no role in other aspects of the study including its design, data collection, data analysis, data	Cross-sectional study	Children (≤59 months of age) admitted to the paediatric ward between January 1st 2007 and December 31st 2016, were eligible if they presented with modified WHO defined syndromic severe or very severe pneumonia as previously described.

					interpretation, or writing of this man.		
Subramoney, 2018	2020/09/22	Human bocavirus, coronavirus, and polyomavirus detected among patients hospitalised with severe acute respiratory illness in South Africa, 2012 to 2013.	South Africa	Journal article	National Institute for Communicable Diseases of the National Health Laboratory Service and the US Centres for Disease Control and Prevention, Grant/Award Number: 5U51IP000155.	Cross-sectional study	Study participants were enrolled in prospective, hospital-based SARI surveillance, from January 2012 through December 2013.
Zar, 2016	2018/11/24	Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: a nested case-control study of the Drakenstein Child Health Study.	South Africa	Journal article	Bill & Melinda Gates Foundation, Medical Research Council South Africa, National Research Foundation South Africa, National Institute of Health, and H3Africa	Case-control study	Signs of respiratory infections.

Zash, 2016	2018/11/24	The aetiology of diarrhoea, pneumonia and respiratory colonization of HIV-exposed infants randomized to breast- or formula- feeding.	Botswana	Journal article	None	Cross-sectional study	Children enrolled in the Mashi study met the WHO definition for pneumonia or were diagnosed with pneumonia by a health-care provider. 2) HIV-exposed infants who presented with acute diarrhoeal illness in a diarrhoeal disease sub-study.
O'Callaghan-Gordo, 2011	2018/11/22	Viral acute respiratory infections among infants visited in a rural area.	Mozambique	Journal article	Health Research Foundation of the Spanish Ministry of Science. Spanish Agency of International Cooperation.	Cross-sectional study	All infants younger than 12 months who visited the OPD of MDH with signs and/or symptoms of Acute lower respiratory tract infection.
Owor, 2016	2018/11/22	Human metapneumovirus epidemiological and evolutionary patterns in Coastal Kenya, 2007-11.	Kenya	Journal article	Welcome Trust, UK [084633, 102975, 077092]	Cross-sectional study	
Pretorius, 2012	2018/11/22	Respiratory Viral Coinfections Identified by a10-Plex Real-Time Reverse-Transcription Polymerase Chain Reaction Assay in Patients	South Africa	Journal article	Co-operative agreement (5U51/IP000155) with the CDC.	Cross-sectional study	

		Hospitalized With Severe Acute Respiratory Illness—South Africa, 2009–2010.					
Pretorius, 2016	2018/11/22	The role of influenza, RSV and other common respiratory viruses in severe acute respiratory infections and influenza-like illness in a population with a high HIV sero-prevalence ,South Africa 2012–2015.	South Africa	Journal article	Co-operative agreement 5U51/IP000155with The Centers for Disease Control and Prevention, Atlanta, Georgia, USA.		

Table 2.3: Results from studies included in systematic review

Author (year)	Type of sample tested	Assay to measure hMPV cases(full details for coinfections)	Definition LRTI/SARI/Pneumonia/bronchiolitis	Total hMPV cases n/N (%)	hMPV incidence per 100,000/1000/100 (95%CI)	Seasonal distribution of hMPV
Ahmed, 2012	NP and OP swabs	RT-PCR	For children > 1 week and < 2 months old, SARI was defined as an admission to the paediatric ward with any of the following: respiratory rate > 60 per minute, severe chest indrawing, nasal flaring, grunting, fever≥38°C,hypothermia < 35.5°C, or pulse oxygenation < 90%. For children 2 months to < 5 years of age, SARI was defined as cough or difficulty breathing and any one of the following: respiratory rate > 50/min for infants 2 months to < 1 year old or > 40/min for children 1 to < 5 years old, chest indrawing or stridor in a	266/4449 (6%)	<1 yr: 10.7 (7.9-14.7); 1-5 yrs 2.1 (1.5-2.9); <5 yrs 3.6 (2.9-4.5) per 1000 children per year.	November and December

			calm child, unable to drink or breast feed, vomiting, convulsions, lethargic or unconscious, or pulse oxygen saturation < 90%. For older children and adults≥5 years of age, SARI was defined as fever≥38°C, and cough or sore throat, and shortness of breath or difficulty breathing. Hospitalisation was a required part of the SARI case definition in all ages. Definitions for ILI and SARI were adapted from those of the WHO.			
Amin, 2013	Swabs	RT-PCR	SALRTI: presence of fever at the onset of the disease and/or current fever or hypothermia, together with at least one of the following symptoms: cough, tachypnoea, sputum, haemoptysis, chest pain, sore throat, and shortness of breath. The presence of working ala nasi, intercostal	14/130 (10.8%)	NR	During late winter through spring months and it is the only virus isolated in May (Feb - May).

			retraction, grunting, inability to drink or breastfeed, persistent vomiting, lethargy, or convulsion was taken as evidence of severe acute lower respiratory tract infection.			
Annamalay, 2016 rhinovirus	NPA	Identification of common respiratory viruses (adenovirus, RSV, bocavirus, coronavirus, parainfluenza viruses, influenza viruses and metapneumovirus) was carried out using two independent multiplex RT-PCR assays	Pneumonia: increased respiratory rate and cough and/or difficulty in breathing (WHO).	25/237 (10.5%) HIV uninfected; 3/38 (8); total 28/275 (10%).	NR	NR
Annamalay, 2016 respiratory virus	NPA	RT-PCR	Pneumonia: respiratory distress and either chest X-ray changes (e.g. consolidation or effusion) or auscultatory findings (e.g. crepitations or bronchial breathing); bronchiolitis: children with respiratory distress and at least one of the following:	Total: 7/105 (6.7%); Pneumonia 5/57 (8.8%)	-	Autumn (March–May) and winter(June–August)

			wheeze, chest-x ray changes (e.g. Hyperinflation) or Hoover's sign (inward movement of the lower ribcage during inspiration).			
Bennet, 2017 microorganisms	NP swabs, blood	Nasopharyngeal swabs were collected using Flexible mini tip flocked swabs. Bacteria were detected using reverse-transcription polymerase chain reaction (RT-PCR; Fast-Track Diagnostics, Respiratory Pathogens 21 Plus, Luxembourg. Whole blood were subjected to blood counts, blood culture, RT-PCR to identify Staphylococcus aureus and S. pneumoniae and Hib, serum C-reactive protein (CRP), and procalcitonin (PCT); pleural effusions were processed as respiratory specimens.	Pneumonia: (1) hospitalized patients aged 2–60 months; (2) presenting with cough and/or dyspnoea; (3) with tachypnoea, as defined by the World Health Organization breathing rate ≥50 cycles/minute at 2–12 months of age; ≥40 cycles/minute at 12–59 months of age); (4) first symptoms appearing in the last 14 days; (5) radiological confirmation of primary end-point pneumonia following WHO guidelines; and (6) informed consent statement signed by parents or legal guardian. Primary end-point pneumonia was confirmed by at least 1 medical reader at	Madagascar cases 7/80 (8.7%) controls 1/60 (1.6%); Mali cases 12/118 (10.2%) controls 1/93 (1.1%).		Nr

			each study site as the presence of end-point consolidation on chest radiograph, or pleural effusion in lateral pleural space associated with pulmonary parenchymal infiltrate, or effusion large enough to obscure such opacity.			
Bennet, 2017 severity of pneumonia	NP swabs/aspirates	RT-PCR	Pneumonia: Cough and/or dyspnoea. Tachypnoea, as delineated by the WHO (in children 2–12 months of age: breathing rate 50 cycles per minute; in children 12–59 months of age: breathing rate 40 cycles per minute). First symptoms appearing within the last 14 days. Radiological confirmation of pneumonia as per WHO guidelines, including primary endpoint pneumonia or other infiltrates.	Hypoxic pneumonia 10/70 (14.3%); non-hypoxic pneumonia 23/335 (6.9%).	NR	
Bennet, 2015	NP swabs, blood	RT-PCR	Pneumonia: - Cough and/or dyspnoea, and- Tachypnoea,	12/118 (10.2%) in pneumonia cases;		Jan to December

			as characterized by the World Health Organization (WHO) in children between 2 and 12 months of age: breathing rate 50 cycles per minute; in children between 12 and 59 months of age: breathing rate 40 cycles per minute, and- Absence of wheezing at auscultation, and- first symptoms appearing within the last 14 days, and- Radiological confirmation of pneumonia as per WHO guidelines.	1/98 (1%) in controls		
Berkley, 2010	NP wash	RT-PCR	'Severe pneumonia' (cough or difficult breathing plus lower chest wall indrawing and no signs of 'very severe pneumonia') or 'very severe pneumonia' (cough or difficult breathing plus at least one of: hypoxia, defined as an oxygen saturation <90% by fingertip pulse oximetry (Nellcor, USA), inability to drink or breastfeed,	23/759(3)	0.20 per 1000 Live Births, Aged 28 days <13yr:21, <1 yr: 138, 1-2 yr:58, 2-5 yr:11, <5 yr: 44, 5-12 yr:6 per 100,000	

			inability to sit or impaired consciousness) at admission, 17 including infants aged <2months.			
Breiman, 2015	NP and OP swabs	RT-PCR	For SARI for children <5 years old used a modification of the WHO Integrated Management of Childhood Illness algorithm for severe and very severe pneumonia, defined as a child with cough or difficulty breathing and any of the following symptoms or signs: unable to drink/breast-feed, vomits everything, convulsions, lethargic or un-conscious, stridor when calm, and lower chest wall indrawing, as well as an additional criterion of oxygen saturation <90%.	97/815(11.9)	0.9 (0.3-1.6) per 100 000	

Abbreviations: Nasopharyngeal aspirates;NP, nasopharyngeal; NR, not reported; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; CRP, C-reactive protein; PCT, procalcitonin; ALRI, Acute lower respiratory infection; SALRTI, Severe acute lower respiratory tract infection; SARI, Severe acute respiratory illness; WHO, World health organisation; RSV, respiratory syncytial virus; ILI, Influenza like illness; LRTI, Lower respiratory tract infection.

2.1.4 Data analysis

For the analysis of pooled prevalence and case fatality risk (CFR), point estimates from individual studies were combined using a random-effects model in case of significant heterogeneity (I2 > 50%) and 95% CIs were reported. Analyses were performed using R statistical software, version 3.6.0.

2.1.5 Quality assessment

The risk of bias was assessed using the Newcastle-Ottawa Scale, grading each study using predefined criteria on a 0–9 score (0 = high risk, 9 = low risk) (129). The overall quality of evidence was evaluated using the AMSTAR tool 2. AMSTAR is a validated tool which rates studies on 16 items and scores the overall results as critically low, low, moderate, or high quality (130).

2.2 Human metapneumovirus-associated hospitalisation in HIV-1 exposed uninfected and HIV-1 uninfected children under five years of age in South Africa

2.2.1 Study design

Study site and population

Chris Hani Baragwanath Academic Hospital (CHBAH) is a large tertiary public hospital that serves the communities of Soweto and the peri-urban areas south of Soweto (census regions D + G of Johannesburg) (131).

2.2.2 Parent study

The BoSS study was undertaken at CHBAH in Soweto, South Africa between 2014 and 2018. Children under five years of age hospitalised with symptoms consistent with either lower respiratory tract infection (LRTI) or diarrhoea, as well as infants under three months with suspected sepsis, were enrolled. Children admitted directly to CHBAH intensive care unit prior to admission to the general ward, and neonates who were never discharged home after birth were excluded. The primary aim of the BoSS surveillance was to characterize the aetiology of LRTI hospitalisation in children less than five years, with specific focus on hMPV, respiratory syncytial virus (RSV), influenza A/B and *B. pertussis*, the identification of which in hospitalised children is strongly associated with disease status (27). In this analysis we specifically focused on the epidemiology of hMPV-associated hospitalisation.

A BoSS research nurse reviewed patient files for the diagnosis during the acute admission. During acute admission, a child would first be assessed in the paediatric admissions ward. If a child qualified for admission, they were sent to one of the four paediatric wards, or to the paediatric overnight ward, if the predicted stay was to be less than three days duration. Research assistants based at the paediatric wards recruited suitable patients, based on the inclusion criteria described above, at these five wards. For children less than 12 months enrolment was done every day. For children more than 12 months enrolment was done every second day. Enrolment occurred during weekdays, weekends and holidays from 2014 until August 2016. After that, enrolment was done during weekdays only (Monday through to Friday).

Parents or legal guardians of identified cases were approached and informed about the surveillance study. If the parent or legal guardian agreed that the child participates in the study, a written informed consent was obtained from the caretakers. Furthermore, a research assistant verbally administered a study questionnaire to the parent or legal guardian with information regarding sociodemographic history, medical history of the case, current admission details, laboratory results from admission, including HIV result, maternal HIV status and particulars of treatment during current admission. In brief, the demographic form had details of both the mother and infant such as name, date of birth, hospital number and contact details. The medical history of child form included information like birth history of the child, prior hospitalisations, HIV status and ART treatment, if applicable, and other medications such as antibiotics or corticosteroids. The patient's hospital file was also reviewed to obtain additional information. The information was collected using the surveillance study case report forms (CRF) which were compiled in a BoSS study file together with copies of the hospital file for auditing and quality control purposes. The BoSS study also kept the patient logs to capture information such as patient's details, clinical diagnosis, enrolment status etc. and this information was periodically reviewed. The patient's missing data was retrieved from the National Health Laboratory Service (NHLS) records and included in the patient's file. Further information such as discharge outcome data were collected at a later stage and added to the patient's file.

2.2.3 Specimen collection and testing

Nasopharyngeal swabs and/or aspirates were extracted for total nucleic acid content and tested for hMPV, RSV types A and B, Influenza types A and B and *B. pertussis* (132) using

a 1-step reverse transcriptase/polymerase chain reaction assay (ThermoFisher Scientific, City and Country where registered).

The HIV-1 status and exposure status (i.e. maternal HIV-1 status) of individual study participants identified with hMPV-associated hospitalisation were taken from the BoSS database, which captured verbal responses from the mother during the study interview, and from the CHBAH clinician's notes. HIV-1 testing was performed routinely on children admitted to CHBAH pediatric wards, and we captured the result if it was included in the CHBAH clinician's notes. If the HIV-1 exposure status conflicted between the BoSS database and the CHBAH clinician's notes (20/183, 10.9%), data from the CHBAH clinician's notes were accepted. For children in the BoSS database with no HIV-1 exposure status recorded (6/183, 3.3%), it was assumed that the maternal HIV-1 prevalence was the same as those tested (by age group and year).

2.2.4 Inclusion into present study

The cases included in this study were children less than five years of age admitted to CHBAH, enrolled in the BoSS surveillance, and diagnosed with laboratory confirmed hMPV-associated LRTI between 01 January 2015 and 31 December 2017.

The BoSS study records for each participant were coded with a unique study ID, therefore the patient's information from different BoSS records was merged into the main STATA file through a common study ID. The data management for our study was handled by the BoSS surveillance study. The information at our disposal was protected with passwords and data was backed up daily into the RMPRU central server.

2.2.5 Exclusion criteria

We excluded children older than five years, those who were admitted directly to CHBAH ICU without ward admission, infants who were not discharged home after delivery and children with parents or legal guardians who declined to participate in the study.

2.2.6 Case definition

Children aged less than five years with physician-diagnosed acute LRTI which included bronchiolitis, pneumonia, bronchitis and pleural effusion were included in the study, if they presented within seven days of the onset of the illness. Infants aged less than three months were enrolled if they were diagnosed by a physician with suspected sepsis, regardless of signs and symptoms, because LRTI can present non-specifically in this age group.

2.2.7 Ethics approval

The BoSS study was approved by the Human Research Ethics Committee of the University of the Witwatersrand Faculty of Health Sciences (M140904). The present study was approved by Human Research Ethics Committee of the University of the Witwatersrand Faculty of Health Sciences (M161149). Written informed consent was obtained from caretakers for inclusion in the study.

2.2.8 Estimation of incidence of hMPV-associated hospitalization

The incidence for hMPV-associated LRTI per 100,000 persons in Soweto was stratified by age groups (<6 months, 6- <12 months, 12- <60 months) and HIV-1 infection and exposure status.

In the numerator we adjusted for days when enrolment did not occur, such as weekends, and according to our recruitment scheme. We also adjusted for eligible cases who met the enrolment criteria, but whose legal guardians refused consent or were unavailable to provide. We further adjusted for children not recruited due to admission to other public hospitals in the catchment area by using the administrative database of admissions at these hospitals, which included age and diagnosis; and assumed similar positivity rates of hMPV as for children admitted to CHBAH. We assumed that 10% of the cases may have been admitted at a private hospital facility (132), and upwardly adjusted the incidence estimate by the same margin.

We estimated the size of the relevant population at risk (i.e. the denominator) on the population size taken from the Department of Health midyear census data estimates of Region D and G. This approximately covers the catchment area for CHBAH. Population size estimates for 2017 were unavailable and extrapolated based on population size from five years prior using the best fit model of several tested, which was a fourth-order polynomial fit (133). Population size estimates for 0-<12 months and 12-59 months of age were taken from published estimates from midyear population estimates from Statistics South Africa based upon census data (134). The proportion for HIV-1 exposed and HIV-1 unexposed infants was calculated based on HIV-1 prevalence rates from the antenatal clinic data (by year) provided by ASSA 2008 data (135). For the stratification for <6 months and 6-<12 months, published population size estimates were not available. We estimated population sizes from the 0-<12 months estimates based upon assumptions from McMorrow et al. (136).

2.2.9 Statistical analysis

We compared children with hMPV-associated hospitalisation who were HIV-1 unexposed HUU and HIV-1 exposed and uninfected HEU. Statistical analysis was performed using the STATA program version 13.1 (StataCorp, TX, USA). Proportions were compared using the χ^2 test and Fisher's exact test and continuous data were compared using the Wilcoxon rank sum test. A p value of < 0.05 was considered significant. 95% confidence intervals (95% CI) were calculated using the binomial exact method.

2.3 Fetal transfer of human metapneumovirus neutralizing antibodies is reduced from mothers living with HIV-1: Short report

2.3.1 Setting

Parent study

This study was nested within a larger cohort, in which mother-newborn dyads were prospectively enrolled from June 2014 to investigate the serologic correlates of protection against invasive Group B Streptococcal disease (ClinicalTrials.gov: NCT02215226) (137). Pregnant women were identified and enrolled in the parent study while attending antenatal clinics attached to Soweto's tertiary hospital, Chris Hani Baragwanath Academic Hospital (CHBAH), during labor or immediately post-delivery.

In brief, a total of 35,000 mother-newborn dyads were enrolled between July 2014 and December 2016. The aim of the study was to study the sero-correlate of protection from infant infection with Group B Streptococcus (GBS) anti-capsular antibody, against invasive GBS disease in newborns aged 0-6 days (early onset disease due to serotypes Ia and III) as well as among infants aged 7-90 days (late onset disease due to serotype III). The pregnant women were identified and enrolled when they were attending antenatal clinics attached to the primary participating study hospital (CHBAH), during the early stages of labour or immediately post-delivery. Six recruitment sites and one delivery site were used as enrolment sites for the cohort. The identification of cases was conducted at CHBAH, Rahima Moosa Mother and Child Hospital and Charlotte Maxeke Johannesburg Academic Hospital in South Africa. Furthermore, a follow up for 90-days after delivery was done for cases and matched controls. This occurred by telephone or through a home visit. The

additional follow up for the birth cohort was carried out at six and 12 months of age. The purpose of this was to confirm that the enrolled participants were not hospitalised without the knowledge of the involved study.

The pregnant mothers who consented to study enrolment had 10-15 millilitres of blood drawn from them and from their infants within 24 hours of delivery. The collected sera was stored at the Respiratory meningeal pathogens research unit (RMPRU) laboratory at -18 degrees Celsius or lower. The sera was also used for future immunological studies to identify other pathogen causing diseases in infants. The mothers were invited to participate in the study if they were above 18 years old regardless of gestational age and underlying medical conditions, although gestational age of >34 weeks was considered ideal. After delivery infants were excluded if they were exposed to intrapartum antibiotics, had severe congenital malformation or conditions, and had received a blood transfusion 30 days prior to delivery. Clinical information about the mother was collected using CRFs regarding demographical aspects, gestational age at enrolment, behavioural information such as smoking and douching during pregnancy, previous number of pregnancies and parities. Other information included general medical history and for HIV positive subjects: HIV status, latest CD4 count and viral load measures (obtained through standard of care) and the antiretroviral treatment status of the mother, including regimens and start dates. The infant's information encompassed demographic data such as date and time of birth, gender and race. The birth information included birth weight, head circumference, Apgar scores at 1, 5 and 10 minutes after birth and presence of congenital malformations or underlying medical conditions.

2.3.2 Inclusion into current study

For the current study, 53 mother-infant dyads in which the mother was HIV-1 negative and 53 mother-infant dyads in which the mother was living with HIV-1 were selected randomly and matched by child gender, birth weight (±50g) and gestational age (term [≥37wks] matched with term, or ±1 week if <37 weeks). The most recent HIV-1 status recorded on the antenatal card or hospital notes was taken as the final HIV-1 status. Pregnant women in South Africa are tested for HIV-1 by a point-of-contact rapid test at the first antenatal care visit, at 32 weeks and/or during admission for delivery.

2.3.3 Identification of hMPV cases in children less than six months of age

Cases of hMPV-associated hospitalisation were identified in a surveillance study, the Babies of Soweto Study (BoSS) (132), which was conducted at CHBAH from November 2014. The

BoSS study recruited children admitted to pediatric wards at CHBAH with symptoms consistent with either LRTI or diarrhea. The primary aim of the BoSS surveillance was to determine the pathogens associated with LRTI hospitalisation in children less than five years. Nasopharyngeal swabs and/or nasopharyngeal aspirates were extracted for total nucleic acid content and tested for hMPV, RSV types A and B, Influenza virus types A and B, and *B. pertussis* (IS481) (132) using a 1-step multiplex RT-PCR assay (ThermoFisher Scientific, City and country of registration).

Infants were included if they had tested positive for hMPV in the BoSS study before March 2018, were <6 months of age at the time of testing, and had a cord blood sample stored as part of the GBS study. They were each matched to a target of three control infants from the GBS study by HIV-1 exposure, gender, date of birth (within 2 weeks) and race. Matching for date of birth was intended to match for hMPV exposure, which is seasonal and varies from year to year (72).

2.3.4 Cell line, media and viruses

Vero-118 cells (138) were maintained as described previously (139). Recombinant hMPV NL/1/00 and HMPV NL/1/99 expressing green fluorescent protein (rHMPV NL/1/00-GFP and rHMPV NL/1/99-GFP) (66) were used as protypes for genotypes A and B. Neutralizing antibody titers were determined using two-fold dilutions from 1:8 (140). The reciprocal of the highest dilution in which no infected cells were observed was taken as the titer. The geometric mean of the scores for duplicate rows of dilutions was used. If infected cells were observed at the 1:8 dilution a titer of four was assigned for calculation purposes.

2.3.5 hMPV neutralising antibody assay

Neutralising antibody titres were measured according to de Graaf et al. (66) as follows: Test sera were heat inactivated for 30 min at 56°C to inactivate complement proteins. Two-fold serial dilutions starting at 1:8 of test sera were diluted in Iscove's Modified Dulbecco's Medium (IMDM) medium containing penicillin and streptomycin (pens/strep) (but no foetal bovine serum) in 96-well plates. Duplicate wells of each dilution were then incubated with 100 (Median Tissue Culture Infectious Dose) ($TCID_{50}$) of hMPV NL/1/00 and hMPV NL/1/99 for one hr at 37°C. The virus/serum mixtures were then added to Vero-118 cells and incubated for two hrs at 37°C and then the cells were washed with phosphate buffered saline (PBS). Infection medium (IMDM+PSG [Penicillin, streptomycin and glutamine] +Trypsin) was added to each well (trypsin to activate the virus). Cultures were given fresh media on

day three and analysed on day six by observation under a fluorescence microscope for detection of infected cells (Green fluorescent protein (GFP)-expressing cells). The neutralisation titre was defined as the reciprocal of the highest dilution in which no infected cells were observed. Positive and negative control sera were included in each experiment. The geometric mean of the scores for duplicate wells were used. Samples for which infected cells were observed at the 1:8 dilution were assigned a titre of 4 for calculation purposes.

2.3.6 Statistical analysis

Statistical analysis was performed using Stata 13.1 (Stata Corp, College Station, USA). The cord to mother ratio (CMR) was the hMPV neutralizing antibody titer of the infant's cord blood divided by that of the mother's blood. Titers, CMRs, maternal ages and number of previous pregnancies were compared using multilevel mixed effects linear regression with a random effect for each group of two matched dyads. Thus, in these comparisons, each woman living with HIV-1 or her child were compared directly only to the matched HIV-1 negative woman or her child. Differences in $\log_2$ values from the models were converted back to fold differences of unlogged values to describe fold differences in titer or CMR.

2.3.7 Ethical approval

The permission to conduct these studies was granted by the University of the Witwatersrand Human Research Ethics Committee (M161149). Written consent was collected from parents under the GBS birth cohort study (M140203), and under the BoSS study (M140904).

2.4 Pulmonary sequelae in children with Human metapneumovirus associated lower respiratory tract infection

2.4.1 Study design

This study was a prospective case-control study. hMPV cases were selected from an existing Babies of Soweto surveillance study (BoSS), (HREC (Med): 140904). The controls were healthy community controls identified from a previous birth cohort study V98_280B (HREC (Med): 140203) and surveillance of pathogen-specific causes of pneumonia and diarrhoea hospitalisation in children (HREC (Med): 131109).

In brief, the BoSS surveillance study was conducted at CHBAH, Soweto, South Africa. It was conducted by the RMPRU between 2014 and 2018. The BoSS surveillance study aimed to gather information on children admitted to the CHBAH paediatric wards with symptoms consistent with either acute LRTI or diarrhoea. The main aim of the BoSS surveillance study was to determine the pathogens associated with LRTI and diarrhoea hospitalisation in children less than five years.

2.4.2 Inclusion into the current study

2.4.2.1 Cases

The cases enrolled in this study were children less than two years of age that were previously admitted to CHBAH as part of the BoSS surveillance study associated with LRTI. The student identified confirmed cases of hMPV daily from the RMPRU laboratory log. Cases that tested positive for hMPV between 01 January 2016 and 31 December 2017 were enrolled into the study. After identification of hMPV cases, the patients' contact details, that included telephone number, physical address, date of birth and identifying names of the legal guardian, were extracted from the BoSS by the student.

The suitable hMPV cases that had been discharged from the hospital were invited via telephone and the purpose of the study was explained, followed by a text message to participate in the study. All the non-hospitalised hMPV cases that agreed to be part of the study were given a date to present at the hMPV PFT clinic situated at the CHBAH paediatric outpatient department. Upon participants' arrival on the given date, signed study informed consent was requested from the parent /legal guardian, and the participant was enrolled into the study. (Appendix 2). The study visit schedule was prepared as shown in Appendix 3. Each case and control were assigned a study identity (ID) to ensure the anonymity of the participant. The patient's identifiers were written on the demographics form, the form was then removed and kept in a secure location with only the main investigator having access to it. The study ID was used to identify each patient's file and CRF during the study period.

hMPV-associated LRTI cases were matched to controls at a ratio of 1:3 according to gender, age (±2months) and race. The cases and controls were tested at one year (10-18 months) for iPFTs and the final iPFTs follow up was done at two years (22-26 months) of age. The case-control matching was done according to age (~2 weeks), gender and race. Our hMPV

study protocol originally stipulated that the cases and controls will be matched by HIV exposure, gender, age, date of birth (within 2 weeks) and race, however, due to a low number of HIV exposure in the cases and control this was not possible. Furthermore, the matching according to date of birth was also not possible because hMPV testing was done within a specified time (age 10-14 months) and it was challenging to get suitable matches coming in for the tests at a given time. Hence matching was done according to age rather than date of birth.

The cases were followed up telephonically. If they were hospitalised, they were followed up three weeks after discharge from hospital. The telephonic follow up was done at six months of age (if discharged before five months of age) and a further telephonic follow up was done at 18 months of age. Refer to scheduled visits: Appendix 3. In case of failure to get a response from a legal guardian within a week, a home visit was organised through RMPRU logistic department to enrol patients from their homes. In instances where home addresses or telephone numbers had changed or were missing, a patient's demographic information from the original Boss file was pulled to cross check the contact numbers. Only the student had access to the participants' identifiers. If the patient's proper contact details could not be reached or the participant had demised, the participant was excluded from the study. The purpose of rigorous follow up was to prevent massive loss to follow up.

2.4.2.2 Controls

Controls were healthy participants, with no significant medical history, and no history of hospitalisation due to LRTI. This study shared controls with an RSV LRTI pulmonary sequelae study (HREC (Med): M160224) which was running parallel to the hMPV study. A RMPRU statistician selected the controls and performed matching. A list of suitable participants was selected from the V98_28OB database to match hMPV LRTI cases enrolled in our study. The potential controls were babies with birth weight above 2500 grams. The controls and cases were matched at a ratio of 1:3 according to gender, age (±2months) and race. Controls were also tested at one year (10-18 months) for iPFTs and the final iPFTs follow up was done at two years (22-26 months) of age. The case-control matching was done according to age (~2 weeks), gender and race. Our hMPV study protocol originally stipulated that the cases and controls will be matched by HIV exposure, gender, age, date of birth (within 2 weeks) and race, however, due to a low number of HIV exposure in the cases and control this was not possible. Furthermore, the matching according to date of

birth was also not possible because hMPV testing was done within a specified time (age 10-14 months) and it was challenging to get suitable matches coming in for the tests at a given time. Hence matching was done according to age rather than date of birth.

The controls were randomly selected using statistical software package R version 3.5.1. A total of 100 babies between ages of 10-14 months were selected towards the end of each lung function testing list. The control list included participants' details such as telephone numbers, physical address and date of birth. The parent / legal guardian of the potential control was contacted via telephone for possible enrolment into the study. The calling of controls in the list was done in a systematic order, starting from first potential control in the list going down. Each suitable participant was contacted at least ten times. In case of failure to establish contact with the potential participant, the next potential participant was contacted. The suitable controls were invited via telephone and the purpose of the study was explained, followed by a text message to participate in the study. During the participant's visit a signed study informed consent was requested from the parent /legal guardian, and the participant was enrolled into the study.

The enrolment of controls using the V98_28OB was stopped on the 31 December 2016, after the study had stopped recruiting new participants. There was no enrolment of controls from the V98_28OB into the hMPV iPFT study after 31 December 2018. Thereafter, additional community controls were enrolled from Surveillance of Severe Childhood Illness in Soweto, South Africa (HREC (Med): M140904). The study was conducted by the RMPRU and documented data of all children born at CHBAH. The information collected included the mother's name and hospital number. Additional information about the mother was accessed through the CHBAH's Medicom system. Ethical clearance was obtained for these new controls via the University of the Witwatersrand Human Ethics committee (HREC (Med): M160224). Permission to use the Medicom system was obtained through the relevant authorities at CHBAH including the database manager. A similar order of calling controls like V98_28OB was also practiced. Participants with previous admission of LRTI were excluded during telephonic enrolment. Briefly, suitable controls and their contact details were generated from Medicom system. The research assistant telephoned the parents/ legal guardians in a sequential order and each potential participant was called at least ten times before moving to the next participant.

The cases and controls' information was collected from the parent/ legal guardian of the child using CRFs tailored for hMPV study. (Appendix 4). The information was collected at the one and two year visits. The adapted International Study of Asthma and Allergies in Childhood Phase Three Core Questionnaire 1-2 years (ISAAC) was used to collect the information on the presence of asthma, wheezing and atopic features in the past year (141). (Appendix 5). The cases and controls were tested at one year (10-18 months) for iPFTs and the final iPFTs follow up was done at two years (22-26 months) of age.

2.4.3 Exclusion criteria

Children were excluded if they were older than two years and if they had a birth weight less than 2500g. Furthermore, premature children (born <37 weeks) and children with an acute or chronic medical diagnosis that includes congenital, cardiac, respiratory, neurological, renal, endocrine and primary immunological conditions were not enrolled. Children were not included in our study if they were hospitalised within three weeks of the enrolment (to exclude nosocomial infections that could subsequently cause acute sequelae of the LRTIs). Parent or legal guardian unwilling to consent for the child to be included in the study were not invited to participate in the study.

2.4.4 Summary of the hMPV iPFT CRF/Questionnaires

- Form 1 contained demographic details of both the infant and the mother. The infant's demographics entailed the name and surname, hospital admission number, study number, date of birth, gender and race. The mother's demographics were name and surname, identity document number, hospital admission number, date of birth, residential address, contact phone number, alternative phone number.
- Form 2 and 3 were consent forms for controls and hMPV cases, respectively. These forms contained information regarding purpose of the study, voluntary participation in the iPFT research study, confidentiality, the risks and possible benefits of the study and the pulmonary function procedure followed during the study. If the parent/legal guardian agreed to participate, the participant's signature and the date was obtained in the same form and filed.

- Form 4 contained hMPV iPFTs general visit details for year one and two. The information were study details such as environment settings such as weather details for the day, equipment calibration details and data recordings for the three iPFTs.
- Form 5 was used for year one and two , and was a modified version of the ISAAC form (141). It was adapted for participants in our study to collected information on the presence or absence of asthma/wheeze and atopic features over the past year. This entailed admissions for wheezing chest, nocturnal coughing episodes, sleep disturbance due to wheezing and treatment received related to wheezing. The eczema related details were appearance of an itchy rash and the age at which the rash appeared. Furthermore, the form contained medical examinations such as growth parameters e.g. weight (kg), length (cm) and head circumference (cm) and vital signs e.g. pulse rate (bpm), respiratory rate (bpm), blood pressure (mmHg), temperature (°C) and pulse oximeter reading in room air (% O_2 saturation).
- Form 6 included the medical history of a child such as birth history of the child, prior hospitalisation, HIV status and ART treatment if applicable, other medications such as antibiotics or corticosteroids, family history and environmental history pertaining to factors that could influence pulmonary function like smoking, housing material and living arrangements. Additionally, it contained Road to Health Chart (RTHC) which shows the vaccinations received by the child.

2.4.5 Infant pulmonary function test procedure

All the cases and controls that consented to being part of the study were given a date to present at the iPFT clinic situated at the CHBAH paediatric outpatient clinic. The parents/legal guardians of the enrolled participants were advised to wake the participant earlier on the day of testing and further not to allow the participant to sleep until testing time. The cases and controls were not paid for the participation in this study, however, all participants that showed up on the day regardless of whether the baby completed testing or not, were reimbursed for out-of-pocket expenses such travel costs and food for the day. The compensation amounted to R150 and this was in accordance with Good Clinical Practice guidelines.

Two days a week were allocated to testing of the hMPV cases, while other days were allocated to testing controls and sharing the clinic with the parallel study on RSV LRTI participants. The iPFT laboratory consists of a single room which does not have a

designated and conducive place for parents to slumber the baby. There was no testing done on weekends and public holidays. The operation times for iPFT testing resumed from 07:30 hrs and the closing time was 4pm.

On arrival of the patient, the anthropometric measures and vital signs were taken from the child as follows:

Weight: The weight of one year old children was recorded using a baby scale (Nagata BW-20 Baby Scale, Nagata Scale Co. (Ltd), Taiwan). The two year old children used an electronic scale (Casa electronic body weight scale, Stingray, Cape Town, South Africa). The weight of the baby was rounded to the closest 100g. Measurement was done while child was wearing very light clothing with shoes taken off.

Height /length: The height was measured in centimetres. Young children (usually <1 year) that could not stand on their own, were positioned supine on a Seca stadiometer for length, without shoes. The baby's head was supported to face upwards. For those that could stand height was measured using Telescopic stadiometer (Seca Telescopic stadiometer 220, Seca GMBH, Hamburg, Germany) in a standing position. The child was positioned in front of the vertical tape in an upright position, face facing forward, the head in mid position, arms on the side of the body with fingers and thumb in extension, and knees and toes pointing forward. In instances where the child was not cooperating, the measurement was taken when the child was sleeping on a supine position using a measuring tape from crown to heel.

After these basic measurements the parents were requested to put the babies to sleep. No medication/sedation was given to the child to help the sleeping process. The parents usually carried the babies on the back or arms while soothing them to sleep. In instances where the baby did not sleep the mother was rebooked for a future date that suited the mother. If a baby failed to sleep on three separate testing dates the baby was excluded from the study.

After the child had fallen asleep, he/she was placed gently on a firm mattress in the iPFT laboratory, in a supported supine position; the head was firmly supported in a sniffing position, on both sides using a soft blanket. The new patient was registered in the database of the two iPFT software programmes. The information entailed names, study number, date of birth, age, gender, weight, height and age. We performed two iPFTs in the following order: TBFVL, and MBW. If the baby woke up during testing further soothing attempts were

made to help the baby to sleep. If this failed, the baby was rebooked a further two times and the remaining tests were then completed on the following date. If during the following two dates baby fails to sleep and complete at least two tests, the baby was excluded from the study. Only the fully completed tests were accepted and used.

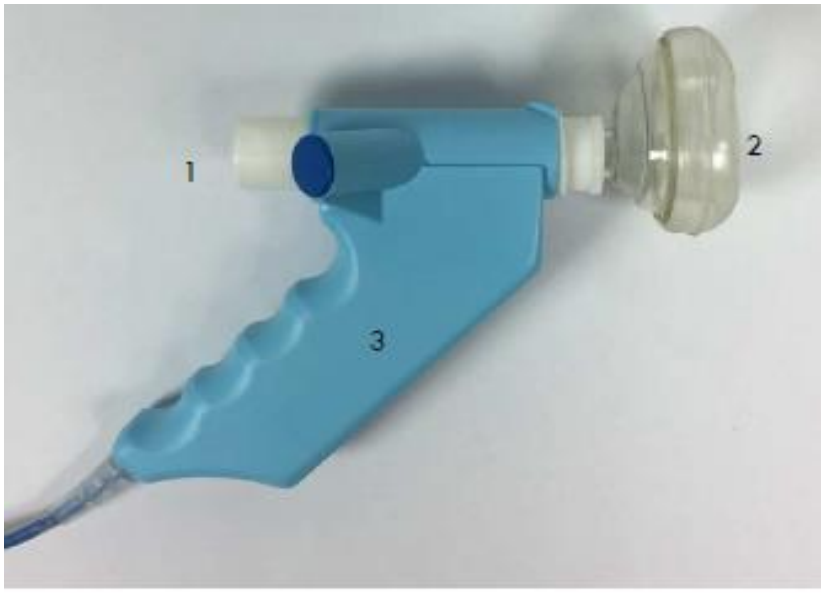
The daily testing of the participants was carried out by the student. A well-fitting face mask was placed directly over the mouth and nose of the baby during tidal breathing. The iPFT measurements were done according to the American Thoracic Society/European Respiratory Society guidelines, which ensure validity and repeatability of the test (142). Training in iPFT techniques were provided to the student by Dr C Verwey. His supervision was constantly available to ensure compliance with the operation procedures, as well as assistance with troubleshooting. Continued support was offered through a multi-national collaborative network with Professor Zoltan Hantos (University of Szeged, Hungary) and Prof Diane Gray (University of Cape Town).

TBFVL and MBW were performed and analysed using the EXHALYSER® D (Ecomedics, Duernten, Switzerland), supported by Spiroware 3.2.1 software.

2.4.6 Tidal breath-flow volume loops and multiple breath inert gas washout technique

The EXHALYSER® D contains an ultrasonic flow measuring system that measures gas flow, molecular mass; carbon dioxide (CO_2) and oxygen (O_2); and an inert gas FRC measurement module for infants and pre-school children. The EXHALYSER® D also contains a flow head which is used as the interface between the patient and the EXHALYSER® D. (Figure 2.1). The light flow head had two ultrasonic transducers which were attached at different ends of the flow channel. The purpose of the transducers was to transmit ultrasonic pulses in opposite directions; the amount of time measured during this transit determined the flow and molecular mass measurements. The sampling frequency could be adjusted to 200Hz and it was acceptable within 2% between 0–50°C.

Figure 2.1: Set-up for TBFVL



1. Dead space reducer (DSR) medium or small and spirette

2. Face mask

3. Flow head

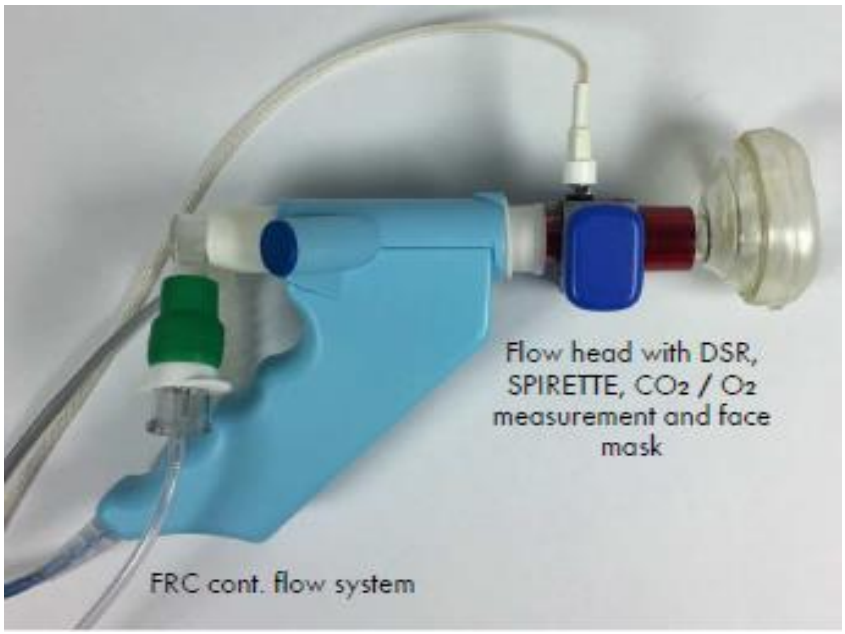
(Picture of TBFVL taken from Ecomedics Operators Manual for Pulmonary Function Testing Device – Permission to use photo)

For TBFVL measurement in our study, 100 tidal breaths were measured and each breath was acceptable if it was within ±10% of the median tidal volume. A minimum of 30 acceptable tidal breaths were necessary for analysis. Although all measurements in our study followed the ATS/ERS guidelines, this deviated from the ATS/ERS guidelines which states that in instances where 10 consecutive tidal breaths are not obtained, the operator may add segments from a different epoch to attain 10 tidal breaths for analysis.

The MBW measures the patient's FRC. (Figure 2.2). The patient breathes in a predetermined gas mixture of air and an inert gas. For MBW measurements in this study, the patient inhaled a mixture of SF_6 (4%), O_2 (21%) and N_2 (75%)(Puregas, Johannesburg, South Africa). The adjustment of gases was controlled by the SPIROWARE® software. The FRC was calculated by measuring the amount of gases inhaled and exhaled by the patient. The amount of CO_2 exhaled by the patient is detected using capnography, through a mainstream sensor that measures CO_2 directly from the patient's breathing circuit with an accuracy of 2mmHg at 0-40mmHg, 5% at 41-70mmHg, 8% at 71-100Hg and 10% at 101-150mmHg, at the sampling rate of 100Hz. The EXHALYSER® D equipment had different

weight dependent measurement set-ups for participants. We utilised set-up 1 and set-up 2, while set-up 3 catered for participants weighing more than 35kg, and as such was not applicable for the children in our study.

Figure 2.2: Set-up for MBW



Picture of FRC taken from Ecomedics Operators Manual for Pulmonary Function Testing Device (Permission to use photo)

2.4.6.1 Set-up 1 (children <15kg)

Children less than 15 kg used set-up 1. A new spirette (breathing filter) (No. M30.8042, ECOMEDICS, Switzerland or No. 2050-7, Amtronix, South Africa) was fitted to the dead space reducer (DSR) (no.M30.8001, ECOMEDICS, Switzerland). DSR comprised of a cylindrical tube with a diameter of 5mm and a resistance of <0.15cmH_2O/l/s at 0.1l/s flow and a dead space of 1.9ml. It was essential that there was perfect alignment between the spirette and the DSR. The attached spirette and DSR were inserted into the flow head of the EXHALYSER® D.

2.4.6.2 Set-up 2 (children 15-35kg)

Children weighing between 15 and 35kg used set-up 2. A spirette was fitted to a medium DSR (no. M30.8002, ECOMEDICS, Switzerland). The DRS had a diameter of 8mm with a resistance of 0.15cmH_2O/l/s at 0.5l/s flow and a dead space of 7.2ml. Similarly the alignment of the spirette and the DSR needed to be perfect. The attached spirette and DSR were inserted into the flow head of the EXHALYSER® D.

For TBFVL a well-fitting facemask (No. M30.8005, ECOMEDICS, Duernten, Switzerland or No. ANCM1512, Intersurgical, Johannesburg, South Africa) was connected to the flow head. For MBW measurement, the combination of the DSR and spirette with the flow head was connected to the CO_2 airway adapter and the Nafion^{TM} tube. These were attached to the anaesthetic face mask. A flow rate of air was set at 200ml/min and delivered to the participant.

2.4.6.3 ExD calibrations

Calibrations were conducted daily by the student using the EXHAYSER® D software. These were done prior the iPFT assessment. The calibrations were performed according to the SOP, supplied by the manufacturer. (Appendix 11). The daily environmental settings were updated with atmospheric temperature (°C), humidity (%) and barometric pressure (hectopascal (hPa)) as recorded by the ACDC dynamics weather station (No. WS1150, ACDC Dynamics, China) supplied by Eco Medics. The measurements were done according to body temperature and pressure saturated (BTPS) standards as required for optimal reporting of lung function data by the ATS/ERS guidelines (142). Temperature at the flow head of the device was manually entered as 30°C with a relative humidity of 60% and patient temperature was assumed to be 37°C with a humidity of 100%. The environment settings were updated daily. If readings were acceptable, they were saved in the EXHAYSER® D software programme and also recorded on the HMPV pulmonary function notebook. The calibrations were performed in this order: flow calibration, channel calibration, and tracer calibration.

2.4.6.4 Flow calibration

This was performed using a 100ml calibration syringe (5510 series, 100ml Cal syringe, Hans Rudolph Inc, USA). Ten simulated test breath strokes were performed by pulling the syringe back and forth, with the reading stopping automatically after the 10^{th} stroke. The EXHALYSER® D equipment had different patient weight dependent calibration set-ups for participants. The calibration syringe was connected to the DSR set-up 1 for children weighing less than 15kg or DSR set-up 2 for children weighing more than 15kg. The readings were acceptable, if the volume offset was within 1% (less than 1.00ml) of the total volume over an average of 10 breaths. If readings were not acceptable, the calibration was repeated until acceptable standards were recorded. The calibration syringe was removed from the flow sensor after the completion of the procedure. The accepted readings were saved in the EXHALYSER® D software, recorded in the hMPV notebook and recorded in the patient data files.

2.4.6.5 Channel calibration

Before starting the channel calibration, the EXHALYSER® D was supplied with medical oxygen at a concentration of 100%. Oxygen calibration was done twice, first at a standard atmospheric O_2 percentage of 20.94% and secondly at a concentration of 100%. The calibrations were acceptable if oxygen range read within 1% of the supplied concentrations. The acceptable readings were saved in the EXHALYSER® D software, recorded in the hMPV notebook and recorded in the patient data files.

2.4.6.6 Tracer calibration

The SF_6 concentration calibration was measured using millimoles (mmol). The lower and higher SF_6 concentrations used for calibrations were 28.94mmol and 35.22mmol respectively. The calibration was started and tests were acceptable if SF_6 readings were within <1% of the supplied SF_6 concentrations. The acceptable readings were saved in the EXHALYSER® D software, recorded in the hMPV notebook and recorded in the patient data files.

After calibration was completed, TBFVL would be performed, followed by MBW. The new patient was registered in the SPIROWARE® software programme. The information entailed names, study number, date of birth, age, gender, weight, height and age. A new spirette was fitted to the fresh DSR and a well-fitting facemask size was connected to the flow sensor. Hundred consecutive tidal breaths were measured with a minimum of 30 non-consecutive acceptable tidal breaths required for a successful test and for subsequent analysis. If the test was acceptable, it was saved in the EXHALYSER® D software file under the patient's study ID number.

For MBW, the CO_2 adaptor with the Nafion^{TM} tube was connected to the flow head and the mask. A tight fitting mask was placed over the child's nose and mouth to prevent air leak. The low flow bypass connection was attached to the rear of the flow head. The medical air flow (bias flow) was supplied at a rate of 200ml/s through the low flow bypass to the participant.

This technique requires stable tidal breathing during the test. After starting the test, the initial recording was a pre-test phase of regular tidal breaths. Once the software noted stable tidal breaths the wash-in phase of the test would initiate the supply of the tracer gas (SF_6) to the participant. The supply of SF_6 stopped after equilibrium of inspiratory and expiratory breaths was reached (4% SF_6 concentration). An additional five breaths were required before the wash-out phase would initiate. In this phase SF_6 would be washed out using the medical air until the final concentration of the tracer gas was 1/40th (2.5%) of the equilibrium SF_6 concentration. Similarly, an additional five breaths were required for an acceptable end to the test. Three acceptable readings were taken from each child and they were considered acceptable if FRC volumes were within 10% of each other and the LCI was within one turnover of each other. In a case where only two successful tests were acquired, they were acceptable if FRC volumes were within 5% of each other and the LCI was within one turnover of each other. Tests were excluded if the child had uneven breathing, sighs, apnoeas or glottic closures. If the test was acceptable, it was concluded and saved in the EXHALYSER® D software under the patient's study ID number.

The TBFVL and MBW results were exported to the participant's study folder in an excel spreadsheet format. The required data was extracted and further stored in the HMPV study database.

At the end of the iPFT tests the vital signs (oxygen saturation at room air and pulse rate) and head circumference were taken as follows;

Vital signs: They were measured using oximeter (Oxypleth pulse oximeter, Novametrix Medical Systems Inc., Wallingford, USA). The measurements were done for 10 seconds. The sensor was placed on the baby's thumb while lying supine until the machine detected the readings. The oxygen saturation was recorded on room air and in percentages while pulse was recorded in beats per minute (bpm).

Head circumference: This was measured in centimetres using a measuring tape. The child was measured while in a sitting position.

2.4.7 Data management

Each participant was assigned a study identity number to ensure the anonymity of the participant and to identify participants in our study. All participant identifiers such as consent forms and demographic forms were excluded from the main file and kept in a separate and secured file accessible only to the student. The CRFs for each patient were then kept in individual files labelled with the assigned study number. The same information was transcribed into the hMPV database designed in alignment with the CRFs. The data entry used a double capture method to enforce entry by different data capturers which minimizes information errors. If errors were detected the data were edited and cleaned to minimise inconsistencies and information error. In case of missing information from the files, the patient was contacted telephonically to obtain the missing information. If the patient was not reached, home visits were arranged. Quality assurance was reinforced by routine data checks performed post data entry. The hMPV database access was protected with passwords and data was backed up daily into the RMPRU central server. Change of information on the database was limited to Lesego Ramocha and the data manager. Once data capturing was finalised, the participants' files were stored in boxes and archived in the RMPRU archiving rooms. The data relating to the iPFTs were exported to the excel spreadsheet as elaborated previously. The spreadsheets were identified with the participant's study number only. During analysis, the CRF database and iPFT databases were merged.

Table 2.4: Sample size

	Case (N1)	Control (N2)	Ratio	Power (%)
1	259	259	1	80
2	194	388	2	80
3	173	518	3	80
4	162	647	4	80
5	156	776	5	80
6	151	905	6	80
Currently achieved	**60**	**306**	**5**	**31.9**

Sample sizes based on airway resistance

The prevalence of pulmonary sequelae in children with hMPV-associated LRTI hospitalisation is unknown. The values in this study were extrapolated from an RSV study (HREC (Med): M160224) and were based on airway resistance as measured by FOT. hMPV positive children had an airway resistance with a mean of 21.91 and a standard deviation of 10.05 cmH2Os.L−1. The controls had airway resistance with a mean of 19.92 and a standard deviation of 5.44 cmH2O.s.L−1. We powered this study to be able to detect a 10% difference of mean airway resistance between the cases and controls, with a power of 80% at a statistical significance threshold of 0.05. The sample size required to detect a 10% difference in mean airway resistance with a case-control ratio of 1:3 required 173 cases and 518 controls. However, we did not reach our sample size due loss to follow up and limited study duration. The final number tested hMPV cases at one year of age is 60 while at two years of age is 39. There were 306 and 206 controls tested at one and two years respectively.

2.4.8 Statistical analysis

We used STATA version 13 for statistical analysis. All analyses were done by the student as follows:

Table 2.5: Statistical analysis

Objective	Data analysis
To compare the clinical sequelae of children hospitalised with hMPV-associated LRTI during the first two years of life to that of healthy controls	The clinical sequelae measurements with normal data distribution were described using means and standard deviation (95% confidence interval). Data with skewed data distribution were described using median and interquartile range. Categorical variables were described using count (% percentage). Comparison of normal continuous variables was done t-test while comparison of skewed continuous variables was done using Mann-Whitney U test. Comparison of categorical variables in hMPV cases and healthy controls was done using chi square test and Fisher's exact test as appropriate. The odds ratios (OR) with 95% confidence intervals (95% CI) were reported. The P value of <0.05 was considered statistically significant.
To compare the pulmonary sequelae of children hospitalised with hMPV-associated LRTI during the first two years of life to that of healthy controls	The pulmonary sequelae measurements with normal data distribution were described using means and standard deviation (95% confidence interval). Data with skewed data distribution were described using median and interquartile range. Categorical variables were described using count (% percentage). Comparison of normal continuous variables was done t-test while comparison of skewed continuous variables was done using Mann-Whitney U test. Comparison of categorical

	variables in hMPV cases and healthy controls was done using chi square test and Fisher's exact test. The P value of <0.05 was considered statistically significant. The odds ratios (OR) with 95% confidence intervals (95% CI) were reported.
To adjust for potential confounders with developing pulmonary sequelae in children less than five years. The confounders that were adjusted for are; birthweight, weight, height, gender, maternal HIV, household smoke exposure, maternal pregnant smoke, maternal use of alcohol during pregnancy, exclusively breastfed, siblings in crèche, attends crèche, severe hMPV LRTI	Regression analysis was performed to adjust for potential confounders with developing pulmonary sequelae in children less than five years. Linear regression was used for continuous outcome variables to analyse the associations between different covariates and lung function outcomes. Logistic regression was used for variables with categorical data. In the first step, we included the variables in the univariate regression model, and variables with a p<0.2 were included in the multivariate regression. model.

2.4.9 Ethical approval

BoSS study was approved by the University of Witwatersrand Medical Ethics Committee (HREC (Med): 140904. The V98_28OB research study was approved by the Human Research Ethics Committee of the University of the Witwatersrand Faculty of Health Sciences (HREC (Med): 140203) for compliance with medical and ethical standards. The ethical clearance for this study was also approved by the University of Witwatersrand Medical Ethics Committee (HREC (Med): 161149).

Chapter Three: Epidemiology of Human metapneumovirus-associated LRTI in African children: Systematic review and meta-analysis

Abstract

Background: Human metapneumovirus (hMPV) has been associated with upper and lower respiratory tract infections in children and adults. This systematic review evaluated the epidemiology of hMPV-associated lower respiratory tract infection (LRTI), including severe acute respiratory infection (SARI) hospitalisation or clinically diagnosed severe pneumonia, in African children under five years of age.

Methods: We searched Science Direct, PubMed, Cochrane Central, Scopus and WHO regional databases using the terms “("Human metapneumovirus" AND "Africa") OR ("hMPV" AND "Africa")” up to 17 September 2020. Other sources included ClinicalTrials.gov to obtain unpublished data. Studies were included if children were less than five years of age and hospitalised with hMPV-associated LRTI, SARI, or if clinically diagnosed with severe pneumonia in the community. The main outcomes were prevalence of hMPV identified among children with hospitalised LRTI or SARI. We further calculated odds ratios for hMPV in cases with LRTI compared to non-LRTI controls. Pooled results were calculated using a random effects model.

Results: Thirty studies were eligible for inclusion in the review. The prevalence of hMPV-LRTI/SARI among hospitalised and severe pneumonia cases was 4.7% (95% confidence interval (CI) 3.9-5.6, I^2=95.0). The case-control studies indicated that hMPV was 2.0-fold (95%CI: 0.9–4.4) more likely to be identified in LRTI-cases (10.3%) than controls (6.0%). Three of five studies reported hMPV-associated LRTI case fatality risk, with a pooled estimate of 1.3% (95%CI 0.3-2.9, I^2=49).

Conclusions: hMPV was associated with approximately 5% of LRTI/SARI hospitalisations or severe pneumonia cases in Africa, however, some of the infections could be incidental as evident by the 6.0% positivity rate in controls.

3.1 Introduction

Acute lower respiratory tract infections (LRTI) are a leading cause of under-five mortality, contributing to 0.809 million [0.747−0.874] deaths globally in 2017 (3). The World Health Organisation (WHO) estimated a crude death rate of 75 per 100,000 population due to LRTI in children under the age of five years from low-middle income countries in 2016 (143). The pathogenesis of LRTI includes infections by bacterial and/or respiratory viruses.

Human metapneumovirus was discovered in 2001 and has been associated with upper and lower respiratory tract infections (34). The circulation of hMPV may be seasonal, including epidemics during winter and spring in temperate climates, albeit some reports indicate perennial circulation (34, 47, 72). Clinical manifestations of hMPV infections include bronchiolitis, pneumonia, croup and asthma exacerbations. The incubation period of hMPV-associated LRTI varies according to an individual, but is commonly 5 to 6 days" (144, 145). hMPV viral shedding is reported to last between 6 and 11 days after acute illness (146, 147).

Infection with hMPV has been associated with hospitalisation, severity of respiratory disease and death in high-risk groups such as children under five years of age, immunocompromised and elderly individuals (26, 33, 62, 72, 73). HIV-exposed children are at heightened risk (incidence rate ratio (IRR) 1.4; 95% confidence interval [CI]: 1.1-2.0) of being hospitalised for hMPV-associated severe LRTI (26).

The multi-country, multi-site Pneumonia Etiology Research for Child Health (PERCH) case-control study in low and middle income African and South Asian countries, attributed hMPV in the pathogenesis of hospitalisation for severe and very severe LRTI in 7.5% (95% CI: 5.9-9.5%) of cases, although identification of hMPV was also evident in controls (2.3%). The identification of hMPV was associated with severe or very-severe pneumonia case status (6.8%; odds ratio 4.6; 95% CI: 3.6-5.8) (27).

There has been limited evaluation of the role of hMPV in LRTI morbidity and mortality in African countries. We undertook a systematic review and meta-analysis to define the epidemiology of hMPV-associated LRTI, including severe acute respiratory infection (SARI) hospitalisation or clinically diagnosed severe pneumonia, in African countries.

3.2 Methods

Search strategy

The systematic review was conducted according to the Preferred Reporting Items for Systematic and Meta-Analyses (PRISMA) guidelines. We searched Science Direct, PubMed, Cochrane Central, Scopus and the WHO regional database using the terms “("Human metapneumovirus" AND "Africa") OR ("hMPV" and "Africa")” and included all identified publications through to 17 September 2020. ClinicalTrials.gov was reviewed for identification of any unpublished trials. There were no limitations to the language or year of publication. The protocol was registered under PROSPERO CRD42018114131.

Inclusion and exclusion criteria

This review included studies involving children less than five years of age with LRTI hospitalisation, SARI or clinically diagnosed severe pneumonia in the community. Eligible study designs were randomised and non-randomised control studies, prospective and retrospective cohort studies, cross sectional and case-control studies. We only included studies conducted over at least, a full calendar year. Excluded from the analyses were systematic reviews, case reports and series, narrative reviews, editorials and animal studies. In instances of multiple publications of the same study, the most comprehensive publication was retained. The Human Research Ethics Committee of the University of the Witwatersrand waived this systematic review (Reference number: W-CBP-190218-2).

Data extraction and management

Two reviewers (LR and EM) independently screened the titles and abstracts of the studies and assessed their eligibility. Full text of eligible studies was obtained and disagreement on eligibility was resolved between the two reviewers. Data were extracted using standardised data extraction forms, including information on hMPV incidence and prevalence, outcomes, study design, study duration, sample size, location, coinfection with other respiratory infections, method of hMPV detection, participant characteristics and study quality. The two reviewers independently extracted data from 10/74 (13.5%) eligible studies. After achieving good concordance (>80%), the remainder was extracted by one reviewer (LR). In the case of lack of clarity during extraction, the second reviewer (EM) was consulted to discuss and resolve issues.

Data analysis

For the analysis of pooled prevalence and case fatality risk (CFR), point estimates from individual studies were combined using a random-effects model in case of significant heterogeneity (I^2 > 50%) and 95% CIs were reported. Analyses were performed using R statistical software, version 3.6.0.

Quality assessment

The risk of bias was assessed using the Newcastle-Ottawa scale (NOS), grading each study using predefined criteria on a 0 to 9 score (0=high risk, 9=low risk) (129). The overall quality of evidence was evaluated using the AMSTAR tool 2. AMSTAR is a validated tool which rates studies on 16 items and scores the overall results as critically low, low, moderate or high quality (130).

3.3 Results

The search strategy identified 1,474 records. After deletion of 168 duplicates, 1,306 articles were included for screening of titles and abstracts (Figure 3.1). Of these, 1,152 articles did not meet the eligibility criteria, and 154 full text articles were reviewed to assess for inclusion eligibility. Finally, 30 individual studies, which were reported in 39 publications, were eligible for inclusion in the systematic review (Table 3.1). Of the 30 studies included, 24 were cross-sectional studies in children presenting with LRTI (28, 41, 62, 72, 73, 148-166) and six were case-control studies (25, 29, 167-170). The median age of study populations ranged between four months and three years. Eighty-percent (24/30) of the studies were hospital-based. Four of the six (67%) case-control studies focused on clinically diagnosed severe or very severe pneumonia and were designed to compare the prevalence of hMPV in children with and without LRTI.

Thirteen of the studies used a WHO clinical classification of pneumonia (171) or SARI (73, 148, 151-154, 162, 165, 168, 170, 172-174); whilst three South African studies in addition to children fulfilling the SARI definition also included any child under three months of age hospitalised for suspected infection (72, 158, 166). The remaining 14 studies had varying case definitions of LRTI.

hMPV seasonality was reported in nine studies, being most prevalent during the winter and spring seasons in most settings (28, 62, 72, 73, 157, 158, 160, 165, 167, 170, 175); albeit being perennial in some areas (Figure 3.2).

Figure 3.1: Flow chart of study selection

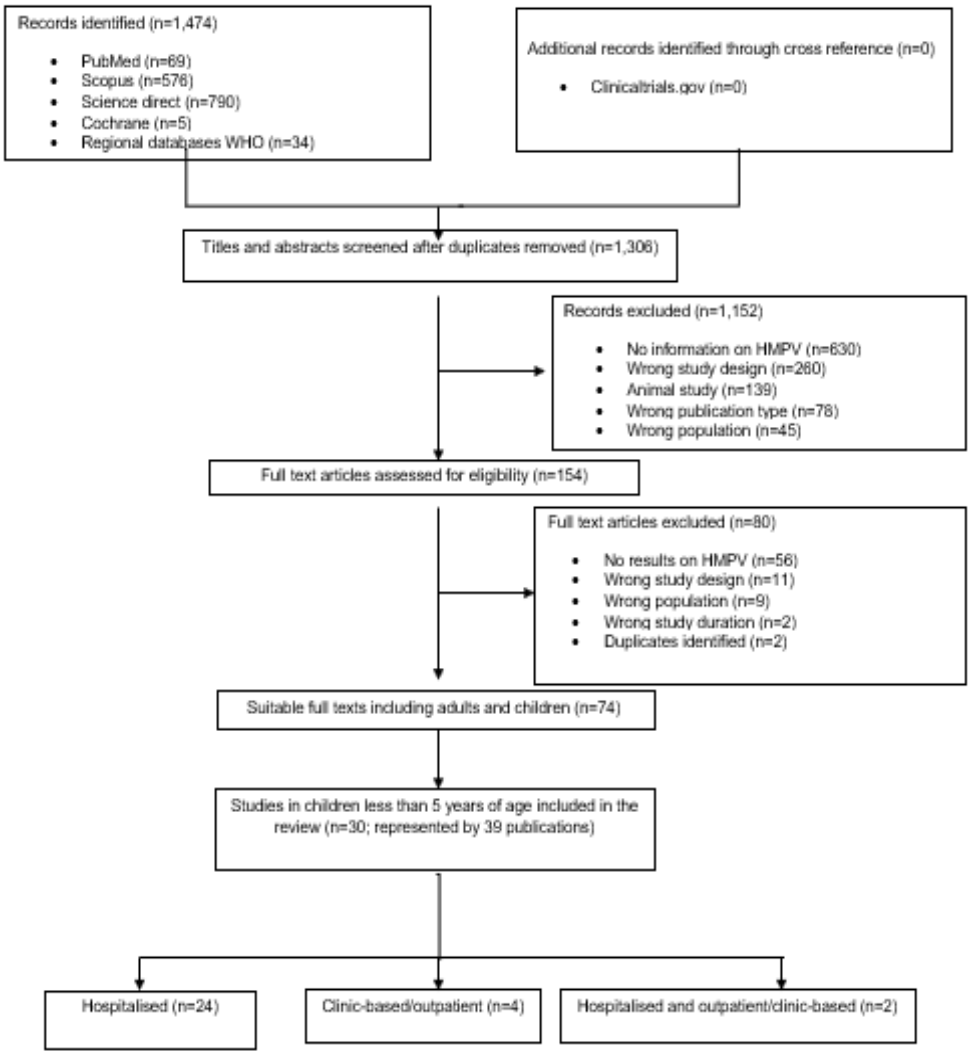


Table 3.1: Studies included in the systematic review

Author, year	Country & site	Type of study	Total hMPV cases n/N (%)	Types of epidemiology outcome measures	Types of clinical outcome measures	Population description (hospital-based, community)	Enrolment period	Study duration (months)	Sampling for hMPV (LRTI hospitalisation, SARI and pneumonia)	Presence of co-infections
Ahmed, 2012	Kenya, Dadaab and Kakuma refugee camps	Cross - sectional study	266/4449 (6.0)	E1,E2	C1	Hospital-based and outpatient	September 2007 - August 2010	36	SARI cases	Nr
Annamalay, 2016[a]	Mozambique, Manhiça	Cross - sectional study	25/237 (10.5) HIV uninfected; 3/38 (8); total 28/275 (10)	E1	C1	Hospital-based	September 2010 - April 2013	31	Clinical pneumonia	Rhino virus-c
Annamalay, 2016[b]	South Africa, Pretoria	Case - control study	Total: 7/105 (6.7); Pneumonia 5/57 (8.8)	E1	C1,C2	Hospital-based	July 2011 - November 2012	17	ALRI cases (pneumonia or bronchiolitis), non ALRI	Nr

Bennet, 2017 [b]	India (2 sites), Madagascar, Mali, and Paraguay	Cross - sectional study	Hypoxic pneumonia 10/70 (14.3); non-hypoxic pneumonia 23/335 (6.9)	E1	C1	Hospital-based	July 2011 - November 2012, in Bamako, Mali	50	Clinical features of pneumonia	Nr
Breiman, 2015	Kenya	Case - control study	97/815 (11.9) SARI; controls 7/115 (6.1)	E1,e2	C1	Clinic based	March 1, 2007 for SARI and January 1, 2009 -February 28, 2011 for control selection	47	Sari	Nr
Berkley, 2010	Kenya, Kilifi District Hospital	Case - control study	23/759 (3.0)	E1, e2	C1	Hospital-based	January 2007 - December 2007	12	(Very) severe pneumonia	Nr
El Kholy, 2014	Egypt, Cairo University Hospital	Cross - sectional study	45/1046 (4.3)	E1,e2	C0	Hospital-based	February 2010 - May 2011	15	Alri	Nr
El Basha, 2019	Egypt, tertiary hospital in Cairo	Cross - sectional study	Preterm infants 4/36 (5.4%); term	E1	C1	Hospital-based	September 2011 - October 2014.	35	Viral bronchiolitis	Nr

			infants 1/53 (1.3%)							
Embarek, 2014	Egypt, Assiut University Hospitals	Cross - sectional study	11/520 (2.2)	E1	C1	Hospital-based	December 2005 - February 2008	26	Lrti	Adenovirus
Feikin, 2013	Kenya	Cross - sectional study	21/408 (5.1)	E1	C1	Cases from households and clinics	March 2007 - February 2010	37	SARI cases, asymptomatic controls	Nr
Feikin, 2017	The Gambia; Kilifi, Kenya; Bamako, Mali; Soweto, South Africa; Lusaka, Zambia	Case - control study	Cases 185/1733 (10.8); controls 206/4986 (4.1)	E1	C1	Hospitalised based and community controls	August 2011 - January 2014	29	Evidence of pneumonia	Nr
Groome, 2015	South Africa, Gauteng Province, Edenvale Hospital ,KwaZulu-Natal Province, Mpumalanga Province,	Cross - sectional study	712/17437 (4.1)	E1,e2	C1,C2	Hospital-based	February 2009 - December 2013	46	SARI	Adenovirus, parainfluenza type I, III , rhinovirus enterovirus, S. Pneumonia

	Klerksdorp Hospital									
Jroundi, 2016 (Jroundi, 2014)	Morocco, Rabat	Cross - sectional study	61/683 (8.9)	E1	C1,C2	Hospital-based	November 2010 - December 2011	14	Respiratory symptoms	Rhinovirus, coronavirus, parainfluenza, adenovirus
Kadjo, 2018	Côte d'Ivoire, Abidjan, San Pedro, Bouake, Korhogo, Man, Agniblekrou	Cross - sectional study	2/142 (1.4) (SARI); 19/917 (6.2) (ILI)	E1	C0	Hospital-based	January 2013 - December 2013	12	SARI	Parainfluenza type I, III
Kelly, 2015	Botswana, Gaborone	Case - control study	20/133 (15.0) pneumonia cases; matched controls 11/133 (8.3)	E1	C0	Hospital-based	April 2012 - August 2014	29	ALRI and children without ALRI	Nr
Kenmoe, 2016	Cameroon, Yaounde	Cross - sectional study	11/347 (3.2)	E1	C0	Hospital-based	September 2011 - September 2013	24	SARI	Nr

Lanaspa, 2015	Mozambique, Manhiça District Hospital	Cross - sectional study	39/806 (4.8)	E1	C0	Hospital-based	September 2006 - September 2007	12	Clinically severe pneumonia	Nr
Ludewick, 2005	South Africa, Soweto	Cross - sectional study	206/2802 (7.4)	E1	C0	Hospital-based	2000 - 2002	36	LRTI	Nr
Majozi, 2017	South Africa, Durban	Cross - sectional study	1/338 (0.3)	E1	C0	Hospital-based	December 2010 - May 2015	53	LRTI	
Mazur, 2016	South Africa, Gauteng, Mpumalanga, KwaZulu-Natal, Northwest	Cross - sectional study	26/2322 (1.1) RSV and hMPV combined	E1	C0	Hospital-based	February 2009 - December 2013	58	SARI	Hrsv
O'Callaghan-Gordo, 2011	Mozambique	Cross - sectional study	22/333 (6.6)	E1	C0	Clinic based	February 1999 and May 2000	15	ALRI	Nr
Oketch, 2019	Kenya, Kilifi County Hospita.l	Cross - sectional study	274/6756 (4.1)	E1	C0	Hospital-based	January 1st 2007 - December 31st 2016	108	Syndromic severe or very severe pneumonia	Nr

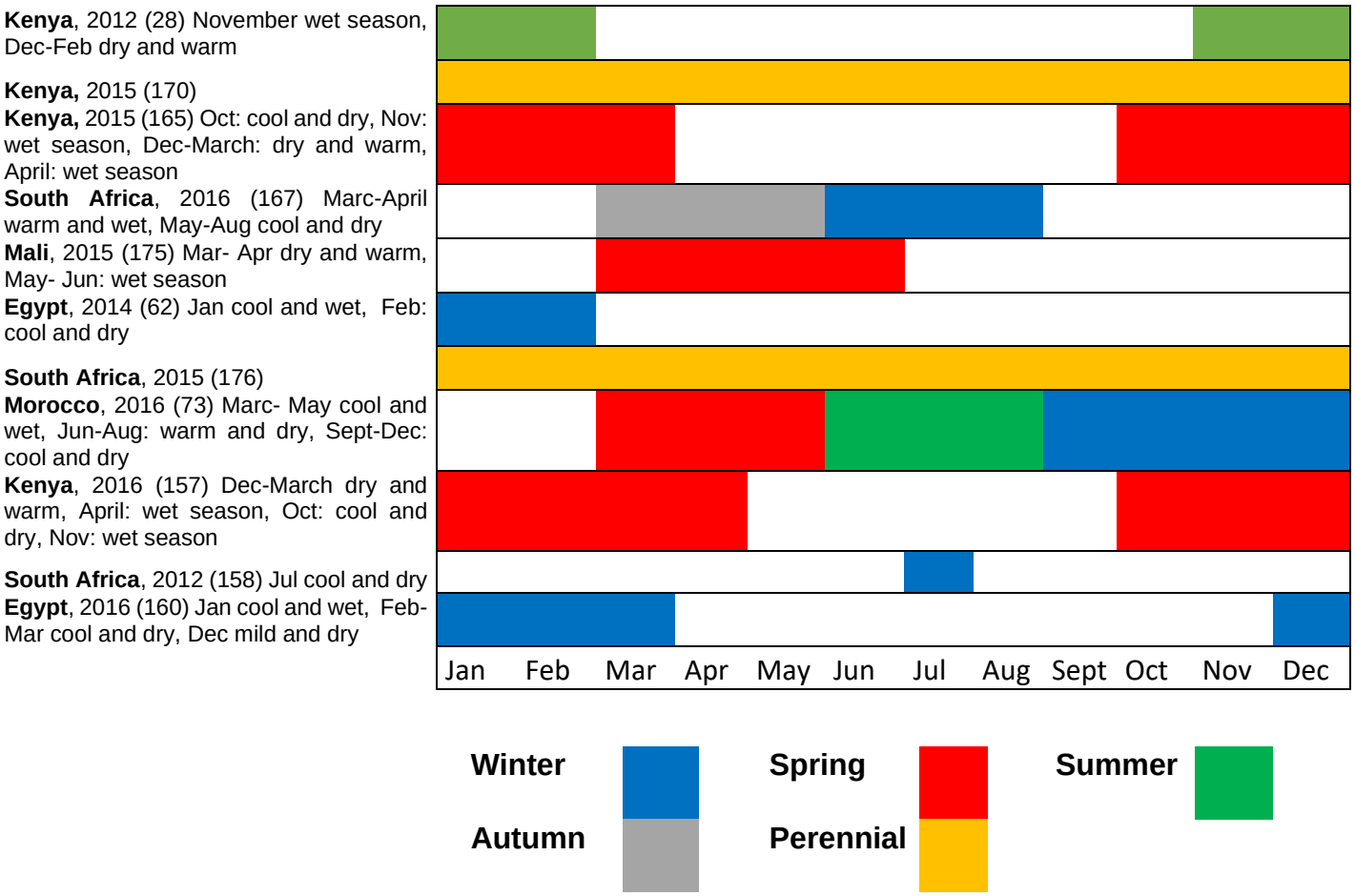
Owor, 2016	Kenya, Kilifi County Hospital.	Cross - sectional study	71/1425 (5.0)	E1	C0	Hospital-based	January 2007 - December 2011	59	Pneumonia cases	Nr
Pretorius, 2012	South Africa, Gauteng Province, Edenvale Hospital, KwaZulu-Natal Province, Mpumalanga Province, Klerksdorp Hospital	Cross - sectional study	303/8173 (3.7) not stratified by age group but included > 5 yrs	E1	C0	Hospital-based	2009 - 2010	20	Sari	Nr
Pretorius, 2016	South Africa, KwaZulu-Natal Province, Northwest Province	Cross - sectional study	79/1431 (5.5) < 5 yrs SARI only	E1	C0	Hospital-based	Feb 2009 & May 2012 -April 2015	36	SARI, ILI, Healthy controls	Nr
Rowlinson, 2016	Egypt, Damanhour district	Cross - sectional study	266/5768 (4.6)	E1	C0	Hospital-based	June 2009 - December 2013	54	ALRI	Nr

Soofie, 2016	South Africa, Soweto	Cross - sectional study	41/1839 (2.2)	E1	C0	Hospital-based	January 2015 - December 2015	12	LRTI	Nr
Subramoney, 2018	South Africa, Gauteng Province, Edenvale Hospital ,KwaZulu-Natal Province, Mpumalanga Province, Klerksdorp Hospital	Cross - sectional study	14/318 (4.4) for < 1 year. 7/144 (4.9) for 1 - 4 years	E1	C0	Hospital-based	January 2012 - December 2013	23	SARI	Nr
Zar, 2016	South Africa, Drakenstein	Case - control study	cases 29/284 (10.2); controls 44/412 (10.7)	E1	C0	Clinic based	May 2012 - December 2014	31	Pneumonia cases, matched controls without pneumonia	Nr
Zash, 2016	Botswana	Cross - sectional study	3/85 (3.5) for	E1	C0	Hospital-based	March 2001 - October 2003	31	Pneumonia and diarrhoea cases	Nr

			pneumonia cases only							

Abbreviations: ALRI, Acute lower respiratory infection; ALRTI , Acute lower respiratory tract infection; ARI, Acute respiratory illness; C0, no clinical characteristics of hMPV cases reported; C1, need for hospitalisation of hMPV cases reported; C2, clinical presentation of hMPV cases reported; E0, Epidemiology not reported; E1, hMPV cases reported; E2, hMPV incidence reported; HRSV, human respiratory syncytial virus; ILI, Influenza like illness; LRTI, Lower respiratory tract infection; NR, not reported; PIV, parainfluenza virus; RTI, Respiratory tract infection; RSV, respiratory syncytial virus; SARI, severe acute respiratory illness; URTI, Upper respiratory tract infection.

Figure 3.2: Seasonal hMPV prevalence



All studies gathered data over multiple years. The colours shows a season when hMPV was detected, whereas the months shows the detection months.

Prevalence of hMPV in hospitalised or non-hospitalised participants

The mean or median age of hMPV-associated LRTI cases varied (range: 3-60 months) between studies. Overall, hMPV was identified in 4.7% (2,908/61,853; 95% CI: 3.9-5.6, I^2=95.0) of children with LRTI, with prevalence ranging from 0.3% (1/338) to 15.0% (20/133) (155, 173) (Figure 3.3). The prevalence of hMPV in children hospitalised with LRTI or SARI was 4.1% (1,420/34,763; 95%CI: 3.9-4.3) and 10.9% (235/2,162; 95%CI: 9.6-12.3) in non-hospitalised children diagnosed with clinically severe pneumonia (clinic and community based studies).

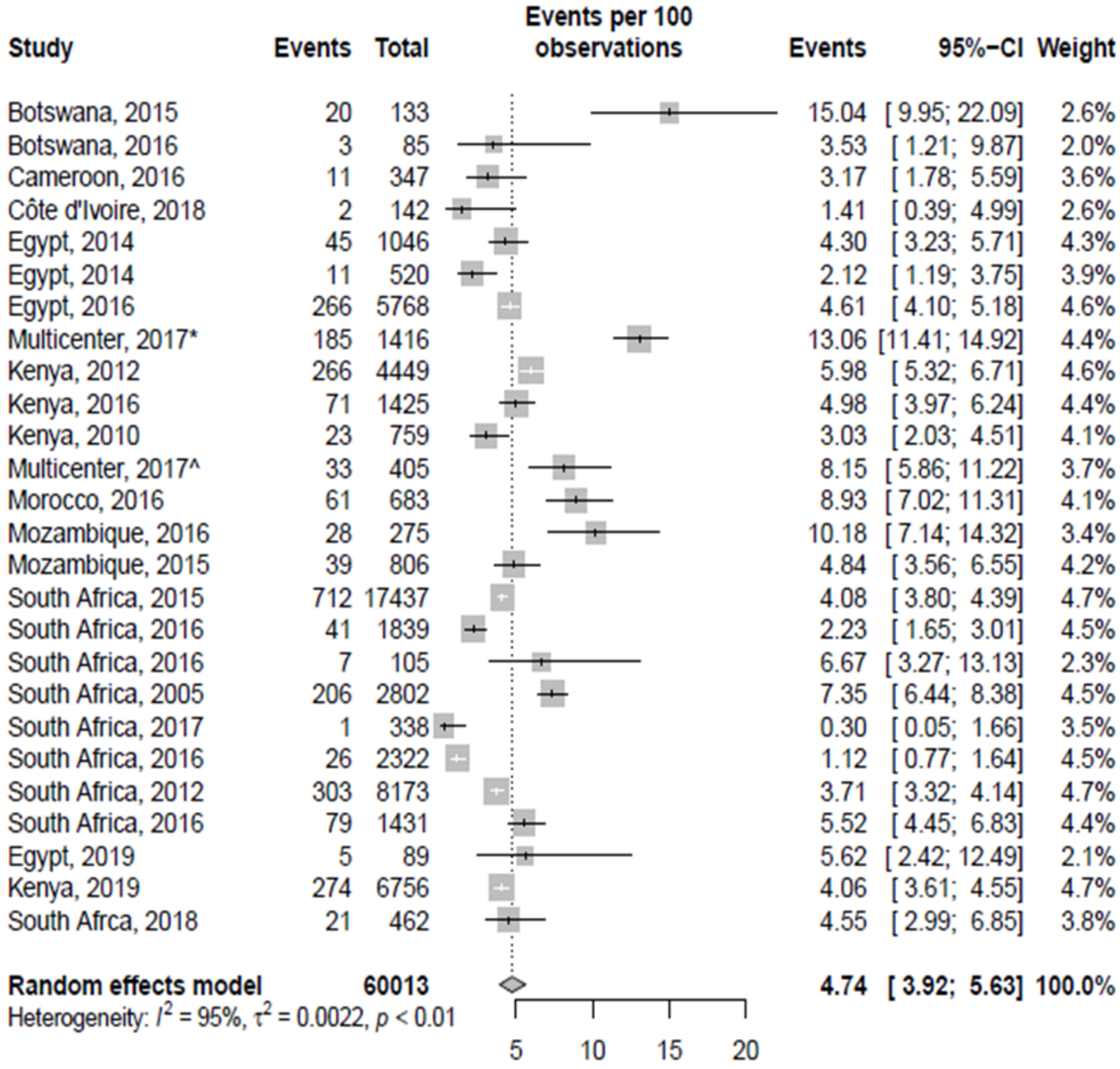
Meta-analysis of six studies indicated a marginal 2.0 fold (95% CI: 0.90-4.4) higher prevalence of hMPV (10.3%, 95%CI: 9.3.-11.3) in children with compared with children without LRTI (6.0%, 95%CI: 5.3-6.8) (Figure 3.4).

Incidence of hMPV in hospitalised or non-hospitalised participants

Four studies (26, 28, 29, 72, 77, 168, 177) reported on incidence of hMPV-associated hospitalisation (Table 3.3). This included one study that analysed hMPV-associated LRTI hospitalisation incidence by HIV status (26, 72, 77) and one that further stratified by age group (72). Notably, however, these studies did not adjust for the attributable fraction of hMPV identification in the causal role of LRTI.

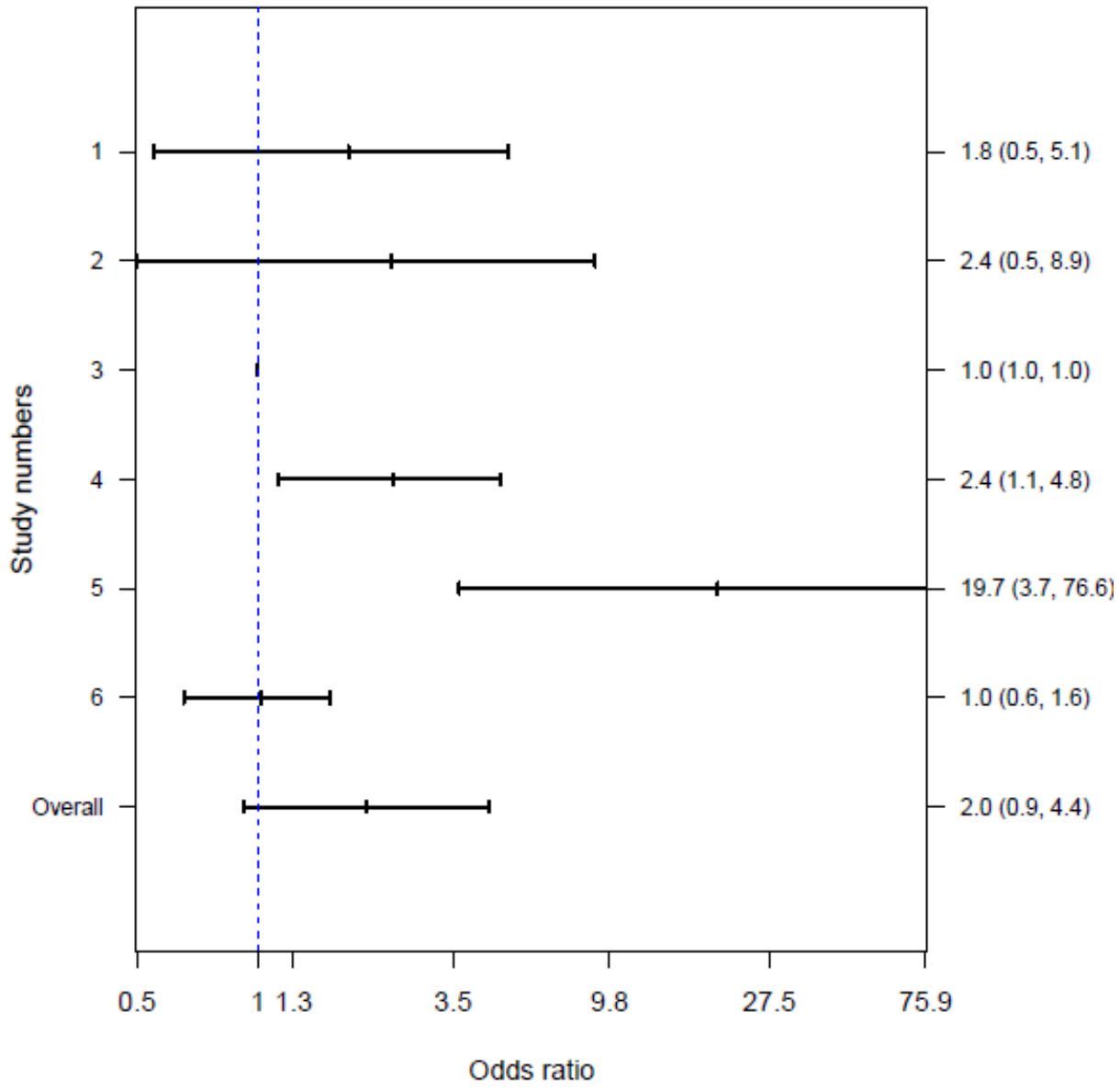
In South Africa, the incidence (per 100,000 population) of hMPV-associated LRTI hospitalisation in children <6 months of age was higher in HIV-exposed uninfected (HEU) children (816; 95% CI: 622–1050; IRR 1.4; 95% CI: 1.1-2.0) and children living with HIV (CLWH); (1,887; 95% CI: 863–3582; IRR 3.3; 95% CI: 1.5-6.5) than HIV-unexposed children (573; 95%CI: 470–692) (26). Two Kenyan studies stratified hMPV incidence by age group, in which infants had a higher incidence of hMPV-associated LRTI hospitalisation than other age groups (28, 29). In the first study, the incidence (per 1,000 children) of hMPV-associated LRTI was 10.7 (95% CI: 7.9-14.7) in infants compared with 2.1 (95% CI: 1.5-2.9) and 3.6 (95% CI: 2.9-4.5) in children 2-5 and >5 years of age, respectively (28). In the second study, hMPV-associated LRTI incidence (per 100,000 children) estimates were 138, 58, 11 and 44 among age groups under 12 months, 1-2 years, 2-5 years and >5 years, respectively (29).

Figure 3.3: hMPV prevalence among hospitalised patients



Legend; References: Botswana 2015 (173), Botswana 2016 (162), Cameroon 2016 (153), Cote D'ívoire 2018 (152), Egypt 2014 (150), Egypt 2014 (62), Egypt 2016 (160), * PERCH multicentre study (Gambia) 2017 (168), Kenya 2012 (28), Kenya 2016 (157), Kenya 2010 (29), ^GABRIEL multicentre study (Mali) 2017 (149), Morocco 2016 (73), Mozambique 2015 (154), Mozambique 2016 (148), South Africa 2015 (72), South Africa 2016 (161), South Africa 2016 (167), South Africa 2005 (41), South Africa 2017 (155), South Africa 2016 (156) ,South Africa 2012 (158), South Africa 2016 (159), Egypt 2019 (164), Kenya 2019 (165), South Africa 2018 (166) .

Figure 3.4: Odds ratios for hMPV in cases with LRTI compared to non LRTI



Legend; study number and references: 1 (167), 2 (29), 3 (168), 4 (170), 5 (173), 6 (169).

Table 3.2: Incidence of hMPV hospitalisation

Author, year	Age	Total hMPV cases n/N (%)	Study duration (months)	Population description	hMPV incidence per 100,000/1000/100 (95%CI)	Adjusted odds ratio 95% CI	Adjusted rate ratio (95% CI)	Was PCV introduced in the country?
Ahmed, 2012	Median:1 year (1 month-84 years)	266/4449 (6.0)	36	Hospitalised	<1 yr: 10.7 (7.9-14.7); 1-5 yrs 2.1 (1.5-2.9); <5 yrs 3.6 (2.9 - 4.5) per 1000 children per year			No
Berkley, 2010	Median: (very) severe pneumonia cases 9.0 months (3.3-20)	23/759 (3.0)	12	Hospitalised	0.20 per 1000 live births, aged 28 days, <1 yr: 138, 1-2 yr: 58, 2-5 yr: 11, <5 yr: 44, per 100,000			No
Breiman, 2015	Age group with highest proportion <12 months old: 34.8%	97/815 (11.9)	47	Clinic	0.9 (0.3-1.6) per 100 person-years of observation in less than 5 years	2.12 (0.96-4.72)		Yes

Cohen, 2016	Nr	78/1410 (1.2)	47	Hospitalised	HUU 573 (470–692), HEU 816 (622–1050), HIV-infected 1887 (863–3582) per 100,000 population in <6 months age group		CLWH/HIV- 1.4 (1.1–2.0); HIV+/HIV- 3.3 (1.5–6.5)	Yes
Groome, 2015	Age group with highest proportion; < 1 year 389/593 (65.6%)	712/17437 (4.1)	46	Hospitalised	CLWH <1 year 664.8 (411.5–1016.2), HIV- 652.9 (600.9-708.2), 1 to <2 years CLWH 94.7 (25.8-242.4), HIV - 174.4 (148.2-203.9), 2-4 years CLWH 30.1 (8.2-77.1), HIV- 33.9 (27.4-41.5) per 100,000 person-years		< 1 yr 1 (0.6–1.6), 1 yr 0.5 (0.1–1.4), 2-4 yrs 0.9 (2–2.3)	Yes

Abbreviations: CLWH, Children living with HIV; HIV-, HIV-uninfected; HEU, HIV-exposed uninfected; NR, not reported; *results of Madhi et al, 2006 not shown in the table because the reduction in incidence was reported

Duration of hospitalisation

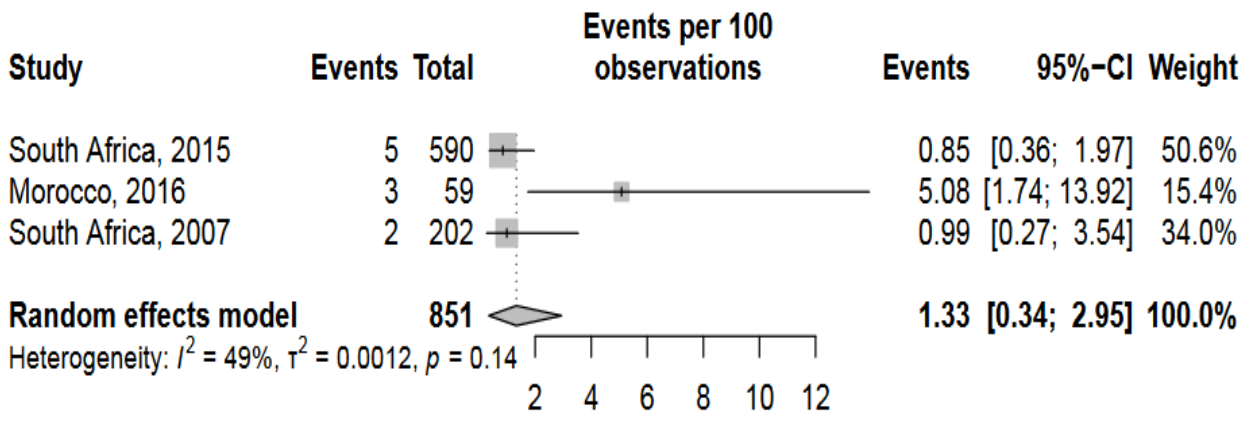
Only four of the 30 studies reported on duration of hMPV-associated hospitalisation (72, 73, 76, 77). The median or mean duration of hospitalisation ranged from two to seven days, and three to 14 days, respectively. Duration of hMPV hospitalisation in children at four sentinel sites in South Africa, reported that CLWH had a longer duration of hospitalisation (5 (interquartile range [IQR]) 2–8] days) than HIV-uninfected children (3 [1–5.5]; p=0.003) (72). This is similar to another study where a longer duration of hospitalisation was also reported in CLWH (mean 5.8 days [standard deviation (SD) 1-21]) than children without HIV (mean 4.1 days [SD 1-3.5]; p=0.003) (77). Furthermore, a longer duration of hospitalisation was also observed in CLWH prior to the routine use of antiretroviral treatment (9 days [SD 1-13]) than HIV-uninfected (2 days [1-21]) children, albeit the difference not being significant (76).

A study from Morocco reported a mean duration of hospitalisation of 7.3 (SD ± 9.8) and 6.0 (SD ± 4.2) days for hMPV and RSV-associated hospitalisation, respectively (73).

Case fatality rate

Three hospital-based studies reported on hMPV-LRTI associated CFR (26, 72, 73, 77), with a pooled CFR of 1.3% (95%CI: 0.3-2.9, I^2=49, n=3); (Figure 3.5). These included a study from Morocco in which the CFR was 5% (3/60) for children aged 1-5 years (73). Two studies stratified CFR according to HIV status (26, 72, 77), with CFR being 0% in HEU (0/52) and CLWH (0/9), and 1% (1/86) in HIV-unexposed children hospitalised for hMPV-associated SARI (26). Low CFR was also observed in an earlier study from 2007 in both CLWH not on anti-retroviral treatment (2/202, 1%) and in HIV-uninfected children (0/154; p=0.05) (77).

Figure 3.5: Pooled hMPV associated case fatality rate



hMPV coinfections

The occurrence of coinfection with hMPV and other respiratory viruses was reported in five studies (62, 72, 73, 148, 156). These studies tested for a varied spectrum of other viruses (Table 3.1) and used different types of specimens, storage and diagnostic tests; (Table 3.4).

The most commonly identified viral coinfections with hMPV were RSV, adenovirus and rhinovirus; (Table 3.1) Mazur et al. tested nasopharyngeal aspirate specimens with reverse transcription polymerase chain reaction (RT-PCR) and detected RSV coinfections with hMPV-associated LRTI in 26/2,322 (1.1%) (156). Groome et al. co-detected other respiratory viruses in children with hMPV-associated SARI in 333/593 (56.2%), including adenovirus 80/593 (13%), enterovirus 15/593 (3%), influenza A 9/593 virus (2%), RSV 19/593 (3%), parainfluenza virus type 1, 2 and 3 8/593 (1%), and rhinovirus 107/593 (18%) (72). A study from Morocco using nasopharyngeal aspirates and multiplex PCR detected parainfluenza coinfections more commonly (14.7%) in children with hMPV LRTI than in those with RSV LRTI (5.6%, p=0.038) (73).

The bacterial coinfection most frequently associated with hMPV was *Streptococcus pneumoniae* (72, 73, 177). All studies on bacterial coinfection were done in South Africa. Groome et al. reported *S. pneumoniae* coinfections identified by lytA quantitative real-time PCR detection using whole blood specimens in 7.3% of children with hMPV-associated SARI hospitalisation (72). *S. pneumoniae* was further identified in blood culture by Madhi et al. in 4/189 (2.1%) of infants with hMPV (76). In a randomised controlled trial comparing an experimental 9-valent pneumococcal conjugate vaccine (PCV) or placebo (77), hospitalisation for hMPV-associated LRTI was 45% (95% CI: 19%–62%, p=0.002) and 53% (95% CI: 3%–77%; p=0.035) lower in PCV-9 compared with placebo recipients in HIV-uninfected children and CLWH (who were not treated with antiretrovirals), respectively.

In the same study, 25% (2/8) of CLWH not on anti-retroviral therapy hospitalised for hMPV-associated LRTI had concurrent *Pneumocystis jiroveci* pneumonia (77).

Risk of bias and quality of evidence

Risk of bias assessment using the NOS showed 21/27 (77.8%) of the included studies to have moderate quality (score >5) (Table 3.5 and 3.6). The overall quality of our review was rated as moderate.

Table 3.3: Table reporting data on the microbiological methods

Author, year	Country & site	Type of sample tested	Assay	Transportation media	Freeze/ storage	Duration until analysis
Ahmed, 2012	Kenya, Dadaab and Kakuma refugee camps.	NP and OP swabs	RT-PCR	1 ml vial of viral transport media.	Cryovials stored in a refrigerator and kept at 2 - 8°C for up to 96 hr.	NR
Annamalay, 2016[a]	Mozambique, Manhiça	NPA	Two independent multiplex RT-PCR assays for adenovirus, RSV, bocavirus, coronavirus, parainfluenza viruses, influenza viruses and hMPV.	NR	-80°C	NR
Annamalay, 2016[b]	South Africa, Pretoria	NPA	RT-PCR	NR	NR	NR
Bennet, 2017 [b]	India (2 sites), Madagascar, Mali, and Paraguay	NP swabs/aspirates	RT-PCR	NR	NR	NR
Breiman, 2015	Kenya	NP and OP swabs	RT-PCR	1 ml of viral transport media without antibiotics. Transported the same day at 2°C - 8°C.	-70°C	NR

Berkley, 2010	Kenya, Kilifi District Hospital.	NP wash	RT-PCR	NR	-80°C	NR
El Kholy, 2014	Egypt, Cairo University Hospital.	NP and OP swabs	RT-PCR	2 ml of viral transport medium and samples. Kept for 4°C for not more than 24 hours.	-70°C until testing	NR
El Basha, 2019	Egypt, tertiary hospital in Cairo.	NP and OP swabs	RT-PCR	viral transport media	NR	NR
Embarek, 2014	Egypt, Assiut University Hospitals	Nasal swabs, throat swabs, nasal aspirates, tracheal aspirates, Broncho alveolar lavages, gargles, and sputum.	RNA Extraction and real-time RT-PCR for hMPV, influenza A and B viruses, RSV A and B, and adenovirus.	Transport medium	-80°C	NR
Feikin, 2013	Kenya	NP and OP swabs	RT-PCR	NR	NR	NR
Feikin, 2017	The Gambia; Kilifi, Kenya; Bamako, Mali; Soweto, South Africa; Lusaka, Zambia.	NP and OP swabs	RT-PCR	3 ml vial of universal transport media.	-70°C.	NR

				Specimens were left at room temperature for no more than 2 hours or at 4°C for no more than 24 hours.		
Groome, 2015	South Africa, Gauteng Province, Edenvale Hospital, KwaZulu-Natal Province, Mpumalanga Province, Klerksdorp Hospital.	NPA (patients <5 years) or NP and throat swab (patients ≥5 years) and whole blood specimen.	Multiplex real-time RT-PCR for respiratory specimens; lytA quantitative real-time PCR detection from whole blood specimens for *S. Pneumoniae.*	Viral transport media. Blood specimens were stored at 4 - 8°C and transported.	NR	NR
Jroundi, 2016 (Jroundi, 2014)	Morocco, Rabat	Nasal, NPA and pharyngeal swabs	Automated blood culture system for blood samples; Phoenix Automated Microbiology System or colony morphology and bio-chemical tests for bacterial isolates; real-time PCR for *S. pneumoniae* in blood samples; multiplex PCR-based automated system for hMPV, RSV (A and B), influenza (A and B), para-	NR	NR	NR

			influenza virus (1–4), RV, adenovirus, enterovirus and coronavirus (229E, NL63 and OC43).			
Kadjo, 2018	Côte d'Ivoire, Abidjan, San Pedro, Bouake, Korhogo, Man, Agniblekrou.	NP swab	Automated blood culture system for blood samples; Phoenix Automated Microbiology System or colony morphology and bio-chemical tests for bacterial isolates; real-time PCR for *S. pneumoniae* in blood samples; multiplex PCR-based automated system for hMPV, RSV (A and B), influenza (A and B), para-influenza virus (1–4), RV, adenovirus, enterovirus and coronavirus (229E, NL63 and OC43).	Viral transport media	-20°C	NR
Kelly, 2015	Botswana, Gaborone	NP swab	RT-PCR	Universal transport media.	-80°C and shipped on dry ice.	NR
Kenmoe, 2016	Cameroon, Yaounde.	NP swab	RT-PCR	NR	- 80°C	NR

Lanaspa, 2015	Mozambique, Manhiça District Hospital.	NPA	PCR, blood culture for bacterial isolates.	NR	NR	NR
Ludewick, 2005	South Africa, Soweto.	NPA	RT-PCR	NR	-70°C until processed for this study.	NR
Majozi, 2017	South Africa, Durban.	NR	PCR	NR	NR	NR
Mazur, 2016	South Africa, Gauteng, Mpumalanga, KwaZulu-Natal, Northwest.	NPA	Multiplex real-time RT-PCR for adenovirus, parainfluenza viruses 1, 2, and 3 (PIV1–3), influenza A and B viruses, hMPV, RV, and enterovirus (EV). Blood culture for Streptococcus pneumoniae and whole blood lytA PCR were performed on blood specimens.	Specimens were trans-ported within 72 hours of collection.	NR	NR
O'Callaghan-Gordo, 2011	Mozambique	NPA	NR	4 ml of Dulbecco's minimal essential medium.	-70°C	NR
Oketch, 2019	Kenya, Kilifi County Hospital.	NP and OP swabs.	One-step in-house Multiplex real-time RT-PCR.	Viral transport medium.	NR	NR

Owor, 2016	Kenya, Kilifi County Hospital.	NP	RT-PCR	3 ml viral transport medium.	-80 °C prior to laboratory screening.	
Perch group, 2019	Bangladesh, The Gambia, Kenya, Mali, South Africa, Thailand, and Zambia.	NP and OP swabs.	PCR	NR	NR	NR
Pretorius, 2012	South Africa, Gauteng Province, Edenvale Hospital, KwaZulu-Natal Province, Mpumalanga Province, Klerksdorp Hospital.	NPA, NP and OP swabs.	RT-PCR	Transported within 72 hours of collection.	-70°C	
Pretorius, 2016	South Africa, KwaZulu-Natal Province, Northwest Province.	NP	RT-PCR for Influenza A and B viruses, PIV1–3, RSV, adenovirus, RV and hMPV.	Viral transport medium.	4 - 8°C	NR
Rowlinson, 2016	Egypt, Damanhour district.	NP and OP swabs	RT-PCR	2 ml of liquid viral transport media. Stored at 4°C in hospital laboratories for a maximum of two days.	Stored and shipped in liquid nitrogen.	

Soofie, 2016	South Africa, Soweto.	NP swab.	PCR	2.5ml of universal transport media. Transported on ice.	NR	NR
Subramoney, 2018	South Africa, Gauteng Province, Edenvale Hospital,KwaZulu-Natal Province, Mpumalanga Province, Klerksdorp Hospital.	NPA	Multiplex real-time RT-PCR.	Viral transport media. Stored at 2 to 8°C within 72 hours of collection.	-70°C	
Zar, 2016	South Africa, Drakenstein.	NP swab	RT-PCR	1 ml of skimmed milk-tryptone-glucose-glycerol (STGG) transport medium. Swabs were transported on ice to the laboratory.	-80°C	
Zash, 2016	Botswana	NPA	RT-PCR	NR for NPA Bacterial pathogens: Swabs placed in bacterial culture transport medium and	-70°C (NPA)	

				kept at room temperature.		

Nasopharyngeal aspirates;NP, nasopharyngeal; NR, not reported; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction.

Table 3.4: Newcastle ottawa scale: Cross sectional studies included

Author	Country	Representativeness of the sample	Sample size	Non-respondents	Ascertainment of the exposure (risk factor)	The subjects in different outcome groups are comparable, based on the study design	Assessment of the outcome	Statistical test	Total
Ahmed, 2012	Kenya	1	0	0	0	1	1	0	3
Annamalay, 2016	Mozambique	1	0	0	1	1	1	1	5
Bennet, 2017	Mali	1	0	0	1	1	1	1	5
El Basha, 2019	Egypt	1	0	0	1	1	1	1	5
El Kholy, 2014	Egypt	1	0	0	1	0	1	1	4
Embarek, 2014	Egypt	1	0	0	1	1	1	1	5
Feikin, 2013	Kenya	1	0	0	1	1	1	1	5
Groome, 2015	South Africa	1	0	0	1	1	1	1	5
Jroundi, 2016	Morocco	1	0	0	1	1	0	1	4
Kadjo, 2018	Coite D'ívoire	1	0	0	1	1	0	1	4
Kenmoe, 2016	Cameroon	1	0	0	1	1	1	1	5
Lanaspa, 2015	Mozambique	1	0	0	1	1	1	1	5
Ludewick, 2005	South Africa	1	1	1	1	0	1	1	6

Majozi, 2017	South Africa	1	0	0	1	1	1	1	5
Mazur, 2016	South Africa	1	0	0	1	1	1	1	5
O'Callaghan-Gordo, 2011	Mozambique	1	0	0	1	1	1	1	5
Oketch, 2019	Kenya	1	0	0	1	0	1	1	4
Owor, 2016	Kenya	1	0	0	1	1	1	1	5
Pretorius, 2012	South Africa	1	0	0	0	0	1	0	2
Pretorius, 2016	South Africa	1	0	0	1	1	1	1	5
Rowlinson, 2016	Egypt	1	0	0	1	0	1	1	4
Soofie, 2016	South Africa	1	0	0	1	1	1	1	5
Subramoney, 2018	South Africa	1	1	0	1	1	1	1	6
Zash, 2016	Botswana	1	1	1	1	1	1	1	7

Our scale was modified from the Newcastle-Ottawa Quality Assessment Scale of cross sectional studies. We coded our assessment using 1 and 0. A 1 denotes that a variable has been fulfilled by the article, 0 denotes lack of fulfilment.

Variable meanings: Sample size was assessed if authors had; a) Justified it and it was satisfactory b) not justified it. Assessment of the outcome; a) Independent blind assessment, b) Record linkage, c) Self report, d) No description. Statistical test was assessed by ; a) the statistical test used to analyse if the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value) b) the statistical test is not appropriate, not described or incomplete.

Table 3.5: Newcastle Ottawa Scale: Case-control studies

		Selection				**Comparability**	**Outcome**			
Author (year)	Country	Is the case definition adequate?	Representativeness of the cases	Selection of Controls	Definition of Controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate (dropouts)	Total
Annamalay, 2016	South Africa	1	1	1	1	1	1	1	0	7
Berkley, 2010	Kenya	1	1	1	0	1	1	1	0	6
Breiman, 2015	Kenya	1	1	1	1	1	1	1	0	7
Feikin, 2017	The Gambia; Kilifi, Kenya; Bamako, Mali; Soweto, South Africa;	1	1	1	1	1	1	1	0	7

	Lusaka, Zambia									
Kelly, 2015	Botswana	1	1	1	1	1	1	1	0	7
Zar, 2016	South Africa	1	1	1	0	1	1	1	0	7

Our scale was modified from the Newcastle-Ottawa Quality Assessment Scale of case control studies. We coded our assessment using 1 and 0. A 1 denotes that a variable has been fulfilled by the article, 0 denotes lack of fulfilment. Variable meanings: Ascertainment of exposure was assessed by; a) Secure record, b) Structured interview where interviewer blind to case/control status c) Interviewer not blinded to case/control status, d) Written self-report of medical record only, e) No description

3.4 Discussion

This systematic review and meta-analysis on the epidemiology of hMPV in children under five years of age indicates that hMPV was associated with 4.7% (95%CI: 3.9-5.6, I^2=95.0) of hospitalised LRTI cases or community treated severe pneumonia cases, with a pooled CFR of 1.3% (95%CI: 0.3-2.9).

The PERCH case-control study reported that hMPV identified in the upper airway could signify a causal role for hMPV in the aetiology of severe or very severe LRTI (168). Further indication that hMPV identification in the upper airway can be attributed a causal role in the aetiology of LRTI is provided by other case-control studies, which too showed a higher prevalence of hMPV in children with LRTI (10.3%, 95% CI: 9.3.-11.3) than in controls (6.0%, 95% CI: 5.3-6.8) (169, 170, 173). Nevertheless, the identification of hMPV in healthy controls without LRTI, indicates that studies in which an adjustment is not made for possible co-incidental identification of the virus, may inadvertently overestimate the contribution of hMPV in the aetiology of LRTI, as well as result in an over-estimation of the incidence of hMPV-associated LRTI hospitalisation.

Studies reported a higher incidence of hMPV-associated LRTI hospitalisation in HEU children and CLWH compared with HIV-uninfected children (26, 72). Most studies were from urban (n=16) rather than rural settings (n=5), which could affect the reported incidence of hMPV hospitalisation due to possible differential access to health care (no data to assess).

Foulongne et al., in a study from France, reported that hMPV and RSV coinfection in children was associated with prolonged hospitalisation and children were more likely to require oxygen support (178). Dual infection of hMPV and RSV has been linked to disease severity (71).

Generally, there were few studies on bacterial or viral codetection with hMPV from Africa. However, Madhi et al. reported hMPV-pneumococcal coinfection using blood culture in LRTI hospitalisation in African children (177). Notably, numerous testing modalities, including enzyme immunoassay, tissue culture, and RT-PCR were used for RSV detection which may have affected the comparability of results on coinfections in children with hMPV-associated LRTI. Severity of hMPV LRTI has also been associated with the different hMPV genotypes, albeit inconsistently (43, 49, 50) . Genotypes were not evaluated in any of the African studies included in this review and further studies evaluating the impact of the different hMPV genotypes, disease severity and co-infection are required from this region.

Using serum *LytA* antigen detection as a proxy for pneumococcal co-infection, 7.3% of South African children hospitalised for hMPV-associated SARI were estimated to have concurrent pneumococcal infection (72). The specificity of the serum *LytA* assay for diagnosing pneumococcal pneumonia, however, remains uncertain. Nevertheless, using a vaccine-probe approach, Madhi et al. reported that hospitalisation for hMPV-associated LRTI was reduced in children vaccinated with a 9-valent PCV compared with placebo recipients among those without HIV, and in CLWH who were naive to anti-retroviral treatment. This difference in the rate of hMPV-associated hospitalisation between 9-valent PCV and placebo recipients suggests that hMPV infection likely predisposes the host to superimposed pneumococcal infection by the vaccine-serotypes, which in turn precipitates hospitalisation with hMPV-associated pneumonia (77).

The PERCH case control study showed the prevalence of respiratory viruses and bacteria in children less than five years of age with severe or very severe LRTI in African and Asian children (27). The most commonly detected virus was RSV with a prevalence of 26.5%, followed by rhinovirus (23.0%) and bocavirus (13.1%). Prevalence of hMPV was reported to be 6.8%. The attributable role of detected viruses in the aetiology of severe or very severe LRTI hospitalisation was 31.1% (95%CI: 28.4-34.2%) for RSV, 7.5% (95%CI: 5.3-10.1%) for rhinovirus, 7.4% (95%CI: 5.8-9.3) for parainfluenza and 7.5% (95%CI: 5.9-9.5%) for hMPV. These results suggest that hMPV is one of the major viruses that contributes to respiratory infectious disease in children.

A meta-analysis based on 69 studies reported hMPV prevalence of 6.4% (95%CI: 5.5–7.4) among hospitalised children with acute respiratory infections globally (179). This higher global prevalence, compared to the findings of our study, may be explained by the inclusion of studies spanning a shorter time period than what was defined by our inclusion criteria.

Head-to-head comparison between studies on the role of hMPV in LRTI is impeded by differences in study methodology, including variability in threshold for investigating, participant's age and spectrum of LRTI severity. Furthermore, studies have used varying analytical methods and adjustment factors.

Limitations of this systematic review and meta-analysis include that all studies had an observational study design, which is prone to selection bias in the recruitment of study participants, sampling from a specified population, lack of comparison groups and (unequal distribution of) confounding factors. Additionally, challenges pertaining to the analysis of

prevalence in case-control studies were the differences in selection of cases and controls between studies due to use of varying definitions of SARI, LRTI and clinically severe pneumonia. Also, analysis errors encountered in original studies, may be passed down to our review potentially invalidating the results. Furthermore, the combination of hospitalised SARI and LRTI cases in the analysis may bias the results, as SARI could be a different phenotype to LRTI. Many studies reporting on hMPV infection were focused on other pathogens that were co- detected with hMPV.

The number of hMPV cases identified between studies may have been affected by different methods used in obtaining respiratory specimens and different diagnostic tools. Furthermore, the storage of specimens, the assays used and the timing of testing of samples differed vastly in the included studies. Nasal swabs have been reported to be less sensitive compared to nasal aspirates in identifying hMPV (180-183). The sensitivity, specificity and reproducibility of different types of PCRs in detecting hMPV has been explored across studies. Feng et al. showed that a multiplex real-time RT-PCR assay used for the simultaneous detection of RSV, HRV and hMPV had very high sensitivity compared to individual real-time quantitative polymerase chain reaction (RT-qPCR) assays (61). Other studies used culture for identification of hMPV and reported higher sensitivity and specificity in RT-PCR (99%) compared to cell culture (68%) (184).

Factors that could have influenced the heterogeneity include differences in study populations and different spectra of respiratory tract organisms investigated across studies, settings and enrolment sites. The majority of subjects were enrolled at the hospital, thereby selecting for more severely ill participants.

Chapter 4.0: hMPV LRTI hospitalisation in HIV-unexposed and HIV-exposed children less than five years in South Africa

Abstract

Background: Human metapneumovirus (hMPV) is a cause of hospitalisation in children, however, data are limited concerning the incidence and clinical course in HIV-1 exposed uninfected (HEU) compared with HIV-1 unexposed uninfected (HUU) children.

Methods: Nasopharyngeal swabs and/or aspirates were collected from children <5 years hospitalised for suspected (LRTI) at Chris Hani Baragwanath Academic Hospital, the main public hospital serving Soweto, South Africa. Samples were tested by multiplex real-time polymerase chain reaction assays for hMPV and other causes of LRTI. We estimated the incidence of hospitalisation-associated hMPV infection, stratified by age and HIV-exposure status during the period 2015 to 2017.

Results: hMPV was identified in 2.8% (183/6422) of hospitalisations for suspected LRTI. The overall annualized incidence (per 100,000 population per year) of hMPV-associated LRTI hospitalisation was higher in children aged 0-<6 months (260.3/100,000/yr) and 6-<12 months (233.4/100,000/yr) compared with the 12-<60 months age group (37.9/100,000/yr). The Incidence Rate Ratio [IRR] of hMPV-associated LRTI hospitalisation in HEU compared with HUU were 1.5 (95%CI 0.9-2.4) for 0-<6 months, 1.4 (95%CI 0.7-2.4) for 6-<12 months and 0.9 (95%CI 0.4-1.9) in children 12-<60 months of age. The case fatality rate for hMPV-associated LRTI was 1.7% (2/116).)

Conclusions: hMPV-associated LRTI hospitalisation is highest in infants <12 months of age, with this age group needing to be targeted with future prevention strategies such as hMPV vaccines.

4.1 Introduction

Human metapneumovirus (hMPV) belongs to the family of Pneumovaridae, along with respiratory syncytial virus (RSV) (34). Studies on hMPV suggest a worldwide distribution, (34, 55, 69-72, 185) however, there is a paucity of information on the incidence of hMPV-associated lower respiratory tract infection (LRTI) hospitalisation in low- and middle- income countries. In an earlier study conducted in South Africa between 2009 and 2013, hMPV was detected in 6.2%, 5.7% and 5.1% of physician-diagnosed severe acute respiratory infections hospitalisation in children in age-groups <1 to 12 months, 12.1 to 24 months and 24.1 to 60 months, respectively (72).

Sub-Saharan Africa has the leading burden of under-5 mortality rate globally, at 76 deaths per 1000 live births, with LRTI a leading cause of death (186). In 2010, the maternal HIV-1 prevalence in South Africa was approximately 30% (16). The maternal to child HIV transmission rate from mothers living with HIV-1 is now less than 1% in South Africa (17). Nevertheless, due to the high HIV-1 infection prevalence among women giving birth, approximately one-third of the birth cohort is HIV-1 exposed uninfected (HEU) (187). Hospitalised LRTI cases in HEU compared with HUU children is associated with longer duration of hospitalisation (21, 188). Furthermore, HEU infants under six months have been reported to be at increased risk (ncidence rate ratio 1.4; 95% confidence interval [CI]: 1.1-2.0) of hMPV-associated LRTI hospitalisation in a study conducted from 2010 to 2013 (26). It is unclear whether HEU remained at greater risk for hMPV LRTI hospitalisation beyond six months of age.

We used data from the Babies of Soweto Study (BoSS) to estimate the incidence of hMPV-associated hospitalisation in HEU and HUU children up to five years of age between 2015 and 2017. The study was done in a setting with high maternal HIV sero-prevalence and an established mother to child HIV-1 transmission program (PMTCT) where pregnant women are treated with antiretroviral treatment regardless of the level of immune suppression (189).

4.2 Methods

BoSS study

The primary aim of the BoSS surveillance was to characterize the aetiology of LRTI hospitalisation in children <5 years of age, with specific focus on hMPV, RSV, influenza A/B

and *Bordetella pertussis*. The identification of these organisms investigated among children hospitalised for pneumonia, was strongly associated with disease status in the Pneumonia Etiology Research for Child Health (PERCH) study (27). In this report, we specifically focus analysis on the epidemiology of hMPV associated hospitalisation in HEU and HUU children.

The surveillance was undertaken at Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, South Africa using data from 2015-2017. CHBAH is a large tertiary public hospital that serves the communities of Soweto and the peri-urban area south of Soweto (census regions G of Johannesburg) (131).

Children under five years of age hospitalised with symptoms consistent with either LRTI, suspected sepsis in infants under three months of age or diarrhea were enrolled. Children admitted directly to CHBAH intensive care unit prior to admission to the general ward, and neonates who were never discharged home after birth were excluded. LRTI was defined as a child presenting with symptoms within seven days of the onset of illness. Children were aged ≥3 months to <5 years with physician-diagnosed acute LRTI, which included bronchiolitis, pneumonia, bronchitis and pleural effusion. Also, infants aged <3 months were enrolled if they were suspected to be suffering from sepsis regardless of signs and symptoms because LRTI could present non-specifically in this age group.

Specimen collection and testing

Nasopharyngeal swabs and/or aspirates were extracted for total nucleic acid content and tested for hMPV, RSV A/B, influenza A and B and *B. pertussis* (132) using a 1-step reverse transcriptase/polymerase chain reaction assay (ThermoFisher Scientific, City, Country). The HIV-1 status and exposure status (i.e. maternal HIV-1 status) of individual participants identified with hMPV-associated hospitalisation was taken from the BoSS database, which captured verbal responses from the mother during the study interview, and from the CHBAH clinician's notes. HIV-1 testing was performed routinely on children as per standard of care. If there was discordance in the HIV-1 exposure categorization status between the BoSS database and the medical records (20/183, 10.9%), the medical records were used to categorize the child's HIV-exposure status.

Statistical analysis

The annual incidence for hMPV-associated LRTI per 100,000 population per year in Soweto was stratified by age groups (0- <6 months, 6 to <12, 12 to <60 months) and HIV-1 infection and exposure status. We assumed that children not tested had the same distribution of HIV status as those who were tested. For children in the BoSS database with no recorded HIV-1 exposure (6/183, 3.3%), we performed a sensitivity analysis assuming that children with unknown HIV status were HIV-unexposed.

In the LRTI case count we adjusted for days when enrolment did not occur such as weekends and using recruitment scheme. We also adjusted for eligible cases who met the enrolment criteria, but whose legal guardians refused consent or were unavailable to provide. Some children were not recruited due to admission to the other public hospital in the catchment area, such as the district level Bheki Malangeni Hospital (BMH). Therefore, we adjusted for admission of children at BMH using the administrative database of admissions at the hospital, which includes age and diagnosis and assumed similar positivity rates of hMPV as for children admitted to CHBAH. We assumed that 10% of cases may have been admitted at a private hospital facility (132), and upwardly adjusted the incidence estimate by the same margin.

Statistical analysis was performed using the STATA program version 13.1 (StataCorp, TX, USA). Proportions were compared using the χ^2 test and Fisher's exact test and continuous data were compared using the Wilcoxon rank sum test. A p value of < 0.05 was considered significant. Confidence intervals (95%) were calculated using the binomial exact method.

4.3 Results

Enrolled infants and HIV-1 exposure status

A total of 6,823 children less than five years age hospitalised for LRTI were enrolled between January 2015 and December 2017, of whom 6,422 (94.1%) had nasal swab/aspirates done. hMPV was identified in 183 (2.68%) LRTI cases, 65.6% (n=120) of whom were HUU, 28.4% (n=52) were HEU and HIV-exposure status was unknown in five (2.7%); (Figure 4.1). Five children infected with HIV who were hospitalised for hMPV-associated LRTI were excluded from further analyses. We assessed coinfection between hMPV and the other organisms

investigated, identifying one case of coinfection with RSV A in an HEU child and two cases of *B. pertussis* co-infection in HUU children.

The most common clinical symptoms were cough (93.6%, 131/140), respiratory distress (71.4%, 100/140%) and wheezing (40.4%, 57/141). Cyanosis was rarely reported (1.47%, 2/136) and 27.5% (38/138) of cases required supplementary oxygen. Only one HEU child with hMPV-associated LRTI required mechanical ventilation. The median duration of hMPV-associated LRTI hospitalisation was two days (IQR 1-5 days). HEU children had a longer duration of hospitalisation (3 [interquartile range [IQR] 1-8 days)] than HUU children (1.5; IQR: 1–4 days). Two (1.7%) of 116 children who had hMPV-associated LRTI died, both of whom were HUU (Table 4.1).

Estimation of the hMPV incidence, in the <5 year old population

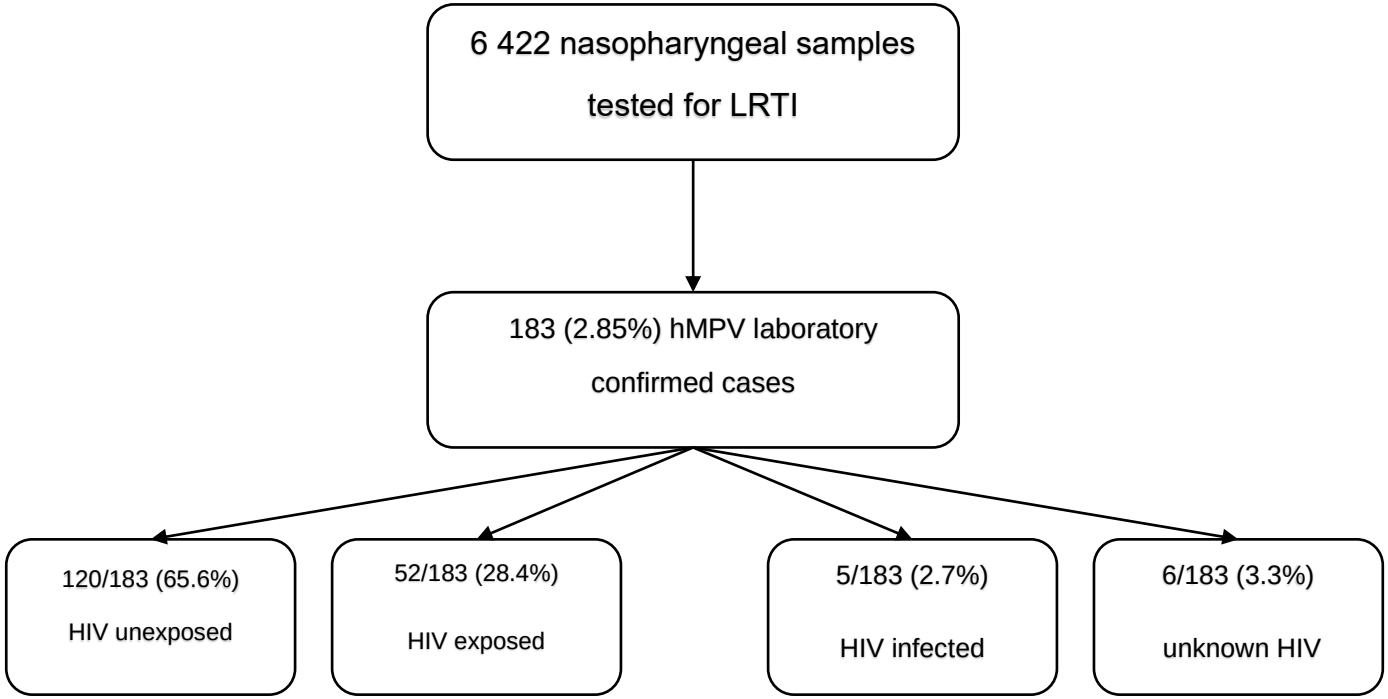
The overall annual hMPV-associated LRTI hospitalisation incidence (per 100,000 population per year) was similar in infants 0 to <6 months (260.34) and 6 to <12 months (233.37), which was higher compared with the 12 to <60 months age group (37.88). The incidence between age groups did not differ substantially year-on-year (Table 4.2).

The incidence rate ratio (IRR) of hMPV-LRTI in HEU compared with HUU children was 1.5 (95% CI 0.9-2.4) among 0 to <6 months old and 1.4 (95% CI 0.7-2.4) in the 6 to <12 months age group, and 0.9 (95% CI 0.4-1.9) in the 12 to <60 months age group; (Table 4.3).

Seasonality

hMPV was detected throughout the year but predominantly during winter and spring season. In 2015, the peak months were August to October (Figure 4.2). In 2016, the cases were detected earlier in the year, from March until September, while 2017 had the highest number of cases detected in the three years with the peak months from June to September.

Figure 4.1: Summary of enrolled children less than 5 years old- 2015-2017



Abbreviations: LRTI, Lower respiratory tract infection; hMPV, human metapneumovirus; HIV, human immunodeficiency virus.

Figure 4.2: Seasonality of hMPV cases: raw case numbers 2015-2017

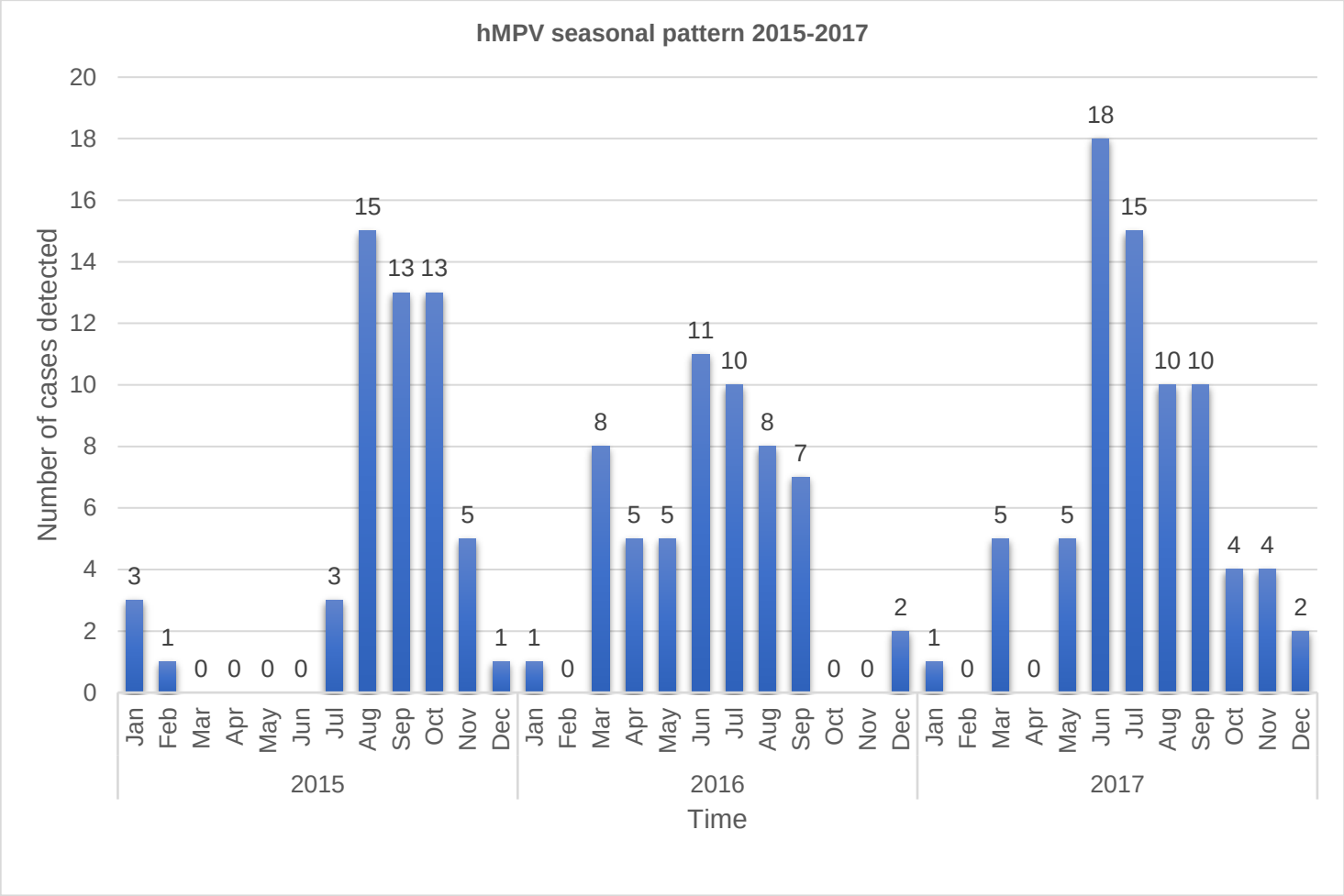


Table 4.1: Table of demographics and clinical outcomes of HIV exposed children < 5 years

Variable	Total (n=172)	HIV-1[a] unexposed (n=120)	HIV-1 exposed (n=52)
Infant demographic			
Age (months), n (%)			
< 6 months	73 (42.4)	49 (40.8)	24 (46.2)
6-<12 months	62 (36.1)	44 (36.7)	18 (34.6)
12-<60 months	37 (18.6)	27 (22.5)	10 (19.2)
Sex, proportion male (%)	94/150 (62.7)	63/105 (60)	31/45 (68.9)
Race, proportion black African (%)	142/148 (95.9)	97/103 (94.2)	45/45 (100)
Birth history			
Birth weight, g, median (IQR)[a]	3000 (2655-3395)	2990 (2635-3460)	3040 (2670-3347.5)
Birth height, cm, median (IQR)	50 (48-52)	50 (48-52)	49 (45.5-52)
Discharged home, n (%)	113/141 (80.1)	79/98 (80.6)	34/43 (79.1)
Admitted to ICU, n (%)	1/92 (1.1)	0/31 (0)	1/61 (1.6)
Underlying or chronic medical condition, n (%)	8/84 (9.5)	6/56 (10.7)	2/28 (7.1)
Clinical presentation			
Respiratory rate, mean (SD)[a]	55.6 (14.8)	55.6 (15.0)	55.47 (14.5)
Body Temperature median(IQR)[a]	36.9 (36.5-37.5)	37 (36.5-37.6)	36.9 (36.5-7.3)
Cough (n (%)	131/140 (93.6)	90/97 (92.8)	41/43 (95.4)

Cyanosis (n (%)	2/136 (1.5)	1/94 (1.1)	1/42 (2.4)
Runny rose/coryza, n (%)	30/134 (22.4)	20/94 (21.3)	10/40 (25)
Wheezing, n (%)	57/141 (40.4)	41/98 (41.8)	16/43 (37.2)
Respiratory distress, n (%)	100/140 (71.4)	67/97 (69.1)	33/43 (76.7)
Supplemental oxygen, n (%)	38/138 (27.5)	27/96 (28.1)	11/42 (26.2)
Hospitalisation duration, days, median (IQR)	2 (1-5)	1.5 (1-4)	3 (1-8)
Case fatality risk, proportion (%)	2/116 (1.7)	2/80 (2.5)	0/36 (0)

[a]Abbreviations: HIV-1, Human immunodeficiency virus; CI, Confidence interval; %, percentage; IQR, interquartile range; SD, standard deviation; ICU, Intensive care unit; n, sample size.

Table 4.2: hMPV annual incidence, by age groups, per 100,000 population per year

	2015				2016				2017			
Age	Raw number cases	Adjusted number of cases[a]	midpoint population estimate[b]	Incidence (95% CI)[c]	Raw number cases	Adjusted number of cases[a]	midpoint population estimate[b]	Incidence (95% CI)[c]	Raw number cases	Adjusted number of cases[a]	midpoint population estimate[b]	Incidence (95% CI)[c]
<6 months	25	43,5	19255	**225,8** (152,7-334,2)	24	38,5	19186	**200,9** (134,6-299,3)	31	67,8	18958	**357,8** (251,7-508,2)
6- <12 months	16	27,6	17693	**155,9** (95,6-254,5)	21	36,7	17630	**208,2** (135,8-319,1)	27	59,2	17420	**339,6** (233,2-495,2)
12-<60 months	12	43,5	146549	**29,7** (16,9-52,3)	12	56,4	146948	**38,4** (21,8-67,6)	15	67,1	147936	**45,4** (27,4-75,2)

[a]Adjustments were made to account for hospitalized hMPV cases whom we did not identify. Adjustments were made for: days when study enrolment did not occur, eligible cases but not tested, admission to the other public hospital in the catchment area and admission at a private hospital facility.

[b]Population size source: Department of Statistics South Africa (134).

[c]Abbreviations: CI, Confidence interval; hMPV, human metapneumovirus.

Table 4.3: Estimated annualised three year (2015-2017) hMPV incidence, by age group, per 100,000 population per year

	HEU				HUU				HEU vs HUU
Age	Raw number of cases	Adjusted number of cases	2016 Midpoint population estimates, averaged	Annualized Incidence (95% CI)	Raw number of cases	Adjusted number of cases	Midpoint population estimate[a]	Annualized Incidence (95% CI)[b])	Incidence Rate Ratio [IRR] (95% CI)
<6 months	24	44.7	4475	**333,0 (223,6-495,7)**	48	96.5	14119	**227,8** 171,9-302,0	**1.5** (0.9-2.4)
6- <12 months	18	36.4	4168	291,1 (183,8-461,1)	44	82.4	12918	**212,6** 158,4-285,4	**1.4** (0.7-2.4)
12-<60 months	10	34.9	36547	31,8 **(17,1-59,1)**	27	109.9	105867	**34,6** 23,7-50.5	**0.9** (0.4-1.9)

[a] Midpoint population estimate for the three year period was used. Population size source; Department of Statistics South Africa (134).

[b]Adjustments were made to account for hospitalized hMPV cases whom we did not identify. Adjustments were made for: days when study enrolment did not occur, eligible cases but not tested, admission to the other public hospital in the catchment area and admission at a private hospital facility

4.4 Discussion

Our study found that a higher incidence of hMPV LRTI hospitalisation in children <6 months (260 per 100,000 population per year) and six to <12 months (233) than in the 12 to <60 months age group (37). Kenyan studies have also reported the higher incidence burden of hMPV LRTI hospitalisation in this 0 to <6 months age group compared with older children (157, 190). The incidence of hMPV LRTI hospitalisation in children under six months of age in our study is lower compared with an earlier report from the same study setting done between 2009 and 2013 (653.3 per 100,000 population per year; 95% CI 602.2–707.6) (72). This could be due to differences in criteria for study inclusion, or due to variation in the severity of hMPV seasons from year to year (72).

Cohen et al. found elevated hMPV-associated LRTI hospitalisation incidence in HEU children <6 months old compared with HUU children <6 months old (26). The incidence rate ratio derived from our analysis for this age group trended in the same direction and was of a similar magnitude. Thus, our analysis corroborates this prior result. There is reduced transplacental transfer of anti-hMPV neutralizing antibody from women living with HIV-1 compared with those without HIV (191), which could explain an increased risk of hMPV-associated hospitalisation, at least up to the first six months of life.

Our findings suggest a seasonal distribution of hMPV LRTI hospitalisation, with a peak during the winter and spring seasons, which is consistent with other reports (72, 170, 192). Also, in agreement with many (62, 73, 193), but not all studies (190), there were few cases of co-infection with the few other respiratory organisms that we evaluated for in children hospitalised for LRTI.

Limitations of our study include that we cannot exclude co-infections with other organisms beyond which we tested. Additionally, we did not evaluate the impact of bacterial coinfection, except for *B. pertussis*. The contribution of bacterial pathogens such as *Streptococcus pneumoniae* to hMPV disease severity and hospitalisation has been previously reported (177). Pneumococcal conjugate vaccines have been reported to reduce the risk of hMPV-associated pneumonia hospitalisation by 45% (95%CI:19-62, p=0.002) (177). However, incidence of *S. pneumoniae* disease has dropped since the successful introduction of vaccination in South Africa (194). Also, children admitted directly to CHBAH intensive care unit prior to admission to the general ward may have been missed, however, direct

admission to the ICU is uncommon at CHBAH and is only allowed for children being transferred in from other facilities.

Our study may have introduced misclassification bias due to the subjective history of the maternal HIV-1 infection status to determine children's HIV-1 exposure status. Also, our study was observational, a design which is prone to selection bias in recruitment of study participants, sampling from a specified population, lack of comparison groups and confounding factors. We made adjustments when calculating incidence estimates and assumed that children not tested had the same incidence as children tested.

Importantly, our study showed that the highest burden of hMPV-associated hospitalisation is in children under one year of age, as previously reported (7, 72). Strategies to prevent hMPV morbidity should be developed. These particularly include immunization. Therapies for severe cases could include monoclonal antibody treatments or maternal immunziation to adress the high burden of hMPV-associated LRTI during early infancy.

Chapter 5: Fetal transfer of Human metapneumovirus neutralizing antibodies from mothers living with HIV-1 is reduced: Short report

Abstract

Background: HIV-1 exposed uninfected infants (HEU) have an increased risk of hospitalisation due to severe human metapneumovirus (hMPV) infection. We evaluated the influence of maternal HIV-1 status on transfer of hMPV neutralizing antibodies to the fetus.

Methods: 53 mother-infant dyads with the mother living with HIV-1 were randomly selected from a birth cohort in Soweto, South Africa and matched to 53 dyads in which the mother was HIV-1 negative. We measured hMPV neutralizing antibody titers against prototype viruses of genotype A and B in maternal and cord blood. We also compared cord blood hMPV neutralizing antibody titers at birth between 18 hMPV cases <6 months and 50 matched controls.

Results: Transplacental hMPV neutralizing antibody transfer was reduced from mothers living with HIV-1. Ratios of the cord blood to mother ratio (CMRs) of women living with HIV-1 to those without HIV-1 were 0.62 (95% CI 0.47-0.84, p=0.0015) and 0.60 (95% CI 0.44-0.82, p=0.0013) for genotype A and genotype B, respectively. The cord blood antibody titers at birth were similar in cases hospitalised with hMPV-associated lower respiratory tract infection (LRTI) before six months of age and matched controls.

Conclusions: Reduced transplacental transfer of hMPV neutralizing antibody from mothers living with HIV-1 could contribute to the previously reported elevated hMPV risk in HIV-1 exposed infants. However, cord blood levels of hMPV neutralizing antibodies were not lower in children subsequently hospitalised with hMPV. This suggests that protection from such antibody could be absent or limited in duration of protection.

5.1 Introduction

hMPV infection is associated with severe respiratory disease resulting in hospitalization (26, 72, 195). HIV-1 exposed uninfected (HEU) infants <6 months are at elevated risk of hMPV-associated hospitalisation (26). Transfer of maternal antibodies targeting a related pathogen, respiratory syncytial virus, is reduced in HEU infants (89, 196). It is unclear if any differences in transfer of maternal antibodies play a role in elevated hMPV risk in HEU children, or to what extent maternally-derived hMPV neutralizing antibodies confer protection against hMPV illness during early infancy.

Generally, maternal antibodies provide immune protection to newborn children before the immune system has fully developed. The transfer of immunoglobulins is dependent on factors such as gestational age of the fetus, the level of maternal IgG and placental integrity (88). A reduction in transfer of maternal antibodies to a related respiratory pathogen, respiratory syncytial virus (RSV), from mother to child has been observed in HEU infants (89, 101), although this did not result in lower cord blood antibody levels at birth in one study (89). A similar reduction in maternal transfer of hMPV antibodies has the potential to explain the increased susceptibility of HEU children to hMPV-associated hospitalisation (26).

The aim of this study was to evaluate the effect of maternal HIV-1 infection on titers of maternal and cord blood hMPV neutralizing antibody, and transplacental transfer. Secondarily, we evaluated whether hMPV-associated hospitalisation at <6 months was associated with lower hMPV neutralizing antibody titers in cord blood at birth. If higher cord blood hMPV neutralizing antibody titers were protective against hMPV, then we expect the cord blood hMPV neutralizing antibody titers to be reduced in subsequent hMPV cases compared with controls.

5.2 Methods

Setting

This study was nested within a birth cohort study at Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, South Africa, in which mother-newborn dyads were enrolled from June 2014 to investigate the serologic correlates of protection against invasive Group B Streptococcal disease (clinicaltrials.gov: NCT02215226) (137).

Mother-infant dyads

Fifty-three mother-infant dyads in which the mother was HIV-1 negative and 53 in which the mother was living with HIV-1 were selected randomly, matched by child gender, birth weight (±50g) and gestational age (term [≥37wks] matched with term, or ±1 week if <37 weeks). The most recent maternal HIV-1 status recorded on the antenatal card or hospital notes was taken as the final HIV-1 status.

Identification of hMPV cases <6 months old and controls

Cases of hMPV-associated hospitalisation were identified in a surveillance study, the Babies of Soweto Study (BoSS) (132), conducted at CHBAH. This study tested 6422 children under five years of age admitted to paediatric wards with symptoms consistent with lower respiratory tract infection (LRTI) from 2015-2018; we included children screened from January 2015 to March 2018. Nasopharyngeal swabs and/or aspirates were extracted for total nucleic acid content and tested for hMPV, RSV types A & B, influenza types A & B, and *Bordetella pertussis* (132) using a 1-step reverse transcriptase/polymerase chain reaction assay (ThermoFisher Scientific, City. Country).

Infants were included if they tested positive for hMPV at <6 months in the BoSS study and had a cord blood sample stored as part of the GBS study. They were each matched to a target of three control infants from the GBS study by HIV-1 exposure, gender, and date of birth (±2 weeks). Matching for date of birth was intended to match for hMPV exposure, which is seasonal and varies from year to year (72).

Sample size calculation

For the comparison of transplacental transfer from mothers living with HIV-1 versus HIV-1 negative mothers, the sample size of 53 pairs per group was based on a power of 90% to detect at 2-fold difference in neutralization titer between HEU infants and HUU infants, assuming that the variability in titer within groups resembled that of a two month old group (197).

The infants hospitalised with hMPV infection at <6 months were effectively a convenience sample, because they were all <6month cases detected in a large hospital surveillance study (the BoSS study) over a 27-month period who also had a prior cord blood sample stored in a large birth cohort (the GBS study). We maximized our power to detect a difference by matching to three controls per case wherever possible, for a total of 50 controls for 18 cases.

Virus neutralization assay

Based on genetic and antigenic variation, hMPV strains are classified in two genotypes, A and B (140). Recombinant hMPV NL/1/00 and hMPV NL/1/99 expressing green fluorescent protein (66) were used as prototypes for genotypes A and B. Neutralizing antibody titers were determined (66, 140) in Vero-118 cells (66) using two-fold dilutions from 1:8. The reciprocal of the highest dilution in which no infected cells were observed was taken as the titer. The geometric mean of the scores for duplicate rows of dilutions was used. If infected cells were not observed at the 1:8 dilution, a titer of four was assigned for calculation purposes.

Statistical analysis

Statistical analysis was performed using Stata 13.1 (Stata Corp, College Station, USA). The cord blood to mother ratio (CMR) was the hMPV neutralizing antibody titer of the infant's cord blood divided by that of the mother's blood. Titers, CMRs, maternal ages and number of previous pregnancies were compared using multilevel mixed effects linear regression with a random effect for each group of two matched dyads. Thus, in these comparisons, each woman living with HIV-1, or her child, were compared directly only to the matched HIV-1 negative woman or her child. Differences in $\log_2$ values from the models were converted back to fold differences of unlogged values to describe fold differences in titer or CMR.

Ethical approval

These studies were approved by the University of the Witwatersrand Human Research Ethics Committee (M161149). Written consent was collected under the parent protocols: GBS (M140203), and BoSS (M140904).

5.3 Results

Demographics

Women living with HIV-1 (median age 30 (IQR 24-34)) were on average 3.57 (95% CI 1.32-5.81) years older than the matched HIV-1 uninfected women (median 25 (IQR 22-30); p=0.0018). Women living with HIV-1 (2 (IQR 1-2.5)) had on average 0.83 (95% CI 0.40-1.26) more previous pregnancies compared with the HIV-1 negative mothers (1 (IQR 0-2); p=0.0001). Viral loads and $CD4^+$ T cell counts were not routinely assessed and were not

recorded. Of the 45 mothers living with HIV-1 (of 53) for whom data were recorded, 44 were on antiretroviral therapy at delivery suggesting they could've been HIV-1 virologically suppressed.

Comparison of hMPV neutralizing antibody titers and CMRs by maternal HIV-1 status

Serum hMPV neutralizing antibody titers were not different between women living with HIV-1 and HIV-1 negative women. Ratios of titers of women living with HIV-1 compared with matched HIV-1 negative women were 1.01 (95% CI 0.74-1.34; p=0.9658) for hMPV genotype A, and 1.18 (95% CI 0.80-1.73; p=0.4029) for hMPV genotype B (Figure 5.1a).

hMPV genotype A neutralizing antibody titers in HEU infants were reduced: they were 0.64-fold (95% CI 0.45-0.92; p=0.0114) of those in matched HUU. There was a non-significant trend in the same direction for hMPV genotype B neutralizing antibody titers, with a ratio of 0.72 (95% CI 0.48-1.08; p=0.1117) (Figure 5.1b).

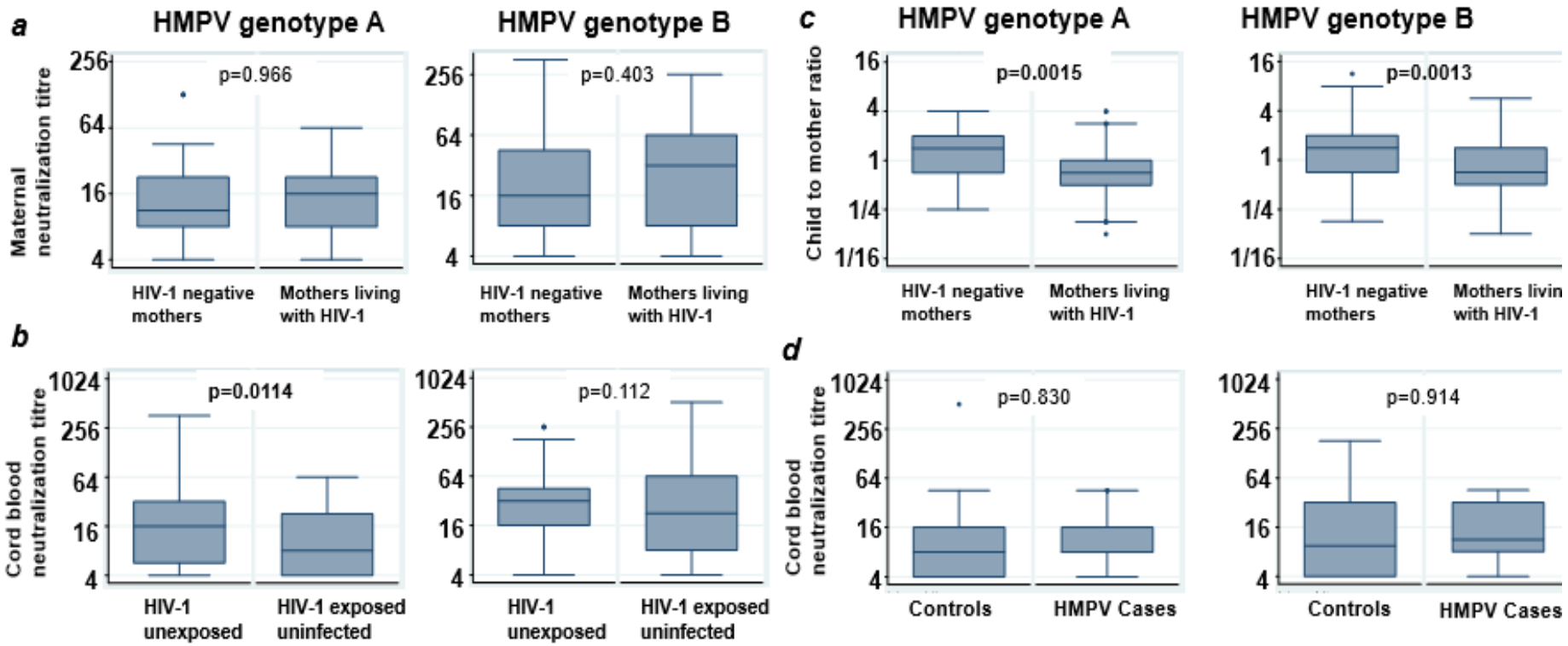
Transplacental transfer of hMPV neutralizing antibodies was significantly lower in women living with HIV-1 compared to that of matched women without HIV-1 (Figure 5.1c). The median cord blood to mother serum ratios (CMRs) were 0.71 (interquartile range [IQR], 0.50-1.0) vs 1.4 [IQR 0.71- 2.0] (genotype A) and 0.71 [IQR 0.50-1.4] vs 1.4 [IQR 0.71- 2.0] (genotype B). Using a mixed effects analysis that accounts for the matching that we employed, ratios of the CMRs of women living with HIV-1 to those without HIV-1 were 0.62 (95% CI 0.47-0.84, p=0.0015) and 0.60 (95% CI 0.44-0.82, p=0.0013) for genotype A and genotype B, respectively.

Cord blood neutralizing antibody titers in children with hMPV-associated hospitalisation at <6 months

We compared cord blood neutralizing antibody titers in 18 hMPV-associated hospitalisation cases at <6 months of age identified in the parent BoSS study for whom there were stored cord blood samples to those of 50 matched controls. No differences in neutralizing antibody titers were found (Figure 5.1d). The ratio of titers of cases to titers of controls was 1.05 (95% CI 0.65-1.71) and 1.03 (95% CI 0.59-1.80) for genotype A and B, respectively. None of the 18 hMPV cases tested positive for influenza A or B, RSV B or *B. pertussis* in the same sample. Only one hMPV sample was positive for RSV A at cut-off threshold (C_t)=38 (not

shown). It thus seems likely that the symptoms resulting in hospitalisation were likely caused by hMPV and not caused by co-infecting pertussis, influenza or RSV.

Figure 5.1: hMPV neutralising antibody titers and CMR



hMPV neutralizing titer and child to mother ratio (CMR) by maternal HIV-1 status or by whether child had an HMPV-associated hospitalization in the first six months of life**.** (a-c) Neutralizing titers to prototype hMPV genotype A and genotype B viruses in maternal blood (a), the cord blood of newborn infants (b) and child to mother ratios (c) are displayed for mother-infant dyads in which the mother was living with HIV-1, compared to matched mother-infant dyads in which the mother was HIV-1 negative. (d) Neutralizing titers to prototype hMPV genotype A and genotype B viruses are displayed for the cord blood are displayed for 18 newborn infants who had a subsequent hMPV-associated hospitalization in the first six months of life, compared to titers of 50 matched controls.

5.4 Discussion

We found a significant decrease in cord blood to mother blood ratios (CMRs) of hMPV neutralizing antibody levels in women living with HIV-1 infection, albeit no detectable difference in maternal serum levels. Transplacental neutralizing antibody transfer was reduced by 60% to newborns of women living with HIV compared with those born to HIV-uninfected women.

Reduction in transplacental transfer of maternal hMPV neutralizing antibodies was postulated as a possible reason for the increased risk hMPV-associated hospitalisation in HEU compared with HUU infants that had been previously described (26). We did not, however, find any difference in cord blood levels of hMPV neutralizing antibodies in children subsequently hospitalised with hMPV compared to matched controls. This suggests that neutralizing antibody at birth is not highly protective and/or that the duration of protection is relatively short lived.

Limitations of our study include that we did not exclude co-infections with other organisms that could have caused symptoms beyond those that we tested. In particular, the contribution of *S. pneumoniae* to hMPV disease severity and hospitalisation has been previously reported (177). Pneumococcal conjugate vaccines have been reported to reduce the risk of hMPV-associated pneumonia hospitalisation by 45% (177). However, incidence of *S. pneumoniae* disease has dropped markedly since the successful introduction of the pneumococcal conjugate vaccine in South Africa (198). Importantly, our results, from a relatively limited number of cases, limited our ability to detect protection that is short lived or of limited magnitude.

In summary, transfer of maternal hMPV neutralizing antibodies was reduced from mothers living with HIV-1. There was, however, no association observed for cord blood hMPV neutralizing antibody and hospitalisation for hMPV0-associated LRTI, albeit having only included a limited number (n=18) cases in this analysis.

Chapter 6: Pulmonary sequelae in children with Human metapneumovirus associated lower respiratory tract Infection

Abstract

Background: Human metapneumovirus (hMPV)-associated lower respiratory tract infection (LRTI) is an important contributor to childhood morbidity. In this study we use infant pulmonary function tests (PFT) to measure and describe the pulmonary sequelae of children hospitalised with hMPV-associated LRTI during the first two years of life.

Methods: We conducted a case-control study describing the pulmonary function in children under two years of age with hMPV-associated LRTI hospitalisation, admitted to Chris Hani Baragwanath Academic Hospital, Soweto, South Africa, between January 2017 and February 2019. hMPV cases were selected from an existing Babies of Soweto surveillance study and controls were healthy community controls identified from a previous birth cohort study. The PFTs performed were tidal breath flow volume loops (TBFVL), and multiple breath washout (MBW). The cases and controls were tested at approximately one and two years of age.

Results: PFT was successful in 365 one year old and 229 two year old children. In the first year of life hMPV-associated LRTI was associated with a higher respiratory rate (27.91; interquartile range [IQR], 25.88-31.78) than controls (27.17 (IQR 24.44-30.72)), (p=0.05). Furthermore, the inspiratory time and lung clearance index was higher in controls (1.00 seconds (IQR 0.89-1.10) vs 0.93 seconds (IQR 0.86-1.04), p=0.03) and 7.27 (IQR 6.79-8.03) vs 6.81 (IQR 6.43-7.59), p<0.0001) respectively. Lung function at two years also showed higher respiratory rates in cases (25.19 (IQR 24.41-28.01)) than controls (24.35 (IQR 22.21-26.89); p=0.05). The tidal volume was higher in controls (122.50ml (IQR 113.88-132.82)) than in cases (117.95 (IQR 107.79-124.090; p=0.01).

Conclusion: Hospitalisation for hMPV-associated LRTI during the first year of life was associated with impaired lung function in children at one and two years of age. These results illustrate the need for interventions to protect against hMPV-associated LRTI during early infancy.

6.1 Introduction

Human metapneumovirus (hMPV) was first identified in 2001 in The Netherlands, as a cause of upper and lower respiratory tract infections (LRTI) (34). Clinical manifestations of hMPV infections include bronchiolitis, pneumonia, croup, and asthma exacerbations. Infection with hMPV has been associated with hospitalisation, increased severity of respiratory disease, and death in high-risk groups, such as children less than five years of age, immunocompromised and elderly individuals (26, 33, 72).

LRTI in children has been reported to cause pulmonary inflammation, leading to long-term lung function abnormalities (199). Pulmonary function tests (PFT) are done to assess and monitor lung function and pulmonary development in children (107, 109, 142) . Spirometry has been commonly used in the past to measure lung function, however children under five years find the complex instructions difficult to follow, compromising the quality and the results of the tests. Evolving technology has introduced PFTs that require minimal cooperation in children under five years and have shown good validity and reproducibility. These tests include multiple breath inert gas washout techniques (MBW), which measures functional residual capacity (FRC) and the lung clearance index (LCI) (117); and tidal breath flow volume loops (TBFVL), which measures airflow and volumes (142).

Gray et al. used MBW to compare lung function between one year old children with and without LRTI, and reported that children with LRTI had an increased respiratory rate (95% confidence interval [CI], 1.01-1.08; P = 0.02), decreased tidal volume (95% CI, 23.3-20.2; P = 0.03), and increased LCI (95% CI, 0.04-0.22; P = 0.006) (104). These results suggest that LRTI acquired within the first year of life influences the subsequent pulmonary development in children.

The impact of hMPV-associated LRTI on lung function has not yet been fully elucidated, with one study reporting that alveolar epithelial cells infected with hMPV secrete increased levels of cytokines and chemokines, which trigger the pulmonary inflammatory cascade (112). A similar response, with long term pulmonary inflammation, was also seen in mice after being infected with hMPV (113, 114). Increased levels of pulmonary inflammation induced by cytokines in children with hMPV has been associated with episodes of recurrent wheezing, exacerbations of chronic obstructive airway disease, asthma and bronchiolitis (113) .

The clinical symptoms of hMPV are similar to those of respiratory syncytial virus (RSV) (36, 51). Studies investigating the risk of impaired lung function post-RSV LRTI, using PFTs, have shown evidence of small airway obstruction, with no bronchodilator reversibility (107, 109) . Because of the similarities between RSV and hMPV, we hypothesized that hMPV infection in the first two years of life would be associated with impaired future lung function.The primary aim of this study was to measure and describe the pulmonary sequelae in children hospitalised with hMPV-associated LRTI during the first two years of life.

6.2 Methods

Study design

This study was a prospective case-control study. hMPV cases were selected from an existing Babies of Soweto surveillance study (BoSS), (HREC (Med): 140904). The controls were healthy community controls identified from a previous birth cohort study V98_280B (HREC (Med): 140203) and surveillance of pathogen-specific causes of pneumonia and diarrhoea hospitalization in children (HREC (Med): 131109).

Cases

The cases enrolled in this study were children less than two years of age, previously admitted to CHBAH with LRTI, and part of the BoSS surveillance study. We enrolled all positive hMPV cases between 01 January 2016 and 31 December 2017. Suitable hMPV cases that had been discharged from the hospital were invited via telephone. Cases were matched to controls at a ratio of 1:3 according to gender, age (±2months) and race.

Controls

Controls were healthy participants, with no significant medical history, and no history of hospitalisation due to any LRTI. The controls were randomly selected from the database of V98_28OB study using statistical software package R version 3.5.1. A total of 100 babies between ages of 10-14 months were selected at each assessment period. There was no enrolment of controls from the V98_28OB into the hMPV iPFT study after 31 December 2018. Thereafter, additional community controls were enrolled from Surveillance of Severe Childhood Illness in Soweto, South Africa (HREC (Med): M140904).

Demographic and clinical details from cases and controls were collected from the parent/ legal guardian of the child at the one and two year visits. The adapted International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three Core Questionnaire was used to collect the information on the presence of asthma, wheezing and atopic features in the past year (141). The cases and controls had pulmonary function testing done at one (10-18 months) and two years (22-26 months) of age.

Exclusion criteria

Children were excluded if they had a birth weight less than 2500g, were premature (born <37 weeks) or had an acute or chronic medical diagnosis, that could adversely affect the pulmonary function, including congenital, cardiac, respiratory, neurological, renal, endocrine and primary immunological conditions. Children were also excluded if they had a LRTI within three weeks before PFTs. Parents or legal guardians unwilling to consent for the child to be included in the study were not invited to participate in the study.

Lung function measures

PFT measurements (TBFVL and MBW) were done according to American Thoracic Society/European Respiratory Society guidelines (142), using an EXHALYSER® D with SPIROWARE® 3.2.1 software (Ecomedics, Duernten, Switzerland). TBFVL and MBW was performed during stable tidal breathing, while in unsedated natural sleep. A well-fitting facemask (No. M30.8005, ECOMEDICS, Duernten, Switzerland or No. ANCM1512, Intersurgical, Johannesburg, South Africa) was connected to the flow head (TBFVL) or combination of a dead space reducer (DSR), spirette, with the flow head, was connected to a CO_2 airway adapter and a Nafion^{TM} tube (MBW). For MBW, a bypass airflow flow of 200ml/min was delivered to the participant. During MBW measurements, the patient inhaled a mixture of gases (SF_6 (4%), O_2 (21%) and nitrogen (75%) (Puregas, Johannesburg, South Africa)), controlled by the SPIROWARE® software.

Ethical approval

The BoSS study was approved by the University of the Witwatersrand Medical Ethics Committee (HREC (Med): 140904), as was the V98_28OB research study (HREC (Med): 140203). The hMPV PFT study was approved by the University of the Witwatersrand Medical Ethics Committee (HREC (Med): 161149) and (HREC (Med): M160224).

Permission to use the CHBAH Medicom system was obtained through the relevant authorities.

Statistical analysis

We used STATA version 13 (City, Country) for statistical analysis. The clinical and pulmonary sequelae measurements with normal data distribution were described using means and standard deviation (95% confidence interval). Data with skewed data distribution were described using median and interquartile range. Categorical variables were described using count (% percentage). Comparison of normal continuous variables was done t-test while comparison of skewed continuous variables was done using Mann-Whitney U test. Comparison of categorical variables in hMPV cases and healthy controls was done using chi square test and Fisher's exact test as appropriate. The odds ratios (OR) with 95% confidence intervals (95% CI) were reported. The P value of <0.05 was considered statistically significant. Regression analysis was performed to adjust for potential confounders with developing pulmonary sequelae in children less than five years. Linear regression was used for continuous outcome variables to analyse the associations between different covariates and lung function outcomes. Logistic regression was used for variables with categorical data. In the first step, we included the variables in the univariate regression model, and variables with a p<0.2 were included in the multivariate regression. model.

6.3 Results

Lung function results for one year old infants

Study population

Between January 2017 and February 2019 59 cases and 306 controls were tested before 18 months of age (Table 6.1). Cases were more likely to be male (69.5%).

Risk factors for developing pulmonary sequelae

Cases were more likely to have been admitted post-delivery (Odds ratio (OR) 4.58 (95% confidence interval (CI): 1.98-10.19), p<0.0001) or have had an admission prior to the hMPV-associated admission (OR 4.00 (95% CI 2.16-7.48), p=0.01) (Table 6.2). Attending

crèche was more common amongst hMPV LRTI cases than healthy controls (OR 2.56 (95% CI 1.19-5.29), p=0.02).

ISAAC questionnaire data

Cases had more episodes of wheezing; were more likely to have been admitted for wheezing or chest infections; had more sleep disturbance due to wheezing or a dry cough at night; had more wheezing during activity; and were more likely to be diagnosed with asthma or to have received treatment for wheezing, in the period following hospitalisation for hMPV-associated LRTI and pulmonary function testing (Table 6.3).

Table 6.1: Basic demographics of one-year-old hospitalised hMPV LRTI cases and controls

Variables	**Cases (N=59)**	**Controls (N=306)**	**p-value**
Age (Months) (Median (IQR)	12 (10-13)	12 (12-13)	**0.001**
Gender Male (N/%)	41/59 (69.49)	160/304 (52.63)	**0.02**
Weight (kg) (Median (IQR)	9.5 (8.5-10.9)	9.7 (8.8-10.5)	0.69
Height (cm) (Median (IQR)	73.65 (71-76.8)	74 (72-77)	0.60
Birth weight (g) (Median (IQR)	3060 (2795-3560)	3112.5 (2887.5-3380) 1450-4875	0.76
HIV status (N/%)			0.41
- Unexposed	36/54 (66.66)	215/298 (72.15)	
- Exposed uninfected	16/54 (29.63)	79/298 (26.51)	
- Infected	0/54 (0)	1/298 (0.33)	
- Unknown	2/54 (3.70)	3/298 (1.00)	

Underlying medical conditions (N/%)	0 (0.0)	0 (0.0)	1.0
Age (Months) at hMPV admission (Median (IQR)	5.61 (2.92- 8.46)	N/A	N/A
Time (months) between hMPV admission and 1 year visit (Median (IQR)	6.49 (4.39-8.43)	N/A	N/A
Duration of hMPV admission (days)(Median (IQR)	1.5 (1-3)	N/A	N/A
Required oxygen during hMPV admission (N/%)	4/39 (10.26)	N/A	N/A

Abbreviations: hMPV, Human metapneumovirus; LRTI, Lower respiratory tract infection; IQR, Interquartile range; N/A, data not available/reported; N, Sample size.

Table 6.2: Risk factors for developing pulmonary sequelae in one-year-old hospitalised hMPV LRTI cases and controls

Variable	Case (N=59)	Control (N=306)	p-value	OR (95% CI)
Admission post-delivery (N/%)	14/47 (29.79)	25/295 (9.83)	**<0.0001**	4.58 (1.98-10.19)
Duration of admission post-delivery (days) (Median (IQR))	2 (1-3)	4 (2-7)	**0.01**	N/A
Required oxygen post-delivery (N/%)	4/39 (10.26)	10/283 (3.53)	**0.05**	3.12 (0.67-11.50)
Duration of oxygen post-delivery (days) (Median (IQR))	3 (2-17)	2 (1-4)	0.44	N/A
Hospitalised prior to hMPV admission (N/%)	36/59 (61.01)	86/306 (28.10)	**0.01**	4.00 (2.16-7.48)
Weight loss in last six months (N/%)	7/53 (13.21)	45/296 (15.20)	0.64	0.85 (0.30-2.06)
TB contact (N/%)	1/52 (1.92)	16/293 (5.46)	0.35	0.34 (0.01-2.29)

Mother's HIV status: Positive (N/%)	17/50 (34.00)	90/293 (30.72)	0.64	1.16 (0.58-2.28)
Maternal atopy (reported) (N/%)	6/52 (11.50)	47/297 (15.82)	0.43	0.69 (0.28-1.72)
Paternal atopy (reported) (N/%)	1/52 (1.92)	30/297 (10.10)	0.09	0.17 (0.02-1.31)
Sibling atopy (reported) (N/%)	3/59 (5.08)	22/206 (10.68)	0.21	0.44 (0.13-1.55)
Smoke exposure within home (N/%)	1/22 (4.55)	13/292 (4.45)	0.98	1.02 (0.02-7.47)
Smoke exposure from primary caregiver during pregnancy (N/%)	0/34 (0.00)	9/295 (3.05)	0.57	0.43 (0.02-7.68)
Does primary caregiver consume alcohol (N/%)?	6/55 (10.91)	61/297 (20.54)	0.09	0 .47 (0.16-1.18)
Alcohol consumption by primary caregiver during pregnancy (N/%)	3/38 (7.89)	8/295 (8.42)	0.09	3.07 (0.50-13.54)
Home construction (Brick) (N/%)	40/55 (72.73)	228/298 (76.51)	0.55	0 .81 (0.41-1.69)

Total rooms in the house (Median (IQR))	4 (1-5)	4 (1-4)	0.64	N/A
Main energy source (Electricity) (N/%)	56/59 (94.92)	298/306 (97.39)	0.42	0 .50 (0.12-3.02)
Main water source of household (Indoor) (N/%)	29/52 (55.77)	160/297 (53.87)	0.87	1.08 (0.57-2.05)
Type of toilet (Flush) (N/%)	46/53 (86.79)	271/297 (91.23)	**0.01**	0.63 (0.25-1.82)
Number of people sleeping in the same room as the child (Median (IQR)	2 (2-2)	2 (1-2)	0.23	N/A
Total number of people living in the house (Median (IQR)	5 (4-6)	5 (3-6)	0.93	N/A
Under-5s attending crèche in the household (Median (IQR)	0 (0-1)	0 (0-1)	**0.01**	N/A
Child attends crèche (N/%)	15/55 (27.27)	38/298 (12.75)	**0.02**	2.56 (1.19-5.29)
Pets in the household (N/%)	8/52 (15.38)	44/297 (14.81)	0.91	1.04 (0.39-2.44)
Exposure to farm animals (N/%)	1/52 (1.92)	6/298 (2.01)	0.97	0.95 (0.02-8.12)

RTHC available (N/%)	50 /51 (98.04)	285/298 (95.67)	0.42	2.28 (0.33-98.81)

Abbreviations: hMPV, Human metapneumovirus; OR, Odds ratio; IQR, Interquartile range; N/A, data not available/reported; TB, Tuberculosis; HIV, Human immunodeficiency virus; RTHC, Road to health card; N, Sample size; Atopy, refers to the development of asthma, eczema and allergic rhinitis.

Table 6.3: ISAAC data for one-year-old hospitalised hMPV LRTI cases and controls

Variable	Case (N=59)	Control (N=306)	p-value	OR (95% CI)
Wheezing before hMPV admission (N/%)	46/55	N/A	N/A	N/A
Wheezing in past year* (N/%)	32/54 (59.26)	70/298 (23.49)	**< 0.0001**	4.74 (2.48-9.10)
Admissions for wheezing episodes after hMPV during last year* (N/%)	32/54 (59.26)	70/298 (23.49)	**< 0.0001**	4.74 (2.48-9.10)
Number of admissions for wheezing episodes during last year* (N/%) -1-2 ->2	 13/19 (68.42) 6/19 (31.58)	 11/17 (64.71) 6/17 (35.29)	 0.81	 1.18 (0.24-5.89) 0 .85 (0 .17-4.23)
Admission for chest infections during last year* (N/%)	 13/19 (68.42) 6/19 (31.58)	 11/17 (64.71) 6/17 (35.29)	 0.81	 1.18 (0.24-5.89) 0 .85 (0 .17-4.23)
Number of admissions for chest infections during last year* (N/%) -1-2 ->2	11/55 (20)	7/298 (2.35)	**< 0.0001**	10.39 (3.43-33.04)
Sleep disturbance due to wheezing during last year* (N/%)	 7/13 (53.85) 6/13 (46.15)	 6/7 (85.71) 1/7 (14.29)	 0.15 0.15	 0 .19 (0.00-2.59) 5.14 (0.39-275.2)

Number of times per week sleep disturbed due to wheezing during last year* (Median (IQR)	38/55 (69.09)	36/298 (12.08)	**< 0.0001**	16.27 (7.93-33.76)
Number of nights with dry cough apart from cold or infection during last year* (N/%)	43/53 (81.13)	76/298 (25.50)	**< 0.0001**	12.56 (5.81-29.19)
Wheezing with activity during last year (N/%)	26/53 (49.06)	N/A	N/A	N/A
Doctor's diagnosed asthma during last year (N/%)	26/53 (49.06)	15/296 (5.07)	**< 0.0001**	18.04 (8.01-40.94)
Received any treatment for wheezing during the past year * (N/%)	5/51 (9.80)	2/297 (0.67)	**< 0.0001**	16.03 (2.50-170.60)
Doctor's diagnosis of eczema (N/%)	19/40 (47.5)	23/294 (7.82)	**< 0.0001**	10.66 (4.7-24.07)
Itchy rash that comes and goes during the past year (N/%)	8/57 (14.04)	50/297 (16.83)	**< 0.0001**	0 .81 (0.31-0.85)
Itchy rash that affects the folds of the elbows, behind the knees, front of the ankles, buttocks, around the eyes during the past year (N/%)	22/57 (38.59)	114/297 (38.38)	0.98	1.01 (0.54-1.87)

* For cases questions apply to the remainder of the year after the hMPV admission. Abbreviations: hMPV, Human metapneumovirus; IQR, Interquartile range; N/A, data not available/reported; N, Sample size.

Main pulmonary function data

Acceptable measurements were obtained in 86% of TBFVL, and 75% of MBW tests.

Cases had a higher respiratory rate than controls (27.91 (IQR 25.88-31.78) vs 27.17 (IQR 24.44-30.72) , p=0.05). The inspiratory time (Ti) was longer in controls, (1.00 seconds (IQR 0.89-1.10) vs 0.93 seconds (IQR 0.86-1.04), p=0.03) (Table 6.4).

The LCI was higher in controls than in cases (7.27 (6.79-8.03) vs 6.81 (6.43-7.59), p<0.0001) (Table 6.4).

Table 6.4: Pulmonary function data for one-year-old hospitalised hMPV LRTI cases and controls

Variables	**Case (N=59)**	**Control (N=306)**	**p-value**
TBFVL completed (N/%)	59/59 (100.00)	255/306 (83.33)	**0.03**
MBW completed (N/%)	47/59 (79.66)	214/306 (69.93)	0.30
Respiratory rate (b/min) (Median (IQR)	27.91 (25.88-31.78)	27.17 (24.44-30.72)	**0.05**
Tidal volume (ml) (VT) (Median (IQR))	95.32 (79.51-107.61)	97.78 (89.63-107.66)	0.08
Inspiratory time (TI)(sec) (Median (IQR))	0.93 (0.86-1.038)	1.00 (0.89-1.10)	**0.03**
Expiratory time (TE)(sec) (Mean (SD))	1.14 (0.97-1.29)	1.20 (1.04-1.37)	0.14
Mean tidal inspiratory flow (MTIF) (ml/s) (Median (IQR))	96.71(81.04-107.48)	117.85 (105.04- 132.01)	0.14

Peak tidal inspiratory flow (PTIF)(ml/s) (Median (IQR))	122.31 (108.36-139.07)	126.67 (115.83-138.55)	0.25
Mean tidal expiratory flow (MTEF) (ml/s) (Median (IQR))	80.44 (75.98-93.79)	82.58 (74.44-92.90)	0.93
Peak tidal expiratory flow (PTEF)(ml/s) (Median (IQR))	118.92 (106.76-139.61)	117.85 (105.04-132.01)	0.22
Ratio of time to peak tidal expiratory flow to expiratory time (TPEF/TE) (%) (Median (IQR)	34.99 (27.88-44.27)	37.68 (30.86-46.84)	0.18
Functional residual capacity (FRC) (ml) (Median (IQR))	167.78 (142.43-199.42)	175.22 (154.24-200.48)	0.39
Lung clearance index @ 2.5% (LCI2.5%) (Median (IQR))	6.81 (6.43-7.59)	7.27 (6.79-8.03)	**<0.0001**
Oxygen saturation (%) (Median (IQR))	96.67 (81.03-107.47)	96.98 (89.39-105.12)	0.14

Abbreviations: TBFVL, Tidal breathing flow volume loop; MBW, Multiple breath washout; IQR, Interquartile range; N, Sample size; b/min, breath per minute; ml, millilitre, Sec, Second; SD, Standard deviation; ml/s, Millilitre per second.

Regression analysis of pulmonary function outcomes and lung function predictors

The results of the multivariate regression analyses for associations between different covariates and lung function outcomes are shown in Appendix 6-10.

Weight at one year was associated with reduced respiratory rate, as was being male. Being male also increased the LCI. Maternal HIV was associated with increased tidal volume and with reduced tPTEF/tE at one year of age. Household smoke exposure and exclusive breastfeeding was associated with a reduced LCI, while children that had siblings that attend crèche were associated with an increased LCI. The hMPV LRTI status was not associated with any of the lung function outcomes in the regression analysis.

Lung function results for two years old children

Study population

A total of 39 cases and 190 controls children aged two years old were tested before 26 months of age (Table 6.5). Cases were more likely to be male (64.1%). The height for cases was 83 (IQR 79-87) and 86 months (IQR 83-89, p<0.0001) for controls. Furthermore the cases had a weight of 10kg (IQR 10-11) and 11 kg (IQR 10-12, p=0.01) for controls. There were no differences in birth and HIV status between the groups

Risk factors

The two year old cases were more likely to have been admitted post-delivery (odds ratio (OR) 4.34(95% confidence interval (CI): 1.62-11.17, p=0.001)) (Table 6.6). There were no differences between the groups.

ISAAC questionnaire data

The ISAAC questionnaire for the two-year-old children showed no difference between hMPV-LRTI cases and controls in the period following hospitalisation for hMPV-associated LRTI and pulmonary function testing. See table 6.7

Table 6.5: Basic demographics of two-year-old hospitalised hMPV LRTI cases and controls

Variable	**Case (N=39)**	**Control (N=190)**	**p-value**
Age (Months) (Median (IQR))	22.15 (21.34-23.13)	23.85 (23.16-24.66)	**<0.0001**
Gender Male (N/%)	25/39 (64.10)	83/190 (43.68)	**0.02**
Race Black African (N/%)	39/39 (100.00)	190/190 (100.00)	**0.43**
Weight (kg) (Median (IQR))	10.9 (10-11.7)	11.6 (10.6 -12.5)	**0.01**
Height (cm) (Median (IQR))	83 (79-87)	86 (83-89)	**<0.0001**
Birth weight (g) (Median (IQR))	3110 (2800-3460)	3185 (2880-3480)	0.47
HIV status (N/%)			0.49
- Unexposed	19/29 (65.52)	139/189 (73.54)	
- Exposed uninfected	10/29 (34.48)	44/189 (23.28)	
- Infected	0/2 (0.00)	1/189 (0.53)	
- Unknown	0/29 (3.70)	5/189 (2.65)	
Underlying medical conditions (N/%)	0 (0.0)	0 (0.0)	1.00

Age (Months) at hMPV admission (Median (IQR))	5.61 (2.92- 8.46)	N/A	N/A
Time (months) between hMPV admission and 1 year visit (Median	6.49 (4.39-8.43)	N/A	N/A
Duration of hMPV admission (days)(Median (IQR))	1.5 (1-3)	N/A	N/A
Required oxygen during hMPV admission (N/%)	4/39 (10.26)	N/A	N/A

Abbreviations: hMPV, Human metapneumovirus; LRTI, Lower respiratory tract infection; IQR, Interquartile range; N/A, data not available/reported; N, Sample size.

Table 6.6: Risk factors for developing pulmonary sequelae in two-year-old hospitalised hMPV LRTI cases and controls

Variable	Case (N=39)	Control (N=190)	p-value	OR (95% CI
Admission post-delivery (N/%)	11/28 (39.29)	24/185 (12.97)	**0.001**	4.34 (1.62-11.17)
Duration of admission post-delivery (days) (Median (IQR))	1 (1-3)	5 (2-7.5)	0.10	N/A
Required oxygen post-delivery (N/%)	4/23 (17.39)	11/187 (5.88)	**0.04**	3.37 (0.71-12.78)
Duration of oxygen post-delivery (days) Median (IQR))	3 (2- 17)	4 (2-7)	0.95	N/A
Hospitalized prior to hMPV admission (N/%)	5/22 (22.73)	6/40 (15.00)	0.45	1.67 (0.35-7.59)
Weight loss in last 6 months (N/%)	5/29 (17.24)	47/186 (25.27)	0.35	0 .62 (0.17-1.78)
TB contact (N/%)	0/27 (0.00)	1/186 (0.54)	0.62	2.25 (0.08-56.59)
Mother's HIV status: Positive (N/%)	10/29 (34.48)	50/177 (28.25)	0.49	1.34 (0.52-3.27)
Maternal atopy (N/%)	3/27 (11.11)	13/188 (6.91)	0.44	1.68 (0.45-6.34)
Paternal atopy (N/%)	2/27 (7.41)	5/188 (2.66)	0.21	2.93 (0.54-15.90)

Sibling atopy (N/%)	2/27 (7.41)	13/188 (6.91)	0.93	1.07 (0.23-5.06)
Smoke exposure within home (N/%)	0/31 (0.00)	17/188 (9.04)	0.19	0.16 (0.009-2.65)
Smoke exposure from primary caregiver during pregnancy (N/%)	0/21 (0.00)	6/189 (3.17)	0.78	0.66 (0.04- 12.13)
Does primary caregiver consume alcohol (N/%)?	1/30 (3.33)	30/188 (15.96)	0.07	0.11 (0.00-0.69)
Alcohol consumption by primary caregiver during pregnancy (N/%)	0/23 (0.00)	6/189 (3.17)	0.73	0.60 (0.03-11.01)
Type of feeding during first 4 months (N/%) -Breastfeeding -Formula -Mixed feeding	 23/31 (74.19) 8/31 (25.81) 0/31 (0.00)	 104/189 (55.03) 52/189 (27.51) 33/189 (17.46)	 0.05 0.84 0.07	 2.35 (0.95-6.37) 0.92 (0.33-2.29) 0.07 (0.004-1.24)
Home construction (Brick) (N/%)	19/30 (72.73)	146/188 (76.51)	0.09	0.49 (0.21-1.26)
Total rooms in the house (Median (IQR))	3.5 (1-5)	3 (1-4)	0.43	N/A
Main energy source (Electricity) (N/%)	23/31 (74.19)	179/189 (94.71)	**0.0005**	0.16 (0.05-0.53)
Main water source of household (Indoor) (N/%)	14/29 (48.28)	174/188 (47.62)	0.34	0.08 (0.03-0.21)
Type of toilet (Flush) (N/%)	24/28 (85.71)	174/188 (92.55)	0.10	0.48 (0.14-2.19)

How many people sleep in the same room as the child (Median)	2 (2-3)	2 (2-3)	0.95	N/A
Total number of people living in the house (Median (IQR)	5 (4-6)	5 (3-6)	0.31	N/A
Under-5s attending crèche in the household (Median (IQR)	0 (0-1)	0 (0-1)	0.13	N/A
Child attends crèche (N/%)	14/30 (46.67)	71/189 (37.57)	0.34	1.45 (0.62-3.39)
Pets in the household (N/%)	4/28 (14.29)	22/187 (11.76)	0.70	1.25 (0.29-4.14)
Exposure to farm animals (N/%)	8/28 (28.57)	4/189 (2.12)	**0.03**	18.5 (4.37-89.20)
RTHC available (N/%)	29 /29 (100.00)	181/189 (95.77)	0.49	2.76 (0.16-49.16)

Abbreviations: hMPV, Human metapneumovirus; OR, Odds ratio; IQR, Interquartile range; N/A, data not available/reported; TB, Tuberculosis; HIV, Human immunodeficiency virus; RTHC, Road to health card; N, Sample size; Atopy, refers to the development of asthma, eczema and allergic rhinitis.

Table 6.7: ISAAC data for two-year-old hospitalised hMPV LRTI cases and controls

Variable	Case (N=39)	Control (N=190)	p-value	OR (95% CI)
Wheezing before hMPV admission (N/%)	27/28 (96.43)	N/A	N/A	N/A
Wheezing in past year* (N/%)	14/28 (50.00)	3/3 (100.00)	0.09	0.1429 (0-1.46)
Number of wheezing episodes during last year* (N/%) -1-3 >3	 13/14 (92.86) 1/14 (7.14)	 2/3 (66.67) 1/3 (33.33)	 0.24 0.24	 6.50 (0.28-151.13) 0.15 (0.01-3.58)
Admissions for wheezing episodes during last year* (N/%)	11/28 (39.28)	3/190 (1.58)	**< 0.0001**	40.33 (10.25-158.69)
Number of admissions for wheezing episodes during last year* (N/%) -1-2 ->2	 10/11 (90.91) 1/11/ (9.01)	N/A	N/A	N/A
Admission for chest infections during last year* (N/%)	4/28 (14.29)	N/A	N/A	N/A

Number of admissions for chest infections during last year* (N/%) -1-2 ->2	 4 /5 (80.00) 1/5 (20.00)	N/A	N/A	N/A
Sleep disturbance due to wheezing during last year* (N/%)	18/28 (64.29)	1/3 (33.33)	0.29	3.6 (0.16-223.92)
Number of times per week sleep disturbed due to wheezing during last year* (N/%) -1-2 ->2	 6/17 (35.29) 11/17 (64.71)	 1/1 (100.00) N/A	 0.33	 0.19 (0.01-5.33)
Number of times per week sleep disturbed due to wheezing during last year* (Median (IQR))	4 (2-4)	0 (0-1)	0.32	N/A
Dry cough at night apart from cold or infection during the last year* (N/%)	19/28 (67.86)	2/3 (66.67)	0.27	1.06 (0.08-13.23)
Number of nights with dry cough apart from cold or infection during last year* (N/%) -1-2 ->2	 4/17 (23.53) 13/17 (76.47)	 2/3 (66.67) N/A	 0.13 N/A	 0.15 (0-4.11) N/A
Number of nights with dry cough apart from cold or infection during last year* (Median (IQR))	4 (4-4)	3 (2-4)	0.47	N/A
Wheezing with activity during last year* (N/%)	9/27 (33.33)	3/3 (100)	0.09	0.07 (0.003-1.572)
Doctor’s diagnosed asthma during last year (N/%)	1/26 (3.85)	N/A	N/A	N/A

Received any treatment for wheezing during the past year * (N/%)	10/24 (41.67)	N/A	N/A	N/A
Doctor’s diagnosis of eczema (N/%)	3/31 (9.68)	N/A	N/A	N/A
Itchy rash that comes and goes during the past year (N/%)	14/31 (45.16)	2/3 (66.67)	0.48	0.41 (0.01-8.92)
Itchy rash that affects the folds of the elbows, behind the knees, front of the ankles, buttocks, around the	12/15 (80.00)	2/2 (100.00)	0.84	0.71 (0.03-18.59)

* For cases questions apply to the remainder of the year after the hMPV admission. Abbreviations: hMPV, Human metapneumovirus; IQR, Interquartile range; N/A, data not available/reported; N, Sample size.

Main pulmonary function data

Two year old cases were successfully tested as follows: TBFVL 39/39 (100.00%) and MBW 39/39 (100.00%). For controls >80% TBFVL 161/190 (84.74) and MBW 140/190 (73.68) were successful.

The hMPV LRTI cases had a higher respiratory rate (25.19 (IQR 24.41-28.01) vs 24.35 (IQR 22.21-26.89), (p=0.05)). Furthermore, the tidal volume was higher in the controls (122.50 ml (IQR 113.88- 132.82) vs 117.95ml (IQR 107.79-124.09), (p=0.001)). See table 6.8 for complete pulmonary function data. More extensive outcomes of pulmonary function data for one-year-old cases and controls are shown in the appendix.

The results of the multivariate regression show no association between lung function outcomes and any of the lung function predictors. See appendix 11-15.

Table 6.8: Pulmonary function data for two-year-old hospitalised hMPV LRTI cases and controls

Variables	Case (N=39)	Control (N=190)	p-value
TBFVL completed (N/%)	39/39 (100.00)	161/190 (84.74)	**0.06**
MBW completed (N/%)	39/39 (100.00)	140/190 (73.68)	**0.02**
Respiratory rate (b/min) (Median (IQR))	25.19 (22.41-28.01)	24.35 (22.21-26.89)	**0.05**
Tidal volume (ml) (VT) (Median (IQR))	117.95 (107.79-124.09)	122.50 (113.88- 132.82)	**0.01**
Inspiratory time (TI)(sec) ((Median (IQR))	1.08 (0.95-1.21)	1.10 (1.00-1.19)	0.39
Expiratory time (TE)(sec) (Median (IQR))	1.36 (1.17-1.58)	1.39 (1.22-1.52)	0.51
Mean tidal inspiratory flow (MTIF) (ml/s) (Median (IQR))	106.88 (96.75-119.82)	111.93 (100.52- 120.00)	0.21
Peak tidal inspiratory flow (PTIF)(ml/s) ((Median (IQR))	138.76 (123.47-156.63)	143.33 (128.5-153.95)	0.35
Mean tidal expiratory flow (MTEF) (ml/s) (Median (IQR))	90.18 (78.75-98.79)	93.18 (84.69-101.03)	0.19

Peak tidal expiratory flow (PTEF)(ml/s) (Median (IQR)) (Median (IQR))	123.52 (107.13-143.20)	128.04 (115.84-142.49)	0.56
Ratio of time to peak tidal expiratory flow to expiratory time (TPEF/TE) (%) (Median (IQR)	36.41 (28.49-44.34)	39.02 (33.11-45.38)	0.12
Functional residual capacity (FRC) (ml) (Median (IQR))	77 (24-140)	93.5 (48-133)	0.40
Lung clearance index @ 2.5% (LCI2.5%) (Median (IQR))	6.79 (6.52-7.01)	6.63 (6.33-7.01)	0.12
Oxygen saturation (%) (Median (IQR))	106.86 (96.74 -119.81)	111.93 (100.51-119.99)	0.21

Abbreviations: TBFVL, Tidal breathing flow volume loop; MBW, Multiple breath washout; IQR, Interquartile range; N, Sample size; b/min, breath per minute; ml, millilitre, Sec, Second; SD, Standard deviation; ml/s, Millilitre per second.

6.4 Discussion

To our knowledge, this is the first longitudinal follow-up study of lung function measurements in children after hMPV-associated LRTI using MBW and TBFVL.

Our study found several differences in respiratory parameters between children at one and two years of age following hospitalisation for hMPV-associated LRTI during infancy compared with healthy controls. We found that at one year of age, respiratory rate was increased and inspiratory time and lung clearance index were decreased in cases compared with controls. There are currently no similar studies investigating hMPV LRTI and pulmonary function testing that allow for direct comparisons with our results. In comparison, findings from a related study using the same testing technique in children with all-cause LRTI at one year of age, also reported a higher respiratory rate for cases (104). Previous studies showed that there is an increase in the respiratory rate and a decrease in tidal volume leading to impaired lung function at one year of age, after an LRTI (104, 200). This was reflected in our study, however, the increase in tidal volume in our study could also be due to differences in age and gender between the groups, which have been reported to influence both lung volumes and capacities (201).

The PFT results in this study showed a long term increase in respiratory rate and lower tidal volume at two years in cases compared with healthy children. Zar et al. reported that LRTI in children was associated with a 3% increase in respiratory rate and a decrease in compliance at two years compared to children without LRTI (202) .

In this study, hospitalisation for hMPV-associated LRTI was associated with more episodes of wheezing in the first year of life, after the initial episode. These results are consistent with a study by Coverstone et al. which reported a higher rate of wheezing episodes after hMPV in children less than five years with LRTI compared with controls who had never had a proven hMPV-associated LRTI (15). However, our study did not show significant difference between cases and controls, with regards to wheezing prevalence, at the two years follow up. In both studies the episodes of wheezing were reported by the mother or the legal guardian, which may overestimate the actual episodes of wheezing, or this might imply that the wheezing improves with time and that the initial increase in wheezing episodes in cases is still an after-effect of the acute hMPV LRTI and not a long-term issue.

Previous studies showed that weight-for-age, length-for-age, maternal smoking, maternal consumption of alcohol and maternal HIV status alter lung function parameters in African

children (7,8). A study on determinants of early-life lung function in African infants at 5-11 weeks of age reported that infants whose mothers smoked had lower tidal volumes and higher lung clearance index than infants unexposed to tobacco smoke. Our study showed that maternal smoking decreased lung clearance index -2.31 (-3.51- -1.12) at one year but had no effect on tidal volume.

Factors affecting lung function in children may differ in lower and middle income countries (LMICs) compared to high income countries. LMICs bear a disproportionately greater burden of morbidity and mortality due to LRTIs (3). LRTIs are strongly associated with impaired lung function (104). A systematic review on pulmonary function sequelae after respiratory syncytial virus lower respiratory tract infection in children in high income countries showed that children who have a confirmed RSV LRTI during the first three years of life are likely to have impaired iPFTs, particularly due to obstructive lung disease, however with varying results across studies (203). Furthermore, the study reported that the presence of impaired pulmonary function increased with the age of testing.

As strengths of our study, we mention a high success rate in all our lung function techniques. Furthermore, this is the first study examining the pulmonary function sequelae after hMPV-associated LRTI hospitalisation from Africa and it contributes to the global pool of data on hMPV. An important limitation of our study concerns low study sample and relatively small sample size with a substantial loss to follow-up. However, these results provides important information needed for prevention of pulmonary sequelae associated with hMPV, and also provides a base for future research into this subject. Also, we did not perform lung function testing before admission for LRTI, therefore we did not have available data on the lung function of the participants prior to the infection. Our study also included a higher proportion of one year old males in the case group, compared to the controls. The disproportionate gender ratio could have biased the results, as males have significantly higher mean values for pulmonary variables such as volumes and flows, and lower resistance values (204). New infections between first and second year follow up were not investigated therefore this may have biased the lung function results.

6.5 Conclusion

We have described the pulmonary function outcomes after hospitalisation for hMPV-associated LRTI in a LMIC. We found that hMPV impairs lung function in the first two years

of life. These results may help in future interventions aimed at reducing the childhood burden of LRTI.

Chapter 7

Integrated discussion and conclusion

For this thesis, we conducted several studies that provided novel results regarding hMPV-associated LRTI in children and its sequelae. The systematic review and meta-analysis study showed that hMPV is an important cause of LRTI in young children. We found that 4.7% (95%CI: 3.9-5.6) of hospitalised LRTI cases or community-treated severe pneumonia cases are associated with hMPV with a pooled CFR of 1.3% (95%CI: 0.3-2.9).

To further understand the epidemiology of hMPV-associated LRTI in our setting (South Africa) we estimated the incidence of hMPV. We found that the highest incidence of hMPV-associated LRTI hospitalisation was in children <6 months (260 per 100,000 population per year), with a lower incidence in 6-12 months (233) and 12-59 months old children (37).

Because of the high prevalence of HIV among South African mothers, we studied hMPV maternal antibodies and found that HIV-1 infection in mothers was associated with a substantial and significant decrease in cord blood to mother blood ratios of antibody levels. Also, HIV-1 exposed children had a higher incidence of hMPV-associated LRTI hospitalisation.

Furthermore, we provided evidence that children infected with hMPV are at a risk of developing pulmonary sequelae in the first two years of life. In the following paragraphs these results will be further discussed.

Our systematic review and epidemiological studies reported a higher incidence of hMPV-associated LRTI hospitalisation in HIV-exposed children and children living with HIV compared to HIV-uninfected children (26, 72). Most studies were from urban (n=16) rather than rural settings (n=5), which could affect the reported incidence of hMPV hospitalisation due to possible differential access to health care. Coinfection with hMPV could increase disease severity of other infectious diseases, for example RSV (71). A study from France by Foulongne et al. showed that hMPV and RSV coinfection in children was associated with prolonged hospitalisation and children were more likely to require oxygen support (178). Generally, there were few studies on bacterial or viral co-detection with hMPV from Africa.

However, Madhi et al. reported hMPV-pneumococcal co-infection using blood culture in LRTI hospitalisation in African children (177). Severity of hMPV LRTI has also been associated with the different hMPV genotypes, albeit inconsistently (43, 49, 50). Genotypes were not evaluated in any of the African studies included in this review. Further studies are required to evaluate the impact of different hMPV genotypes, and co-infections on disease severity from the African region. A meta-analysis based on 69 studies reported hMPV prevalence of 6.40% (95%CI: 5.51–7.35) among hospitalised children with acute respiratory infections globally (179). This higher global prevalence, compared to the findings of our study, may be explained by the inclusion of studies with a shorter time span than what was defined in our inclusion criteria.

To further examine the epidemiology of hMPV-associated we estimated the incidence of hMPV in Johannesburg, South Africa. We found that the highest incidence of hMPV-LRTI hospitalization was in children < 6m. The incidence rate of hMPV-LRTI hospitalization in children under 6 months of age in our study (260 per 100,000 population per year) is lower compared with an earlier report from the same study setting done between 2009 and 2013 (653.3 per 100,000 person years; 95% CI 602.2–707.6) (72) (26). The difference in incidence between the studies could be due to differences in study inclusion criteria, the use of a different analytical approach in our study, when adjusting the incidence estimate or it could reflect inter-year differences in hMPV infections and severity of illness (72).

To better understand the influence of maternal HIV status and maternal hMPV antibodies, we conducted a study to understand the protective role of maternal hMPV neutralizing antibodies in children. We showed a reduction in transfer of maternal hMPV neutralizing antibodies among mothers living with HIV-1, compared to HIV-1 negative mothers. It is possible that the reduced transfer of maternal hMPV neutralizing antibodies explains the previously observed increased risk in HEU children for hMPV-associated hospitalization (26), although in our hMPV incidence study we could not replicate the elevated risk in HEU children. Our inability to detect an association between reduced levels of neutralizing antibodies at birth and subsequent hMPV-associated hospitalization at <6 months suggests that the neutralizing antibody at birth is not highly protective and that this deficiency might not be the primary cause of the increased hMPV hospitalization risk in HIV-1-exposed, uninfected (HEU) infants. Elevated vulnerability beyond six months suggests explanations other than those connected to maternally-derived antibody levels, which would be expected

to wane in all children by 6 months. The data are thus valuable despite the limited sample size.

In addition to the epidemiology of hMPV and examination of maternal hMPV antibody status, we conducted the first study that performed infant pulmonary function tests (PFT) to assess the pulmonary sequelae following hMPV infection in the first two years of life. The hMPV PFT results in this study showed an increase in the respiratory rate at one year and two years testing. Further, hMPV was further associated with more episodes of wheezing in the first year and at the two years follow up compared with controls. Both increased respiratory rate and wheezing have been previously associated with abnormal lung function in other studies (104, 110). The findings of our study suggest that hMPV infection during infancy contributes to impaired lung function later in life.

7.1 Limitations

The studies conducted for this thesis had several limitations. All studies included in the systematic review had an observational study design, this type of design is prone to selection bias in the recruitment of study participants, sampling from a specified population and lack of comparison groups. Also, most of the included studies were focused on other viruses co-detected with hMPV. The majority of subjects were hospitalized, thereby selecting for more severely ill participants. The severity of hMPV has also been associated with the different hMPV genotypes and RSV coinfection, although the findings varies across studies. Our study and majority of African studies did not evaluate the genotypes and coinfection and their influence on disease severity.

When comparing hMPV-associated LRTI hospitalisation between HEU and HUU children less than five years, hMPV was detected using less sensitive test suggesting the possibility of under detection in our study. Similar to the studies included in the systematic review, our incidence study was observational, and thus prone to the limitations stated above. To calculate the hMPV incidence estimates, we made adjustments and assumed that children not tested had the same incidence as children we tested. For the study on fetal transfer of hMPV neutralizing antibodies the infants hospitalized with hMPV infection at <6 months were selected from a prior study using convenience sample. This limited our study power and the ability to generalise the results. During our study on the pulmonary sequelae of

hMPV we experienced a large loss to follow up, especially during the second year of testing. Therefore, head to head comparison of results in the first and second year of life was not possible.

7.2 Strengths

The studies included also had several strengths. Our systematic review and meta-analyses was the first to describe the epidemiology of hMPV among children in the African continent, using strict and reproducible criteria for study inclusion. We emphasized the risk of acquiring hMPV in children less than six months. Further, we used a robust measure of antibodies, a neutralisation assay, to show increased risk in HEU children for hMPV-associated hospitalisation. Our study also demonstrated abnormalities in pulmonary function in children hospitalised for hMPV-associated LRTI after one and two years follow-up. For this, the pulmonary function study in children used robust PFT measurements and provided reference data of lung function measurements in one and two year old children.

7.3 Future research

We identified several areas to strengthen research and hopefully, prevention and clinical treatment of hMPV infection. There is still paucity of hMPV data in the African continent. Due to lack of studies, our systematic review was not able to adequately describe the occurrence of hMPV in African children. More longitudinal studies are needed to further understand the epidemiology and clinical characteristics of hMPV. Unfortunately, hMPV does not have specific treatment and there are no vaccines to reduce its burden. Funding and immunology studies are needed to develop safe and effective vaccines. Our study was not able to link the lower levels of hMPV neutralizing antibody in cord blood and acquiring hMPV at less than 6 months old. It is possible that other factors contribute to the cause of infection in these young children therefore, to understand immunity in children we need further research with an adequate sample size to detect possible differences in antibody levels.

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Appendices

Appendix 1: Epidemiology of Human metapneumovirus-associated lower respiratory tract infections in African Children: Systematic review and metanalysis

Epidemiology of Human Metapneumovirus-associated Lower Respiratory Tract Infections in African Children: Systematic Review and Meta-analysis

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Background: Human metapneumovirus (hMPV) has been associated with upper and lower respiratory tract infections (LRTI) in children and adults. This systematic review evaluated the epidemiology of hMPV-associated LRTI, including severe acute respiratory infection (SARI) hospitalization or clinically diagnosed severe pneumonia, in African children under 5 years of age.

Methods: We searched Science Direct, PubMed, Cochrane Central, Scopus, and WHO regional databases using the terms "("Human metapneumovirus" AND "Africa") OR ("hMPV" AND "Africa")" up to September 17, 2020. Other sources included ClinicalTrials.gov to obtain unpublished data. Studies were included if children were less than 5 years of age and hospitalized with hMPV-associated LRTI, SARI or if clinically diagnosed with severe pneumonia in the community. The main outcomes were prevalence of hMPV identified among children with hospitalized LRTI or SARI. We further calculated odds ratios for hMPV in cases with LRTI compared with non-LRTI controls. Pooled results were calculated using a random-effects model.

Results: Thirty studies were eligible for inclusion in the review. The prevalence of hMPV-LRTI/SARI among hospitalized and severe pneumonia cases was 4.7% [95% confidence interval (CI): 3.9–5.6, I^2 = 95.0]. The case-control studies indicated that hMPV was 2.0-fold (95% CI: 0.9–4.4) more likely to be identified in LRTI cases (10.3%) than controls (6.0%). Three of 5 studies reported hMPV-associated LRTI case fatality risk, with a pooled estimate of 1.3% (95% CI: 0.3–2.9; I^2 = 49).

Conclusions: hMPV was associated with approximately 5% of LRTI/SARI hospitalizations or severe pneumonia cases in Africa.

Key Words: hMPV, Africa

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Acute lower respiratory tract infections (LRTI) are a leading cause of under 5 mortality, contributing to 0.809 million (0.747–0.874) deaths globally in 2017.[1] The World Health Organization (WHO) estimated a crude death rate of 75 per 100,000 population due to LRTI deaths in children from low-middle income countries in 2016 under the age of 5 years.[2] The pathogenesis of LRTI includes infections by bacterial and respiratory viruses.

Human metapneumovirus was discovered in 2001 and has been associated with upper and LRTI.[3] The circulation of hMPV may be seasonal, including epidemics during winter and spring in temperate climates, albeit some reports indicate perennial circulation.[3–5] Clinical manifestations of hMPV infections include bronchiolitis, pneumonia, croup, and asthma exacerbations.

Infection with hMPV has been associated with hospitalization, severity of respiratory disease, and death in high-risk groups such as children less than 5 years of age, immunocompromised, and elderly individuals.[3–9] Also, HIV-exposed children are at heightened risk [IRR 1.4; 95% confidence interval (CI): 1.1–2.0] of being hospitalized for hMPV-associated severe LRTI.[7]

The multicountry, multisite Pneumonia Etiology Research for Child Health (PERCH) case-control study in low- and middle-income African and South Asian countries, attributed hMPV in the pathogenesis of hospitalization for severe and very-severe LRTI in 7.5% (95% CI: 5.9–9.5%) of cases, although identification of hMPV was also evident in controls (2.3%). The identification of hMPV was associated with severe or very-severe pneumonia case status (6.8%; odds ratio 4.6; 95% CI: 3.6–5.8).[10]

There has been limited evaluation of the role of hMPV in LRTI morbidity and mortality in African countries. We undertook a systematic review and meta-analysis to define the epidemiology of hMPV-associated LRTI, including severe acute respiratory infection (SARI) hospitalization or clinically diagnosed severe pneumonia, in African countries.

METHODS

Search Strategy

The systematic review was conducted according to the Preferred Reporting Items for Systematic and Meta-analyses guidelines. We searched Science Direct, PubMed, Cochrane Central, Scopus and the WHO regional database using the terms "("Human metapneumovirus" AND "Africa") OR ("hMPV" and "Africa")" and included all identified publications through to September 17, 2020 (Full search strategy and terms used are detailed in Table, Supplemental Digital Content 1, http://links.lww.com/INF/E291). ClinicalTrials.gov was reviewed for identification of any unpublished trials. There were no limitations to the language or year of publication. The protocol was registered under PROSPERO CRD42018114131.

Inclusion and Exclusion Criteria

This review included studies involving children less than 5 years of age with LRTI hospitalization, SARI, or clinically diagnosed severe pneumonia in the community. Eligible study designs

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were randomized and nonrandomized control studies, prospective and retrospective cohort studies, cross-sectional and case-control studies. We only included studies conducted over at least a full calendar year. Excluded from the analyses were systematic reviews, case reports and series, narrative reviews, editorials, and animal studies. In instances of multiple publications of same study, the most comprehensive publication was retained. The Human Research Ethics Committee of the University of the Witwatersrand approved this systematic review (Reference number: W-CBP-190218-2).

Data Extraction and Management

Two reviewers (LR and EM) independently screened the titles and abstracts of the studies and assessed their eligibility. Full text of eligible studies was obtained and disagreement on eligibility was resolved between the 2 reviewers. Data were extracted using standardized data extraction forms, including information on hMPV incidence and prevalence, outcomes, study design, study duration, sample size, location, coinfection with other respiratory infections, method of hMPV detection, participant characteristics, and study quality. The 2 reviewers independently extracted data from 1074 (13.5%) of eligible studies. After achieving good concordance (at least 80%), the remainder was extracted by 1 reviewer (LR). In case of lack of clarity during extraction, the second reviewer (EM) was consulted to discuss and resolve issues.

Data Analysis

For the analysis of pooled prevalence and case fatality risk (CFR), point estimates from individual studies were combined using a random-effects model in case of significant heterogeneity (I^2 >50%) and 95% CIs were reported. Analyses were performed using R statistical software, version 3.6.0.

Quality Assessment

The risk of bias was assessed using the Newcastle-Ottawa Scale, grading each study using predefined criteria on a 0–9 score (0 = high risk, 9 = low risk).[11] The overall quality of evidence was evaluated using the AMSTAR tool 2. AMSTAR is a validated tool which rates studies on 16 items and scores the overall results as critically low, low, moderate, or high quality.[12]

RESULTS

The search strategy identified 1474 records. After deletion of 168 duplicates, 1306 articles were included for screening of titles and abstracts; Figure 1. Of these, 1152 articles did not meet eligibility criteria, and 154 full text articles were reviewed to assess for inclusion eligibility. Finally, 30 individual studies, which were reported in 39 publications, were eligible for inclusion in the systematic review (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/E292). Of the 30 studies included, 24 were cross-sectional studies in children presenting with LRTI[5,6,9–11,13–33] and 6 were case-control studies[34–39] (see Figure, Supplemental Digital Content 3, http://links.lww.com/INF/E293). The median age of study populations ranged between 4 months and 3 years. Eighty percent (24/30) of studies were hospital-based. Four of the 6 (67%) case-control studies focused on clinically diagnosed severe or very-severe pneumonia and were designed to compare the prevalence of hMPV in children with and without LRTI.

Thirteen of the studies used a WHO clinical classification of pneumonia[40] or SARI,[6,14,17–20,25,28,32,34,38,41,42] while 3 South African studies in addition to children fulfilling the SARI definition also included any child under 3 months of age hospitalized for suspected infection.[5,28,38] The remaining 14 studies had varying case definitions of LRTI.

hMPV seasonality was reported in 9 studies, being most prevalent during the winter and spring seasons in most settings[5,6,9,13,24,25,27,32,34,39,43]; albeit being perennial in some areas (see Figure, Supplemental Digital Content 4, http://links.lww.com/INF/E294).

Prevalence of hMPV in Hospitalized or Nonhospitalized Participants

The mean or median age of hMPV-associated LRTI cases varied (range: 3–60 months) between studies. Overall, hMPV was identified in 4.7% (2908/61,853; 95% CI: 3.9–5.6; I^2=95.0) of children with LRTI, with prevalence ranging from 0.3% (1/338) to 15.0% (20/133)[22,42] (Figure 2). The prevalence of hMPV in children hospitalized with LRTI or SARI was 4.1% (1420/34,763; 95% CI: 3.9–4.3) and 10.9% (235/2162; 95% CI: 9.6–12.3) in nonhospitalized children diagnosed with clinically severe pneumonia (clinic and community-based studies).

Meta-analysis of 6 studies (including the multicountry PERCH study as a single study) indicated a marginally 2.0-fold (95% CI: 0.90–4.4) higher prevalence of hMPV (10.3%, 95% CI: 9.3–11.3) than children without LRTI (6.0%, 95% CI: 5.3–6.8) (see Figure, Supplemental Digital Content 5, http://links.lww.com/INF/E295).

Incidence of hMPV in Hospitalized or Nonhospitalized Participants

Four studies[5,7,13,35,36,44,45] reported on incidence of hMPV-associated hospitalization (Table 1). This included 1 study that analyzed hMPV-associated LRTI hospitalization incidence by HIV status[5,7,44] and one that further stratified by age group.[5] Notably, however, these studies did not adjust for the attributable fraction of hMPV identification in the causal role of LRTI.

In South Africa, the incidence (per 100,000 population) of hMPV-associated LRTI hospitalization in children <6 months of age was higher in HIV-exposed uninfected children (816; 95% CI: 622–1050; incidence rate ratio [IRR] 1.4; 95% CI: 1.1–2.0) and children living with HIV (CLWH; 1887; 95% CI: 863–3582; incidence rate ratio 3.3; 95% CI: 1.5–6.5) than HIV-unexposed children (573; 95% CI: 470–692).[7] Two Kenyan studies stratified hMPV incidence by age group, in which infants had a higher incidence of hMPV-associated LRTI hospitalization than other age groups.[13,35] In the first study, the incidence (per 1000 children) of hMPV-associated LRTI was 10.7 (95% CI: 7.9–14.7) in infants compared with 2.1 (95% CI: 1.5–2.9) and 3.6 (95% CI: 2.9–4.5) in children 2–5 and >5 years of age, respectively.[13] In the second study, hMPV-associated LRTI incidence (per 100,000 children) estimates were 138, 58, 11, and 44 among age groups under 12 months, 1–2 years, 2–5 years, and >5 years, respectively.[35]

Duration of Hospitalization

Only 4 of the 30 studies reported on duration of hMPV-associated hospitalization.[5,6,44,45] The median and mean duration of hospitalization ranged from 2 to 7 days and 3 to 14 days, respectively. Duration of hMPV hospitalization in children at 4 sentinel sites in South Africa, reported that children living with HIV (CLWH) had a longer duration of hospitalization [5 (interquartile range [IQR]) 2–8] days] than HIV-uninfected children [3 (1–5.5); P = 0.003].[5] This is similar to another study where a longer duration of hospitalization was also reported in CLWH [mean 5.8 days (SD 1–21)] than children without HIV [mean 4.1 days (SD 1–3.5); P = 0.003].[44] Furthermore, a longer duration of hospitalization was also observed in CLWH before the routine use of antiretroviral treatment [mean 9 days (SD 1–13)] than HIV-uninfected [2 days (1–21)] children, albeit the difference was not significant.[44]

A study from Morocco reported a mean duration of hospitalization of 7.3 days (SD±9.8) and 6.0 (SD±4.2) for hMPV

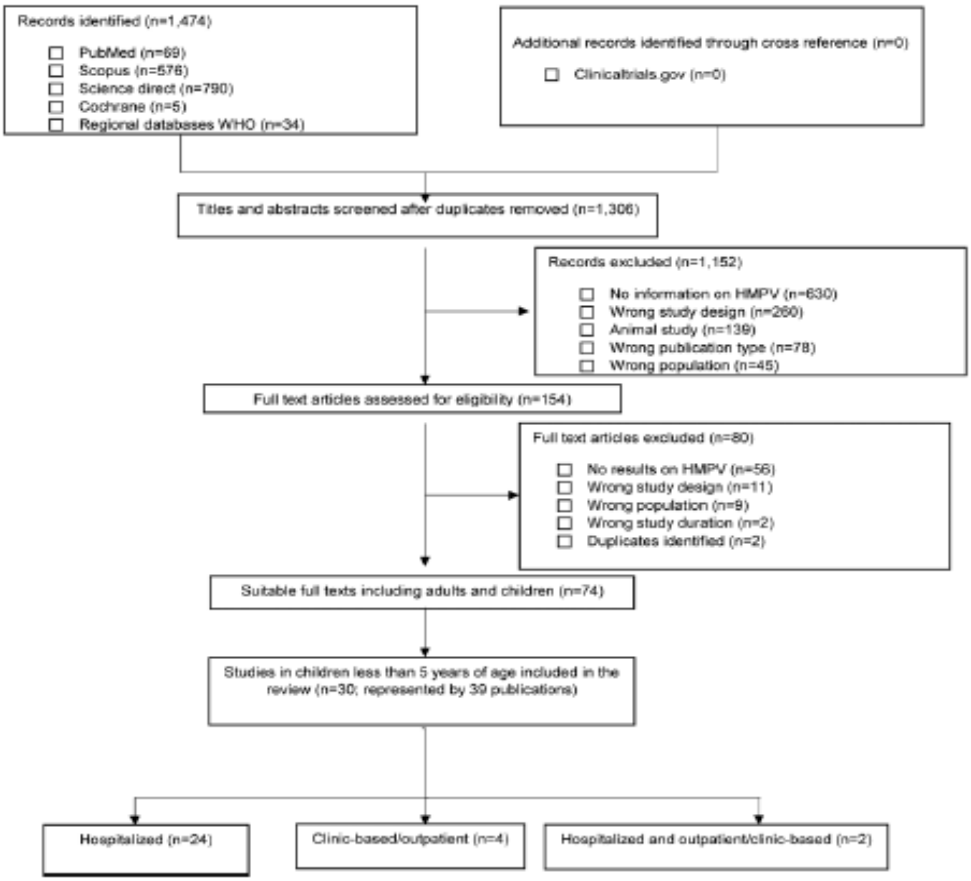

FIGURE 1. Flow chart of study selection.

and respiratory syncytial virus (RSV)-associated hospitalization, respectively.[6]

Case Fatality Rate

Three hospital-based studies reported on hMPV-LRTI associated CFR,[3-7,44] with a pooled CFR of 1.3% (95% CI: 0.3–2.9, I^2=49, n=3) (Figure 3). These included a study from Morocco in which the CFR was 5% (3/60) for children 1–5 years of age.[6] Two studies stratified CFR according to HIV status,[5,7,44] with CFR being 0% in HIV-exposed uninfected (0/52) and CLWH (0/9), and 1% (1/86) in HIV-unexposed children hospitalized for hMPV-associated SARI.[7] Low CFR was also observed in an earlier study from 2007 in both CLWH not on antiretroviral treatment (2/202, 1%) and in HIV-uninfected children (0/154; P=0.05).[44]

HMPV Coinfections

The occurrence of coinfection with hMPV and other respiratory viruses was reported in 5 studies.[5,6,9,14,25] These studies tested for a varied spectrum of other viruses (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/E292) and used different types of specimen, storage, and diagnostic tests (see Table, Supplemental Digital Content 6, http://links.lww.com/INF/E296).

The most commonly identified viral coinfections with hMPV were RSV, adenovirus, and rhinovirus (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/E292). Mazur et al tested nasopharyngeal aspirate specimens with reverse transcription polymerase chain reaction (RT-PCR) and detected RSV coinfections with hMPV-associated LRTI in 26/2322 (1.1%).[25] Groome et al codetected other respiratory viruses in children with hMPV-associated SARI in 333/593 (56.2%), including adenovirus 80/593 (13%), enterovirus 15/593 (3%), influenza A 9/593 virus (2%), RSV 19/593 (3%), parainfluenza virus type 1, 2, and 3 8/593 (1%), and rhinovirus 107/593 (18%).[5] A study from Morocco using nasopharyngeal aspirates and multiplex PCR detected parainfluenza coinfections more commonly (14.7%) in children with hMPV-LRTI than in those with RSV LRTI (5.6%, P=0.038).[6]

The bacterial coinfection most frequently associated with hMPV was *Streptococcus pneumoniae*.[5,6,45] All studies on bacterial

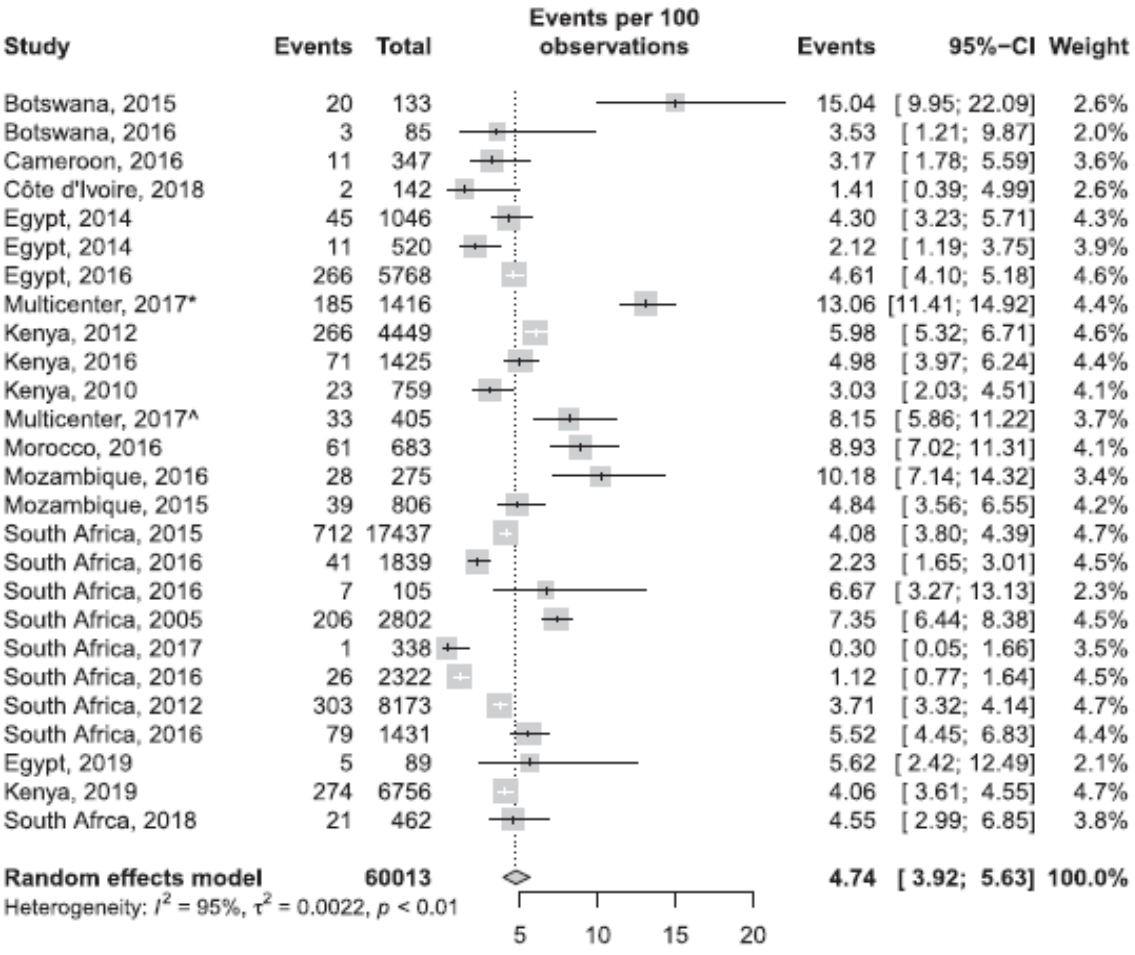

FIGURE 2. hMPV prevalence inpatient.

coinfection were done in South Africa. Groome et al reported *S. pneumoniae* coinfections identified by lytA quantitative real-time PCR detection using whole blood specimens in 7.3% of children with hMPV-associated SARI hospitalization.[5] *S. pneumoniae* was further identified in blood culture by Madhi et al in 4/189 (2.1%) of infants with hMPV.[46] In a randomized controlled trial comparing an experimental 9-valent pneumococcal conjugate vaccine (PCV) or placebo,[44] hospitalization for hMPV-associated LRTI hospitalization was 45% (95% CI: 19%–62%, $P = 0.002$) and 53% (95% CI: 3%–77%; $P = 0.035$) lower in PCV-9 compared with placebo recipients in HIV-uninfected children and CLWH (who were not treated with antiretrovirals), respectively.

In the same study, 25% (2/8) of CLWH not on antiretroviral therapy hospitalized for hMPV-associated LRTI had concurrent *Pneumocystis jiroveci* pneumonia.[44]

Risk of Bias and Quality of Evidence

Risk of bias assessment using the Newcastle-Ottawa Scale showed 21/27 (77.8%) of the included studies to have a moderate quality (score >5) (see Table, Supplemental Digital Content 7, http://links.lww.com/INF/E297). The overall quality of our review was rated as moderate (see Table, Supplemental Digital Content 8, http://links.lww.com/INF/E298).

DISCUSSION

This systematic review and meta-analysis on the epidemiology of hMPV in children under 5 years of age indicates that hMPV was associated with 4.7% (95% CI: 3.9–5.6, I^2=95.0) of hospitalized LRTI cases or community-treated severe pneumonia cases, with a pooled CFR of 1.3% (95% CI: 0.3–2.9).

The PERCH case-control study reported that hMPV identified in the upper airway could signify a causal role for hMPV in the etiology of severe or very-severe LRTI.[34] Further indication that hMPV identification in the upper airway can be attributed a causal role in the etiology of LRTI is provided by other case-control studies, which too showed a higher prevalence of hMPV in children with LRTI (10.3%, 95% CI: 9.3–11.3) than in controls

TABLE 1. HMPV Hospitalization Incidence

Author, Yr	Age	Total HMPV Cases n/N (%)	Study Duration (mo)	Population Description	HMPV Incidence Per 100,000/1000/100 (95% CI)	Adjusted Odds Ratio, 95% CI	Adjusted Rate Ratio (95% CI)	Was PCV Introduced in the Country?
Ahmad, 2012	Median: 1 yr (1 mo–84 yrs)	266/4449 (6.0)	36	Hospitalized	<1 yr: 10.7 (7.9–14.7); 1–5 yrs 2.1 (1.5–2.9); <5 yrs 3.6 (2.9–4.5) per 1000 children per yr			No
Berkley, 2010	Median: (very) severe pneumonia cases 9.0 mo (3.3–20)	29/759 (3.0)	12	Hospitalized	0.20 per 1000 live births, 28 d, <1 yr: 138, 1–2 yr: 68, 2–5 yr: 11, <5 yr: 44, per 100,000			No
Breiman, 2015	Age group with highest proportion <12 mo old: 34.8%	97/815 (11.9)	47	Clinic	0.9 (0.3–1.6) per 100 person-years of observation in less than 5 yrs	2.12 (0.96–4.72)		Yes
Cohen, 2016	NR	78/1410 (1.2)	47	Hospitalized	HIU 573 (470–892), HEU 816 (622–1050), HIV-infected 1887 (863–3682) per 100,000 population in <6 mo age group		CLWH/HIV– 1.4 (1.1–2.0); HIV+/HIV– 3.3 (1.5–6.5)	Yes
Groome, 2015	Age group with highest proportions; <1 yr 389/593 (65.6%)	712/17,437 (4.1)	46	Hospitalized	CLWH <1 yr 666.8 (411.5–1016.2), HIV– 652.9 (600.9–708.2), 1–<2 yrs CLWH 94.7 (25.8–242.4), HIV– 174.4 (148.2–203.9), 2–4 yrs CLWH 30.1 (8.2–77.1), HIV– 39.9 (27.4–41.5) per 100,000 person-years		<1 yr 1 (0.6–1.6), 1 yr 0.5 (0.1–1.4), 2–4 yrs 0.9 (2–2.3)	Yes

*Results of Madhi et al 2006 not shown in the table because the reduction in incidence was reported.
CLWH indicates children living with HIV; HIV+, HIV-uninfected; HEU, HIV-exposed uninfected; NR, not reported.

(6.0%, 95% CI: 5.3–6.8).[34,39,42] Nevertheless, identification of hMPV in healthy controls without LRTI, indicates that studies in which an adjustment is not made for possible coincidental identification of the virus, may inadvertently overestimate the contribution of hMPV in the etiology of LRTI, as well as result in an over-estimation of the incidence of hMPV-associated LRTI hospitalization.

Studies reported a higher incidence of hMPV-associated LRTI hospitalization in HIV-exposed children and CLWH compared with HIV-uninfected children.[5,7] Most studies were from urban (n = 16) rather than rural settings (n = 5), which could affect the reported incidence of hMPV hospitalization due possible differential access to health care (no data to assess).

Foulongne et al[47] in a study from France reported that hMPV and RSV coinfection in children was associated with prolonged hospitalization and children were more likely to require oxygen support.[48] Dual infection of hMPV and RSV has been linked to disease severity.

Generally, there were few studies on bacterial or viral codetection with hMPV from Africa. However, Madhi et al[46] reported hMPV-pneumococcal coinfection using blood culture in LRTI hospitalization in African children. Notably, numerous testing modalities, including enzyme immunoassay, tissue culture, and RT-PCR were used for RSV detection, which may have affected the comparability of results on coinfections in children with hMPV-associated LRTI. Severity of hMPV LRTI has also been associated with the different hMPV genotypes, albeit inconsistently.[49–51] Genotypes were not evaluated in any of the African studies included in this review and further studies evaluating the impact of the different hMPV genotypes, disease severity, and coinfection are required from this region.

Using serum *LytA* antigen detection as a proxy for pneumococcal coinfection, 7.3% of South African children hospitalized for hMPV-associated SARI were estimated to have concurrent pneumococcal infection.[5] The specificity of the serum *LytA* assay for diagnosing pneumococcal pneumonia, however, remains uncertain. Nevertheless, using a vaccine-probe approach, Madhi et al[44] reported that hospitalization for hMPV-associated LRTI was reduced in children vaccinated with a 9-valent PCV compared with placebo recipients among those without HIV, and in CLWH who were naive to antiretroviral treatment. This difference in rate of hMPV-associated hospitalization between 9-valent PCV and placebo recipients suggests that hMPV infection likely predisposes the host to superimposed pneumococcal infection by the vaccine-serotypes, which in turn precipitates hospitalization with hMPV-associated pneumonia.

The PERCH case-control study[10] showed the prevalence of respiratory viruses and bacteria in children less than 5 years of age with severe or very-severe LRTI in African and Asian children. The most commonly detected virus was RSV with a prevalence of 26.5%, followed by rhinovirus (23.0%) and bocavirus (13.1%). Prevalence of hMPV was reported to be 6.8%. The attributable role of detected viruses in the etiology of severe or very-severe LRTI hospitalization was 31.1% (95% CI: 28.4–34.2%) for RSV, 7.5% (95% CI: 5.3–10.1%) for rhinovirus, 7.4% (95% CI: 5.8–9.3) for parainfluenza, and 7.5% (95% CI: 5.9–9.5%) for hMPV. These results suggest that hMPV is one of the major viruses that contributes to respiratory infectious disease in children.

A meta-analysis based on 69 studies reported hMPV prevalence of 6.40% (95% CI: 5.51–7.35) among hospitalized children with acute respiratory infections globally.[52] This higher global prevalence, compared with the findings of our study, may be explained by the inclusion of studies spanning a shorter time period than what was defined by our inclusion criteria.

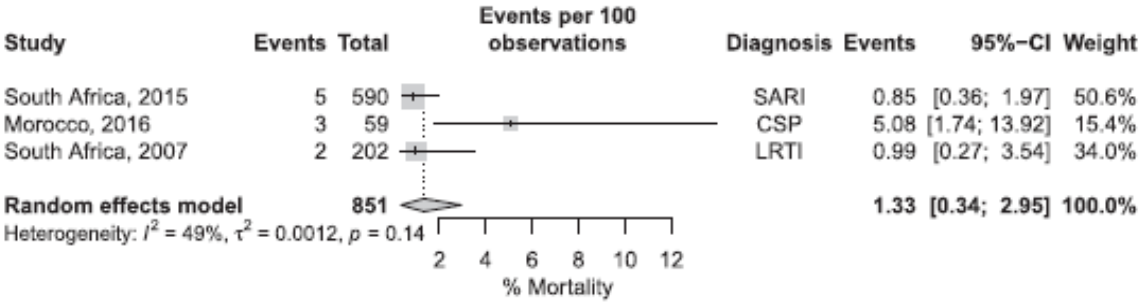

FIGURE 3. Pooled hMPV-associated case fatality rates.

Head-to-head comparison between studies on the role of hMPV in LRTI is impeded by differences in study methodology, including variability in threshold for investigating, participant's age and spectrum of LRTI severity. Furthermore, studies have used varying analytical methods and adjustment factors.

Limitations of this systematic review and meta-analysis include that all studies had an observational study design, which is prone to selection bias in recruitment of study participants, sampling from a specified population, lack of comparison groups and (unequal distribution of) confounding factors. Additionally, challenges pertaining to analysis of prevalence in case-control studies were the differences in selection of cases and controls between studies due to use of varying definitions of SARI, LRTI, and clinically severe pneumonia. Also, analysis errors encountered in original studies may be passed down to our review potentially invalidating the results. Furthermore, the combination of hospitalized SARI and LRTI cases in the analysis may bias the results, as SARI could be a different phenotype to LRTI. Many studies reporting on hMPV infection were focused on other pathogens that were codetected with hMPV.

The number of hMPV cases identified between studies may have been affected by different methods used in obtaining respiratory specimens and different diagnostic tools. Furthermore, the storage of specimens, the assays used and the timing of testing of samples differed vastly in the included studies. Nasal swabs have been reported to be less sensitive compared with nasal aspirates in identifying hMPV.[53–56] The sensitivity, specificity, and reproducibility of different types of PCR's in detecting hMPV has been explored across studies. Feng et al showed that a multiplex real-time RT-PCR assay used for simultaneous detection of RSV, HRV, and hMPV had very high sensitivity compared with individual real-time quantitative polymerase chain reaction (RT-qPCR) assays.[57] Other studies used culture for identification of hMPV, such as Matsuzaki et al, and reported higher sensitivity and specificity in RT-PCR (99%) compared with cell culture (68%).[58]

Factors that could have influenced the heterogeneity include differences in study populations and different spectra of respiratory tract organisms investigated across studies, settings, and enrollment sites. The majority of subjects were enrolled at the hospital, thereby selecting for more severely ill participants.

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Appendix 2: FORM 3 Epidemiology and pulmonary sequelae development in children with Human metapneumovirus

Investigator

Name: Lesego M Ramocha

Department: Clinical Microbiology and infectious Disease

Phone: 0765574306

E-mail:lramocha@gmail.com

General Information

You and your child are being invited to participate in a clinical research study. Participation is fully voluntary and will not affect your quality of routine care. To decide whether or not you and your child are willing to be part of this study, this form explains the things you will be asked to do as a study participant as well as any risks and possible benefits of the study. Please read this form carefully and ask any questions that might help you decide if you would like to take part in this study. If you decide to take part in this study, you will be asked to sign this consent form. A copy of this form will be given to you to keep.

The study has been approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) for compliance with medical and ethical standards. In addition, the study will be conducted according to the Declaration of Helsinki (last updated October 2013) and Good Clinical Practice Guidelines (SA GCP and ICH GCP).

Background:

You and your child are being invited to participate in a clinical research study. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information

carefully. Please ask the researcher if there is anything that is not clear or if you need more information.

The purpose of this study is:

This study is being done to describe the causes and effects of Human Metapneumovirus (hMPV) which is the common cause of lung disease and sometimes death in young babies. The virus usually enters the body through touching the secretions of an infected person. This virus infects the lungs and causes symptoms such as bronchiolitis, Pneumonia, croup, wheezing and increase in severity of asthma and other flue like illnesses. Currently, there is no treatment for the disease. Treatment procedure is currently supportive and it involves giving oxygen and antibiotics. It is not the fault of the mother or the hospital if the baby gets the germ. The results of this research will give valuable information on the treatment of the disease and prevention of future abnormal function that may result.

Study procedure:

There will be characteristics that participants have to meet to be included in the study. This includes all hospitalized children less than 2 years with lower respiratory tract infections. They will be selected from an ongoing surveillance study (BOSS).Children whose mothers refuse to take part in the study and have chronic illness will not be included in the study. We expect to identify and enrol babies with HMPV infection. The measurements of lung function in these children over the next two years will be compared

Your expected time commitment for this study is: approximately 2 hours.

Follow-up visits will be done at 12 months and 24 months of age where special tests will be done to check the strength of your child's lungs. These tests are done while your child is sleeping. There is no pain involved and you can be present all the time through the test. The test lasts around 45 minutes.

In every follow up visit, the participants are expected to give answers to 2 separate questionnaires that will be completed by interview. One questionnaire is the modified version of the International Study of Asthma and Allergies in Childhood Phase Three Core Questionnaire (ISAAC) for collecting the information on the presence of wheeze, allergy or hypersensitivity features in the past year. While the other one is the Case Report File (CRF) for collecting information regarding sociodemographic history, medical history of the case,

current admission details and laboratory results from admission, including HIV result, and particulars of treatment during current admission. The information will be collected using the case report form.

Performing lung function testing: We will be performing lung function tests on your child during this visit. These tests check the strength of the lungs and whether the lungs have been damaged at all by the RSV infection. All lung tests will be performed while your child is sleeping naturally. The tests are performed by allowing your child to breathe normally while a facemask is held over his / her face. There is no pain or discomfort involved. At specific stages during the test, your child will be breathing in a mixture of gases, oxygen, helium, nitrogen or sulphur hexafluoride. These gases are not dangerous to your child. Testing takes about 30 – 45 minutes. After testing the results will be available to us with the aid of a computer programme that will do all the sums to work out the lung function. All lung functions will be done by Lesego Ramocha.

Risks:

There are no risks related to this study. You may decline to answer any or all questions and you may terminate your involvement at any time if you choose.

Discomfort during lung function testing: There is a small theoretical chance that the child's breathing can be obstructed during the lung function testing. Lesego Ramocha and her team will be around the child at all times during the testing. Furthermore, the child will be monitored with a machine that measures pulse rate and the amount of oxygen in the blood (sats monitor) and all the masks used are soft and transparent. If there is any suggestion of discomfort or obstruction to breathing the testing will be interrupted immediately.

Benefits:

There will be no direct benefit to you for your participation in this study. However, we hope that the information obtained from this study may be beneficial to the public as a whole.

Though there may be no direct medical benefit to you or your child as a result of study participation prior to and at birth, information from this study will help us to learn more about the HMPV germ and contribute to developing an effective vaccine.

The study health worker will advise you on the symptoms of potential illness in your child. As soon as you think that your child is developing any of those symptoms, you should bring your child to a hospital/clinic to ensure that he/she gets timely medical care.

If your child is admitted to hospital, the results of the nasal aspirate will be reported to the doctors looking after him/her. This may benefit your child, as the attending doctors may change the medicines your child is receiving, to something more effective against the germ causing illness.

Alternative Procedures:

If you do not want to be in the study, you may choose not to participate and leave your answers blank.

Confidentiality:

Please do not give any identifying information on your questionnaire. Your responses will be anonymous.

Every effort will be made by the researcher to preserve your confidentiality including the following:

- Assigning code names/numbers for participants that will be used on all researcher notes and documents.
- Notes, interview transcriptions, and transcribed notes and any other identifying participant information will be held by RMPRU. To ensure that your personal information is kept confidential, yours and your child's name, and any other information that allows either of you to be identified, will not be entered on the case report forms or included in any records the study health worker provides to RMPRU. When no longer necessary for research, all materials will be destroyed. Good Clinical

Practice, the guidelines governing research, requires that study records be kept at RMPRU for 15 years.

- All biological samples (blood, nasopharyngeal swabs) taken from you and your child will be processed for use in study-related testing and stored at RMPRU. Samples obtained in the study will be labelled with a study code, but not yours or your child's name. Good Clinical Laboratory Practice guidelines require that all biological samples are kept at RMPRU for up to 15 years. The nasopharyngeal samples collected in this study could also be used for other future testing for allergens and genetic tests used for the diagnosis of asthma. Any such further testing will involve RMPRU requesting approval for such testing from the University of the Witwatersrand Human Research Ethics Committee.
- The researcher and the members of the researcher's committee will review the researcher's collected data. Information from this research will be used solely for the purpose of this study and any publications that may result from this study. All participants involved in this study will not be identified and their anonymity will be maintained.
- Each participant has the opportunity to obtain a transcribed copy of their interview. Participants should tell the researcher if a copy of the interview is desired.

Person to Contact:

Should you have any questions about the research or any related matters, please contact the researcher at lramocha@gmail.com or 0765574306.

Institutional review board:

If you have questions regarding your rights as a research subject, or if problems arise which you

do not feel you can discuss with the Investigator, you may contact Professor Cleaton-Jones, Chairperson of University of the Witwatersrand, Human Research Ethics Committee (HREC) which is an independent committee established to help protect the rights of research participants at (011) 717-2301.

Voluntary participation:

Your participation in this study is voluntary. It is up to you to decide whether or not to take part in this study. If you do decide to take part in this study, you will be asked to sign a consent form. If you decide to take part in this study, you are still free to withdraw at any time and without giving a reason. You are free to not answer any question or questions if you choose. This will not affect the relationship you have with the researcher.

Unforeseeable risks:

There are no risks related to this study. However every effort will be made to minimize any unforeseeable risks. If your child is injured as a direct result of a study related procedure, appropriate medical care for the treatment of the illness or injury will be given to you. If any RMPRU staff causes you or your child to be injured, please contact us as soon as possible. If you did not follow study instructions, RMPRU will not pay your medical bill. The study site is covered by an insurance policy for clinical studies, and ABPI guidelines for an injury due to the study itself.

If you experience an injury or have any questions or concerns, you should contact the study investigator:

Lesego Ramocha

E-mail: lramocha@gmail.com

Phone: 0765574306 (24 hours)

Costs to subject:

There are no costs to you for your participation in this study.

Compensation:

You will not be paid for your participation in this study. You will be reimbursed for out-of-pocket expenses such as additional time spent at the study site to provide information for the study. You will receive R150.00 for each completed study visit.

Consent:

By signing this consent form, I confirm that I have read and understood the information and have had the opportunity to ask questions. I understand that my participation is voluntary

and that I am free to withdraw at any time, without giving a reason and without cost. I voluntarily agree for my child(ren) to take part in this research study. I understand that I will be given a copy of this consent form. I voluntarily agree to take part in this study.

Please indicate below, whether you want your personal doctor or your specialist to be informed of your participation in this study:

□ YES, I want you to inform my personal doctor / specialist of my participation in this study.

□ NO, I do not want you to inform my personal doctor / specialist of my participation in this study.

□ I do not have a personal doctor / specialist

_____

___

Full name and surname of maternal participant (print)

_____ _____

Signature/thumbprint (if illiterate) of participant Date

_____

___

Full name and surname of person obtaining consent (print)

Signature _____

Appendix 3: Visit schedule

Cases

Visit	**1**	**2**	**3**	**4**	**4**	**6**
Study period	Admission to Hospital	1 month after discharge from hospital	6 months after discharge from hospital2	12 months of age (10 – 18)	18 months of age (16 – 20)	24 months of age (22 – 26)
Screening	X					
Written informed consent		X				
Case Report File	X					
Telephonic Follow-up		X	X		X	
iPFTs				X		X
iPFTs form				X		X
ISAAC Form				X		X

Controls

Visit	**1**	**2**	**3**
Study period	12 months of age (10 – 18)	18 months of age (16 – 20)	24 months of age (22 – 26)
Screening	X		
Written informed consent	X		
Case Report File	X		

Appendix 4 : Form 1 hMPV demographics

Form 1

hMPV demographics

1. Infant's Demographics

1.1 I – Given Name _____

1.2 I – Surname _____

1.3 I – Hospital Number G [___]-[___][___][___][___][___][___][___][___]

1.4 I – Alternative Hospital Number _____

1.5 I – Date of Birth [___][___] / [___][___][___]/ 201 [___]

1.6 I – Gender ○ Male ○ Female ○ Ambiguous gender

1.7 I – Race ○ Black ○Coloured ○ Caucasian ○ Asian ○ Other _____

2. Mom's Demographics

2.1 M – Given Name _____

2.2 M – Surname _____

2.3 M – ID Number _____

2.4 M – Passport Number _____

2.5 M – Hospital Number G [___]-[___][___][___][___][___][___][___][___]

2.6 M – BW Number BW-[___][___][___][___][___][___][___][___]

2.7 M – Date of Birth [___][___] / [___][___][___]/[___] [___][___][___]

2.8 M – Address _____

(House number, street name and suburb) _____

(Please remember to query a landmark close to the home – for identification purposes)

2.9 M – Current Cell Number [___][___][___][___][___][___][___][___][___][___]

2.10 M – Alternative Contact Number [___][___][___][___][___][___][___][___][___][___]

3. Other studies infant <u>was previously</u> enrolled on

3.1 GBS (28OBTP) [___]-[___]-[___][___][___][___][___]

3.2 EPI-RSV_005 [___][___][___][___]

3.3 BOD BOD [___][___][___][___]

3.4 PET PET [___][___][___][___]

3.5 Other: _____

3.6 Other: _____

Appendix 5 : Form 5 Wheeze specific and exam visit 1

HMPV iPFT Study

1. Demographics

1.1. hMPV iPFT study No:
PET[___][___][___][___]-[___][___]-[___][___][___][___]

1.2. BoSS ID No:
PET[___][___][___][___][___]

1.3. V98_28OB No: [___]-[___]-[___][___][___][___][___]

1.4. Gender o Male o Female o Ambiguous

1.5. Race o Black o Coloured o Caucasian o Asian o Other____

2. Asthma and Wheezing

2.1. Has your child been admitted for a HMPV lower respiratory tract infection in the first year of life?

o Yes (See section 4.1.1) o No (See section 4.1.2)

2.1.1. If yes,

2.1.1.1. Did your child experience any wheezing or whistling in the chest before the hMPV admission?

o Yes oNo o Unknown

2.1.1.2. Has your child experienced any wheezing or whistling in the chest over the past year (after the HMPV admission)?

o Yes o No o Unknown

2.1.1.2.1. If yes, how many episodes of wheezing or whistling in the chest has your child had in the last year (after the HMPV admission)? o 1 – 3 o 4 – 6 o> 6

2.1.1.3. Has your child had any admissions for wheezing or whistling in the chest in the last year (after the HMPV admission)?

o Yes o No o Unknown

2.1.1.3.1. If yes, how many admissions for wheezing or whistling in the chest has your child had in the last year (after the HMPV admission)? o 1 o 2 o 3 o>3

2.1.1.4. Has your child had any admissions for a chest infection in the last year (after the HMPV admission)? o Yes o No o Unknown

2.1.1.4.1. If yes, how many admissions has your child had for chest infections in the past year (after the HMPV admission) ? o 1 o 2 o 3 o>3

2.1.1.5. Has your child's sleep ever been disturbed due to wheezing or whistling in the chest in the last year (after the HMPV infection)?

o Yes o No o Unknown

2.1.1.5.1. If yes, how many times per week has your child's sleep been disturbed due to wheezing or whistling in the chest in the past year (after the HMPV infection)?

o 1 o 2 o 3 o>3

2.1.1.6. Has your child ever had a dry cough at night, apart from when he has a cold or an infection, during the last year (after the HMPV admission)?

o Yes o No o Unknown

2.1.1.6.1. If yes, how many times per week does your child have a dry cough, apart from when he has a cold or an infection, in the past year (after the HMPV infection)?

o 1 o 2 o 3 o>3

2.1.1.7. Has your child ever had wheezing or whistling in the chest during activity in the past year (after the HMPV infection)?

o Yes o No o Unknown

2.1.1.8. Has your child been diagnosed with asthma by a doctor in the past year (after the HMPV admission)? o Yes o No o Unknown

2.1.1.9. Has your child received any treatment for wheezing or whistling in the chest in the last year (after the HMPV admission)?

o Yes o No o Unknown

2.1.1.9.1. If yes, please tick all the treatments received:

□ Beta-2 agonist (MDI) □ Beta-2 agonist (Nebulisation)

□ Inhaled corticosteroid □ Oral corticosteroids □ Antihistamines

□ Montelukast (Singulair) □ Cough medicines □ Other: _____

2.1.2. If no,

2.1.2.1. Has your child experienced any wheezing or whistling in the chest over the past year? o Yes o No o Unknown

2.1.2.1.1. If yes, how many episodes of wheezing or whistling in the chest has your child had in the last year? o 1 – 3 o 4 – 6 o> 6

2.1.2.2. Has your child had any admissions for wheezing or whistling in the chest in the last year?

o Yes o No o Unknown

2.1.2.2.1. If yes, how many admissions for wheezing or whistling in the chest has your child had in the last year? o 1 o 2 o 3 o>3

2.1.2.3. Has your child had any admissions for a chest infection in the last year?

o Yes o No o Unknown

2.1.2.3.1. If yes, how many admissions has your child had for chest infections in the past year? o 1 o 2 o 3 o>3

2.1.2.4. Has your child's sleep ever been disturbed due to wheezing or whistling in the chest in the last year?

o Yes o No o Unknown

2.1.2.4.1. If yes, how many times per week has your child's sleep been disturbed due to wheezing or whistling in the chest in the past year? o 1 o 2 o 3 o>3

2.1.2.5. Has your child ever had a dry cough at night, apart from when he has a cold or an infection, during the last year?

o Yes o No o Unknown

2.1.2.5.1. If yes, how many times per week does your child have a dry cough, apart from when he has a cold or an infection, in the past year? o 1 o 2 o 3 o>3

2.1.2.6. Has your child ever had wheezing or whistling in the chest during activity in the past year?

o Yes o No o Unknown

2.1.2.7. Has your child been diagnosed with asthma by a doctor in the past year?

o Yes o No o Unknown

2.1.2.8. Has your child received any treatment for wheezing or whistling in the chest in the last year? o Yes o No o Unknown

2.1.2.8.1. If yes, please tick all the treatments received:

□ Beta-2 agonist (MDI) □ Beta-2 agonist (Nebulisation)

□ Inhaled corticosteroid □ Oral corticosteroids □ Antihistamines

□ Montelukast (Singulair) □ Cough medicines □ Other _____

3. Eczema

3.1. Has your child ever been diagnosed with eczema by a doctor? o Yes o No o Unknown

3.2. Has your child ever had an itchy rash that comes and goes? o Yes o No o Unknown

3.3. Has your child had an itchy rash that comes and goes over the past 12 months?

o Yes o No o Unknown

3.3.1. If yes to either of the above two questions:

3.3.1.1. Has the itchy rash affected any of the following places (folds of elbows, behind the knees, in front of the ankles, under the buttocks, around the neck, eyes or ears)?

o Yes o No o Unknown

3.3.1.2. At what age did the rash first appear? o< 6 months o 6 – 12 months o> 12 months

3.3.1.3. Has the rash ever disappeared completely?

o Yes o No o Unknown

3.3.1.4. In the past 12 months, how often has your child been kept awake at night by the itchy rash?

o Never o ≤ 1 per week o> 1 per week

4. Examination

4.1. Growth parameters

4.1.1. Weight

[___][___],[___]kg o Unknown

4.1.2. Height

[___][___][___]cm o Unknown

4.1.3. Head circumference

[___][___]cm o Unknown

4.2. Vital signs

4.2.1. Pulse rate

[___][___][___] per min o Unknown

4.2.2. Respiratory rate

[___][___][___] per min o Unknown

4.2.3. Blood pressure

[___][___][___]/[___][___][___]o Unknown

4.2.4. Temperature

[___][___].[___]oC o Unknown

4.2.5. Pulse oximeter reading

[___][___][___]% o Unknown

4.2.5.1. Pulse oximeter reading in o Room air o Oxygen: [___][___][___]%

4.3. Physical exam

4.3.1. General appearance: □ Normal □ Malnourished □ Syndromic: _____

4.3.2. General: □ Jaundice □ Pale □ Cyanosis □ Clubbing □ Oedema □ LA

4.3.3. Head and neck: □ Normal □ LA □ Congestion □ Allergic shiners □ Polyps

□ Inflamedturbinates □ Other: _____

4.3.4. CVS: □ Normal □ Pulmonary HT □ Murmurs □ Cardiac failure

4.3.5. Resp: □ Normal □ Respiratory distress □ Hyperinflation □ Chronic signs

□ Wheezing □ Crackles □ Stridor

4.3.6. GIT: □ Normal □ Hepatomegaly □ Splenomegaly

□ Ascites □ Masses

4.3.7. Skin: □ Normal □ Eczema □Other: _____

Appendix 6: Linear regression of determinants of respiratory rate at one year

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	-0.001695 (-0.00280- -0.0005869)	0.003	-0.0010105 (-0.0067772- 0.0047563)	0.701
Weight at 1 year (kg)	-0.3098229(-0.6321827-0.0125369)	0.060	-2.162968 (-4.209463--0.1164742)	0.040
Height at 1 year (cm)	-0.1861087 (-0.3029309--0.0692865)	0.002	-0.1101552 (-0.5663626-0.3460522)	0.598
Gender (Male) (N/%)	0.1340579 (-0.9020353-1.170151)	0.799	-7.212396 (-13.80488--0.6199101)	0.035
Maternal HIV	-0.007 (-0.054-0.040)	0.775	-7.753966 (-14.47531--1.032622)	0.028
Household smoke exposure	0.040 (0.178-0.259)	0.950	-3.177618 (-10.66187-4.306633)	0.362
Maternal pregnant smoke	0.035 (-0.085-0.155)	0.565	omitted	
Maternal use of alcohol during pregnancy	0.221 (0.114-0.328)	0.000	10.16891 (-4.701648-25.03946)	0.156
Exclusively breastfed (N/%)	-0.7202507 (-1.996315-0.5558134	0.268	6.407527 (-3.10821-15.92327)	0.162
Siblings in crèche (N/%)	0.482576 (-0.332341-1.297493)	0.245	-1.787288 (-8.264057- 4.689482)	0.548
Attends crèche (N/%)	-0.0268697 (-0.0909931- 0.0372537)	0.410	0.5470683 (-3.679482- 4.773618)	0.776
Severe hMPV LRTI (N/%)	-2.725553 (-7.013032-1.561925)	0.207	1.238549 (-8.969886- 11.44698)	0.790

Appendix 7: Linear regression of determinants of tidal volume at one year

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	0.0110779 (0.0074896-0.0146662)	0.000	0.0008552 (-0.0197939- 0.0215043)	0.927
Weight at 1 year (kg)	5.208471 (4.269161-6.14778)	0.000	4.271901 (-3.056032- 11.59983)	0.220
Height at 1 year (cm)	1.550453(1.185279-1.915626)	0.000	0.2086632 (-1.42489-1.842216)	0.779
Gender (Male) (N/%)	-6.672374 (-10.1229 - -3.221847)	0.000	17.4204 (-6.185474 - 41.02628)	0.129
Maternal HIV	0.9128673 (-2.940666-4.7664)	0.641	30.45156 (6.384276- 54.51884)	0.019
Household smoke exposure	-9.234119 (-16.14851--2.31973)	0.009	9.434522 (-17.36452- 36.23357)	0.446
Maternal pregnant smoke	-10.27083 (-20.58283-0.0411627)	0.051	omitted	
Maternal use of alcohol during pregnancy	-14.25961 (-23.51399--5.005234)	0.003	-25.96499 (-79.21236- 27.28237)	0.299
Exclusively breastfed (N/%)	4.075687 (-0.3151527-8.466527)	0.069	-16.8974 (-50.97064- 17.17584)	0.291
Siblings in crèche (N/%)	0.0543979 (-0.1671503-0.275946)	0.629	-7.174584 (-30.36611-16.01695)	0.502
Attends crèche (N/%)	-1.068636 (-3.885013-1.747741)	0.456	5.623748 (-9.510365-20.75786)	0.422
Severe hMPV LRTI (N/%)	5.182006 (-11.54137-1.90538)	0.536	4.153227 (-32.40037- 40.70682)	0.803

Appendix 8: Linear regression of determinants of FRC at one year

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	0.0000235 (0.0000144-0.0000326)	0.000	-0.000039 (-0.0001168-0.0000388	0.274
Weight at 1 year (kg)	0.0073238 (0.004746-0.0099016)	0.000	-0.0071974 (-0.0456564-0.0312617)	0.671
Height at 1 year (cm)	0.0041608 (0.0033067-0.0050149)	0.000	-0.0008944 (-0.0097724-0.0079837	0.819
Gender (Male) (N/%)	-0.021469 (-0.0296636--0.0132743)	0.000	0.0095748 (-0.0902878-0.1094374)	0.827
Maternal HIV	-0.0013157 (-0.0109667- 0.0083354)	0.789	-0.0113387 (-0.0999755-0.0772982)	0.771
Household smoke exposure	-0.0170375 (-0.0320303--0.0020447)	0.026	0.0584164 (-0.0910959- 0.2079286)	0.386
Maternal pregnant smoke	-0.0200798 (-0.043986- 0.0038265)	0.099	omitted	
Maternal use of alcohol during pregnancy	-0.0149906 (-0.0376718- 0.0076907)	0.194	omitted	
Exclusively breastfed (N/%)	0.0007139 (-0.0100699-0.0114978)	0.896	0.0313463 (-0.2084259-0.2711185)	0.766
Siblings in crèche (N/%)	0.0001405 (-0.0003502- 0.0006311)	0.573	-0.048656 (-0.1112862- 0.0139741)	0.109
Attends crèche (N/%)	0 .0018 (-0.0047523- 0.0083522)	0.589	0.0240936 (-0.0604911- 0.1086784)	0.522
Severe hMPV LRTI (N/%)	-0.0087708 (-0.0558824- 0.0383409)	0.708	0.0572126 (-0.1722607-0.2866858)	0.574

Appendix 9: Linear regression of determinants of LCI 5% at one year

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	-0.0000118 (-0.0001764- 0.0001527)	0.888	0.0003099 (-0.0003128-0.0009325)	0.278
Weight at 1 year (kg)	-0.0070914 (-0.0543878-0.0402049)	0.768	-0.0936618 (-0.4014862- 0.2141626	0.495
Height at 1 year (cm)	-0.0221691 (-0.0392023--0.0051358)	0.011	-0.0596443 (-0.1307035-0.011415)	0.088
Gender (Male) (N/%)	0.2339536 (0.087612- 0.3802952)	0.002	1.039576 (0.2402806- 1.838871)	0.018
Maternal HIV	-0.0136592 (-0.1755766- 0.1482583)	0.868	-0.2906316 (-1.000077- 0.4188137)	0.365
Household smoke exposure	0.1071583 (-0.1532611-0.3675777)	0.418	-2.312679 (-3.509367- -1.11599)	0.003
Maternal pregnant smoke	0.029279 (-0.3932902- 0.4518482)	0.892	omitted	
Maternal use of alcohol during pregnancy	-0.1596689 (-0.5568137-0.2374758)	0.429	omitted	
Exclusively breastfed (N/%)	0.1657924 (-0.0172685-0.3488533)	0.076	2.049341 (0.1302158- 3.968467)	0.040
Siblings in crèche (N/%)	-0.004914 (-0.0132676 -0.0034395)	0.248	0.7985222 (0.2972332-1.299811)	0.007
Attends crèche (N/%)	-0.051545 (-0.1629854- 0.0598954)	0.363	0.4672145 (-0.209798 -1.144227	0.147
Severe hMPV LRTI (N/%)	-.5193719 (-1.158547- 0.1198032)	0.108	0.5671228 (-1.26957- 2.403816)	0.489

Appendix 10: Linear regression of determinants of time to peak tidal expiratory flow over total expiratory time (tPTEF/tE) at one year

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	-0.001041 (-0.0040763- 0.0019943)	0.500	-0.0060273 (-0.026549-0.0144943)	0.523
Weight at 1 year (kg)	-0.6445571 (-1.513984- 0.2248703)	0.146	3.753847 (-3.528848- 11.03654)	0.274
Height at 1 year (cm)	-0.2728285 (-0.5908847- 0.0452277)	0.092	-0.4502183 (-2.073687- 1.173251)	0.546
Gender (Male) (N/%)	1.822495 (-0.9593689- 4.604358)	0.198	6.337453 (-17.1227- 29.7976)	0.556
Maternal HIV	1.595347 (-1.498124- 4.688818)	0.311	5.237715 (-18.68099- 29.15642)	0.632
Household smoke exposure	1.156312 (-4.369672-6.682296)	0.681	4.015265 (-22.61834- 30.64887)	0.741
Maternal pregnant smoke	5.752025 (-2.379582-13.88363)	0.165	omitted	
Maternal use of alcohol during pregnancy	1.598705 (-5.818646- 9.016056)	0.672	-23.32322 (-76.24187-29.59543)	0.345
Exclusively breastfed (N/%)	-0.2776511 (-3.765164-3.209862)	0.876	-8.270674 (-42.13357- 25.59222)	0.594
Siblings in crèche (N/%)	0.0095821 (-0.1655667-0.1847309)	0.914	3.941175 (-19.10719-26.98954)	0.708
Attends crèche (N/%)	-0.6394635 (-2.849765-1.570838)	0.570	-9.370041 (-24.41073-5.670643)	0.192
Severe hMPV LRTI (N/%)	-0.4168362 (-13.01175-12.17808)	0.947	-16.78976 (-53.1177-19.53817)	0.323

Appendix 11: Linear regression of determinants of respiratory rate at two years

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	0.0001252 (-0.0010556-0.001306)	0.835	-0.0008514 (-0.0048253-0.0031225)	0.606
Weight at 1 year (kg)	-0.0446531 (-0.3481254- 0.2588193)	0.772	-1.714256 (-4.666995-1.238483)	0.196
Height at 1 year (cm)	-0.0387715 (-0.1357882-0.0582453)	0.432	0.2540825 (-0.3622717-0.8704366)	0.338
Gender (Male) (N/%)	0.7129255 (-0.2871217-1.712973)	0.161	2.106359 (-8.801288-13.01401)	0.641
Maternal HIV	-0.1975645 (-1.344963-0.9498344)	0.734	-6.483308 (-14.65702-1.6904)	0.097
Household smoke exposure	-0.1881522 (-2.43808-2.061776)	0.869	0.2463449 (-7.465098-7.957787)	0.938
Maternal pregnant smoke	2.622001 (-1.382324-6.626326)	0.198	Omitted	
Maternal use of alcohol during pregnancy	1.203405 (-1.943673-4.350483)	0.452	Omitted	
Exclusively breastfed (N/%)	-0.3184854 (-1.468196-0.831225)	0.585	11.18338 (-7.914973-0.28173)	0.193
Siblings in crèche (N/%)	1.445744 (0.4282742-2.463214)	0.006	3.740431 (-5.867816-13.34868)	0.363
Attends crèche (N/%)	0.6636315 (-0.1594671-1.48673)	0.113	1.733264 (-3.150947-6.617476)	0.403
Severe hMPV LRTI (N/%)	-1.406077 (-5.290048-2.477894)	0.463	6.093807 (-11.4423-23.62991)	0.413

Appendix 12: Linear regression of determinants of tidal volume at two years

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	0.0053298 (0.0000764-0.0105833)	0.047	0.0101267 (-0.0118507-0.0321041)	0.289
Weight at 1 year (kg)	4.091331 (2.843163- 5.3395)	0.000	7.039137 (-9.290653-23.36893)	0.318
Height at 1 year (cm)	1.189669 (0.7827129-1.596624)	0.000	2.04403 (-1.364647-5.452707)	0.184
Gender (Male) (N/%)	-3.470092 (-7.99604-1.055856)	0.132	-36.33099 (-96.6545-23.99252)	0.182
Maternal HIV	-2.528614 (-7.786907- 2.72968)	0.344	16.65432 (-28.54945-61.8581)	0.387
Household smoke exposure	2.167733 (-8.220695-12.55616)	0.681	16.11686 (-26.5304-58.76413)	0.376
Maternal pregnant smoke	-7.110614 (-25.50257-11.28134)	0.447	Omitted	
Maternal use of alcohol during pregnancy	-0.4683389 (-14.84774-13.91106)	0.949	Omitted	
Exclusively breastfed (N/%)	1.694899 (-3.593263-6.983061)	0.528	-75.10498 (-180.7263-30.51631)	0.127
Siblings in crèche (N/%)	-2.85519 (-7.632923-1.922542)	0.240	-38.36717 (-91.5045-14.77016)	0.123
Attends crèche (N/%)	-2.606851 (-6.413977-1.200275)	0.178	-16.74181 (-43.75339-10.26977)	0.172
Severe hMPV LRTI (N/%)	3.111237 (-15.93868-22.16115)	0.740	-62.54426 (-159.5257-34.4372)	0.158

Appendix 13: Linear regression of determinants of FRC at two years

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	0.0000101 (-0.0000108-0.000031)	0.341	0.0000223 (-0.0000525-0.0000971)	0.455
Weight at 1 year (kg)	0.0071336 (0.0020723-0.0121948)	0.006	0.0083519 (-0.0574877-0.0741914)	0.742
Height at 1 year (cm)	0.0033026 (0.001736-0.0048693)	0.000	-0.0097901 (-0.024991- 0.0054107)	0.148
Gender (Male) (N/%)	-0.0317577 (-0.0486195- -0.0148959)	0.000	0.2181992 (0.0110499-0.4253485)	0.043
Maternal HIV			-0.1247381 (-0.2830304- 0.0335541)	0.094
Household smoke exposure	-0.0104038 (-0.0414004-0.0205929)	0.508	-0.0869225 (-0.225927-0.0520821	0.158
Maternal pregnant smoke	-0.0033064 (-0.0517129-0.0451001)	0.893	Omitted	
Maternal use of alcohol during pregnancy	-0.0453994 (-0.0885436--0.0022552)	0.039	Omitted	
Exclusively breastfed (N/%)	0.0041075 (-0.0131237-0.0213387)	0.638	0.2659517 (-0.0783172-0.6102205)	0.099
Siblings in crèche (N/%)	0.0014526 (-0.0120176-0.0149227)	0.832	0.125991 (-0.0545906-0.3065727)	0.125
Attends crèche (N/%)	-0.0057457 (-0.0162965-0.0048051)	0.284	0.0554778 (-0.0325676-0.1435231)	0.155
Severe hMPV LRTI (N/%)	-0.0521209 (-0.1358565-0.0316147)	0.212	0.2114318 (-0.1077889-0.5306524)	0.140

Appendix 14: Linear regression of determinants of LCI 5% at two years

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	0.0000788 (-0.0000307-0.0001883)	0.157	0.0000747 (-0.0006547- 0.0008041)	0.790
Weight at 1 year (kg)	-0.0001871 (-0.0273223-0.026948)	0.989	-0.4842113 (-1.126272-0.157849)	0.104
Height at 1 year (cm)	-0.0026307 (-0.0111536-0.0058921)	0.543	0.0985377 (-0.0496995-0.2467748	0.139
Gender (Male) (N/%)	-0.0024915 (-0.0941999-0.0892169)	0.957	-1.313324 (-3.333422-0.7067743)	0.145
Maternal HIV	-0.0163841 (-0.1284121-0.0956438)	0.773	-0.5398238 (-2.083473-1.003826)	0.387
Household smoke exposure	0.0665872 (-0.1397378-0.2729121)	0.525	0.238331 (-1.117227-1.593889)	0.651
Maternal pregnant smoke	-0.2414791 (-0.6706294-0.1876712)	0.268	Omitted	
Maternal use of alcohol during pregnancy	0.2659547 (-0.0089044-0.5408137)	0.058	Omitted	
Exclusively breastfed (N/%)	0.0143765 (-0.0973692- 0.1261222)	0.800	0.1167856 (-3.240489-3.47406)	0.928
Siblings in crèche (N/%)	-0.0327866 (-0.1314538- 0.0658806)	0.513	-0.5506791 (-2.311693-1.210335)	0.434
Attends crèche (N/%)	0.0014731 (-0.0753273-0.0782734)	0.970	-0.1138454 (-0.9724545- 0.7447638)	0.731
Severe hMPV LRTI (N/%)	0.2577651 (-0.1149581-0.6304883)	0.167	0.3944424 (-2.718565-3.50745)	0.743

Appendix 15: Linear regression of determinants of time to peak tidal expiratory flow over total expiratory time (tPTEF/tE) at one year

Respiratory rate	Univariate analysis		Multivariate analysis	
Independent Variables	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birthweight (g)	-0.0003351 (-0.0039278-0.0032575)	0.854	0.0086397 (-.0084576-0.025737)	0.251
Weight at 1 year (kg)	-0.3117928 (-1.218461- 0.5948758)	0.498	9.53018 (-3.173566- 22.23393)	0.112
Height at 1 year (cm)	-0.1831535 (-0.4725948- 0.1062878)	0.214	-1.39273 (-4.044507-1.259047)	0.235
Gender (Male) (N/%)	1.935344 (-1.057959- 4.928648)	0.204	23.9946 (-22.93402-70.92322)	0.246
Maternal HIV	-0.3227866 (-3.868765-3.223192)	0.858	8.632562 (-26.53367-43.79879)	0.556
Household smoke exposure	2.836612 (-4.062609-9.735832)	0.418	13.77298 (-19.40442- 46.95038)	0.335
Maternal pregnant smoke	2.237598 (-9.669442-14.14464)	0.711	Omitted	
Maternal use of alcohol during pregnancy	-2.270592 (-11.62073-7.079549)	0.632	Omitted	
Exclusively breastfed (N/%)	2.314882 (-1.194361- 5.824124)	0.195	-7.190798 (-89.35879-74.97719)	0.831
Siblings in crèche (N/%)	-0.1237834 (-3.298947-3.05138)	0.939	2.404834 (-38.9333-43.74297)	0.887
Attends crèche (N/%)	-1.404633 (-3.929567-1.120302)	0.274	-7.436965 (-28.4506-13.57667)	0.405
Severe hMPV LRTI (N/%)	-2.002592 (-16.60814-12.60296)	0.780	-32.58162 (-108.0283-42.86502)	0.317

Appendix 16: Ethics clearance

R14/49 Miss Lesego Matlhogonolo Ramocha et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M161149

NAME: (Principal Investigator)	Miss Lesego Matlhogonolo Ramocha et al
DEPARTMENT:	Clinical Microbiology and Infectious Disease, School of Pathology Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Epidemiology and Pulmonary Sequelae Development in Children with Human Metapneumovirus
DATE CONSIDERED:	25/11/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shabir Madhi
APPROVED BY:	Professor P Cleaton-Jones,Chairperson, HREC (Medical)
DATE OF APPROVAL:	13/01/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in November and will therefore be due in the month of November each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date