A prospective study investigating the role of respiratory viral infections in Sudden Unexpected Death in Infancy (SUDI) at Tygerberg Medico-legal Mortuary

by
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Thesis presented in fulfilment of the requirements for the degree of Master of Science in the Faculty of Medicine and Health Sciences at Stellenbosch University

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March 2020

Declaration

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Janca Ferreira

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March 2020

Dedication

To every parent who lost an angel

Tomorrow will come. The pain will ease,

But you will never forget your precious child.

It takes hope, time and love for the healing to take place.

Remember along the way to accept, but never forget.

Love lives longer than grief or pain...

All other things pass, but love will remain.

A bond that nothing can sever,

Because love lasts forever.

Our hearts are broken. Our world seems like it has ended.

Our dreams, our hopes and our future with this child are over.

Our precious child has died.

God sends his little angels, in many forms and guises,

They come as lovely miracles that God alone devises.

What we have once enjoyed and deeply loved; we can never lose.

For all that we love deeply becomes a part of us.

Helen Keller

Acknowledgements

This thesis became a reality with the kind support and help of many individuals.

I would like to extend my sincere appreciation to all of them.

Foremost, I want to offer this endeavour to our God almighty, for the wisdom He bestowed upon me. The strength, peace of mind and good health in order to finish this research.

My supervisor, Dr Corena de Beer, for her guidance, support, encouragement and motivation during the completion of this project.

My co-supervisor, Prof Johan Dempers, for his essential input, assistance and for teaching me everything I know about histology during the completion of my MSc.

Dr Björn Swigelaar, for his willingness to always help and encourage me wherever he could and for helping me understand concepts of forensic pathology.

The forensic pathologists (Dr I. Nieuwoudt, Dr H. Okkers, Dr L.J. Mostert, Dr J. Roman, Dr C. Wilscott-Davids, Dr S.E. Alfonso, Dr G. Uren and Dr J. Verster) and Forensic Pathology Officers who assisted me during sample collection at the Mortuary.

Ms Prudence Flaendorp, Mr Wayne Claassen and Mr Rameez Sulaiman for notifying me of the SUDI cases every morning.

Mr André du Toit and his colleagues Mr John Segole and Mr Marshall Hartzenberg for the processing of tissue specimens and preparation of slides for histological.

The staff from the Division of Forensic Pathology for assisting me with data capturing.

Prof Martin Kidd for his assistance during statistical analysis.

The Harry Crossley Foundation, the Poliomyelitis Research Foundation and the National Health Laboratory Services Research Trust for funding this project.

Ms Robin- Lee Burger from the South Africa Weather Services for providing all weather data used during this study.

Ms Michaela Venter, Ms Danielle Cupido, Ms Olivette Varathan and Mr Emmanuel Obasa for your friendship, guidance through difficult times, helping me make important decision, for making me laugh when I was down and making this whole experience worthwhile.

My parents Mr Kobus Ferreira, Mrs Amanda Ferreira and my husband Mr Reinhardt Pieters for their endless love, guidance, support, patience and prayer during this thesis.

Abstract

Background: The current South African infant mortality rate is 22.1 per 1 000 live births with respiratory infections, pneumonia, influenza and interstitial lung infections being responsible for most infant deaths. Sudden Unexpected Death in Infancy (SUDI) includes all infant deaths between the age of 7 days and 1 year without an apparent cause before any investigation has occurred. However, cases that remain unexplained following thorough investigation are classified as Sudden Infant Death Syndrome (SIDS). SIDS is regarded as a disease in search of a cause with several interlinking risk factors. Numerous respiratory viruses have been detected from SUDI autopsy specimens, therefore, viral infections could contribute to some SUDI cases as an exogenous trigger on a vulnerable infant during a specific developmental stage. This might be due to the infants' vulnerability to infections due to immaturities of their immune systems. Nonetheless, the exact contribution of respiratory viruses preceding death still needs further investigation.

Objectives: The primary aim of this study was to investigate the role of major respiratory viruses, found in the lungs and trachea as either single or co-infections of all SUDI cases admitted to Tygerberg Medico-legal Mortuary (MLM) over a 1-year period. The secondary aim entailed collecting all the epidemiological information and other relevant laboratory data from the retrospective cases from the Tygerberg MLM (2015-2016) to assess any trends or differences between the 2 studies and to evaluate how risk factors associated with SUDI cases at the Tygerberg MLM might have differed or remained constant over 2 study periods. Finally, laboratory results from all infants aged between 7 days and 1 year admitted to Tygerberg Hospital (TBH) due to respiratory infections during the study period were retrieved in order to identify if similar single and multiple viruses were circulating in both populations**.**

Methods: Between March 2018 and March 2019 samples were collected from 173 SUDI cases admitted to Tygerberg MLM. As part of the mandatory routine investigations into SUDI cases bacterial culture swabs were collected from the lower left and right lung lobes at autopsy to investigate the role of single and co-infections of viruses associated with SUDI. The Seegene Allplex™ RV Essential Assay one-step multiplex, real-time Polymerase Chain reaction (PCR) assay was used for the detection of 6 ribonucleic acid (RNA) respiratory viruses, Influenza A (Flu A), Influenza B (Flu B), Human Metapneumovirus (HMPV), Human Parainfluenza virus (HPIV), Respiratory syncytial virus A and B (RSVA/B) and Human Rhinovirus (HRV) from RNA extracted from lower left and right lung lobe and tracheal swabs.

Tissue sections from the lower left and right lung lobes were also assessed for histology signs of infection. TIBCO Statistica® version 13.5.0 was used to identify any similarities or differences between the current prospective study and retrospective study, as well as the comparison group of infants admitted to TBH.

Results: During this study multiple known demographic risk factors for SUDI, such as age (12.1 ± 9.8 weeks), male predominance, prematurity, low birthweight, cold season, bedsharing, prone sleeping position and ventilation were observed. With the Allplex™ RV Essential real-time PCR assay between 1 and 5 viruses were detected in 90.2% (156/173) of cases. RSV A/B (31.7%) and HRV (24.8%) were the most commonly detected viruses. The majority of PCR-positive cases were detected in the cold season, with a statistical significance observed for Flu A ($p = 0.04$), Flu B ($p = 0.04$), HPIV ($p = 0.03$) and RSV ($p = 0.02$) and cold season. The most frequently detected co-infection was between RSVA/B and HRV. Thirty-three cases had 2 viruses detected, 5 had 3 viruses and 1 case had 5 different viruses (Flu A - Flu B - HMPV - RSV A/B - HRV). The majority of cases had a cause of death (COD) of *Infection.* Furthermore, Flu A was significantly associated with the COD *Infection* and Flu B with *SIDS*. In 4 *SIDS* cases with positive histology, positive viral PCR results were observed leading to a change in COD to *Infection*. Major differences between the prospective and retrospective studies included female predominance, COD of *SIDS,* HRV being the most frequently detected virus and co-infection only being observed in 3 cases (Flu A - HRV, Flu B - HRV; HPIV - HRV - RSV A/B). The same viruses were circulating in SUDI cases and the comparison group.

Conclusion: In cases that had a COD of *SIDS*, the PCR viruses detected cannot be ignored, especially when it is supported by histological signs of infection as seen during this study Therefore, the use of real-time PCR could alter a COD Classification from *SIDS* to *Infection*. However, the role of single or co-infection with respiratory viruses in SUDI cases wherein no histological sign of infection was observed requires further investigation. Future research is needed to determine the exact role of co-infections in those who succumb to SUDI, more specifically how viral interactions play a role in disease progression and severity in a vulnerable infant. Finally, research should be aimed at determining the effect of PCR-positive viral results in the absence of histology to identify the true cause of vulnerability in infants.

Opsomming

Agtergrond: Die huidige baba sterftesyfer in Suid-Afrika is 22.1 per 1 000 lewendige geboortes. Die meeste sterftes kan toegeskryf word aan respiratoriese infeksies, longontsteking, griep en interstisiële longinfeksies. Die skielike onverwagte dood van ‘n baba, of beter bekend as wiegiedood (SUDI), sluit alle onverwagte sterftes van babas tussen die ouderdom van 7 dae en 1 jaar in, voor enige ondersoek plaasgevind het. Gevalle wat na 'n deeglike ondersoek steeds onverklaarbaar bly, word geklassifiseer as “Sudden Infant Death Syndrome” (SIDS). Verskeie risikofaktore kan bydra tot wiegiedood, byvoorbeeld virale respiratoriese infeksies. Die toename kan toegeskryf word aan babas se kwesbaarheid vir infeksies as gevolg van hul onderontwikkelde immuunstelsel, maar die presiese bydrae van respiratoriese virusse in wiegiedood moet egter nog verder ondersoek word.

Doelstellings: Die primêre doel van hierdie studie was om die rol van respiratoriese viruses wat alleen of in kombinasies in die longe en lugweë aangetref word, te ondersoek. Alle SUDI-gevalle wat gedurende 'n periode van 1 jaar by Tygerberg Geregtelik-geneeskundige Lykshuis (GGL) opgeneem is, was ondersoek. Die sekondêre doelwit was om alle epidemiologiese en laboratorium resultate van ‘n vorige studie wat tussen 2015 en 2016 by Tygerberg GGL plaasgevind het, te vergelyk met die huidige studie in 'n poging om ooreenkomste of verskille tussen die 2 studies te identifiseer en te ondersoek hoe risikofaktore geassosieer met SUDI gevalle by die Tygerberg GGL oor die 2 studieperiodes verskil of konstant gebly het. Laastens is laboratorium resultate van alle babas tussen 7 dae en 1 jaar wat tydens die studieperiode in Tygerberg-hospitaal (TBH) opgeneem is as gevolg van respiratoriese infeksies, verkry om te bepaal of dieselfde virusse in beide populasies voorgekom het.

Metodes: Tussen Maart 2018 en Maart 2019 is monsters versamel van 173 SUDI-gevalle wat by Tygerberg GGL opgeneem is. As deel van die verpligte roetine-ondersoeke na SUDI-gevalle is bakteriële kultuurdeppers tydens die lykskouing van die linker- en regterlonge versamel. Om die rol van enkel- en mede virusinfeksies wat met SUDI verband hou, te ondersoek, is die “Seegene AllplexTM RV Essential Assay, one-step, multiplex, real-time PCR” toets gebruik vir die opsporing van 6 ribonukleïensuur (RNS) respiratoriese virusse. Die virusse sluit in Influenza A (Flu A), Influenza B (Flu B), Human Metapneumovirus (HMPV), Human Parainfluenza virus (HPIV), Respiratory syncytial virus A/B (RSVA/B) en Human Rhinovirus (HRV) vanaf RNS wat uit die longe en lugweë versamel is. Weefsel van

die onderste linker- en regterlong lobbe is geëvalueer vir histologiese tekens van infeksie. TIBCO Statistica® weergawe 13.5.0 is gebruik om enige ooreenkomste of verskille tussen die huidige prospektiewe studie en retrospektiewe studie te identifiseer, sowel as die vergelykingsgroep wat in TBH opgeneem is.

Resultate: Tydens hierdie studie is verskeie bekende demografiese risikofaktore vir SUDI waargeneem, soos ouderdom (12.1 ± 9.8 weke), meerderheid manlike geslag, prematuriteit, lae geboortegewig, koue seisoen, bed-deling, slaapposisie op die maag en ventilasie. Die PKR (polymerase kettingreaksie) toets het tussen 1 en 5 virusse in 90.2% (156/173) van die gevalle opgespoor; RSV A/B (31.7%) en HRV (24.8%) het die meeste voorgekom. Die meerderheid PKR-positiewe gevalle is in die koue seisoen opgespoor, met statisties-beduidende assosiasies vir Flu A ($p = 0.04$), Flu B ($p = 0.04$), HPIV ($p = 0.03$) en RSV ($p = 0.02$) en koue seisoen. RSV A/B en HRV is die kombinasie wat die meeste voorkom het. In 33 gevalle is 2 virusse opgespoor, 5 gevalle het 3 virusse gehad en 1 geval het 5 verskillende virusse gehad (Flu A - Flu B - HMPV - RSV A/B en HRV). Die oorsaak van dood (OVD) in die meeste gevalle was *Infeksie,* Flu A was statisties-beduidend geassosieer met die OVD-*infeksie* en Flu B met *SIDS.* In 4 *SIDS*-gevalle met positiewe histologie, is mede-infeksie ook waargeneem. Die teenwoordigheid van hierdie mede-infeksies het gelei dat die OVD verander is na *Infeksie.* Aspekte waar die retrospektiewe studie van die prospektiewe studie verskil het, was die meerderheid vroulike geslag, meer gevalle het ‘n OVD van *SIDS* gehad*,* HRV was die virus wat die meeste opgespoor is en mede-infeksie is slegs in 3 gevalle waargeneem (Flu A - HRV, Flu B - HRV; HPIV - HRV - RSV A/B). Dieselfde virusse het voorgekom in die SUDI-gevalle en in die vergelykingsgroep.

Gevolgtrekking: In gevalle met 'n OVD van *SIDS*, kan die virusse wat met PKR opgespoor is nie geïgnoreer word nie, veral nie as dit ondersteun word deur histologiese tekens van infeksie, soos waargeneem tydens hierdie studie nie. Die gebruik van real-time PKR analises het dus die vermoë om die verandering van ‘n OVD-klassifikasie van *SIDS* na *Infeksie* te ondersteun. Die rol van enkel- of veelvuldige infeksies met respiratoriese virusse in SUDI-gevalle waarin geen histologiese teken van infeksie waargeneem is nie, vereis egter verdere ondersoek. Meer navorsing is nodig om die rol van mede infeksies te ondersoek in SUDI gevalle, meer spesifiek die rol van virale interaksies in progressie en erns van siektes by 'n kwesbare baba. Laastens moet navorsing gerig wees op die bepaling van die effek van PKR-positiewe virale resultate in die afwesigheid van histologie om die werklike oorsaak van die kwesbaarheid van babas te identifiseer.

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List of Abbreviations

AEC	Airway Epithelial Cells
ANOVA	Analysis of Variance
ARTI	Acute Respiratory Tract Infection
CDC	Centre for Disease Control
COD	Cause of Death
CPE	Cytopathic Effect
DC	Dendritic Cells
DNA	Deoxyribonucleic acid
DOEA	Department of Environmental Affairs
E. coli	*Escherichia coli*
Flu	Influenza
H&E	Haematoxylin and Eosin
HMPV	Human Metapneumovirus
HPIV	Human Parainfluenza Virus
HREC	Health Research Ethics Committee
HRV	Human Rhinovirus
IC	Internal Control
ICU	Intensive Care Unit
IFN	Interferon
Ig	Immunoglobin
IMR	Infant Mortality Rate
IP	Interstitial Pneumonitis
K. pneumoniae	*Klebsiella pneumoniae*
kb	Kilobase pair
LRT	Lower Respiratory Tract
LRTI	Lower Respiratory Tract Infection
MLM	Medico-Legal Mortuary
NC	Negative Control
NHLS	National Health Laboratory Service
NZ	New Zealand
PC	Positive Control
PCR	Polymerase Chain Reaction
PMI	Post-mortem Interval

QCMD	Quality Control for Molecular Diagnostics
RNA	Ribonucleic acid
RSV	Respiratory Syncytial Virus
SADHS	South Africa Demographics Health Survey
SIDS	Sudden Infant Death Syndrome
ss	Single Stranded
Staph	*Stahylococcus*
StatsSA	Statistics South Africa
Strep	*Streptococcus*
SUDI	Sudden Unexpected Death in Infancy
SVC	Shell Vial Culture
TBH	Tygerberg Hospital
UK	United Kingdom
UN	United Nations
UNICEF	United Nations International Children's Emergency Fund
URT	Upper Respiratory Tract
URTI	Upper Respiratory Tract Infections
USA	United States of America
UTM	Universal Transport Media
WHO	World Health Organisation

CHAPTER ONE:
INTRODUCTION

In 2018, a total of 5.3 million infants and children under 5 died worldwide, with Sub-Saharan Africa having the highest infant mortality rate (IMR) and under-five mortality rate (United Nations [UN] Sustainable Development, 2019). In South Africa, the current under-five mortality rate and IMR are 28.5 and 22.1 per 1 000 live births respectively (Statistics South Africa [StatsSA] , 2019c). According to the UN sustainable goals, the reduction in infant and child under-five mortality is the third most important goal to end or reduce by 2030 (UN Sustainable Development, 2019). The number of infants dying before the age of 1 year is an important indicator of the overall health of a community or population with a high IMR indicating inadequate human health needs in sanitation, medical care, nutrition and education. Preserving the lives of infants has been a long-standing issue in public health with many humanitarian endeavours and campaigns been established to decrease IMR. The leading causes of infant mortality globally are preterm birth complications, acute respiratory infections, congenital anomalies and sudden infant death syndrome (SIDS) (Goutas et al., 2011).

The term SIDS was first proposed in 1969 to describe a group of infants with similar clinical characteristics who died unexpectedly in the postnatal period (Beckwith, 1970). Although this definition was used interchangeably with sudden unexpected death in infancy (SUDI) amongst the research communities, today there is a clear distinction between these 2 terms, following the implementation of the 2004 San Diego definition. SUDI replaced the original 1969 definition (it describes all cases that involve the sudden and unexpected death of an infant under the age of 1 year where all intentional and unintentional fatal injuries can be excluded), while SIDS refers to death in a seemingly healthy infant younger than 1 year of age whose death remains unexplained after a thorough case investigation including a complete autopsy, review of medical and clinical history and death scene investigation (Weber et al., 2008). However, SUDI still remains a medical mystery with no single cause; it is proposed that the mechanisms of death in infants involve a complex interaction of individual vulnerabilities with developmental stages and environmental factors (Spinelli et al., 2017).

In Australia the number of SUDI cases reduced from 500 per year in 1988 to 134 per year in 1999 and from 1.1 per 1 000 live births in 1990 to 0.47 per 1 000 live births in 1998 in the

United States of America (USA) (Krous et al., 2004; Duncan & Byard, 2018). In 2014, there were approximately 3 500 SUDI cases reported in the USA and 44.0% of them (1 500 deaths) were attributed to SUDI (Duncan & Byard, 2018) In the same year the United Kingdom (UK) experienced a SUDI rate of 0.3 deaths per 1 000 live births .

Malteno et al. (1989) collected infant mortality data from 1983 to 1984 and found that Cape Town had a SUDI rate of 3.8 per 1 000 live births, almost double that of the 1.7 to 2.1 found in Australia during the 1970s and 1980s (Williams et al., 1984; Molteno et al., 1989). In 2016 the South African SUDI incidence was 3.41 per 1 000 live births (Dempers et al., 2016).

In South Africa, all unnatural, sudden and unexpected or unexplained deaths as defined by the Regulations Regarding the Rendering of Forensic Pathology Service, National Health Act [No. 61 of 2003] [N.R.929], must be subjected to a full medico-legal investigation in terms of the Inquests Act [No. 58 of 1959] (Du Toit-Prinsloo et al., 2013; Justice, 2019). All SUDI cases are therefore admitted to Medico-legal Mortuaries (MLM) for further investigation. Cases from the Tygerberg MLM are investigated according to an institutional investigation protocol, because currently no national investigation protocol exists for these cases. Briefly, the current protocol includes a review of the clinical history, circumstances surrounding the death, an autopsy, laboratory analysis (virology, microbiology and toxicology - depending on the case) and occasional investigation of the death scene by the attending pathologist.

Environmental stressors, such as viral infections, can play a vital role in contributing to the death of an infant, especially if it occurs during a vulnerable period. Viruses are common in infants and can lead to acute respiratory tract infections (ARTI) and fatalities (Lau et al., 2007; Athanasakis et al., 2011; Ben-Shimolet al., 2013). Numerous ribonucleic acid (RNA) respiratory viruses have been isolated from SUDI cases and new viruses are continuously being discovered (Dettmeyer et al., 2004; Álvarez-Lafuente et al., 2008). Furthermore, research has recently elucidated that when these viruses co-infect, symptoms can be more severe; this however, may depend on several factors (virus, population type and age, season, etc.). Since infants who succumb to SUDI are especially vulnerable, it is important to establish if co-infection occurs in these cases and if they could contribute to death (Yoshida et al., 2013; Asner et al., 2014). Therefore, is it important to identify both single and co-infection caused by respiratory viruses to ensure that infections contributing to SUDI are not overlooked.

The primary aim of this study was to investigate the role of major respiratory RNA viruses, i.e. influenza virus (Flu) A, Flu B, human metapneumovirus (HMPV), human parainfluenza virus (HPIV), respiratory syncytial virus (RSV) A/B and human rhinovirus (HRV), found in the lungs and trachea as either single or co-infections of all SUDI cases admitted to Tygerberg MLM over a 1-year period. The secondary aim was to retrieve all the epidemiological information and other relevant laboratory data from the retrospective cases from the Tygerberg MLM (2015-2016) to identify any trends or differences between the 2 studies and to evaluate how risk factors associated with SUDI cases at the Tygerberg MLM might have changed or remained constant over the 2 study periods. Finally, laboratory results from all infants aged between 7 days and 1 year admitted to Tygerberg Hospital (TBH) due to respiratory infections during both study periods were retrieved to investigate if similar single and multiple viruses were circulating in both living and SUDI populations.

CHAPTER TWO:
LITERATURE REVIEW

CHAPTER CONTENT

2.1 Infant Mortality

The World Health Organisation (WHO) defines the under-five mortality rate as the number of deaths of children under the age of 5 per 1 000 live births and IMR as the number of infants dying under the age of 1 year per 1 000 live births. Globally, in 2018, 85% of deaths among children occurred in the first 5 years of life, accounting for 5.3 million deaths, of which 2.5 million occurred in the first month of life, 1.5 million at age 1–12 months and 1.3 million at age 1−4 years. In South Africa, the current under-five and IMRs are 28.5 and 22.1 per 1 000 live births respectively (StatsSA, 2019c). Sub-Saharan Africa remains the region with the highest under-five mortality rate in the world and in 2018, it was as high as 78 deaths per 1 000 live births which is 16 times higher than in high-income countries. Therefore, the under-five and IMR are considered a global health concern (UN International Children's Emergency Fund [UNICEF], 2018).

Following the end of Millennium Goals Development in 2015, child mortality remained a global health concern. Therefore, the UN adopted the Sustainable Goals or Global Goals to end poverty, protect the planet and ensure people enjoy peace and prosperity by 2030. Seventeen goals were set, with the third focusing on good health and wellbeing of the population. This goal encompasses and emphasises the need to reduce under-five and IMRs by the year 2030. More specifically the focus is to reduce the IMR to as low as 12 per 1 000 live births and under-five mortality rate to 25 per 1 000 live births in every country (UN Sustainable Development, 2019).

The leading causes of death (COD) worldwide in infants and children under 5 are pneumonia or other acute respiratory infections. This is also true for South Africa where the main COD in infants younger than 1 year is respiratory infections, pneumonia, influenza and interstitial lung infections (StatsSA, 2018a). According to the Centre for Disease Control (CDC), Flu infections and associated complications are one of the top 10 COD and result in millions of hospitalisations, costing over $10 billion each year in the USA (CDC, 2019a). In 2018, pneumonia was the leading COD amongst children under 5 years of age (Childmortality, 2019).

2.2 Classification of SUDI and SIDS

In the 1960s there was no consensus amongst the medical and scientific communities regarding the definition of SIDS. The first definition was proposed in 1969 in order to

describe a subgroup of infants whose deaths occurred unexpectedly in the postnatal period and the term SIDS was used interchangeably with SUDI (Beckwith, 1970).

In more recent years the San Diego Definition was established, which provides a definite distinction between the terms SUDI and SIDS (Krous, 2010). SUDI describes all cases that involve the sudden and unexpected death of an infant under the age of 1 year where all intentional and unintentional fatal injuries can be excluded (Weber et al., 2008). However, if a SUDI case remains unexplained following a complete investigation (a full autopsy, history of gestation, medical history examination, death scene investigation, etc.) it is classified as SIDS. Thus, SIDS is a subcategory of SUDI that is used when the evidence fails to provide the pathologist with a COD (Alfelali & Khandaker, 2014). If a case is classified as SIDS, it can furthermore be divided into subcategories, namely Category IA SIDS, Category IB SIDS, Category II SIDS and finally Unclassified Sudden Infant Death (Table 2.1) (Du Toit-Prinsloo et al., 2013; Hunt et al., 2015).

Table 2.1: The different categories of SIDS (Krous et al., 2004)

Category	Description
Category IA SIDS	Infants aged between 21 days and 9 months at time of death Death scene provided no explanation for death Findings observed during autopsy were not lethal Infants with a normal clinical history Safe sleeping environment No evidence of any unexplained trauma, abuse, neglect or unintentional injury A full-term infant (> 37 weeks) with no substantial thymic stress Normal toxicology, radiology, microbiology, chemistry and metabolic screening No similar death in family
Category IB SIDS	Meets the same criteria as Category IA, but excludes one of the following: • Death scene investigation is absent • Only certain of the screening processes where completed
Category II SIDS	This category meets all requirements of Category IA and IB but excludes the following: • Age range between 0 and 21 days and/or up to 1 year • History of SIDS in the family but excluding possible infanticide or genetic disorders • Neonatal or perinatal conditions such as prematurity that had resolved prior to death • Possible asphyxia or suffocation • Abnormal growth and development and inflammatory disorders insufficient to contribute to death
Unclassified Sudden Infant Death (USID)	Infants whose clinical history does not meet criteria for classification into Category I and II SIDS; alternative diagnoses of natural or unnatural death are unclear and an autopsy was not performed

Although the literature often uses the term SIDS as an all-encompassing term to describe unexpected death in infants, mostly in the absence of investigations, this thesis will use the term SUDI to describe all infant deaths before any investigations have been conducted and SIDS for those cases where no COD could be found.

2.3 The Medico-legal Examination of Sudden Infant Death Cases

The performance of a forensic autopsy forms a part of the medico-legal death investigation process, but the process differs globally and may even differ within a country. For instance, in India, either the magistrate or the police conduct the medico-legal death investigation. In some regions of the UK (England, Wales and Northern Ireland) the coroner investigates these deaths (Mitchell et al., 2017). Furthermore, it is not mandatory in all countries for a SUDI case to be referred to a medico-legal facility due to social challenges, for example Pakistan and other Islamic countries (Cassum, 2014).

Unnatural, sudden and unexpected or unexplained deaths as defined by the Regulations Regarding the Rendering of Forensic Pathology Service, National Health Act [No. 61 of 2003] [N.R.929] are deaths where physical injuries, trauma, abuse, homicide, suffocation, aspiration or choking amongst others are present. SUDI cases are therefore admitted to MLM for further investigation. In South Africa, all unnatural deaths must be subjected to a full medico-legal investigation in terms of the Inquests Act [No.58 of 1959] (Du Toit-Prinsloo et al., 2013; Justice 2019). Upon completion of medico-legal investigations, a death is classified as natural when genetic abnormalities, abnormalities of any organs or infections are diagnosed or recorded by the attending pathologist (Du Toit-Prinsloo et al., 2013). Cases from the TBH referral area are admitted to the Tygerberg MLM and are investigated according to an institutional investigation protocol, because currently no national investigation protocol exists for these cases. The current protocol includes a review of the clinical history, circumstances surrounding the death, an autopsy and occasional investigation of the death scene by the attending pathologist.

Between 2000 and 2004 a South African study was conducted to evaluate and compare the assessment protocols for SUDI cases at the Tygerberg MLM in the Western Cape and the Pretoria MLM in Gauteng. This study revealed prominent discrepancies between the standard SUDI investigation protocols at these 2 institutions. At Tygerberg a full macroscopic autopsy was performed in majority of the SUDI cases with detailed histological analysis conducted in 81.0% of the cases. At Pretoria a full macroscopic autopsy was performed in only 60.0% of the cases and histological analysis in only 29.0% of the cases.

Furthermore, at Tygerberg 60.0% of cases were screened for bacteria and viruses, whereas Pretoria only performed viral screening in less than 3.0% of the cases (Du Toit-Prinsloo et al., 2011). In contrast, in South-Australia it is mandatory to take blood, urine, cerebral spinal fluid, throat and skin swabs for microbiology investigations, blood and urine for clinical chemistry and biochemistry, blood for cytogenetics, as well as throat swab and nasopharyngeal aspartate for virology analysis (Duncan & Byard, 2018).

Du Toit-Prinsloo et al. (2013) also conducted an audit over a 5-year period (2005-2009) on all infants younger than 1 year of age that were admitted to the 5 MLMs linked to academic institutions (Bloemfontein [University of the Free State], Cape Town [University of Stellenbosch and University of Cape Town], Durban [University of KwaZulu Natal], Johannesburg [University of the Witwatersrand and Pretoria [University of Pretoria]). During this study it became evident that the Tygerberg MLM conducted the most virology and microbiology investigations (57.0% of cases), followed by Pretoria MLM (virology in 31.0% and microbiology in 29.0% of cases). This study also indicated that University of Stellenbosch was the academic institution in South Africa where SUDI cases were most extensively and thoroughly investigated at the time. The investigation of the death scene is an import element in SUDI investigation, which was limited at all facilities. Death scene investigations may, under certain conditions, reveal the COD. Furthermore, the investigations can be crucial for identifying hazardous environments, which may put other siblings at risk (Du Toit-Prinsloo et al., 2013). Thus, SUDI investigations and determination of death strongly rely on ancillary investigations, such as virological and microbiological screening and histological examination of body organs (Fernández-Rodríguez et al., 2006; Douglas, 2012).

The discrepancies between institutional protocols may affect the final classification of the COD (Du Toit-Prinsloo et al., 2011; 2013). Once a standardised national infant death investigation protocol is implemented, it will ensure accurate assessment and the possibility of inter-institutional comparison of SUDI data. This would contribute to identifying and verifying risk factors and improve the understanding of the pathophysiological processes involved in these infant deaths (Weber & Sebire, 2009).

2.4 The Global Impact of SUDI

SUDI is a global problem affecting both developing and developed countries contributing to half of all post-neonatal deaths (Byard & Krous, 2003; Carpenter et al., 2004; Toro et al., 2015). Unfortunately, due to SUDI cases being investigated and certified differently across

the world and some not being analysed at all, it is difficult to compare regions and obtain an accurate global estimated incidence for SUDI and SIDS.

The highest rate of sudden infant deaths occurred in the middle of the 20th century, but a decreased incidence followed after the global implementation of various campaigns promoting safe sleeping environments and creating awareness about SUDI amongst parents and caregivers. Toro et al. (2015) investigated the occurrence of SUDI from 2002 to 2010 in Australia, Canada, UK, Wales, Germany, Japan, the Netherlands, New Zealand (NZ) and the USA. During this period the highest mortality rates were recorded in Japan (0.46 per 1 000 live births) and the lowest rate occurred in the Netherlands (0.18 per 1 000 live births). Additionally, in 2002 a high SUDI rate of 1.01 deaths per 1 000 live births was detected in NZ. Toro et al. (2015) further analysed the total occurrence of SUDI in Hungary for a period of 1979 until 2012. The male to female ratio of SUDI deaths between 1979 and 1989 was 1:0.62, with a slight increase to 1:0.72 between 1990 and 1999 and 1:0.75 between 2000 and 2012 (Toro et al., 2015).

2.5 The Impact of SUDI in South Africa

Socio-economic status and demographics play a crucial role in the occurrence of SUDI. According to the UN World Economic Situation and Prospects 2014 report, South Africa is classified as a developing country with an extremely high overall unemployment rate (27.6%), but more specifically the burden of unemployment is concentrated amongst the youth (15-34 years), as they account for 63.4% of the total number of unemployed individuals (StatsSA, 2019b; UN, 2014). Additionally, this group also accounts for the peak ferility and thus child-bearing age (StatsSA, 2018b). In 2017, 58.0% of individuals aged between 15 and 34 only attended secondary school (StatsSA, 2017). Twenty-nine percent of working individuals in South Africa earn less than the national minimum wage of R3 500 per month (South African Board for Personnel Practice [SABPP], 2018; StatsSA, 2019a). It is therefore fair to assume that the incidence rates of SUDI in South Africa are bound to be very high as low income is a known risk factor (Du Toit-Prinsloo et al., 2013).

A total number of 1 030 SUDI cases were admitted to Tygerberg MLM between 2015 and 2018. Early incidence figures suggest that Cape Town has one of the highest SUDI rates in the world, being 3.41 per 1 000 live births (Dempers et al., 2016).

2.6 Risk Factors Associated with SUDI

SUDI has been defined as a syndrome in search of a cause and several contributing factors, both modifiable (extrinsic) and not (intrinsic), have been found to have significant associations (Malloy, 2004). This led to the Triple Risk Model, described by Filiano and Kinney in 1994, stating that SUDI is the interface of 3 major factors; i.e. (i) a critical developmental stage; (ii) a vulnerable infant; and (iii) an exogenous trigger (Kinney et al., 2001). Therefore, the risk of SUDI is at its highest when an underlying vulnerability in homeostatic control in the central nervous system or immune system overlaps with a specific exogenous trigger (environment such as viral infection) at a critical developmental stage of the infant (Samuels, 2003; Opdal & Rognum, 2004). Most of these risk factors can be clustered into socio-demographic, environmental, pregnancy and infection-related risks.

2.6.1 Socio-demographic Risks

The socio-demographic risk factors include, amongst others, seasonal variation, age and gender of the infant, low birthweight, prematurity and poor socio-economic status.

SUDI is more common in colder than warmer climates, likewise it is more common during the colder than months of the year (Ponsonby et al., 1992). A male predominance is evident in SUDI cases and it might be attributed to lower pro-inflammatory cytokine levels in males than females. This inability to produce sufficient cytokines against a bacterial or viral antigen can contribute towards vulnerability to infection (Moscovis et al., 2014). Another hypothesis is that an increase in testosterone levels in male infants might affect the dysregulation of inflammatory responses to ‘mild’ infections (Moscovis et al., 2014; Kruger et al., 2018). The high rate of SUDI at 2-4 months might be indictive of a critical developmental period that coincides with the weakening of maternally obtained immune protection (Balduzzi & Greendyke, 1966; Du Toit-Prinsloo et al., 2011; Alfelali & Khandaker, 2014).

Several studies also highlighted the impact of ethnic differences and traditions on SUDI. Some of these discrepancies may be due to higher levels of poverty, harmful environmental factors found in these communities, infant care practices and genetics. The USA Black, Indian and Alaskan native infants have been shown to be 2-3 times more likely to succumb to SUDI than their Caucasian counterparts (Moon, 2016). This is also true for other ethnic groups, such as the Aboriginal Australians and NZ Mãori, where the incidence of SUDI is higher than in Caucasian populations (Duncan & Byard, 2018).

Low socio-economic conditions include factors such as (i) limited or lack of education of caregivers; (ii) restricted access to transport, healthcare facilities and medication; (iii) suboptimal nutritional status of infants and caregivers; and (iv) poor living conditions, such as informal housing, poor sanitation, inadequate ventilation, overcrowding and suboptimal sleeping conditions. Infants born to mothers with no education face almost twice the risk of dying during the neonatal period than infants born to mothers with at least a secondary education (UNICEF, 2018). In Cape Town, 5.3 % of individuals aged 20 years and older have completed Grade 5 or less, while 50.2% left school before completing Grade 12. Twenty nine percent competed Grade 12 and 18.8% post Grade 12. The majority of the population has only some primary and some secondary education (StatsSA, 2017; 2019b). The City of Cape Town consists of 1 068 572 households according to the South African National Census of 2011. There were 129 918 informal structures in informal settlements. Nearly 36.0% of the households lived below the poverty line and earned less than R3 500 per month; 3.7% of the households did not have access to electricity for lighting or cooking and 8.8% had no access to sanitation on site (StatsSA, 2019b). The situation has not changed much since.

Poor socio-economic conditions are often accompanied by other social welfare problems associated with parents or caregivers, such as child abuse or neglect, domestic violence, absence of parent(s) and substance abuse, which can all contribute to increased vulnerability of the infants (Mitchell & Krous, 2015).

2.6.2 Sleeping Position and Pregnancy-related Risks

The risks associated with the infant's sleeping position is a well-known. Infants sleeping in the supine (back) position are at a lower risk of dying than those who sleep in the prone (stomach) position (Vennemann et al., 2007). Prone sleeping exposes the infant to ingestion of several microorganisms present in and on the sleeping environment and the introduction of bacterial toxins (bacterial toxin hypothesis) (Morris, 1999). Infants in the prone position also have a higher risk of regurgitation and chocking than those in a supine position. In South Africa, a study conducted in the early 1990s found that 50.0% of Caucasian, 59.0% of Black and 70.0% of Mixed-Race infants slept in the prone position. Additionally, 94.0% of the Black and 74.0% of the Mixed-Race infants slept with their mothers, compared to only 4.0% of Caucasian infants further increasing the risk of SUDI (Potgieter & Kibel, 1992). Additionally, excessive clothing or bedding (soft bedding or crib covers), head-covering and the presence of other objects, such as soft toys, might further increase the possibility of

SUDI. Excessive bedding can be associated with increased body temperature and fever, increasing the chances of SUDI. The combination of prone sleeping, excessive bedding and increased body temperature increases the risk of SUDI 2-6 times (Athanasakis et al., 2011; Duncan & Byard, 2018). Since this risk was identified, several campaigns have been raising awareness regarding its association with SUDI. The American Academy of Paediatrics and National Institutes of Health implemented the “Back to Sleep” campaign in 1994 aimed at educating mothers and caregivers on the dangers of prone sleeping and bedsharing (Adams et al., 2009).

Pregnancy-related risks include infants born to young mothers and those with a short interval between pregnancies. In 2017 a total of 119 645 teenage mothers aged between 15 and19 gave birth in South Africa (StatsSA, 2018b).Exposure to second-hand cigarette smoke both prior to and after birth also have an impact on the infant’s wellbeing (Treyster & Gitterman, 2011). In South Africa, the Western Cape has the highest tobacco smoking prevalence amongst all provinces (32.9%) with more female smokers. The peak age for female smoking coincides with the peak age of fertility (South Africa Demographics Health Survey [SADHS], 2016).

Infants depend on passively acquired maternal antibodies for protection against infections. However, only a limited number of antibodies are passed on to the infant during the first and second trimester of pregnancy. It is only after 32 weeks gestation that the infant starts acquiring maternal immunoglobulin (Ig) G (Crowe & Williams, 2003). As such, preterm infants are at a greater risk of developing more severe symptoms when suffering from infection compared to full-term infants. Lack of breastfeeding and non-use of pacifiers are also regarded as risk factors (Horn & Smout, 2003; Hakeem et al., 2014).

2.6.3 Infections

Viral infections have been recognised as an important co-factor which may trigger the events leading to SUDI, but whether it can be regarded as the direct aetiological agent is still unclear (Blackwell et al., 2001). Viral infections are of importance especially when it occurs during a vulnerable period of the infant’s development and the infant still relies on maternal immunity and does not have a fully developed immune system capable of combating infections (Alfelali & Khandaker, 2014; Burger et al., 2014). Even though the role of infection in SUDI is acknowledged, only a hand-full of possible viruses have been confirmed in autopsy samples from SUDI cases (Table 2.2). Therefore, the role of infections still requires further investigation and should not be overlooked. Quite a few bacterial species and their toxins

have also been isolated from SUDI cases. These include *Staphylococcus (Staph) aureus*, *Escherichia coli* (*E. coli*) *and Streptococcus (Strep) pneumonia*, amongst others. These bacterial pathogens induce their effect through the release of endotoxins that have various effects on the body's physiology (Blackwell et al., 2001; Highet, 2008; Morris, 2008).

Table 2.2: Respiratory viruses detected from autopsy samples of SUDI cases

Viruses	References
HRV	Uren et al., 1980; Williams et al., 1984; Patrick et al., 1989; La Grange, 2013; Matshazi, 2017
Flu A & B	Uren et al., 1980; Williams et al., 1984; Patrick et al., 1989; Bajanowski et al., 2003; La Grange, 2013; Matshazi, 2017
RSV A/B	Uren et al., 1980; Williams et al., 1984; Patrick et al., 1989; La Grange, 2013; Matshazi, 2017
HPIV	Uren et al., 1980; Patrick et al., 1989; La Grange, 2013; Matshazi, 2017

2.7 Respiratory Viral Pathogens

Since the scientific communities worldwide started to acknowledge the existence of SUDI and SIDS, substantial financial investments have been made over the years to focus on identifying the COD and especially the role of respiratory infections (Athanasakis et al., 2011; Alfelali & Khandaker, 2014).

2.7.1 Human Influenza virus A and B

Flu A was first discovered in 1933 and Flu B in 1940 (Smith et al., 1933; Francis, 1940) and belong to the family *Orthomyxoviridae* and genus *Influenzavirus* A and *Influenzavirus* B respectively. These viruses are enveloped, single-stranded (ss) negative-sense RNA viruses with 13.5 kilobase pair (kb) genome (Ghedin et al., 2005). Evidence from epidemiological studies and experimental infection of human volunteers suggests that Flu infection can occur by inhalation of aerosols or direct contact transmission (Alford et al., 1966; Moser et al., 1979; Bean et al., 1982). The incubation period varies from person to person but is usually between 1 and 4 days. Annual Flu epidemics occur during the winter months in temperate climates; however, infections can be detected year-round in tropical climates with a high prevalence during the rainy seasons (CDC, 2019a). Flu is one of the most important respiratory infections of humans, responsible for 250 000 to 500 000 deaths annually (WHO, 2019).

Symptoms associated with uncomplicated Flu illness is characterised by fever, chills, headache, sore throat, cough and rhinitis (CDC2019a). Flu A has been detected in between 5.4% and 13.4% of children presenting with the aforementioned symptoms, whereas Flu B only in 1.4% to 7.8% (Do et al., 2011). Annual Flu detection rates in children vary from year to year but can be as high as 223 infections per 1 000 children (Heikkinen et al., 2004). Flu infections has also been associated with a variety of ARTIs, including croup, bronchiolitis and pneumonia amongst others. Furthermore, Flu viruses are responsible for between 4.0% and 16.0% of all cases of community acquired pneumonia in children younger than 2 years. Flu A and Flu B infections lead to between 36 and 135 hospitalisations per 100 000 children annually, with the highest rate in children younger than 6 months of age (275 per 100 000). Approximately 12.0% of children are admitted to the intensive care unit (ICU) with up to 5.9% requiring mechanical ventilation (Coffin et al., 2007; Silvennoinen et al., 2011).

2.7.2 Human Metapneumovirus

HMPV was first discovered in the early 2000s from nasopharyngeal samples of 28 children who suffered from respiratory illness (Van den Hoogen et al., 2001). HMPV belongs to the family *Pneumonoviridae* and genus *Metapneumovirus.* It is a negative-sense, non-segmented, ssRNA virus consisting of a 13 kb genome (Panda et al., 2014). HMPV is transmitted by infectious airborne droplets through direct contact or close contact with an infected individual or objects (Vicente et al., 2006; Panda et al., 2014). The incubation period varies but is commonly between 3 and 5 days (Vargas et al., 2004; Wyde et al., 2005). HMPV has been isolated on all continents and have a distinct seasonal distribution that is similar to other respiratory viruses. Outbreaks mainly occur between January and March in the northern hemisphere and June to July in the southern hemisphere. A recent study reported that the peak of the HMPV seasonal cases was observed between March and April, following the RSV and Flu infection seasons. Another study reported that the HMPV infection season overlapped with that of the RSV infection season (Bastien et al., 2003; Mullins et al., 2004; Williams et al., 2004; Manoha et al., 2007).

HMPV infects the airway epithelial cells (AEC) of both the upper respiratory tract (URT) and lower respiratory tract (LRT) and displays symptoms such as fever, cough, hypoxia and wheezing amongst others (Panda et al., 2014). However, some may be severe enough to require hospitalisation (in cases of bronchitis, bronchiolitis and pneumonia) or even admission to a paediatric ICU, because of acute respiratory failure. HMPV is commonly found in the paediatric population, with high susceptibility rates in children less than 2 years

old. Seroprevalence studies have shown that a high percentage (90-100%) of children have been infected by the time they are 5-10 years old (Panda et al., 2014). HMPV mortality rate is exceptional and may occur in only 5-10% of HMPV-positive children admitted to the ICU (Principi & Esposito, 2014). The highest incidence rates of HMPV are observed among children during the first few months of life. Overall, in the USA annual rates of hospitalisation associated with HMPV infection were 3 per 1 000 infants younger than 6 months of age, 2 per 1 000 children 6-11 months of age and 1 per 1 000 children younger than 5 years of age. Children hospitalised with HMPV infection, as compared to those hospitalised without HMPV infection, are more likely to receive a diagnosis of pneumonia or asthma, require supplemental oxygen and have a longer stay in the ICU. Premature birth and asthma are more frequently associated with hospitalised children with HMPV infection than those without HMPV infection. Younger age, prematurity and the nosocomial acquisition of viral infection have been found to be risk factors for severe HMPV infections (Mullins et al., 2004).

2.7.3 Human Parainfluenza Virus

Four different serotypes of HPIV are currently circulating in the population worldwide. These viruses were discovered between 1956 and 1960 from children displaying various URT symptoms (Chanock & Finberg, 1956; Johnson et al., 1960; Tyrrell & Bynoe, 1969). All 4 HPIVs were proven to cause common cold symptoms, but with a longer incubation period than normally observed in HRV infections. The HPIVs belong to the *Paramyxoviridae* family and genus *Respirovirus* (HPIV-1,3) and *Rubulavirus* (HPIV-2,4) respectively and are negative-sense ssRNA viruses with a 15 kb genome. HPIVs usually spread by direct contact with infectious droplets or by airborne route through coughs or sneezes (CDC, 2019b). The seasonal circulation of the 4 types of HPIVs differ, each displaying a distinct seasonal pattern. HPIV-1, 2 and 4 display autumn and winter peaks, while HPIV-3 peaks in summer and spring (Maree, 2012).

HPIVs can cause a variety of diseases ranging from common cold to severe URT infection (URTI) and LRT infection (LRTI), requiring hospitalisation. HPIV-3 and HPIV-4 are detected most often in children less than 1 year. HPIV-3 is often associated with pneumonia and bronchiolitis and has been detected in up to 73.6% of children presenting with pneumonia. Approximately 18 000 infants and children are hospitalised each year in the USA as a result of LRTI caused by HPIV-3 (Henrickson, 2003). HPIV-1 can cause LRTIs in young infants but is rare in those younger than 1 month. However, recently an outbreak of HPIV-3 infection among 6 preterm infants was reported in a neonatal nursery (Ben-Shimol et al., 2013). Most

children will contract HPIV infection for the first time before they are 5 years old. More males are usually affected than females. Bronchiolitis occurs in winter and spring with a peak in infants aged between 2 and 6 months. Chances of obtaining a HPIV increase with overcrowding, for example day care attendance and the presence of siblings. Re-infections can occur after the first infection, but are usually less severe (Childrenshospital, 2019).

2.7.4 Respiratory Syncytial Virus A/B

RSV was first discovered in 1957 by Chanock & Finberg (1956) from several infants with mild illness. RSV is an enveloped, negative sense ssRNA virus belonging to the family *Paramyxoviridae* and genus *Pneumovirus* with a 15.2 kb genome. Based on antigenic variability RSV is divided into 2 distinct subgroups, RSV A and RSV B (Mufson et al., 1985). Typically, the incubation period for RSV is 2-8 days, however, it can survive for an extended period on the skin and clothes facilitating the ease with which it spreads (Hall, 2001). The seasonality of RSV infection varies across the globe. The temperate zones tend to experience epidemics during late autumn, winter and spring, whereas the tropical and arctic climates do not have such a well-defined seasonality and might even experience year-round disease (Hui, 2016). In northern tropical areas seasonality of infection is associated with a decreased temperature and increased rainfall, in contrast to the more tropical areas that experience epidemics during both warm and rainy seasons. In Europe, infections peak in December and January and the Mediterranean experiences peaks in March (Constantopoulos et al., 2002; Simoens, 2003). The increased RSV infection rates in declining temperatures can be attributed to increased indoor crowding, which leads to enhanced viral transmission. The lower temperatures furthermore lead to increasing viral stability and host susceptibility or activation of dormant viruses (Griffiths et al., 2017).

RSV infects ciliated epithelial cells of both the URT and LRT. The clinical presentation of RSV includes symptoms such as coryza, cough and a febrile illness, rhinitis, pharyngitis in association with conjunctival signs and erythema (Smyth & Openshaw, 2006; Tregoning & Schwarze, 2010; Wu et al., 2011). RSV infection in children causes a variety of clinical manifestations, depending on the patient's age, comorbidities, environmental exposures and history of RSV infections. Most often infection will lead to diseases, such as bronchitis and pneumonia, requiring hospital admission and mechanical ventilation. RSV causes significant morbidity and mortality worldwide and is currently the leading cause of pneumonia and bronchitis in infants. It is projected that RSV causes 30 million ARTI and more than 60 000 childhood deaths around the globe annually (Nair et al., 2010).

At the age of 18 months 87.0% of children will have antibodies to RSV and by age of 3 years, virtually all children have been infected (Simoens, 1999). In the USA, it has been estimated that RSV accounts for 20.0% of hospitalisation due to ARTI in children under the age of 5, i.e. 17 per 1 000 children under 6 months and 3 per 1 000 children under 5 years. However, children are at the highest risk of death in the first 6 months of life (Thompson et al., 2003). Hospitalisation due to RSV infection increases when the infant has a low birthweight, young age and was born prematurely or close to the start of RSV season (Collins & Graham, 2008; Moyes et al., 2013). Environmental risk factors, such as tobacco smoke exposure, air pollution and indoor crowding (e.g. attendance of a day care centre, hospital or presence of siblings) might further increase the risk of RSV infection (Simoens, 2003).

2.7.5 Human Rhinovirus

HRV, also known as the causative agent for the common cold, is a member of the family *Picornaviridae a*nd the genus *Enterovirus*. These viruses are small, non-enveloped, ssRNA viruses consisting of a 7.2 kb genome. HRV infects AEC and require a relatively low optimal temperature for growth (33°C), which may in turn reflect their adaptation to the human nasopharynx and association with URTIs and LRTIs (Lau et al., 2007). It has an incubation period of 2 days and is symptomatic for 7 - 14 days (Makela et al., 1998; Brownlee & Truner, 2008). HRV is most often transmitted from person to person via contact, either direct or through contaminated objects, aerosols generated by coughing, talking or sneezing. Infection is established by intranasal and conjunctival inoculation, but not via the oral route. Transmission occurs routinely in temperate countries primarily between April and May with a second peak between September and October (Peltola et al., 2008). In South Africa outbreaks occur annually in the winter (June-September) and autumn (March-May) months.

Large epidemiological studies have reported that all children have experienced at least 1 HRV infection by the age of 2 years (Hayden, 2004). Common symptoms include rhinorrhoea, nasal congestion, sore throat, cough, headache, fever and malaise (Winther et al., 1986; Jacobs et al., 2013). HRV causes both URTIs and LRTIs in infants, including bronchiolitis, pneumonia and acute exacerbations of chronic lung diseases, which often result in hospitalisation or even death (Busse et al., 2010). Bronchiolitis causes inflammation and congestion in the small airways (bronchioles) of the lungs and symptoms might mimic those of the common cold, but might progress to coughing, wheezing and sometimes difficulty breathing (Piralla et al., 2012). A study evaluating the incidence of bronchiolitis and low birthweight infants in Argentina found that HRV was detected in 40.0% of bronchiolitis

episodes, by far exceeding the common respiratory viral agent RSV (7.0%) (Kainulainen et al., 2010; Jacobs et al., 2013). HRV infections have been shown to induce cell damage, as well as alter immune responses. Lung development starts at 4 weeks of gestation and continues through early childhood. Therefore, HRV infection may have severe direct and indirect effects on lung tissue leading to chronic lung disease (Jacobs et al., 2013). Moreover, wheezing illness due to HRV infection in the first 3 years of life is associated with an almost 10-fold increase in the risk of developing asthma by the age of 6, in comparison to the only 2.6-fold increased risk associated with RSV infection (Piedimonte, 2013).

2.8 Co-infections

Flu A, Flu B, HMPV, HPIV, RSVA/B and HRV are responsible for respiratory tract infections in infants and young children. Recent trends in molecular technology, such as multiplex PCR, has made it possible to detect multiple respiratory viruses in a single sample and has revealed that multiple or co-infections in ARTI are not uncommon. Furthermore, research has recently elucidated that when these viruses co-infect, symptoms can be more severe; this however may depend on several factors (virus, season, population type and age) (Franz et al., 2010; Yoshida et al., 2013; Asner et al., 2014).

Yohida et al. (2013) completed a comprehensive population-based study on the incidence and effect of single and multiple infections with respiratory viruses and the risk of developing a LRTI in infants. Their results indicate that a co-infection with RSV increased the risk of developing a LRTI. The risk was significantly increased when co-infections occurred between RSV and HRV, as well as RSV and HMPV, however, this was not the case for RSV and Flu A. Semple et al. (2005) focused on the effect of dual infection between RSV and HMPV in children younger than 2 years and the severity of bronchiolitis. During this 1-year study (2001-2002) at the Alder Hey Children's Hospital, Liverpool, UK, it was found that dual infection with HMPV and RSV was associated with severe bronchiolitis and conferred a 10-fold increase in relative risk of admission to a paediatric ICU for mechanical ventilation. Da Silva et al. (2013) evaluated how viral co-infections affect the severity of clinical LRTI in children younger than 3 years in a tertiary hospital in Brazil. A total of 260 episodes of LRTI were analysed with a viral detection rate of 85.0% (n = 222). Co-infection was observed in 65.0% of all virus-positive episodes. The most prevalent single virus was RSV (54.0%), followed by HMPV (32.0%) and HRV (21.0%). Co-infection between HRV and RSV leads to a significant increase in the hospital stay period (4.5 extra days), when compared to those with single infection. The same trends were observed for the outcome of days of

supplemental oxygen use. Goto et al. (2015) analysed the association between Flu A and other respiratory viral agents and during this study it became evident that Flu A's growth was enhanced when co-infection occurred with HPIV, causing more severe infection. Furthermore, HPIV is known to co-infect with HRV and RSV (Linster et al., 2018). Flu B also has the ability to co-infect with RSV, HRV, Flu A, HMPV and HPIV in paediatric patients and cause severe and chronic disease (Stefanska et al., 2012). In a study on 1 335 paediatric patients diagnosed with LRTI in Yuying Children's Hospital, Zhejiang, China, from December 2013 to June 2015, RSV, HRV, HPIV and HMPV respiratory pathogens were detected most frequently. HPIV co-infection was associated with a runny nose, shortness of breath and oxygen support compared to HPIV single infection (Zhong et al., 2019). Co-infections and their contribution to SUDI, have not yet been investigated.

2.9 Respiratory Immune Defence

In the URT a cough drives foreign material up and out of the trachea and bronchi, assisted by the beating of cilia on the AEC lining the URT. The secretion of mucus-containing IgA leads to the neutralisation of foreign pathogens and prevents the attachment of microorganisms to the epithelium. The AECs secrete chemokines and increase the expression of adhesion proteins during inflammation. Adhesion proteins slow down the immune cells in circulation by permitting adherence of neutrophils, monocytes, macrophages and lymphocytes to blood vessels and facilitate their migration from the blood stream to the area of inflammation. The recruited alveolar macrophages serve as a first line of defence in the lungs by neutralising foreign particles and recruiting more neutrophils and mononuclear cells to the site of infection and inflammation. The respiratory mucosa houses dendritic cells (DCs) that are able to detect, capture and transport foreign microorganisms to the lymph nodes of the body, where the adaptive immune system can be stimulated. The DCs phagocytose epithelial cells that are infected with viruses. They then display viral particles on the outer membrane receptors to attract and stimulate naïve T lymphocytes into effector T cells. These effector T cells play an important role in viral clearance (Nicod, 2005).

2.10 Immaturity of Infant Immune Defences

Despite the respiratory immune defence mechanisms, the immune system of infants is immature, in comparison to older children and adults. Before birth, the uterine cavity offers little antigen exposure and infants are thus initially dependent on their innate immune mechanisms, trans-placentally transferred maternal antibodies and the protective immune components of breastmilk for protection against pathogens (Fuchs & Blaas, 2010).

Neutrophils function as part of the first line of defence during the innate immune response and their primary function is phagocytosis of pathogens through the release of enzymes from cytoplasmic granules (Segal 2005; Fuchs & Blaas, 2010). Fewer neutrophils are present in infants, compared to older children and adults. Furthermore, neutrophils of infants respond sub-optimally, resulting in inadequate recruitment of immune cells to the site of infection (Fuchs & Blaas, 2010). Similar to neutrophils, the monocytes and macrophages in infants are also less sensitive to chemo-attractants and infant monocytes generate lower levels of interferon (IFN)-α and IFN-β than those of older children and adults. These 2 cytokines are key components of the regulation of both innate and adaptive immune responses to viral infections through interference with viral entry and replication (Fuchs & Blaas, 2010).

2.11 Viral Detection Methods

A variety of different methods and techniques for the detection of respiratory viruses have been developed over the years. Viral culture is regarded as the gold standard for the detection of most viruses, especially studies that focus on the characterisation and disease pathogenesis of specific viruses. However, this procedure is laborious and take a long time for isolation and identification. Cell culture can take up to 14 days before a presumptive identification can be made based on the cytopathic effect (CPE) caused by the virus. However, the CPE caused by certain viruses might resemble each other resulting in ineffective identification (Jacobs et al., 2013). Viral culture depends highly on viral viability and a long post-mortem interval (PMI) may influence viral viability negatively. Additionally, effective culturing may be dampened by low viral loads, tissue autolysis and post-mortem bacterial contamination of autopsy specimens (Fernández-Rodríguez et al., 2006).

Since conventional viral culture techniques are extremely time-consuming and labour-intensive, the shell vial culture (SVC) method was developed. This technique involves inoculation of a clinical specimen onto a cell monolayer grown on a coverslip in a SVC tube, followed by low speed centrifugation and incubation (Shelhamer et al., 1996). In terms of SUDI cases it had been established that SVC only detects a limited number of viruses in both tissue samples and swabs and is therefore suboptimal in detecting respiratory viruses in a post-mortem setting (La Grange, 2013). This method is increasingly being replaced by molecular methods, such as PCR. Although these early PCR-based assays required confirmation with the use of Sanger sequencing due to cross-reactivity with human deoxyribonucleic acid (DNA), it still shortened the diagnosis time considerably from weeks

to days. Reverse transcriptase PCR, multiplex PCR and real-time PCR assays are now commercially available for viral detection in just a few hours (Jacobs et al., 2013). Additionally, numerous studies have elucidated that these molecular methods are effective in detecting viruses in post-mortem samples and PMI seems to have a limited effect on the detection rate in comparison to viral culture (Weber et al., 2010). Hurtado et al. (2018) determined the effect of PMI on the detection of viruses with molecular detection methods and found no relationship between the PMI and estimated detection rates of viruses between 24 hours and 4 days. Using non-culture methods for bacterial and viral detection assays, a PMI of four hours to four days still yielded significant bacterial and viral isolates in 85.0% of SUDI cases (Rambaud et al., 1999).

2.12 Study Aims and Objectives

The primary aim of this study was to investigate the role of major respiratory viruses, i.e. Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV, found in the lungs and trachea as either single or co-infections of all SUDI cases admitted to Tygerberg MLM from March 2018-March 2019.

As a secondary aim all the epidemiological information and other relevant laboratory data of the retrospective cases from the Tygerberg MLM (2015-2016) were retrieved and used for statistical analyses to identify any trends or differences between the 2 studies in order to evaluate how risk factors associated with SUDI cases at the Tygerberg MLM changed or remained constant over the 2 study periods.

Thirdly, all laboratory results of all infants aged between 7 days and 1 year admitted to TBH due to respiratory infections between 2015 and 2019 were retrieved from the National Health Laboratory Service (NHLS) laboratory information system and used as a comparison group for the SUDI cases to identify if similar single and multiple viruses were circulating both populations at the time of this study.

The specific objectives of this study are:

i. To collect swabs from the lungs and trachea in prospective SUDI cases admitted to the Tygerberg MLM from March 2018 to March 2019 and establish the presence of Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV.

ii. To identify confirmed Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV cases with multiplex PCR in lung or trachea swabs from cases collected between 2015 and 2016 for retrospective analyses.

iii. To identify specific co-infections in all positive samples of both retrospective and prospective studies.

iv. To correlate the socio-demographic data from these cases to identify possible associations between Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV infection and known risk factors for SUDI (e.g. age, gender, season, clinical signs of respiratory distress in the days preceding death, etc.).

v. To compare viral results with histological assessment of lung tissue.

vi. To compare results obtained from prospective study with results from the retrospective study.

vii. To compare viral results with the comparison group, in order to determine if the same viruses were circulating both the SUDI and hospital infant population at the time of this study.

viii. To communicate the findings to the Division of Forensic Pathology to consider in formulating the final COD.

CHAPTER THREE:
MATERIALS AND METHODS

CHAPTER CONTENT

3.1 Study Design

This study comprised both a retrospective and prospective arm designed at profiling Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV infections and co-infection in cases presented as SUDI at Tygerberg MLM in the Western Cape. All data of cases positive for Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV from retrospective studies (2015-2016) conducted at the Tygerberg MLM were retrieved from the Tygerberg SUDI database and all single and multiple infections recorded. Prospective autopsy samples were collected from all SUDI cases over a 1-year period from March 2018 to March 2019 to include all four consecutive seasons.

3.2 Ethics

The study was originally approved for 1 year by the Health Research Ethics Committee (HREC) of Stellenbosch University on 7 March 2012 (protocol number N12/02/007) (Appendix 1) and it is renewed annually with amendments if necessary.

The samples that were collected during the current study did not differ from the standard autopsy procedure followed during SUDI investigations at Tygerberg MLM. Therefore, a waiver of consent was granted by HREC for both collection and subsequent laboratory testing of samples. All swabs collected were analysed with the aim of reporting all results to the Division of Forensic Pathology to assist in the process of determining the final COD, and consent was provided for by Section 3(a) of the Inquests Act [No 58 of 1959] and the Criminal Procedure Act [No 51 of 1977]. All specimens were labelled with the death register number (WC/14/number/year) in order to de-identify cases and ensure that no personal information was available to laboratory personnel or the research team.

3.3 Study Population

During the 1-year study, only cases admitted to Tygerberg MLM between the ages of 7 days (excluding perinatal deaths) and 1 year were included in this study. The only inclusion criterion was cases that comply with the SUDI definition according to the literature (all cases that involve the sudden and unexpected death of an infant under the age of 1 year where intentional and unintentional injury can be excluded) (Krous, 2010). All cases that initially presented as SUDI but were subsequently classified as unnatural COD (child abuse, homicide, poisoning, etc.), either during the autopsy or after further investigations, were

excluded from the study. Additionally, all known natural causes of infant death, life-threatening disease or deformities were also excluded.

3.4 Post-mortem Dissection Process

The routine post-mortem process starts with a Y-shaped incision that is made by the forensic pathologist or forensic pathology officer assisting with the body dissection. Two incisions start behind the ears and continue to the top of the sternum. The cut is then extended all the way down to the pubic bone. Finally, the sternum and ribs are cut open to remove the chest plate and expose the chest cavity.

3.5 Sample Collection and Analysis

During the study period routine mandatory samples were collected for microbiological testing by the NHLS, TBH, as well as subsequent samples required for investigations in this study. All specimens collected were labelled with the unique death register number and date of collection.

3.5.1 Routine Microbiological Sample Collection and Analysis

During sample collection, the lower lobes of both lungs were surface sterilised with a spatula heated in a direct flame to limit contamination by post-mortem flora or any other contaminants. After sterilisation a small incision was made into the lower lobes of the lungs with a sterile surgical blade (Lasec, South Africa). The microbiology culture swab (Lasec, South Africa) was then inserted into the incision and rotated in order to ensure optimal sample collection. Samples were sent to the Medical Microbiology laboratory, NHLS, TBH, for routine microbiological culture. Each sample was analysed for bacterial growth on 4% blood agar, MacConkey agar and Chocolate agar (Sigma Aldrich, South Africa) and incubated at ± 35°C for 24 - 18 hours in order to select for the growth of clinically relevant Gram-positive and Gram-negative bacteria, such as *E. coli, Klebsiella pneumoniae (K. pneumoniae), Staph aureus, Strep pneumoniae, Haemophilus influenzae* and *Salmonella* spp., amongst others.

3.5.2 Research Sample Collection and Analysis

Since Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV are found in both the URT and LRT, samples were collected from both lungs and the trachea. During autopsy the infant's neck tissue was exposed as previously described and dissected with a sterile surgical blade

(Lasec, South Africa) to expose the trachea. A flocculated swab (COPAN Diagnostics, USA) was inserted into a small incision between the sternal notch and thyroid gland and pushed downwards as far as possible. Each swab was rotated inside the trachea several times before being removed. Separate flocculated swabs were then inserted into the incisions previously made in both lower lobes of the lungs and rotated numerous times. Each individual swab was placed into a container with 3 ml universal transport media (UTM) (COPAN Diagnostics, USA), labelled with the death register number of the particular case, date of collection and transported to the Medical Virology laboratory, Tygerberg Campus for storage and further analysis. The UTM containers contain multiple glass beads at the bottom which facilitate maximum sample recovery by constantly beating the flocculated swab during pulse vortexing. Each swab was vortexed for 30 seconds to ensure maximum specimen recovery. The recovered samples were aliquoted into 3 different 1.5 ml microcentrifuge tubes (Lasec, South Africa) and stored at -80°C until analysis.

3.6 Nucleic Acid Extraction

RNA was extracted from both lung and tracheal swabs using the Zymo Quick RNA™ Viral Kit according the manufacturer's instructions (Inqaba Biotechnical Industries, South Africa). Quality Control for Molecular Diagnostics (QCMD) samples for each virus were obtained from the routine NHLS Medical Virology laboratory, Tygerberg Campus, and used as positive controls (PC). UTM served as the negative control (NC) and RV-EA (included in the multiplex real-time PCR RV Essential Assay) as the internal control (IC).

In brief, samples were removed from the -80°C freezer and placed on ice to thaw. Once samples were completely thawed, 190 µL of each sample and 10 µL of the IC were aliquoted into a 1.5 mL microcentrifuge tube. To lyse the samples, 400 µL of Viral RNA buffer was added to each sample and mixed thoroughly. This mixture was then transferred to a Zymo-Spin™ IC collection tube and centrifuged for 2 minutes at 13 000 g to disrupt and homogenise the sample. The lysate was then transferred to a clean Zymo-Spin™ IC collection tube and 500 µL of Viral Wash Buffer was added, followed by centrifugation of 30 seconds at 13 000 g. This step was repeated twice. Several wash steps with 95-100% ethanol were performed to remove residual contaminants. Lastly, the purified RNA was eluted with buffer containing RNAse-free water into a clean 1.5 mL microcentrifuge tube. A final volume of 15 µL was obtained and extracted RNA was stored at -80°C.

3.7 RV Essential Assay Multiples Real-time PCR Assay

The Seegene Allplex™ RV Essential one-step multiplex, real-time PCR assay (Inqaba Biotechnical Industries, South Africa) was used for the detection of Flu A, Flu B, HMPV, HPIV, RSV A/B, HRV per manufacturer's instructions. The kit contained IC, PC and NC that were included in every real-time PCR assay. The IC was designed to monitor the process of nucleic acid extraction and detect any possible PCR inhibition. The PC contained a mixture of the 6 respiratory viruses and IC clones, whereas the NC consisted of only RNAse-free water. The total reaction volume was 20 µL and the reaction mixture contained 15 µL of the master mix and 5 µL of template RNA (Table 3.1). Real-time PCR was performed in a 96 well skirted PCR plate and sealed with optical flat 8-cap strips (Bio-Rad, USA). Subsequently each plate was centrifuged to eliminate all air bubbles and collect all residual liquid at the bottom of the tubes. Each reaction performed included the testing of the supplied PC and NC.

Table 3.1: Reaction mixture for real-time PCR analysis

Volume	Reagent
5 µL	RV-EA MOM
5 µL	EM5
5 µL	EM5 Buffer
15 µL	Total volume of PCR Master mix

Volume	Reagent
15 µL	Reaction mixture
5 µL	Template

The CFX96™ Real-time PCR System (Bio-Rad, USA) was used for analyses and thermal cycling conditions for each reaction are outlined in Table 3.2:

Table 3.2: Thermal cycling conditions for real-time PCR analysis

Step	No. of cycles	Temperature	Duration
1		50°C	20 min
2	1	95°C	15 min
3		95°C	10 s
4*	45	60°C	15 s
5*		72°C	10 s
6		**REPEAT STEP 3, 44 TIMES**	

*Plate Read at Step 4 and 5.

Four fluorophores were used during analysis: FAM, HEX, Cal Red 610 and Quasar 670, with fluorescence being detected at 60°C and 72°C. FAM was used for the detection of HMPV and RSV, HEX for HPIV and Flu B, Cal Red 610 for Flu A and finally Quasar 670 for HRV and the IC. Data were analysed with Seegene Viewer software Version 3.1. C_t values are inversely proportional to the amount of target nucleic acid in the sample; therefore, all clinical samples producing a C_t value of ≤ 40 were reported as “Detected” and > 40 “Not detected” if all the criteria in Table 3.3 were met:

Table 3.3: Criteria for positive PCR result

Control	Seegene Viewer Results (C_t)							
	FAM		HEX		Cal Red 610	Quasar 670		Auto Interpretation
	HMPV	RSVA/B	HPIV	FLU B	FLU A	HRV	IC	
	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	PC
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	NC

3.8 Histology

All histology slide preparation of lung sections was completed by medical technologists at the Division of Forensic Pathology, Faculty of Medicine and Health Sciences, University of Stellenbosch. Both left and right lungs were dissected through the middle to obtain a 1-3 mm thick slice with a Tissue-Tek® Accu-Edge® trimming knife handle. After dissection the tissue samples were placed into separate histology cassettes and submerged in 10.0% formalin (BASF, South Africa). The cassettes were labelled with the unique death register number and “2X L” (left lung) or “2Y R” (right lung).

3.8.1 Histology Slide Preparation

The Tissue-TeK® VIPTM 5 Jr. Vacuum Infiltration Processor (Sakura Finetek, The Netherlands) was used to prepare the wax blocks. This automated tissue processor utilises pressure and vacuum cycles to facilitate penetration of the formalin, ethanol (Illovo, South Africa) and xylene (BASF, South Africa) into tissue sections. The process starts with 10.0% formalin and continues with increasing series of ethanol concentrations starting at 70.0%, 80.0%, 96.0% and finally 100%. This ethanol concentration gradient prevents the precipitation of salts from the formalin and serves as a dehydrating solution to remove both water and residual formalin from the tissues. Finally, xylene was used to mix the tissue sections with wax. The Tissue-TeK® TEC embedding console system was used to create

wax blocks of the tissue. Once hardened, facing was followed by cutting on a Microm HM 335E microtome Rotary (Thermo Fisher Scientific, South Africa) to acquire 4-5 µm thick tissue sections. This section was then placed in a 60°C water bath (Amos Scientific, Australia) to remove any folds and melt excess paraffin wax. Afterwards the sections were mounted on glass microscope slides (Starfrost®, UK) and incubated (Scientific Series 9000) at 77°C for 30 minutes to facilitate the attachment of tissue to slide. The tissue was then subjected to Haematoxylin and Eosin (H&E) (Sigma-Aldrich, South Africa) staining for visualisation under light microscope (Titford, 2005) (Table 3.4).

Table 3.4: Protocol for Haematoxylin and Eosin staining

Step	Medium	Time
1	Xylene	5 min (3 cycles)
2	Ethanol 100%	5 min
	Ethanol 96.0%	5 min
	Ethanol 70.0%	5 min
3	Rinse with tap water	
4*	Mayer's-Haematoxylin	5 min
5	Rinse with tap water	
6	Scott's tap water	1 min
7	Rinse with tap water	
8[†]	Eosin	2 min
9	Rinse with tap water	
10	Ethanol 70.0%	5 min
	Ethanol 85.0%	5 min
	Ethanol 96.0%	5 min
	Ethanol 100%	5 min
11	Xylene	1 min
12	Mount cover slip (DPX mounting medium)	

*Haematoxylin is a basic dye that stains the nuclei of the cells a dark purple blue.

[†] Eosin is an acidic dye that stains the cell cytoplasm in various shades of pink while staining red blood cells an orange-red colour.

3.8.2 Histology Slide Analysis

Histological evaluations of the lung sections were completed with an Olympus BX41 light microscope and included the documentation of interstitial pneumonitis (IP), bronchitis, bronchiolitis, bronchopneumonia, pneumonia and any other unusual observations (Table 3.5). All observations were conducted together with Prof Johan Dempers, Principal Specialist Forensic Pathologist, Division of Forensic Pathology, Faculty of Medicine and Health Sciences, University of Stellenbosch. All descriptions of images were evaluated and

confirmed by Dr Björn Swigelaar, Senior Forensic Pathologist, Division of Forensic Pathology, Faculty of Medicine and Health Sciences, University of Stellenbosch (Swigelaar, 2019).

Table 3.5: Grades of IP as defined by Krous et al. (2003)

Grade	Description
Grade 1	Very mild (barely present, often focal and with no overall alteration of the alveolar septa)
Grade 2	Mild (relatively diffuse and mild thickening of alveolar septa)
Grade 3	Moderate (diffuse involvement and associated with interstitial oedema)
Grade 3^{+}	Severe (diffuse infiltration associated with significantly widened alveolar septa)

3.9 Review of Prospective SUDI Case Files and Data Collection

All socio-demographic and epidemiological data were collected from the SUDI case files included in this study and transcribed into a Microsoft™ Excel spreadsheet. Information collected included gender, dates of birth, death and autopsy, time of death, sleeping position, type of bedding used, bedsharing, type of housing, birthweight and prematurity, as well as medical history and presence of any clinical symptoms or illnesses prior to demise, the final concluding diagnosis and any additional comments from the presiding pathologist.

3.10 Review of Retrospective SUDI cases and Data Collection

All socio-demographic and epidemiology data were retrieved from the Tygerberg SUDI database. This included viral PCR-positive results for both lungs and trachea, as well as microbiology culture results. This information was collected to compare retrospective and prospective results in order to identify possible similarities and differences to obtain a better understanding of SUDI risk factors and whether these factors stayed constant over these specific time periods. The retrospective results were combined with the prospective results and compared to living infants admitted to TBH.

3.11 Evaluation of NHLS Respiratory Viral Results of Infants Admitted to TBH

After obtaining approval for data mining from the NHLS (Appendix 2), data of all living infants aged between 7 days and 1 year that were admitted to TBH with respiratory infections (July 2015-2016; March 2018-2019) were retrieved from the NHLS laboratory information system, TBH, to utilise as a comparison group for the SUDI cases (retrospective and prospective). This data included age, gender and respiratory viruses detected with Seegene, Anyplex™

II RV16 Detection Kit (Inqaba Biotechnical Industries, South Africa). All records were anonymised to protect the infants' identity.

3.12 Weather Data Retrieval from Tygerberg Referral Area

Temperatures (maximum and minimum) on the days that all SUDI cases died were obtained from the South African Weather Service, Cape Town International Airport Station (GPS co-ordinates 33.9715° S, 18.6021° E). This was performed to assess any association between the maximum, minimum or difference in daily temperature and the occurrence of SUDI cases. Cape Town International Airport Station was chosen as it encompasses all areas that refer cases to Tygerberg MLM and would closely resemble the direct environment on the days that infants from this study died. According to our knowledge this would be the first study in South Africa analysing the presence or absence of an association with temperature.

3.13 Statistical Analysis

Statistical analysis was conducted by Professor M. Kidd at the Centre for Statistical Consultation, Department of Statistics and Actuarial Sciences, University of Stellenbosch. TIBCO Statistica® version 13.5.0 was used for data analysis. One-way Analysis of Variance (ANOVA) was completed for all quantitative data, such as age, birthweight and temperature. Categorical and qualitative data were analysed with Kruskal-Wallis and Pearson Chi-square tests.

CHAPTER FOUR:
RESULTS

CHAPTER CONTENT

4.1 Study Population

A total of 173 SUDI cases with a male to female ratio of 1:0.9 were admitted to the Tygerberg MLM during the study period. The average post-mortem interval (PMI) was 6.5 ± 2.8 days. The average age was 12.1 ± 9.8 weeks with the most deaths occurring between 1 and 12 weeks with a peak incidence between 5 and 8 weeks (Figure 4.1).

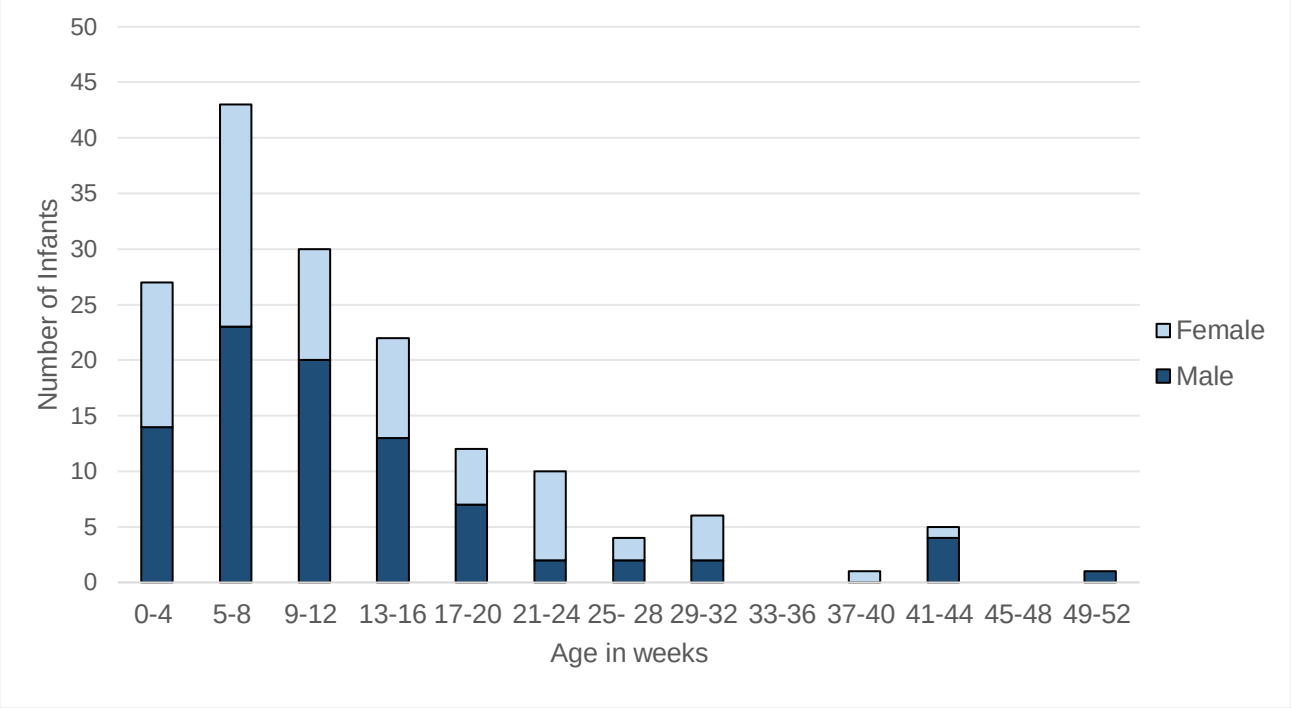

Figure 4.1: Age and gender distribution of SUDI cases admitted to Tygerberg MLM

4.2 Socio-demographic Information

The seasonal distribution of the 173 cases admitted to Tygerberg MLM is depicted in Figure 4.2. Cold and warm seasons were defined according to the equinox and solstice with September to February being classified as the warm season and March to August regarded as the cold season. More than half of the cases occurred during the cold months of the year 61.8% (107/173). Similarly, more male infants died during the colder months compared to females. Additionally, younger infants (1-12 weeks) succumbed more often in the colder months.

All information regarding the death of the infant was not captured for every case due to a variety of reasons, amongst other parents, caregivers or guardians being too distressed to answer questions, while others were unavailable. Risk factors (sleeping environment, housing and ventilation) were only available for 161 cases and only these were included in the statistical analyses.

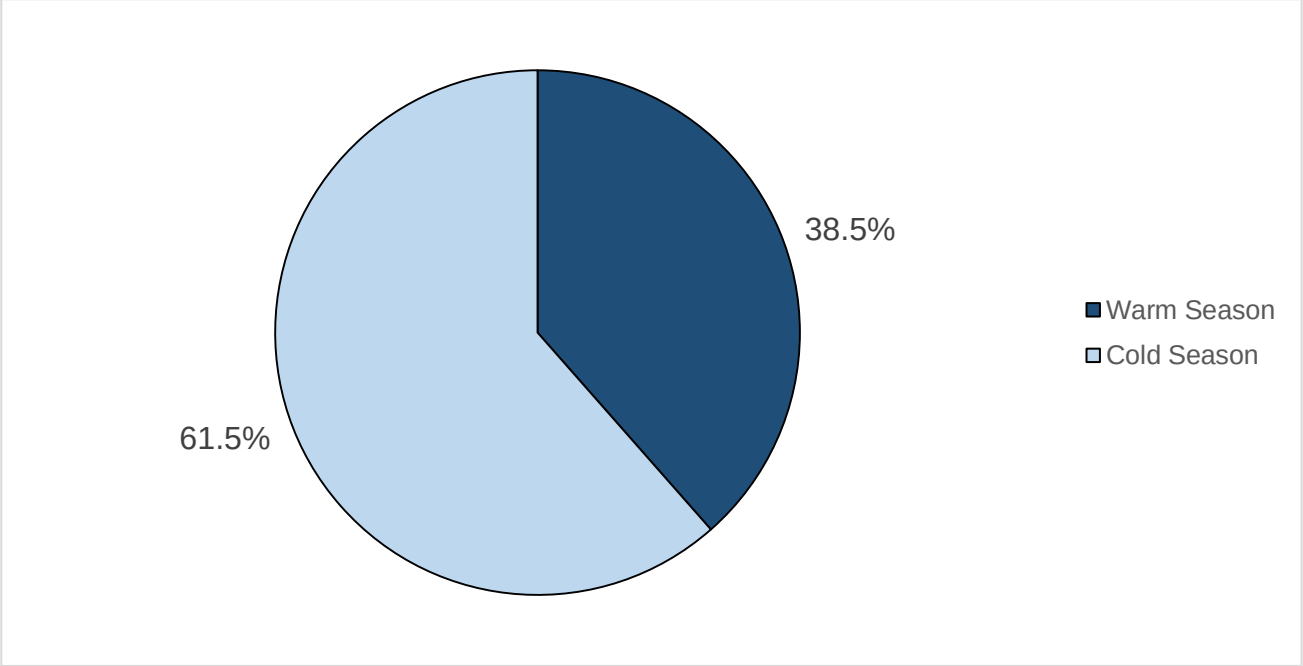

Figure 4.2: Seasonal distribution of 173 cases admitted to Tygerberg MLM

In 88.8% (143/161) of the SUDI cases bedsharing was reported. Fifty-four percent (87/161) of infants were put to bed on their sides, 31.1% (50/161) on their stomachs and 12.4% (20/161) on their backs. A total of 42.2% (68/161) of infants were born prematurely and 50.0% had a birthweight less than 2500 g, therefore being classified as having a low birthweight. Sleep-related risk factors are shown in Figure 4.3. The majority of cases lived in formal housing, with 47.2% (76 /161) families having confirmed ventilation (open window) in the room where the infant slept.

4.3 Cause of Death Classification

At the time of submitting this thesis only a 161 of the SUDI cases were signed out by attending pathologists. These cases were classified as *SIDS*, where evidence failed to reveal a definite COD, in 31.7% (51/161), *Infection* in 60.9% (98/161) and *Other* in 7.5% (12/161) cases. Diagnoses in the *Other* group included cardiac causes, an interrupted aortic arch anomaly, abnormalities of the brain, aspiration of gastric content, congenital cardiac defects, cardiopulmonary problems and hypoxic ischemic encephalopathy**.** More infant males succumbed to *SIDS*, *Infection* and *Other* deaths, in comparison to their female counterparts. The age at which the majority of *SIDS* and *Infections* cases occurred was 5-8 weeks with most of these deaths occurring during the colder months.

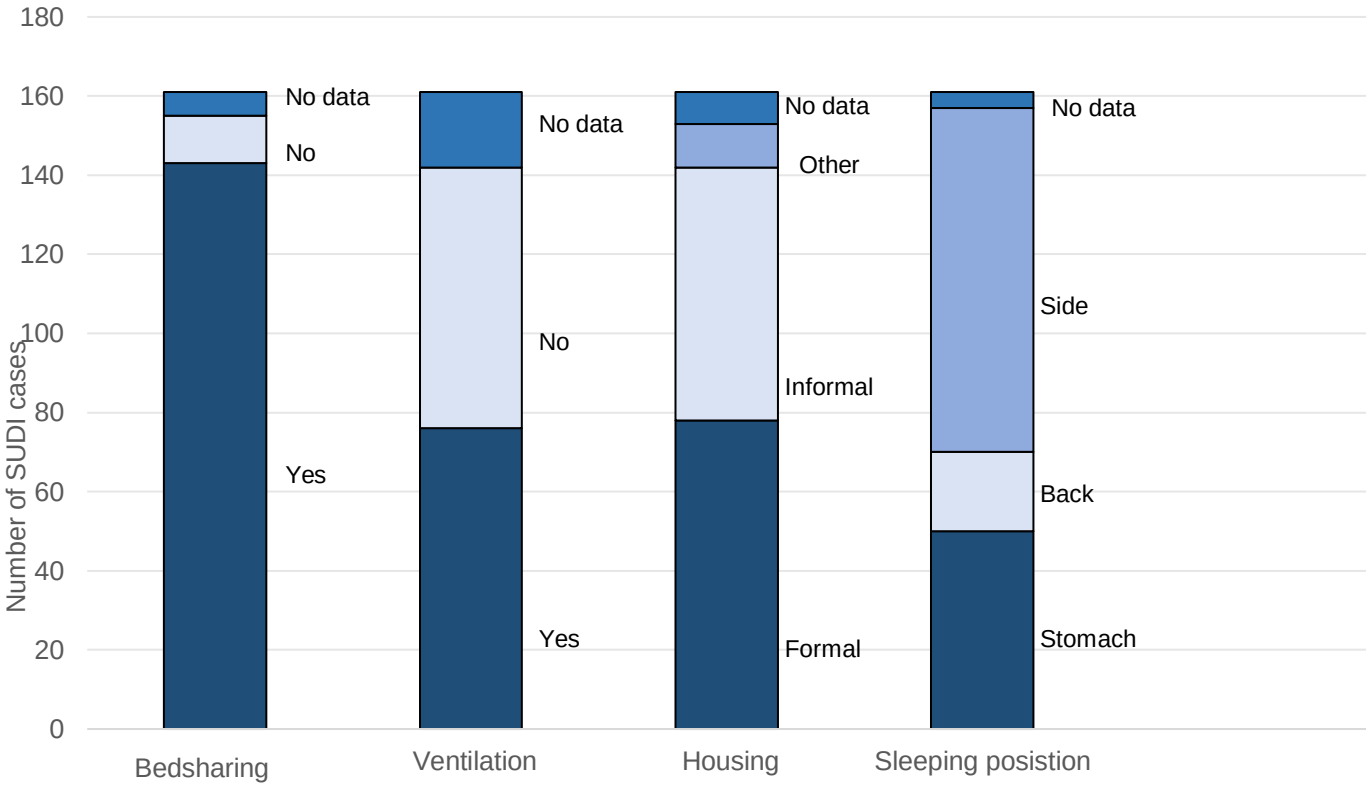

Figure 4.3: Sleep-related risk factors in 161 SUDI cases admitted to Tygerberg MLM

4.4 Detection of Respiratory Viral Pathogens in Prospective Study

Respiratory virus detection in the prospective study utilised the Allplex™ RV Essential multiplex real-time PCR assay to test lung and trachea samples obtained from the 173 SUDI cases. A total of 156 PCR-positive viral results were obtained. The trachea and lungs had almost similar number of positive viral samples in 46.2% (81/173) and 43.3% (75/173) cases respectively. In the 173 cases RSV A/B was the most commonly detected virus (31.7%), followed by HRV (24.8%). Flu A, HMPV, Flu B and HPIV were detected in 13.8%, 12.4%, 11.8% and 3.1% of all cases respectively (Table 4.1). The majority of the 156 viral PCR-positive cases occurred in the cold season in 73.1% (114/156), compared to the 26.9% (42/156) in the warm season. RSV A/B and HRV had the highest prevalence during the cold season and HRV and HMPV in warmer seasons (Figure 4.4). More males produced viral PCR-positive results. Additionally, 38 infants (23.6%) that were premature produced viral PCR-positive results.

Co-infections were more commonly found in the trachea than the lungs. The different combinations of viruses including multiple site infections (virus present in both lungs and trachea) observed during the prospective study are depicted in Table 4.2. Co-infection

between 2, 3 and 5 were detected with RSV A/B and HRV being detected most often. Furthermore, co-infections occurred more often in males than females.

Table 4.1: Number of respiratory viruses detected in 173 SUDI cases

Respiratory Virus	Positive in Lungs	Positive in Trachea	Positive in both sites
Flu A	4.6%	6.9%	0.6%
Flu B	4.6%	4.0%	2.3%
HMPV	4.0%	5.2%	2.3%
HPIV	0.6%	1.2%	1.2%
RSV A/B	9.8%	5.8%	13.9%
HRV	7.5%	11.0%	4.6%

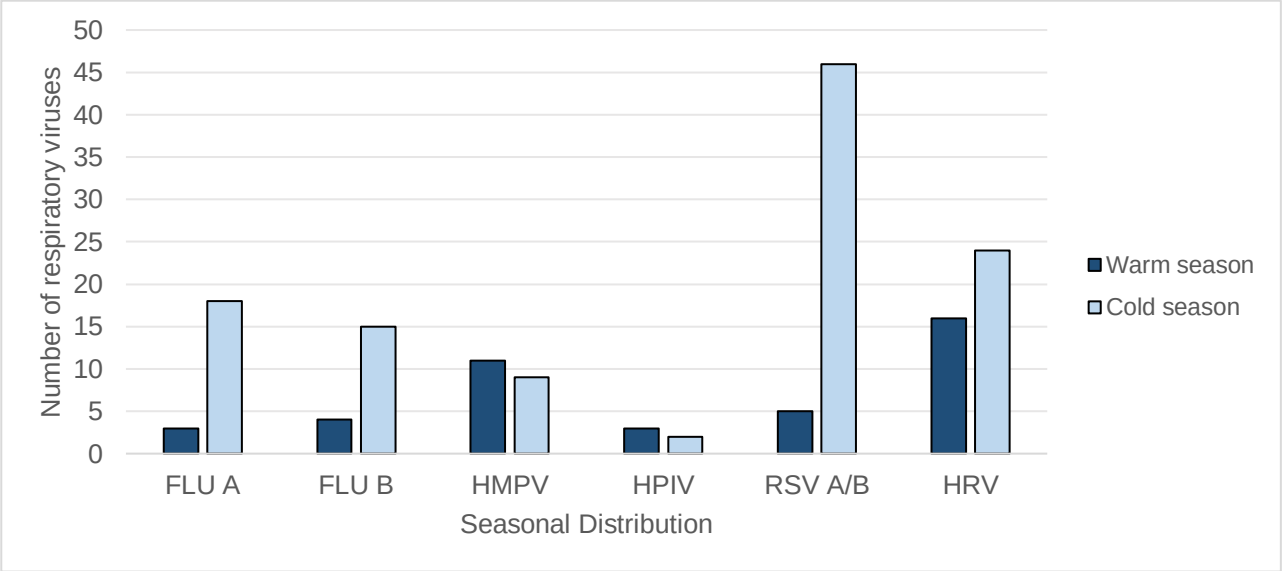

Figure 4.4: The seasonal distribution of respiratory viruses observed in this study

4.5 Detection of Microbial Pathogens

Only 169 of 173 microbiology swabs were analysed for bacterial growth (Table 4.3). Bacterial culture results were positive in 79.2% (134/169) of cases. A single bacterium was detected in 85.1% (114/134) of the cases, while 14.2% (19/134) had 2 bacterial species and 0.7% (1/134) had 3. Of the 134 bacterial culture-positive cases, 32.8% (44/134) also produced PCR-positive viral results. Co-infection with a single virus and multiple viruses was confirmed in 47.7% (21/44) and 52.3% (23/44) of these cases respectively. *Staph aureus* was most often associated with co-infection. The percentages of bacterial species detected during routine bacterial culture from SUDI cases are shown in Table 4.4. *E. coli, K. pneumoniae* and *Staph aureus* were the most frequently detected bacterial pathogens.

Table 4.2: The different combinations of co-infections observed in 173 SUDI cases

Co- infection combinations observed in the lungs	Number observed
RSV A/B - HRV	8
Flu A - Flu B	4
Flu A - RSV A/B	2
HMPV - RSV A/B	2
Flu A - Flu B - HMPV - RSV A/B - HRV	1
Flu B - HMPV - RSV A/B	1
HPIV - RSV A/B - HRV	1
Co-infection combinations observed in the trachea	
Flu A - Flu B	5
RSV A/B - HRV	4
Flu A - RSV A/B	2
HMPV - HRV	2
Flu A - HMPV - RSV A/B	1
Flu A - Flu B - RSV A/B	1
Flu A - Flu B - HMPV - RSV A/B - HRV	1
Flu B - RSV A/B	1
HMPV - HRV - RSV A/B	1
HMPV - HPIV	1
Flu A - HPIV	1
HPIV - HRV	1

Table 4.3: Routine bacterial culture results for lungs in 173 SUDI cases

	Right Lung Swab	Left Lung Swab
Bacterial Growth	70 (40.5%)	64 (37.0%)
No Growth	52 (30.1%)	60 (34.9%)
Post-mortem Flora	47 (27.2%)	45 (26.0%)
Not Tested	4 (2.3%)	4 (2.3%)

Table 4.4: The presence of bacterial species found in lungs during the study period

Bacterial Isolate	Prevalence in the study (n= 169)
E. coli	12.4% (21/169)
K. pneumoniae	11.8% (20/169)
Staph aureus	11.2% (19/169)
Strep pneumoniae	4.7% (8/169)
Proteus mirabilis	3.5% (6/169)
***Raoutella* spp.**	3.5% (6/169)
Haemophilus Influenza	2.9% (5/169)
Strep agalactiae	2.9% (5/169)
Strep pyogenes	2.9% (5/169)
Enterococcus faecalis	2.4% (4/169)
Klebsiella oxytoca	2.4% (4/169)
Staph pneumoniae	1.7% (3/169)
Aeromonas hydrophila	1.8% (2/169)
Candida albicans	1.8% (2/169)
***Haemophilus Influenza* B**	1.8% (2/169)
***Leuconostoc* spp.**	1.8% (2/169)
***Salmonella* D**	1.8% (2/169)
***Strep* group C**	1.8% (2/169)
Strep mitis	1.8% (2/169)
Strep oralis	1.8% (2/169)
Strep salivarius	1.8% (2/169)
Coagulase negative staphylococcus	0.6% (1/169)
Enterococcus faecium	0.6% (1/169)
Enterococcus gallinarum	0.6% (1/169)
Lactococcus garvieae	0.6% (1/169)
Lelliottia amnigena	0.6% (1/169)
Leuconostoc citreum	0.6% (1/169)
Leuconostoc pseudomesenteroides	0.6% (1/169)
Serratia liquefaciens	0.6% (1/169)
Serratia marcescens	0.6% (1/169)
***Strep* group B**	0.6% (1/169)

4.6 Histology

Microscopic signs of infection present in the left and right lower lung lobes were detected in 70.8% (114/161) of cases and included IP in 65.2% (105/161), pneumonia in 5.6% (9/161), bronchopneumonia in 4.3% (7/161), bronchitis in 1.9% (3/161) and bronchiolitis and neutrophil and lymphocyte infiltrate in 1.2% (2/161) of cases each. In 20 cases, IP and signs of infection were present. An overview of the number of cases that displayed histological

signs of infection in PCR positive viral cases is presented in Figure 4.5. RSV A/B had the highest prevalence of viral PCR-positive cases and signs of histological infection, while HPIV had the lowest.

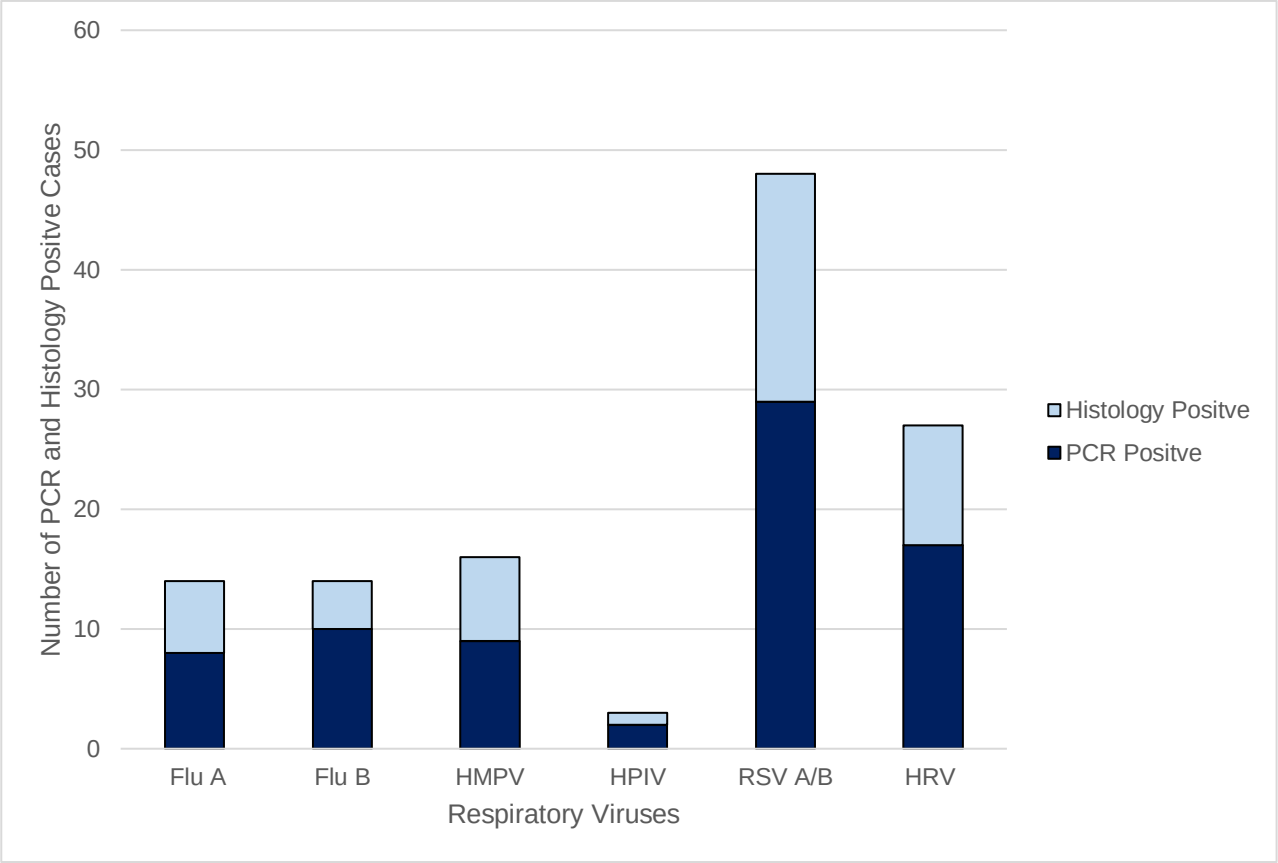

Figure 4.5: Overview of viral PCR positive cases and histological sign of infection

A normal post-mortem lung is depicted in Figure 4.6A and B at 40x and 100x magnification respectively. Normal pulmonary architecture, comprising of relatively normal sized alveoli spaces and thin alveoli septa are present. In some areas the alveoli septum has ruptured. This is a normal post-mortem feature. Furthermore, the alveolar spaces are clear, with no collection of fluids, cells or proteins. The interstitium is not hypercellular with normal type 1 and type 2 pneumocytes present and no inflammatory cells present.

In Figure 4.7A and B histological signs of bronchopneumonia are present. At 40x magnification (Figure 4.7A) alveolar collapse and congestion is present in the capillaries. Additionally, in the alveolar spaces, intra-alveolar collection of neutrophils and protein extrudate are present. At 100x magnification (Figure 4.7B) all aforementioned observations are present and sieving off, of the alveolar walls are evident.

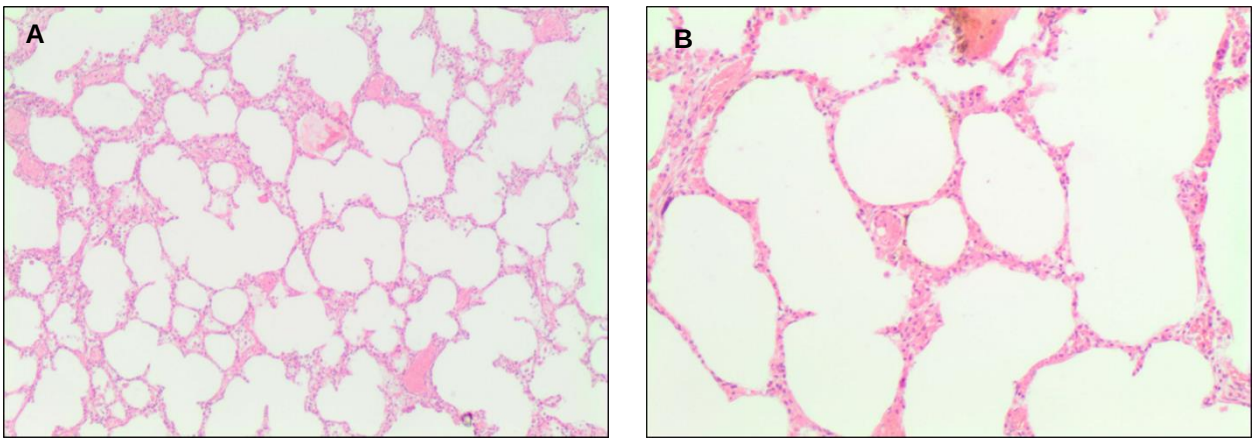

Figure 4.6: A normal post-mortem lung

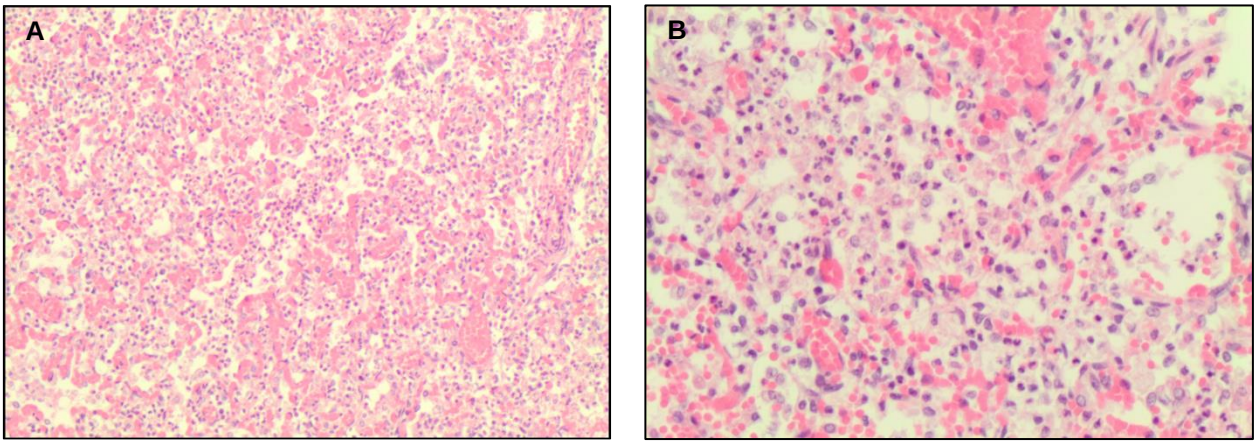

Figure 4.7: Bronchopneumonia

In Figure 4.8A and B Grade 3 IP is present. At 40x magnification (Figure 4.8A) and 200x magnification (Figure 4.8B) thickening of the alveolar walls and areas of alveolar collapse are evident, as well as a thickened and hypercellular interstitium. The interstitium is expanded by mononuclear inflammatory cells, such as lymphocytes or macrophages. The airway labelled with a red arrow in Figure 4.8C is consistent with a bronchiole. In the bronchiole sloughing of bronchiolar epithelium and a dense neutrophilic collection are present. These observations are constant with an acute bronchiolitis.

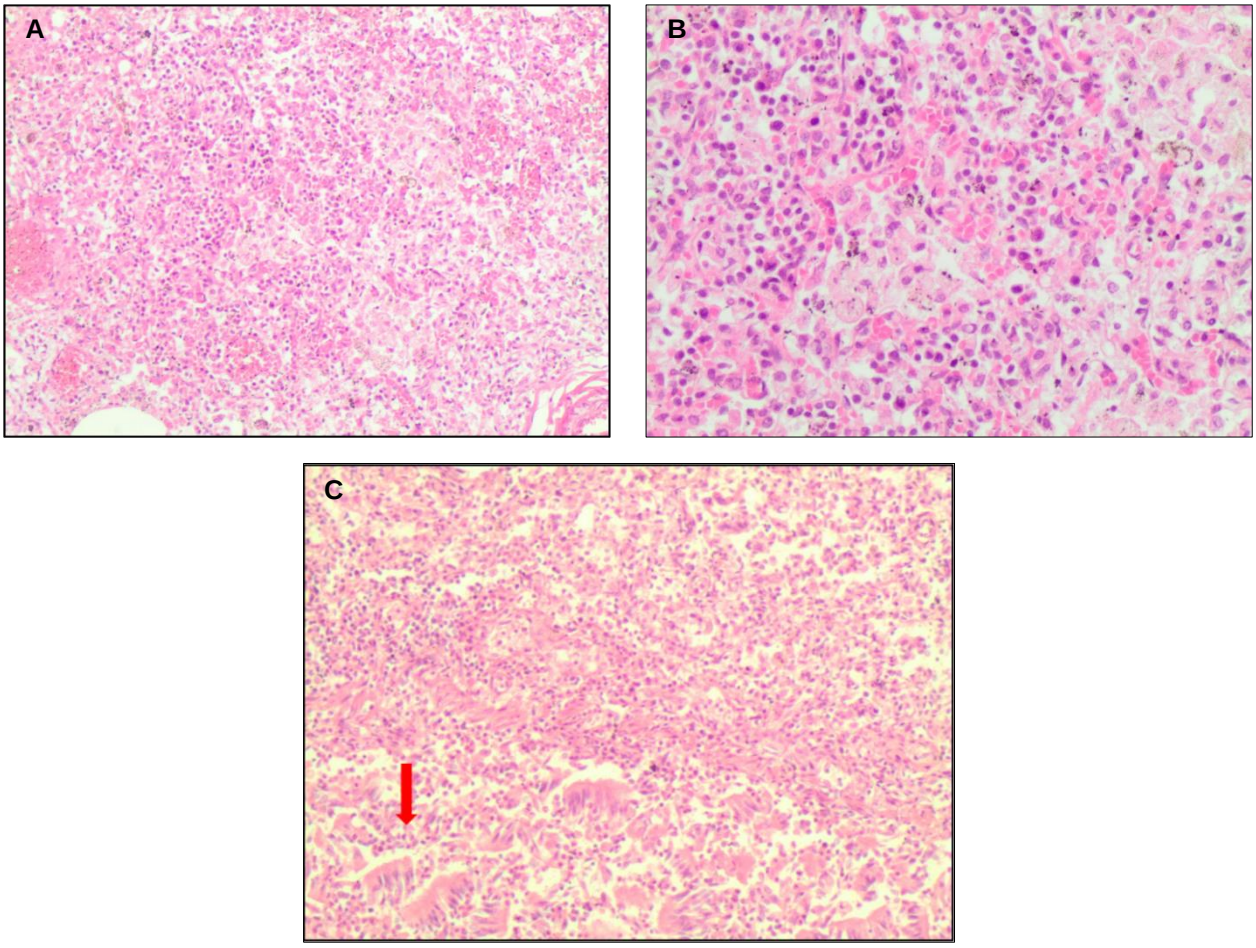

Figure 4.8: Bronchiolitis and Grade 3 Interstitial Pneumonitis

4.7 Description of Retrospective Study Population (2015-2016)

A total of 177 SUDI cases admitted to the Tygerberg MLM between 2015 and 2016 were reviewed in this section of the study. The male to female ratio for this study was 1:1.01. The average age was 13.5 ± 12.8 weeks and average PMI was 4.6 ± 2.6 days. A total of 66.7% (118/177) infants died during the cold season, compared to 33.0% (59/177) in the warm season. Interestingly, more females than males died in the colder months, while more males than females died in the warmer months. Thirty-nine percent (77/177) weighed less than 2 500 g at birth, which was regarded as low birthweight. Sleep-related information was only available for 101 of these cases; 36.6% (37/101) of infants were put to sleep on their stomach, 53.5% (54/101) on their sides and 10 9.9% (10/101) on their backs. At the time of final data analyses, only 66.7% (118/177) cases were signed out with a final COD by the forensic pathologists. *SIDS* was assigned as the final COD in 48.3% (57/118) of cases, while 24.5% (29/118) were a result of *Infection* and 22.9% (27/118) were classified as *Other* (Matshazi, 2017).

4.7.1 Respiratory Viral Detection

Respiratory virus detection in the retrospective study utilised the Seeplex RV15 ACE multiplex PCR detection kit. The detection rate of viruses in 183 cases was as follows: Flu A 2.2% and Flu B 0.5%, HPIV 7.1%, RSV A/B 4.9% and HRV 35.5%. Not a single HMPV was detected during this study. The majority of PCR-positive samples were obtained from tracheal swabs. If respiratory viruses were present in both lungs and trachea, it was classified as a multiple site infection. Co-infections were only detected in the trachea. The different combinations observed were as follows; Flu A with HRV, Flu B with HRV and HPIV with HRV as well as RSV A/B.

4.8 Evaluation of NHLS Respiratory Viral Results of Infants Admitted to TBH

From March 2015 to March 2019 a total of 808 living infants between the ages of 7 days and 1 year were admitted to TBH due to respiratory infections. This data was used to compare to the SUDI cases evaluated in the prospective and retrospective studies. A male to female ratio of 1:0.6 was observed and average age of 18.2 ± 13.4 weeks. HRV was the most prevalent virus followed by RSV A/B (Table 4.5). Co-infections between 2 and 4 viruses were observed in 22.2% (179/808) of cases. The majority of co-infections were observed between RSV A/B and HRV in 47.5% (85/179) of these cases.

Table 4.5: Viruses detected from 808 living infants admitted to TBH from March 2015-March 2019

Respiratory Virus	Number of Infants admitted	Prevalence in 808 cases tested
Flu A	32	4.0%
Flu B	13	1.6%
HMPV	41	5.1%
HPIV	99	12.3%
RSV A/B	290	35.9%
HRV	415	51.4%

4.9 Review of South African Weather Service Data

During the study period the maximum temperature observed was 36.4°C, the minimum 1.9°C and the largest difference in daily temperature was 24.9°C. A trend was observed that on the days an infant died there was a very large difference in the maximum and minimum temperatures in comparison to days where no infants died.

4.10 Statistical Analyses

4.10.1 Prospective Study Socio-demographics and SUDI Risk Factors

Significantly more infants in the *SIDS* group were premature compared to those in the *Infection* or *Other* groups. Infants who slept in a room where ventilation was present were more likely to be assigned a final COD of *Infection*. In contrast, infants in the *SIDS* group died more often when no ventilation was present in the room. Additionally, infants in the *SIDS* group also had a significantly lower birthweight in comparison to infants in the *Infection* and *Other* groups ($p < 0.01$) (Figure 4.9). No significant associations were found when comparing COD classification to factors such as sleeping position, bedsharing, gender and seasonality (Table 4.6).

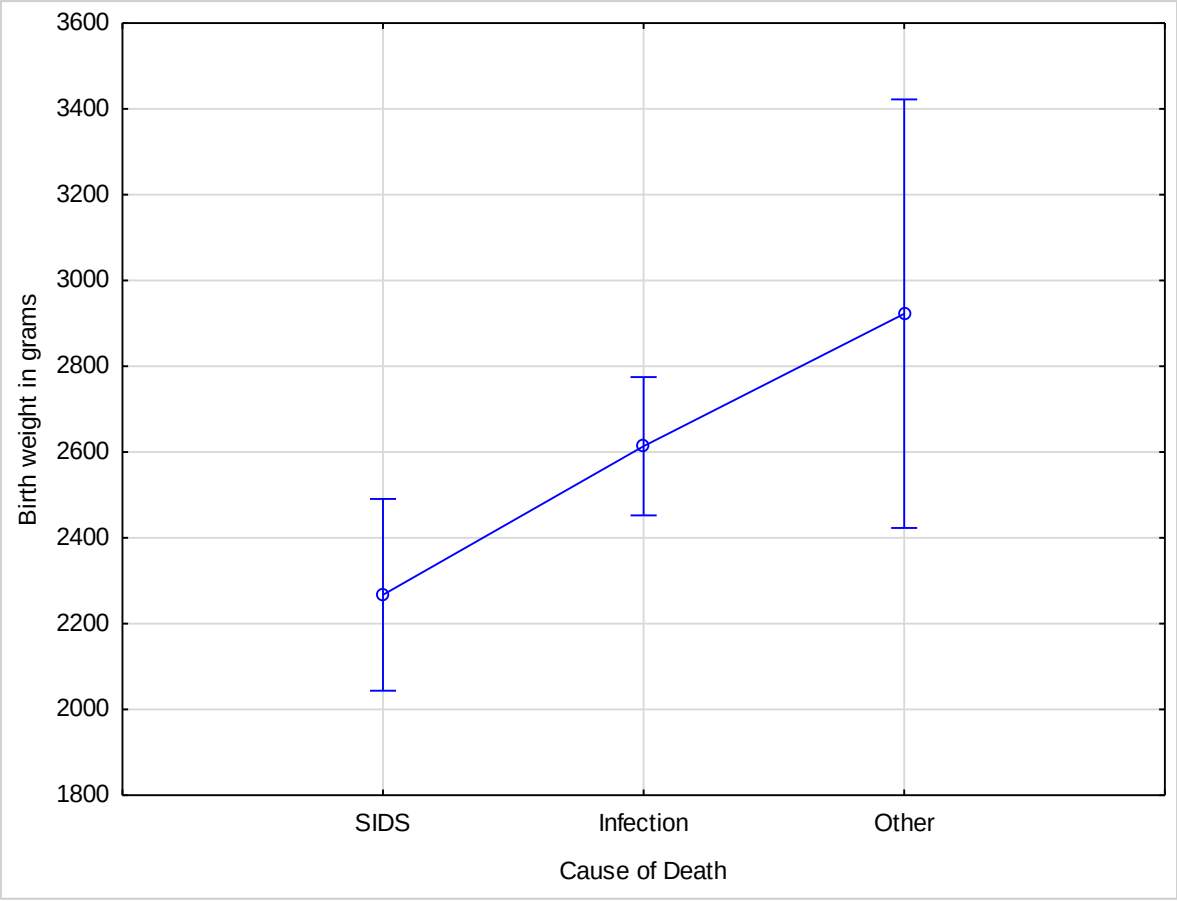

Figure 4.9: The average birthweight of infants in the *SIDS*, *Infection* and *Other* groups

There was no significant difference in the age of infants from the different COD classification groups. The average age for the *SIDS* group was 10.9 ± 7.5 weeks, *Infection* was 12.6 ± 10.4 and *Other* was 13.0 ± 13.4 weeks. Although infants in the *Other* group seemed to be older than those in the *SIDS* group, the difference did not reach statistical significance ($p = 0.78$).

Table 4.6: Socio-demographic factors associated with the COD in SUDI cases

Demographic	Cause of Death			p-value
	***SIDS* %**	***Infection* %**	***Other* %**	
Gender				p = 0.96
Male	32	60	8	
Female	32	62	7	
Season				p = 0.45
Warm	37	55	8	
Cold	28	65	7	
Prematurity				**p = 0.02**
Yes	43	54	3	
No	24	66	10	
Bedsharing				p = 0.80
Yes	32	61	7	
No	25	67	8	
Ventilation				**p = 0.02**
Yes	21	72	7	
No	41	50	9	
Sleeping Position				p = 0.20
Stomach	22	74	4	
Back	40	50	10	
Side	36	56	8	

4.10.2 Respiratory Virus Detection

A 2-way summary table was utilised to compare gender, season and COD to any viral PCR-positive result, a viral PCR-positive result in the lungs only and finally a viral PCR-positive result in the trachea (Table 4.7). A one-way ANOVA was further used to determine the association between age and all viral PCR-positive outcomes. Males had significantly more multiple site infections than females (Table 4.7). However, no further associations could be demonstrated.

The same analysis was completed for co-infections present in the lungs and trachea (Table 4.8). Co-infection in the lungs displayed a significant association with both cold season and COD of *Infection*. Subsequently, co-infections in the trachea only displayed an association with the cold season. No association between age and gender were identified.

Table 4.7: PCR-positive results and association with age, gender, seasonality and COD

	Age	Gender	Season	COD
All Viral PCR Positive	p = 0.57	**p < 0.01**	p = 0.12	p = 0.82
Lung Viral PCR Positive	p = 0.98	p = 0.51	p = 0.24	p = 0.33
Trachea Viral PCR Positive	p = 0.39	p = 0.19	p = 0.13	p = 0.99
Virus positive and culture positive	p = 0.35	p = 0.61	p = 0.11	p = 0.19

Table 4.8: Co-infection between respiratory viruses and association with age, gender, seasonality and COD

	Age	Gender	Season	COD
Multiple Lung	p = 0.58	p = 1.00	**p = 0.04**	**p = <0.01**
Multiple Trachea	p = 0.93	p = 0.49	**p = 0.09**	p = 0.15

The associations between each individual virus and season, gender and COD were analysed with a 2-way summary table. Following analysis, it became evident that Flu A (p = 0.04), Flu B (p = 0.04), HPIV (p = 0.03) and RSV (p = 0.02) were significantly associated with the cold season. HMPV and HRV displayed no association with either season. No virus was associated with a certain gender either.

In the lungs, Flu A was significantly associated with *Infection* (p = 0.08). In the trachea Flu B was significantly associated with *SIDS* (p = 0.02) (Figure 4.10). Correspondence analyses was used to visualise graphically the association between COD and individual viruses. The closer a specific virus is to a COD on the chart, the stronger the association. The closer it is to the origin; it does not display a specific association with a COD. If a virus occurs in only 1 quadrant it is only associated with that COD.

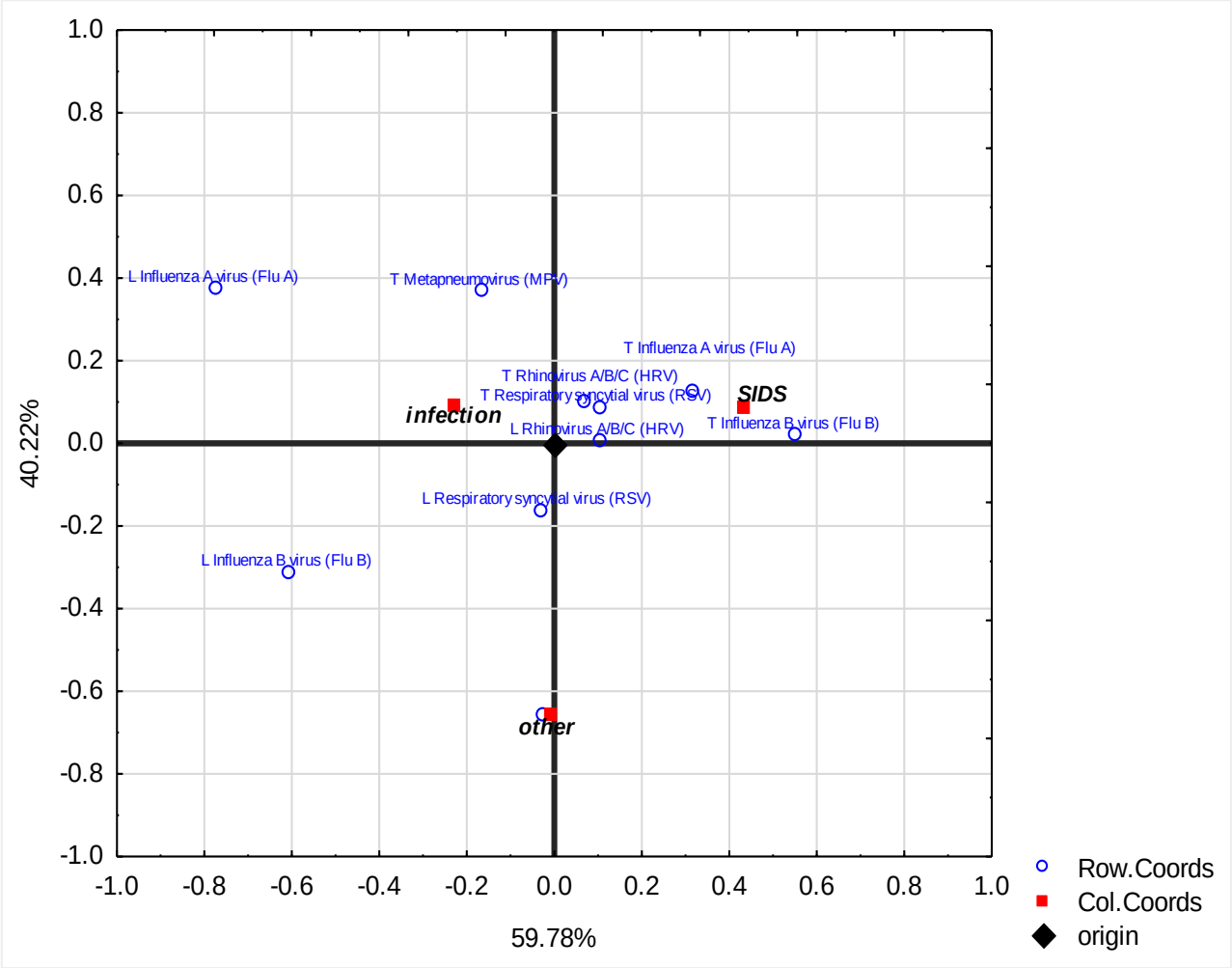

Figure 4.10: Correspondence analysis of COD and specific viruses

4.10.3 Histology

Although more SUDI cases with PCR-positive results had histology signs of infection compared to PCR-negative cases, the difference was not significant (p = 0.88).

Histological signs of infection could also not be associated with viral PCR-positive results in the lungs (p = 0.49) or co-infections in lungs (p = 0.61). The same is true for single (p = 0.87) and co-infection (p = 1.00) in the trachea.

No associations could be established between histological signs of infection and positive bacterial pathogens (p = 0.75) or between histology signs of infection, viral PCR-positive results and detection of bacterial pathogens (p = 0.55).

A significant association was found between histological signs of infection and a COD of *Infection* and *SIDS* (p = 0.03) (Figure 4.11). However, no significant associations could be demonstrated for COD, histology and viral PCR-positive results (p = 0.59).

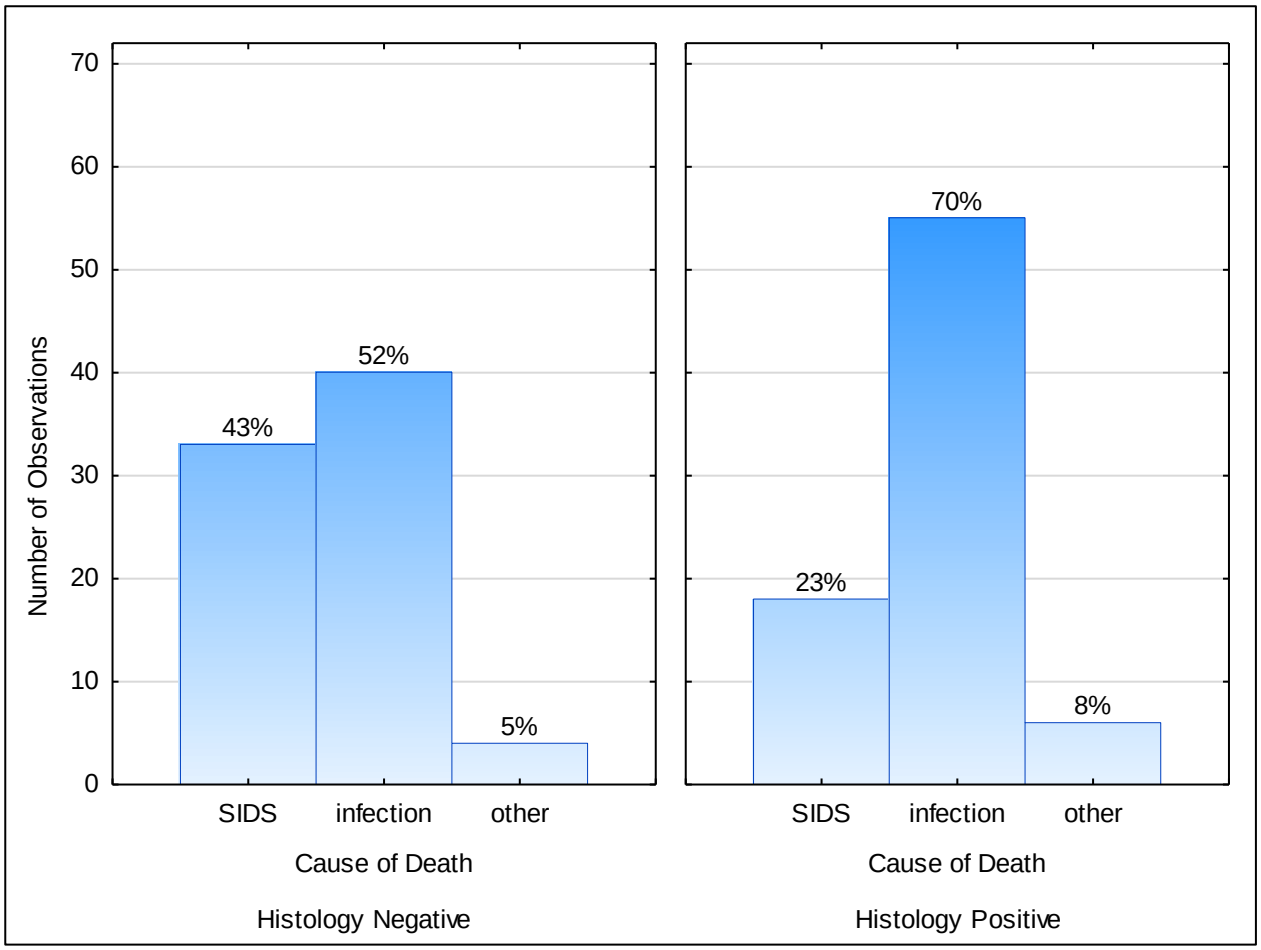

Figure 4.11: COD classification and histological signs of infection

4.11 Retrospective Study Socio-demographics and SUDI Risk Factors

In the retrospective study there was no significant association between bedsharing, gender, birthweight, season or ventilation. The infants in the *Other* group were significantly older than those in the *SIDS* and *Infection* groups ($p = 0.004$). Prematurity was not analysed during this study (Matshazi, 2017).

4.11.1 Respiratory Virus Detection

The same statistical analysis methods employed during prospective study were applied to data in retrospective study. None of the viral PCR-positive outcomes had any association with age, gender, season and COD (Table 4.9). Males had a higher prevalence of co-infections in the trachea compared to females (Table 4.10). The trachea had significantly more viruses than the lungs ($p < 0.01$). While there was no association between any viruses in the lungs and COD, HRV in the trachea was significantly associated with *Infection* ($p = 0.02$).

Table 4.9: Retrospective study: PCR-positive results and association with age, gender, seasonality and COD

	Age	Gender	Season	COD
All viral PCR-positive results	p = 0.87	p = 0.17	p = 0.892	p = 0.65
Lung viral PCR-positive results	p = 0.56	p = 0.753	p = 0.39	p = 0.47
Trachea viral PCR-positive results	p = 0.21	p = 0.123	p = 0.71	p = 0.41

Table 4.10: Retrospective study: Co-infection between respiratory viruses and association with age, gender, seasonality and COD

	Age	Gender	Season	COD
Multiple Trachea	p = 0.65	**p = 0.03**	p = 0.58	p = 047

4.12 NHLS Comparison group

During both retrospective and prospective study periods the same viruses were detected in SUDI cases and in the comparison group. However, throughout the prospective study period Flu B was significantly more prevalent in SUDI cases ($p < 0.01$), while HPIV, RSV and HRV were significantly more prevalent in the comparison group ($p < 0.01$). Flu A and HMPV displayed no significant differences.

4.13 Review of South African Weather Service Data and the Cause of Death

Although a trend was observed for temperature and the days the infants died, it was not statistically significant (maximum temperature $p = 0.82$; minimum temperature $p = 0.72$; temperature difference $p = 0.62$).

CHAPTER FIVE:
DISCUSSION

CHAPTER CONTENT

5.1 Overview

In 1994, Filiano and Kinney proposed the Triple Risk Model and it is still the model that best describes SUDI . The Triple Risk Model proposes that SUDI is not a result of a single common pathway, but rather overlapping factors of a critical developmental period (i.e. the first year of life), the presence of an underlying vulnerability that increases susceptibility (i.e. unrecognised pathology) and exposure to an exogenous stressor (i.e. being placed in a prone position for sleep or infection). When these factors coincide, the risk for SUDI is the greatest. The factors can either be intrinsic (non-modifiable) or extrinsic (modifiable and usually experienced around the time of death). While SUDI is not exclusive to infants with intrinsic or extrinsic risk factors, the importance of their role is demonstrated by the fact that one or more risk factor was present in approximately 90% of all SUDI cases, with very few cases reported where no extrinsic risk factors are present (Trachtenberg et al., 2012).

Although the Triple Risk Model has aided in obtaining a better understanding of SUDI and helped in identifying risk factors that can be altered to reduce the number of cases (for instance prone sleeping and the Back to Sleep campaign), attempts to identify a single causative agent is still unsuccessful with the COD remaining a medical mystery (Koelher, 2010). Despite many different institutional protocols in South Africa, including a review of the clinical history and the circumstances surrounding the event, as well as an autopsy, laboratory investigations and occasional investigation of the death scene, *SIDS* still make up a large proportion of SUDI cases (Du Toit-Prinsloo et al., 2011; 2013). In this prospective descriptive study, the aim was to investigate the prevalence of 6 major respiratory viruses (Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV) found in the lungs and trachea of all SUDI cases admitted to Tygerberg MLM over a 1 year period. Furthermore, co-infections were analysed between these viruses to obtain a better understanding of the role of single and multiple viral infections in SUDI. Additionally, all the epidemiological information and other relevant laboratory data from the retrospective cases (2015-2016) were included to identify any trends or differences between the 2 study periods. This was completed in order to evaluate if risk factors associated with SUDI cases at the Tygerberg MLM might have changed over the 2 study periods. Infants aged between 7 days and 1 year admitted to TBH with respiratory infections were used as a comparison group against SUDI cases in order to identify if similar single and multiple viruses were circulating in both populations.

All virology results were interpreted alongside a clinical history (the presence or absence of infection prior to death) and histology signs of respiratory infection. During this study 90.2%

of SUDI cases had viral PCR-positive results and co-infections occurred in 40 cases, the highest prevalence of co-infection since the start of this research at the Tygerberg MLM in 2009. Using the RV Essential multiplex real-time PCR assay viruses were detected that directly contributed to the infant's death. Incorporating this real-time PCR assay into the standard protocol could potentially alter the final diagnostic categories for SUDI cases at the Tygerberg MLM, or at the least, highlight the possibility of viruses contributing to fatal events. In the sections to follow, demographic risk factors, the role of co-infection and how risk factors have changed or stayed constant will be discussed and further emphasis will be placed on the respiratory viruses detected.

5.2 Intrinsic Risk factors

Of the total 173 SUDI cases included in this study, 53.8% were males. This is the second lowest male predominance since the start of this research in 2009. The lowest record of males (49.1%) was documented by Matshazi (2017), while Burger (2011) found 59.0% (2009-2011) and La Grange (2013) 60.1% (2012-2013) of males in their respective studies. This difference might be due to the increase in female births in South Africa over the last 6 years (StatsSA, 2018b).

Although this study failed to demonstrate any statistical significance between gender and COD classification, male gender remains a well-established risk factor for SUDI and is suggested to have a biological origin. Moscovis et al. (2014) found that male peripheral blood mononuclear cells produced less pro-inflammatory cytokines compared to females. They suggested that a reduced pro-inflammatory response could limit damage during pathogenic stimulation and lead to a higher susceptibility to initial invasion of pathogens. An additional factor affecting dysregulation of the inflammatory responses might also be the increase in testosterone in male infants (Kruger et al., 2018). Paterson et al. (2006) found more male SUDI cases with medullary abnormalities than females, which could reduce the ability to respond to homeostatic stressors while sleeping. Thus, the male predominance may stem from inherent vulnerabilities, which seem to be multifactorial, as is the case with SUDI. While some suggest male vulnerability is influenced by gender differences in genetic and biological makeup, it is not clear why the incidence of SUDI is higher in males and this may simply be a reflection of the fact that male infants are more vulnerable to illness and disease than females, with males having a generally greater mortality rate overall (Pongou, 2012).

The average age for SUDI cases in this study was consistent with the 2-4 month peak age for SUDI reported in the literature (Balduzzi & Greendyke, 1966) where the peak age is reported to be between 5 and 12 weeks. Previous research at Tygerberg MLM reported similar peak age ranges (La Grange, 2013; Du Toit-Prinsloo et al., 2013; Matshazi, 2017). While death can occur at any time during the first year, approximately 90.0% of cases die in the first 6 months of life, with a well described increase between 2 and 4 months of age. This is also the period when the infant's brain is undergoing dramatic neurodevelopmental changes, especially to systems controlling homeostatic control (Fleming et al., 2015). The first few months of life are considered a particularly vulnerable period for infants because their immune responses to pathogens are still developing, their maternally transferred IgG antibodies have begun to decline and all vaccinations have not yet been completed (Gans et al., 2004; Palmeira et al., 2012; Reikie et al., 2012; 2013). This suggests that immature immune responses play a contributory role in infant susceptibility to SUDI in early life.

The seasonality of SUDI is well described in the literature and confirmed to be markedly more common during the colder seasons (Ponsonby et al., 1992). During this study period the same trend was observed, and even though more deaths were observed in the cold season, no specific COD (*SIDS*, *Infection* or *Other*) was significantly associated with either season. This might be attributed to the small study population (only 1 year) and future research should include sampling over a longer time period.

Another interesting aspect to consider is the possible effect of global warming leading to seasonality changes. South Africa is not safe from the effects of climate change. In 2015, South Africa recorded its lowest annual rainfall since 1904 (Western Cape Government, 2019). In the same year, Cape Town recorded its highest temperature in the last 100 years at 42°C. In 2017/2018, the Western Cape experienced its worst drought in decades. According to the Western Cape Department of Environmental Affairs and Development Planning, climate change is effecting the Western Cape (Saving Electricity, 2019) in the following ways: higher average and maximum annual temperatures, more heat waves, higher minimum temperatures, reduced average rainfall, increased frequency and intensity of extreme weather events, including floods, droughts and storm surges. Combining all these events leads to changes in seasonality.

Infants in the *SIDS* group were significantly more prone to prematurity and low birthweight than those in the *Infection* and *Other* groups. This is in agreement with the findings of Malloy (2013). The role of prematurity and low birthweight in SUDI is well established and may be

due to an immature autonomic system, with impaired arousal mechanisms and an increased risk for hypercarbia (Duncan & Byard, 2018).

Prenatal smoke exposure plays a very important role in the occurrence of SUDI. Even though no information regarding parental smoking was collected during this study, the information provided by StatSA regarding smoking in Western Cape, age and female majority, strongly suggest that the infants who succumbed to SUDI could have been exposed to parental cigarette smoke to some extent, either prenatally or second-hand (Treyster & Gitterman, 2011). The role of nicotine in SUDI has been described before. It is hypothesised that nicotine can cross the placenta into the foetal circulation where it binds to endogenous neuronal nicotinic acetylcholine receptors present in the foetal brain. These receptors are widely expressed from as early as 4 to 5 weeks' gestation. Exogenous nicotine may bind to and inappropriately stimulate the function of these receptors, possibly leading to impaired arousal mechanisms, changes to the apnoeic index during sleep and altered parasympathetic control of heart rate (Duncan & Byard, 2018). It has also been suggested that exposure to cigarette smoke in utero reduces lung capacity, thus resulting in chronic hypoxia after birth, or alternatively increases the risk of respiratory tract infection, both increasing infant vulnerability (Duncan & Byard, 2018).

5.3 Extrinsic Risk Factors

Extrinsic risk factors represent physical stressors experienced around the time of death and often relate to the direct environment that the infant faces. These factors include sleeping position (especially prone sleeping position), bedsharing and ventilation, amongst others.

In this study, the majority of infants were reported to be put to sleep on their sides, followed by stomach and then back. The same pattern was observed from the data obtained from the retrospective study and it can be assumed that this is a typical custom of the study population. Even though there was no significant association between sleeping position and COD in either of the studies, it remains alarming how many parents/caregivers are not aware of the risk of non-supine sleeping. The role that prone sleeping position plays in infant death was first reported in 1944 and is still estimated to increase the risk of SUDI by up to 14-fold (Abramson, 1944; Duncan & Byard, 2018).

The face-down position of prone sleeping results in oxygen deprivation leading to hypoxia, rebreathing of carbon dioxide causing hypercarbia, reduced arousal responses and increased waking thresholds (especially to exogenous stimuli). It also compromises cerebral

blood flow, causes airway obstruction, altered cardiovascular capacity and increased body temperature (Chong, 2000; Galland et al., 2002; Duncan & Byard, 2018).

Side sleeping position also increases the risk for SUDI and some studies reported the risk to be similar as the prone position. This is often attributed to the ease with which infants can roll onto their stomachs, as many infants who were placed on their side to sleep were subsequently found prone at the time of death (Li et al., 2003).

It is important to note that even after the safe sleeping awareness campaigns led to decreased SUDI numbers, some healthcare workers continue to promote incorrect sleeping positions to parents. Patton et al. (2015) reported “fear of aspiration” during sleep as the primary reason given by caregivers for not choosing supine positioning despite the fact that the incidence of deaths associated with the aspiration of gastric contents has not changed since the recommendation of supine sleep position (Byard & Beal, 2000; Patton et al., 2015).

Bedsharing had been repeatedly investigated in SUDI and was also reported in the vast majority of cases in the current and retrospective studies. This might be due to parents or caregivers bringing infants to bed for feeding and comforting, but then not returning the infant back to his/her own sleeping space (McGarvey, 2006; Duncan & Byard, 2018). Bedsharing could lead to overheating and re-breathing of CO_2 from others in the same bed (Sullivan et al., 2001).

Ventilation in the home environment seemed to have led to an increase in the number of *Infection* cases in this study, while it was not the case for the *SIDS* cases. This was an unexpected finding, as it would be fair to assume that when no ventilation was present, a COD of *Infection* would be significant, as the spread of infection is associated with crowded and enclosed areas. A possible explanation of this finding could be the presence of air pollution, considering the environment where SUDI cases occurred (industrial areas, close to an airport and in informal settlements) where pollution is very high (Department of Environmental Affairs, [DOEA, 2018). Fine particulate matter air pollution of 2.5 µm or less in diameter has been associated with an increased risk of respiratory disease. A study conducted at one of the regions where most of our cases resided, displayed an increased fine particle matter air pollution of 2.5 µm (DOEA, 2018). Despite limited existing information, airflow and ventilation seem to play a role in respiratory virus infectivity and transmission. Schulman & Kilbourne (1962) demonstrated that the transmissibility of influenza transmission decreased with increasing ventilation (Schulman & Kilbourne, 1962; Pica & Bouvier, 2012). A similar phenomenon was observed with HRV. Vandini et al. (2013)

published results concurring with many other authors which also showed a positive correlation between air pollution, and morbidity of respiratory infections and other respiratory conditions, such as asthma and chronic obstructive pulmonary disease (Barnett et al., 2005; Bedeschi et al., 2007; Karr et al., 2009). This correlation is supported by increased respiratory symptoms, reduced lung function and bronchial reactivity related to air pollution exposure. Moreover, these effects are increased in the paediatric population, especially young infants, because their higher respiratory rate increases air pollutants per kilogram of body weight exposure. The sleeping environment seems to be very important. One study found that bedroom heating increased the odds ratio of SUDI by 4.5 times, while another study showed a decrease of SUDI in a well-ventilated bedroom (Moon, 2016).

5.4 Respiratory Viral Pathogen Detection in Prospective Study

Using the Seegene Allplex^{TM} RV Essential, one-step multiplex, real-time PCR assay for the analysis of lung and tracheal swab samples, between 1 and 5 viruses were detected per case. Overall, a significant association between a viral PCR-positive result and male gender was observed during this study. This corresponds to what La Grange (2013) found. Moreover, literature has well established that males are more likely to succumb to SUDI and is more susceptible to viral infection due to an immature immune system. Multiple viral PCR-positive results in the lungs had a significant association between cold season and the COD of *Infection*. This observation is expected, as all viruses studied during this period are known to have peak incidences in the cold season and can cause LRTI. More than half of the PCR-positive cases suggest a viral aetiology, confirmed by the COD of *Infection* and histological signs of infection. On the contrary, viruses were also confirmed in cases with a COD of *SIDS* and *Other*. In these cases where viral detection occurred in the absence of clinical or morphological signs of infection, may indicate asymptomatic carriage, or viral shedding and may not have any direct contribution to the COD. However, in a number of *SIDS* cases histological signs of infection were present and these cases were further discussed with the attending pathologist.

5.4.1 Human Influenza A and B

Since this research started in 2009, this study was the first to find such a high prevalence of Flu A and Flu B. This could be attributed to the fact that this was the first study to use real-time PCR for viral detection, which is known to be far more sensitive and specific in comparison to conventional PCR. Although the impact of Flu A and Flu B are well documented in living infants, their contribution in SUDI is still limited. Bajanowski et al. (2003)

found Flu B in only 5.4% of their cases, which is much lower than this study, possibly because of the specific epidemic periods of Flu viruses. Flu A and Flu B usually infect infants less than 6 month of age, which agrees with the average age for Flu A and Flu B positive cases in this study (11.7 ± 9.6 weeks). There was no difference in the detection rate between male and female infants, but both Flu A and Flu B were significantly associated with cold season, which coincides with the season in which the majority of infections occur. Furthermore, Flu A was significantly associated with COD of *Infection* and histological signs of infection which included IP grade 2-3 and pneumonia, while Flu B was significantly associated with a COD of *SIDS*. This is the first time these associations had been demonstrated in a SUDI population at the Tygerberg MLM. A possible explanation would be that Flu A and Flu B are known to have seasonal epidemics. Furthermore, Flu A causes more severe infections in infants than Flu B, explaining the association with the COD *Infection.* The association between Flu B and a COD of *SIDS* may be attributed to the detection of an asymptomatic infection, dormant viruses or latent viral shedding which did not directly contributed to the infant's demise.

5.4.2 Human Metapneumovirus

HMPV was detected in both lung and trachea samples. This study identified the highest prevalence of HMPV in SUDI populations at Tygerberg MLM since the start of the research in 2009. This virus was only discovered in 2001 and it is therefore reasonable to assume that prevalence would increase over time. The seasonality of HMPV was not significant as it was detected in almost similar frequencies in both the warm and cold season. Even though it usually peaks during the cold season, studies have identified peaks in warmer months, especially during spring (Oketch et al., 2019). This study did not find a difference between the infection rate of males and females. The highest incidence rates of HMPV are usually observed among children in the first few months of life, which agrees with the average age found for HMPV positive cases in this study (11.8 ± 9.4 weeks). Histological signs of infection were observed in cases with PCR-positive results for HMPV and included pneumonia, bronchopneumonia and IP (grade 2-3^{+}).

5.4.3 Human Parainfluenza Virus

HPIV was the least frequently detected virus in this study, which agrees with results from Ann et al. (1993). The average age for HPIV positive cases in this study (9.9 ± 1.3 weeks) does correspond to age in literature where it is reported that most HPIV infections occur between the age of 4 and 9 months. HPIV was significantly associated with the cold season,

but since only 2 cases were positive for HPIV in this study, this should be interpreted with caution. Both positive cases were males and HPIV is known to infect more males than females (Maree, 2012). A final COD of *SIDS* were assigned to both cases, as there were no histological signs of infection in either of the 2.

5.4.4 Respiratory Syncytial Virus A/B

RSV A/B was the most frequently detected virus in both the lungs and trachea and caused multiple site infections. The fact that RSV A/B was found in both the lungs and trachea simultaneously can be indicative of a severe infection, because infection usually starts in the nasopharynx and then moves down into the LRT. RSV A/B has often been implicated in the SUDI cases (La Grange, 2013; Matshazi, 2017). Typically, RSV A/B infects infants that are over 1 month old and spares those younger, which is similar to the age distribution of SUDI in the literature (Lindgren, 1993). Some studies found similar frequencies of RSV A/B in lung specimens from SUDI and control infants (Cubie et al., 1997), while others detected it in more SUDI cases compared to live control infants (Williams et al., 1984). It is likely that both SUDI and control infants undergo similar levels of exposure to RSV A/B in the first 3 months of life, as anti-RSV IgG antibodies are found in similar frequencies in both groups. There is also a similar age distribution between infants admitted to hospital with RSV bronchiolitis and the age range of SUDI (Scott et al., 1978; Williams et al., 1984).

RSV A/B was significantly associated with the colder months, which corresponds to the literature where the temperate zones tend to experience epidemics during the cold seasons (Zambon et al., 2001). The increased RSV A/B infection rates in declining temperatures can be attributed to increased indoor crowding, which leads to enhanced viral transmission. The lower temperatures furthermore lead to increasing viral stability and host susceptibility or activation of dormant viruses (Griffiths et al., 2017). In this study RSV A/B was found most often in the *Infection* group with an average age of 12.1 ± 9.8 weeks, which is the most vulnerable age (first 6 months of life) for infection to occur in infants (Thompson et al., 2003).

The independent risk factors, such as tobacco smoke exposure, air pollution and indoor crowding (e.g. attendance of a day care centre, hospital or presence of siblings), are some of the most frequent environmental risk factors, which might lead to the development of RSV disease in infants (Simoens, 2003). As previously discussed, infants in this study who succumbed to SUDI were most likely exposed to smoke, pollution and overcrowding. The link between tobacco smoke and RSV infection is well documented in literature. In 2012, DiFranza and colleagues (2012) did a systematic review of 30 studies analysing the effect

of tobacco smoke and RSV infection. This review concluded that tobacco smoke places infants and young children at an increased risk of hospitalisation for RSV-associated LRTI and further increases the severity of the infection (Phaybouth et al., 2006). There is also a strong association between environmental pollution and severe RSV infection. Previous studies have shown that diesel engine emissions cause more pronounced peribronchial and/or bronchiolar inflammation with increased mucous cell metaplasia in an RSV infection (Harrod et al., 2003).

5.4.5 Human Rhinovirus

HRV was the second most frequently detected virus during this study, with the majority of PCR-positive samples obtained from the trachea, and multiple site infections. Patrick et al. (1989) detected the virus in only 16.0% of viral-positive SUDI cases, which is much lower than the prevalence found in this study. Such discrepancies could be attributed to differences in the study populations and/or detection techniques.

HRV PCR-positive samples were more prevalent in male than female infants. The susceptibility of males to infection has been established previously and discussed under Intrinsic factors. The seasonality of HRV observed during this study period corresponds to the literature, where it is confirmed to be 5-10-fold more likely to occur during the winter months ($p < 0.01$) compared to the warmer seasons (Lee et al., 2012).

More than half of the positive HRV cases in this study were in the *Infection* group, followed by *SIDS* and only a few *Other*. The average age for HRV infection in this study (13.0 ± 11.4 weeks) still coincides with the peak incidence age reported for symptomatic HRV infection (3-6 months) (Kieninger et al., 2012).

Caution should be exercised when interpreting the detection rates of viruses in other countries compared to South Africa, or even between regions within South Africa, as population dynamics and methods of viral detection might differ. However, it provides an overview of circulating viruses, but until a standardised investigation protocol for SUDI is established, including collection methods and analysis, results and the possible implications thereof should be interpreted with caution.

5.5 Co-infections

Co-infection between viruses in living infants has been studied to some extent, however, the exact role with regard to the severity of single and multiple infection is still a matter of debate.

Some studies have found that co-infection will increase disease severity, while others did not. Co-infection in SUDI has not been investigated adequately and still needs extended research to elucidate the impact of single and multiple infection on the COD. Since the start of the SUDI research at Tygerberg MLM in 2009, the current study found the highest prevalence of co-infections, as well as different combinations of viruses.

In the lungs co-infection between HRV and RSV A/B was most often observed, displayed histological signs of infection and these deaths occurred during the cold season. The co-infection between Flu A and Flu B affected more females, occurred in both seasons and all cases were signed out as *Infection*. Although 1 of the 4 cases did not display histological evidence of infection, macroscopic signs of infection during autopsy and medical history were sufficient to confirm *Infection* as the COD. Cases with co-infection between the 5 different viruses, Flu A - Flu B - HMPV - RSV A/B - HRV, all displayed positive histology signs of infection and were signed out as *Infection*.

The case that had co-infection between Flu B, HMPV and RSV A/B was a premature male, displayed histology positive for infection, but was assigned a COD of *Other* due to a congenital abnormality of the brain. In another case HPIV, RSV A/B and HRV co-infection was present in a premature male that died in the cold season but was signed out as *SIDS* due to the absence of histological evidence of infection. Even though viruses were present they did not contribute directly to the infant's demise.

More co-infections were observed in the trachea, as the virus would first inoculate the trachea before moving down into the LRT as previously described. Flu A and Flu B co-infection in the trachea was significantly associated with a COD of *SIDS* and only males were affected. Co-infection between Flu A and Flu B are not uncommon, but while this dual infection does have the ability to cause severe infections, it is not always the case. This might explain why the COD was *SIDS* and not *Infection* in these particular cases. In this case the virus might have indirectly aided in death (Cunha et al., 2014). Co-infections between RSV A/B and HRV were present in the *Infection*, *SIDS* and *Other* groups. In both *SIDS* cases no medical history was present, raising the possibility that the viruses might have been present due to a previous infection, or caused an asymptomatic infection. If a medical history prior to death was present, these viruses might not have been the ultimate COD, but could have contributed to it. The same can be true for co-infections with Flu A, Flu B and RSV A/B, HPIV and HRV, as well as Flu A and RSV A/B combinations. The remaining combinations displayed a COD of *Infection* and medical history that was significant and it is

therefore safe to conclude that these co-infections could have actively contributed to the death of the infant.

The co-infection with the 5 different viruses caused a multiple site infection (present in both lungs and trachea) had significant histology signs of infection and a COD of *Infection*. This combination of viruses seemed to have played an important role in the COD of this infant.

5.6 The Value of PCR-positive Viral Results

5.6.1 Cases with a COD Classification – *Infection*

The majority of cases in the *Infection* group were PCR-positive and this association was significant. The real-time PCR assay used can identify a specific aetiological agent, especially in cases with a COD of *Infection*. It may also be relevant in cases where histology does point to a clear infectious COD, especially when routine culture does not reveal any aetiological agents.

5.6.2 Cases with a COD Classification – *SIDS*

In this study, Flu A, Flu B, HMPV, HPIV, RSV A/B and HRV were all detected in *SIDS* cases. Some of these cases displayed co- infections and/or positive histological signs of infection, but were still classified as *SIDS*. Therefore, these cases were re-evaluated with the pathologist after presenting the viral PCR-positive results to reconsider or confirm the COD.

Case 1417 produced PCR-positive results for RSV A/B and HRV, however the lung histology was negative. The infant had a cough, but in the absence of histology, the true significance of the viruses cannot be determined and the COD remained unchanged as *SIDS*.

However, in case 0128, HRV and HMPV were detected in the lungs, as well as IP grade 3 and a medical history of lung infection, but negative bacterial culture. The presence of the 2 viral aetiological agents was sufficient for the pathologist to change the COD from *SIDS* to *Infection*.

Similarly, in Case 0295, RSVA/B and Flu B were detected in the lungs, IP grade 3 was present on histology and the infant had a slight cough before demise, but no bacterial culture could be confirmed. Again, the histology and presence of the 2 viruses were enough to change the COD from *SIDS* to *Infection.*

In Case 2087, HRV was present in the lungs and trachea, histology displayed bronchopneumonia and the infant had a fever and cough prior to death. HRV has the ability to cause bronchopneumonia and the diagnosis was changed to *Infection.*

In Case 3914, HMPV and RSV A/B were present in both lungs, the histology displayed an IP grade 3 and the infant had a slight cough and runny nose. In this instance both the viral aetiological agents were suspected of contributing to the infant's demise and the COD was changed to *Infection*.

On the contrary, Case 1845 had RSV and HRV present in the lungs, histology showed IP grade 3 and the infant had a runny and blocked nose prior to death. This case would have been classified as *Infection* during the initial investigation, however a skull fracture was present and the case was classified as *Other*.

5.7 Microbial Pathogens

Bacterial pathogens have also frequently been detected and considered to contribute to SUDI (Pryce et al., 2011). Bacterial pathogens were detected in the vast majority of SUDI cases in this study and a third of these cases also produced PCR-positive viral results. Co-infection with a single virus and multiple viruses were confirmed in about half of the cases. In 3 cases a combination of multiple bacteria and viruses were found, with *Staph aureus* being most often associated with co-infection. The toxins produced by bacteria, such as *Staph aureus* and *E. coli*, are absorbed in mucosal membranes, creating "channels" in the membranes that disrupt the transport of smooth ions. This in turn affects the function of the cardiovascular and respiratory systems. Due to the immature immune system, an infant with a mild viral infection can be at risk for colonisation and toxin production by bacteria which can lead to sudden death (Kruger et al., 2018). These observations add to the body of evidence for the common bacterial toxin hypothesis as a possible mechanism in some SUDI cases. The most frequently detected bacterial cultures were *E. coli, K. pneumoniae* and *Staph aureus*, which have been significantly associated with SUDI cases with a COD of *Infection*.

5.8 The Retrospective Study

When comparing results obtained from the prospective study with the retrospective study, clear differences can be identified. One major difference is that during the retrospective study more females succumbed to SUDI than males; this does not follow the known male

predominance in SUDI cases. A possible explanation for this could be the year in which sampling took place, as it can vary from year to year. Also, the difference was not significant, as it was 90 males versus 93 females. There was a decrease in bedsharing and number of infants put to sleep on their stomachs and an increase in the number of infants put to sleep on their backs. This might be due to more parents becoming aware of a safe sleeping environment. Infants with low birthweight were more prominent during the prospective study. COD classification changed dramatically over the 2 different study periods. In the retrospective study more infants were signed out as *SIDS* and *Other*, whereas during this study period there was a significant increase in *Infection* and a decrease in *SIDS* cases. The most plausible explanation for this difference is the fact that the histology of *SIDS* cases was re-evaluated during the prospective study, providing the opportunity of considering additional laboratory results, such as the viral PCR results and possibly change the COD if sufficient evidence can be found. This also means that the continued research is adding value in terms of increasing the number of *Infection* cases where there was sufficient evidence of infection, thereby decreasing the number of *SIDS* cases.

In terms of the detection of viruses, HMPV was absent from retrospective study and one of the most frequently detected viruses during the prospective study. The same was observed for Flu and Flu B. More co-infections were also detected in the prospective study, as well as microbial pathogens. These observations can be attributed to the following; different sampling periods (the prevalence of viruses have been shown to fluctuate between years), seasonality changes and different viral detection methods.

5.9 The Comparison Group

The same viruses were found to be circulating in the population of hospitalised infants and in the same ratio as what was observed in the SUDI cases. This result was expected and can be attributed to the fact that both groups consisted of infants from the same geographical area over the same population time period and season.

More research is needed to elucidate the reason(s) why the same viruses are found in living infants and SUDI cases and why only certain infants succumb to the effect of these infections. This emphasises the fact that the mere presence of viruses in the SUDI cases cannot unequivocally be confirmed as the sole COD, but should be interpreted against the background of an overlap of a vulnerable time period, intrinsic and extrinsic factors and an exogenous trigger.

5.10 Temperatures and the Occurrence of SUDI

There was no significant association between temperature and the COD of the infants, even though a trend was observed and the literature also reported that temperature could play a role in the death of the infant (Jhun et al., 2017). In future, larger studies should be conducted over extended periods to obtain the true significance of temperature.

5.11 General Observations

Several cases were signed out as *SIDS,* despite positive histology. This observation questions histology as the gold standard in the diagnosis and confirming the COD in SUDI cases. Histology has significant observation bias, based on the experience, knowledge and skills of the pathologist analysing the results. Also, at Tygerberg MLM only sections of the lower lobes of the lungs are taken, this leads to incompleteness of sample. In order to overcome these potential limitations, sections of both upper and lower lobes should be taken. Furthermore, histology should be subjected to peer-review where findings are ratified by a senior consultant or more experienced colleague. However, due to the workload of the pathologists at the Tygerberg MLM and possibly other institutions as well, this would not be a viable option and other methods should be developed to ensure a standardised evaluation system for histological analysis.

The true value and contribution of histology should not be underestimated and this study has shown the importance of histology, supplemented by other laboratory investigations, such as bacterial culture, virological assays, etc., when formulating the COD.

Finally, SUDI cases requires an interdisciplinary approach to obtain a final COD. The current collaboration between Medical Virology and Forensic Pathology at the Faculty of Medicine and Health Sciences of Stellenbosch University should ideally be extended to include other disciplines, such as Medical Microbiology, Chemical Pathology, Medical Biochemistry and Human Genetics. The ideal would be to have a designated team, encompassing all these disciplines, to investigate SUDI cases and collectively determine the COD.

South Africa is in desperate need of a standard protocol to investigate SUDI cases systematically. The South African infant mortality panel is involved in analysing unnatural deaths of infants (abuse, murder, assault, etc), however, SUDI cases are not discussed by the panel. It would be beneficial to include SUDI in these discussion as it will aid in

determining the true incidence of these cases and perhaps aid in establishing and implementing standardised investigation protocols.

In this study, the association found with ventilation was unexpected. Environmental case-control studies for longer periods (more than 1 year) could be performed in different geographical areas to evaluate the possible effect of air pollution in households in order to elucidate the importance of ventilation in SUDI in a Western Cape and South African context.

The link between low socio-economic status and SUDI has been noted repeatedly in the literature and is not different in South Africa (Pickett et al., 2005; Athanasakis et al., 2011). Socio-economic status represents a constellation of factors reflecting social position and social circumstances including income, occupation and education. The majority of SUDI cases were from families that come from areas with a low socio-economic status, which is characterised by young and uneducated mothers, poor living conditions and general poverty amongst others.

Several of the risk factors defined by the Triple Risk Model can and should be modified in these communities to decrease the occurrence of SUDI, similar to the “Back to Sleep” campaigns, which reduced the occurrences of SUDI cases dramatically in the rest of the world. Bedsharing, education, maternal smoking and young mothers are some of the factors that can be reduced.

It is extremely important to educate these communities on the harms of bedsharing. The bassinet-like wahakura is an indigenous initiative for the prevention of SUDI developed by a Mãori, which lead to a 29.0% decrease in infant mortality in NZ from 2009 to 2015 due to the reduction in bedsharing (Tipene-Leach & Abel, 2019). The same concept can be implemented in South Africa, if the government and community outreach programs are willing to work with the different ethnic groups to find a safe alternative sleeping environment for the infant, but still observe and respect their beliefs and culture.

Low or the lack of education is a significant problem in South Africa, even with campaigns such as the Zero Dropout initiative. The National Department of Education should introduce more campaigns to decrease the number of children leaving school before finishing Grade 12. If learners, especially girls, leave school early they are not educated about the harms of maternal smoking and the importance of family planning. Currently in South Africa, the National Education curriculum is constructed in such a manner that pregnancy and related factors are only taught in grade 9 and not in much depth. It is thus taught after some learners

may have left school. Due to an increase in teen pregnancy the National Education Department has decided to change the curriculum to start teaching children from grade 4 about pregnancy and family planning with the hope that this will decrease teen and unwanted pregnancy (Department of Education 2019; Familyplanning2020.org, 2019).

Even though prematurity is regarded as an intrinsic factor, it is extremely important that mothers of premature infants are aware of all the risks associated with prematurity. Extra postnatal care and support should be available to these mothers to ensure that they are aware of the risk surrounding their vulnerable premature infant and how to care for him/her. Also, new mothers should be educated on identifying any probable symptoms indicative of an illness or disease.

Finally, all the risks identified during this study should be communicated to social services, so that vulnerable communities can be identified and necessary awareness, education and support be provided.

The development of vaccines for RSV A/B and HRV is important as it might provide infants who are already predisposed to succumbing to SUDI with some form of extra protection, especially during the critical developmental stage. It is also vital that expecting mothers receive the annual flu vaccine as the antibodies are transferred trans-placentally, further boosting the infant's immunity (CDC, 2019c). There is currently no vaccine for RSV A/B, however, palivizumab may prevent RSV infections and protect high-risk infants from serious complications of RSV infection. This can be administered since birth and every month after and is specifically beneficial for premature infants. It is important that clinics, hospitals and doctors inform mothers of this option.

5.12 Strengths

This was the first study at Stellenbosch University to use such a sensitive assay for the detection of respiratory viruses and assessment of single and co-infections in SUDI cases. This study further compared 2 different study periods of SUDI cases since the start of the research in 2009. Another novel aspect of this study was the investigation of the probable role of temperature in the occurrence of SUDI in South Africa. Re-evaluating the histology after PCR results were available, enabled pathologists to change or amend the COD from *SIDS* to *Infection* where sufficient evidence became available.

5.13 Limitations

The main limitation of this study is the absence of a suitable control group. However, due to the lack of ethical approval for specimen collection from infants who died of unnatural causes (motor vehicle accidents, abuse, neglect, etc.), this limitation cannot be addressed. The value of a control group would be to asses if the same viruses and histological signs of infections are detected by the real-time PCR assay as in the SUDI cases. In this study, the closest comparison group was living hospitalised infants during the same period. The information collected from the living infants is valuable, as it describes viral prevalence rates in the vulnerable population.

The short study duration can be regarded as an additional limitation. If more data is collected over a longer period, more significant epidemiological risk factors might emerge. A larger sample size over more consecutive seasons could provide a better profile of respiratory viruses, identifying possible seasonal variation in our setting and aid in determining patterns between viruses circulating in hospitalised infants and those in SUDI cases. This limitation was partly addressed in this study by comparing 2 different study periods, identifying several differences.

Only the left and right lower lung lobes were assessed for histology signs of infection. If more representative histology sampling was conducted including the upper and lower left lung lobes and the upper, middle and lower right lung lobes and trachea, it could have ensured that underlying disease processes were not missed.

Recall bias of the parents is a major limitation. All events leading up to death, as well as events on the day/night of the death, are based on what parents can remember and this is seriously jeopardised by the trauma caused by the death of their infant. An ideal solution would be to implement a team (social services and pathologists), which visits the site of infant death and walk through all events that occurred during the time surrounding the death of the infant with the parents or caregivers. Not only will this reduce recall bias, it might identify the actual cause of death (such as accidental suffocation) and any other hazardous environments for siblings.

The information captured by the FPO when a case is admitted to the Tygerberg MLM (Appendix 3) includes a wide range of information on the infant, mother, household, circumstances of death, etc. However, due to the waiver of consent granted by HREC, we were only allowed to use information that had a direct bearing on the infant's health and

diagnosis. Several well-known risk factors (parental smoking, education, exact location, etc.) that could have directly affected the SUDI cases in this study, could therefore not be used in the analysis.

CHAPTER SIX:
CONCLUSION

In this study we aimed to determine the prevalence of 6 major respiratory viruses in single and co-infection in both the lungs and trachea from SUDI cases admitted to the Tygerberg MLM. Furthermore, data from 2 SUDI study periods were compared in order to identify similarities and differences during the 2 study periods. Finally, data from prospective and retrospective studies were combined and compared to the comparison group of hospitalised infants at TBH.

We found that the majority (90.7%) of our SUDI cases produced a viral PCR-positive result. Some of the viruses, e.g. RSV A/B and HRV, were more often associated with multiple site infections. Different combinations of co-infections of between 1 and 5 viruses were observed. The prevalence of co-infection is the highest since the start of this research in 2009. *Staph aureus* and *E. coli* were the bacteria most often detected during this study and could have contributed to colonisation of the viruses. In some of our SIDS cases, the viral PCR-positive results cannot be ignored, especially when histology provided evidence of infection.

In some of the cases that were re-evaluated with pathologists, the COD classifications were changed from *SIDS* to *Infection* after PCR results were added to information to determine the COD. This finding is of great significance, as it displays the importance of detecting respiratory viruses and how it can aid in assigning the correct COD in all SUDI cases. Therefore, this data must be made available to pathologists. This study has shown that when viral agents are present with evidence of histology, the COD classification can be altered by pathologists. This is the first study where this occurred.

This study displayed an extremely high prevalence of co-infections. However, future research is required to determine the exact role of co-infections, more specifically how viral interactions play a role in disease progression and severity and in what way viral combinations affect the vulnerable infant. Additionally, to evaluate the effect that asymptomatic or viruses with lower virulence have on vulnerable infants, with an underdeveloped immune system and whether these viruses could contribute to death in some manner. Subsequently, the role of positive viral results in the absence of histology should be investigated, by means of serological testing for certain immune markers, in order for infection to be confirmed. SUDI research should move towards identifying why certain

infants are more vulnerable than others (genetic abnormalities, brainstem development etc.).

Finally, when 2 different SUDI study periods were compared, it became evident that differences do arise during different study periods, such as the detection of viruses, COD classification, intrinsic and extrinsic factors. This stresses the need for longer continuous investigation periods and including different study populations, but the current non-standardised investigation protocols used at different institutions make it impossible to compare results and project it to wider communities. This will only be possible with a drastic increase in the Department of Health budget for SUDI investigations.

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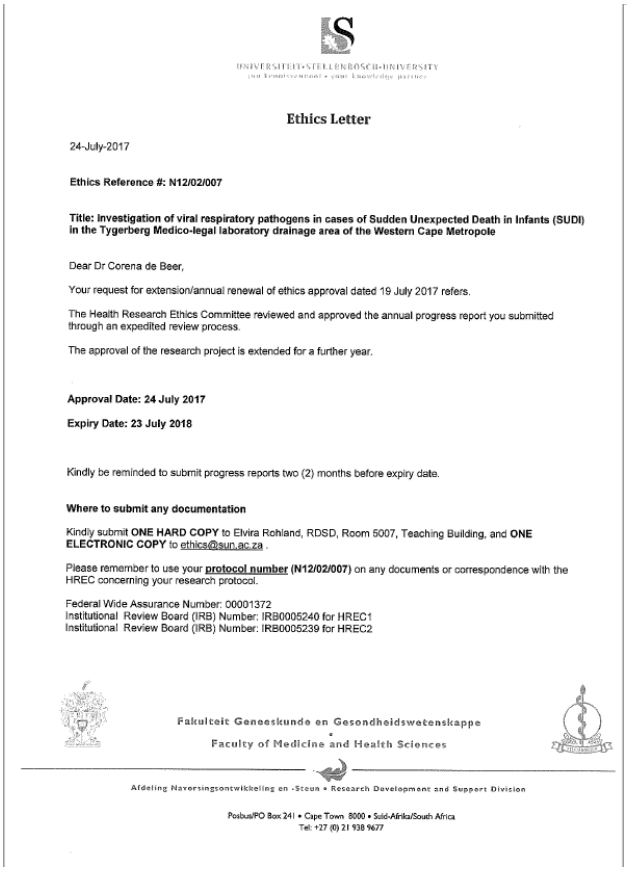
APPENDICES

Appendix 1: Approval and renewal forms from the Health Research Ethics Committee of Stellenbosch University (2017-2019)



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Ethics Letter

24-July-2017

Ethics Reference #: N12/02/007

Title: Investigation of viral respiratory pathogens in cases of Sudden Unexpected Death in Infants (SUDI) in the Tygerberg Medico-legal laboratory drainage area of the Western Cape Metropole

Dear Dr Corena de Beer,

Your request for extension/annual renewal of ethics approval dated 19 July 2017 refers.

The Health Research Ethics Committee reviewed and approved the annual progress report you submitted through an expedited review process.

The approval of the research project is extended for a further year.

Approval Date: 24 July 2017

Expiry Date: 23 July 2018

Kindly be reminded to submit progress reports two (2) months before expiry date.

Where to submit any documentation

Kindly submit **ONE HARD COPY** to Elvira Rohland, RDSD, Room 5007, Teaching Building, and **ONE ELECTRONIC COPY** to ethics@sun.ac.za .

Please remember to use your **protocol number (N12/02/007)** on any documents or correspondence with the HREC concerning your research protocol.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005240 for HREC1
Institutional Review Board (IRB) Number: IRB0005239 for HREC2

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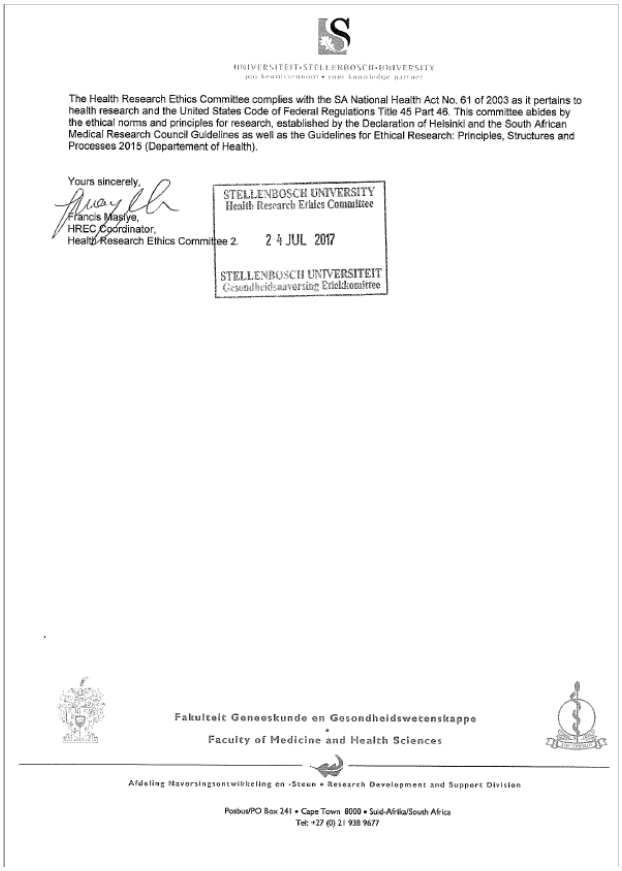


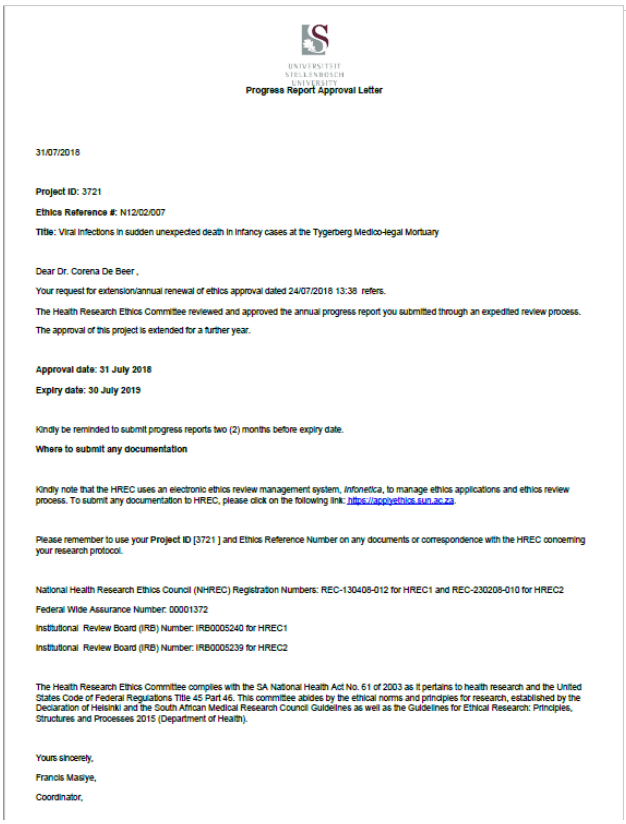
UNIVERSITEIT•STELLENBOSCH•UNIVERSITY
jou kennisvennoot • your knowledge partner

The Health Research Ethics Committee complies with the SA National Health Act No. 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki and the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles, Structures and Processes 2015 (Departement of Health).

Yours sincerely,
Francis Masiye,
HREC Coordinator,
Health Research Ethics Committee 2.

STELLENBOSCH UNIVERSITY
Health Research Ethics Committee
24 JUL 2017
STELLENBOSCH UNIVERSITEIT
Gesondheidsnavorsing Etiekkomitee

Fakulteit Geneeskunde en Gesondheidswetenskappe
Faculty of Medicine and Health Sciences
Afdeling Navorsingsontwikkeling en -Steun • Research Development and Support Division
Posbus/PO Box 241 • Cape Town 8000 • Suid-Afrika/South Africa
Tel: +27 (0) 21 938 9677



UNIVERSITEIT STELLENBOSCH UNIVERSITY
Progress Report Approval Letter

31/07/2018

Project ID: 3721

Ethics Reference #: N12/02/007

Title: Viral infections in sudden unexpected death in infancy cases at the Tygerberg Medico-legal Mortuary

Dear Dr. Corena De Beer ,

Your request for extension/annual renewal of ethics approval dated 24/07/2018 13:38 refers.

The Health Research Ethics Committee reviewed and approved the annual progress report you submitted through an expedited review process.

The approval of this project is extended for a further year.

Approval date: 31 July 2018

Expiry date: 30 July 2019

Kindly be reminded to submit progress reports two (2) months before expiry date.

Where to submit any documentation

Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.

Please remember to use your **Project ID** [3721] and Ethics Reference Number on any documents or correspondence with the HREC concerning your research protocol.

National Health Research Ethics Council (NHREC) Registration Numbers: REC-130408-012 for HREC1 and REC-230208-010 for HREC2

Federal Wide Assurance Number: 00001372

Institutional Review Board (IRB) Number: IRB0005240 for HREC1

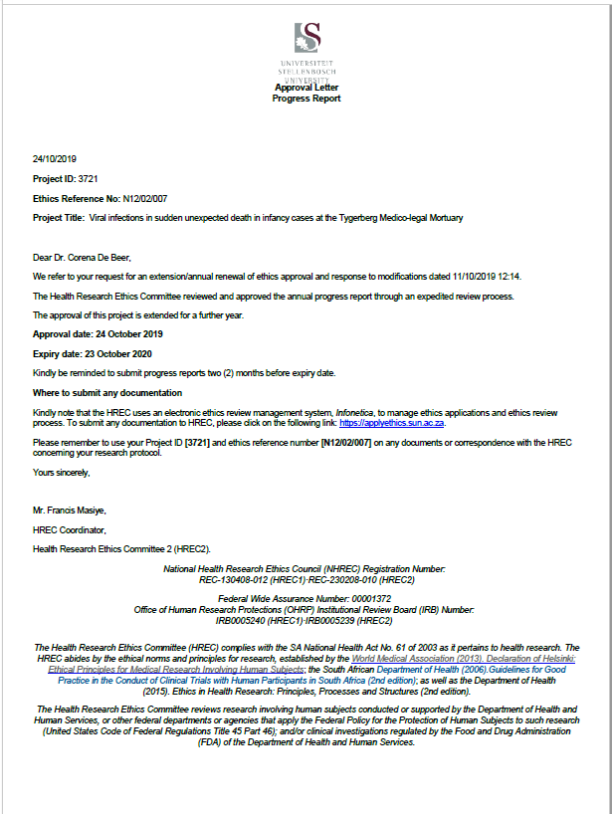
Institutional Review Board (IRB) Number: IRB0005239 for HREC2

The Health Research Ethics Committee complies with the SA National Health Act No. 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki and the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles, Structures and Processes 2015 (Department of Health).

Yours sincerely,

Francis Masiye,

Coordinator,



UNIVERSITEIT STELLENBOSCH UNIVERSITY
Approval Letter
Progress Report

24/10/2019

Project ID: 3721

Ethics Reference No: N12/02/007

Project Title: Viral infections in sudden unexpected death in infancy cases at the Tygerberg Medico-legal Mortuary

Dear Dr. Corena De Beer,

We refer to your request for an extension/annual renewal of ethics approval and response to modifications dated 11/10/2019 12:14.

The Health Research Ethics Committee reviewed and approved the annual progress report through an expedited review process.

The approval of this project is extended for a further year.

Approval date: 24 October 2019

Expiry date: 23 October 2020

Kindly be reminded to submit progress reports two (2) months before expiry date.

Where to submit any documentation

Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.

Please remember to use your Project ID **[3721]** and ethics reference number **[N12/02/007]** on any documents or correspondence with the HREC concerning your research protocol.

Yours sincerely,

Mr. Francis Masiye,

HREC Coordinator,

Health Research Ethics Committee 2 (HREC2).

National Health Research Ethics Council (NHREC) Registration Number:
REC-130408-012 (HREC1)/ REC-230208-010 (HREC2)

Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)/IRB0005239 (HREC2)

The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by the World Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects; the South African Department of Health (2006).Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition); as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes and Structures (2nd edition).

The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46); and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.

Appendix 2: NHLS Data Mining Request Form to retrieve virology results of infants for the comparison group

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TYGERBERG ACADEMIC LABORATORY

TAL.FORM.0101.1
Replaces 101.0

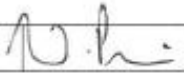
REQUEST FOR DATA MINING

Please use this request form for extractions to be done on the Tygerberg Hospital Disa*Lab Database

REQUEST DETAILS			
Full Name of Requestor	Dr Corena de Beer		
Contact Address	Division of Medical Virology, Tygerberg Campus	E-mail	cdeb@sun.ac.za
Date	30 January 2018	Tel No	021 938 9453
Name of Department and/or Organisation	Division of Medical Virology, Department of Pathology, Stellenbosch University	Signature	

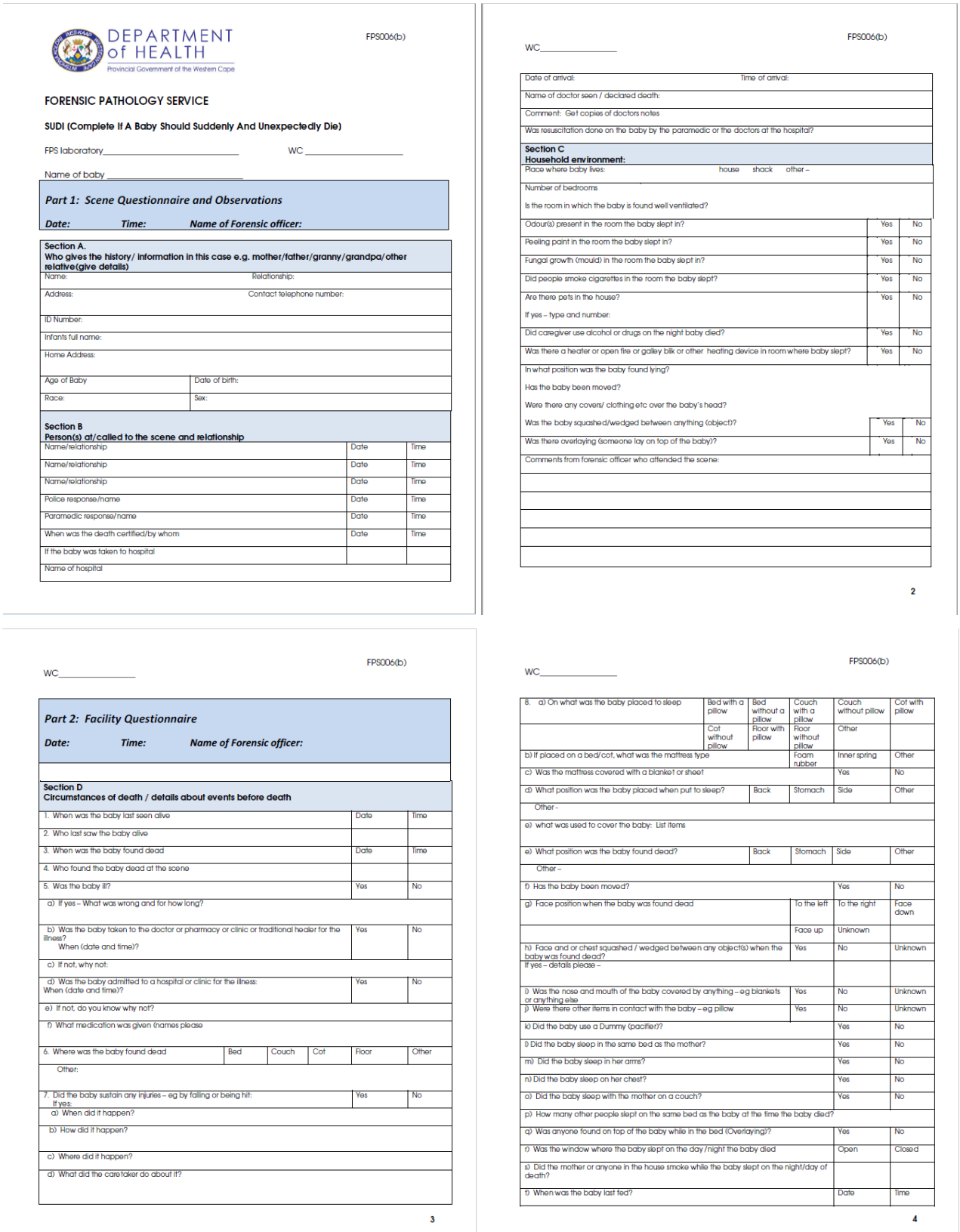
Brief description of search to be done (aim)
RV PCR results from 1 January 2015 for 3 years for infants younger than 1 year
The data is required to assess specific criteria in living infants and compare it to what is known as risk factors in SUDI, e.g. gender, season, age, etc
Full Description of search
A retrospective audit will be done over the last 3 years to establish the prevalence of respiratory viruses in infants (< 1 year old) and compare it to research RV PCR results from SUDI cases over a similar, but not continuous period. This information will be used to identify specific viruses to be investigated in prospective studies where the type and prevalence of respiratory viruses that are found in living infants are compared to SUDI cases as well.
The total number of positive results per virus will be calculated for the following: • Total positive results per virus included in the RV PCR • Gender distribution • Seasonal distribution • Age distribution • Severity in terms of in- or outpatient requests in this population

ADDITIONAL INFORMATION	
Laboratory Data Required	RV PCR results
Date range of search	01/01/2015 to date
Fields required i.e. Patient name, Patient ID etc	RV PCR results, Gender, Date of specimen, Age (or date of birth in order to calculate the age), and Ward requesting the tests

AUTHORISATION (HOD)			
Authorised by	Prof Wolfgang Preiser		
Date	30/01/2018	Signature	

DATA MINING COMPLETED			
Completed by			
Date		Signature	

Appendix 3: Provincial Government of the Western Cape, Forensic Pathology Service, SUDI Questionnaire, FPS006(b)



DEPARTMENT of HEALTH
Provincial Government of the Western Cape

FPS006(b)

FORENSIC PATHOLOGY SERVICE

SUDI (Complete If A Baby Should Suddenly And Unexpectedly Die)

FPS laboratory_____ WC _____

Name of baby _____

Part 1: Scene Questionnaire and Observations

Date: Time: Name of Forensic officer:

Section A.
Who gives the history/ information in this case e.g. mother/father/granny/grandpa/other relative(give details)

Name:	Relationship:
Address:	Contact telephone number:
ID Number:	
Infants full name:	
Home Address:	
Age of Baby	Date of birth:
Race:	Sex:

Section B
Person(s) at/called to the scene and relationship

Name/relationship	Date	Time
Name/relationship	Date	Time
Name/relationship	Date	Time
Police response/name	Date	Time
Paramedic response/name	Date	Time
When was the death certified/by whom	Date	Time
If the baby was taken to hospital		
Name of hospital		

FPS006(b)

WC_____

Date of arrival:	Time of arrival:	
Name of doctor seen / declared death:		
Comment: Get copies of doctors notes		
Was resuscitation done on the baby by the paramedic or the doctors at the hospital?		

Section C
Household environment:

Place where baby lives: house shack other –		
Number of bedrooms		
Is the room in which the baby is found well ventilated?		
Odour(s) present in the room the baby slept in?	Yes	No
Peeling paint in the room the baby slept in?	Yes	No
Fungal growth (mould) in the room the baby slept in?	Yes	No
Did people smoke cigarettes in the room the baby slept?	Yes	No
Are there pets in the house? If yes – type and number:	Yes	No
Did caregiver use alcohol or drugs on the night baby died?	Yes	No
Was there a heater or open fire or galley blik or other heating device in room where baby slept?	Yes	No
In what position was the baby found lying?		
Has the baby been moved?		
Were there any covers/ clothing etc over the baby's head?		
Was the baby squashed/wedged between anything (object)?	Yes	No
Was there overlaying (someone lay on top of the baby)?	Yes	No
Comments from forensic officer who attended the scene:		

2

FPS006(b)

WC_____

Part 2: Facility Questionnaire

Date: Time: Name of Forensic officer:

Section D
Circumstances of death / details about events before death

1. When was the baby last seen alive				Date	Time
2. Who last saw the baby alive					
3. When was the baby found dead				Date	Time
4. Who found the baby dead at the scene					
5. Was the baby ill?				Yes	No
a) If yes – What was wrong and for how long?					
b) Was the baby taken to the doctor or pharmacy or clinic or traditional healer for the illness? When (date and time)?				Yes	No
c) If not, why not:					
d) Was the baby admitted to a hospital or clinic for the illness: When (date and time)?				Yes	No
e) If not, do you know why not?					
f) What medication was given (names please					
6. Where was the baby found dead	Bed	Couch	Cot	Floor	Other
Other:					
7. Did the baby sustain any injuries – eg by falling or being hit: If yes:				Yes	No
a) When did it happen?					
b) How did it happen?					
c) Where did it happen?					
d) What did the caretaker do about it?					

3

FPS006(b)

WC_____

8. a) On what was the baby placed to sleep	Bed with a pillow	Bed without a pillow	Couch with a pillow	Couch without pillow	Cot with pillow
	Cot without pillow	Floor with pillow	Floor without pillow	Other	
b) If placed on a bed/cot, what was the mattress type			Foam rubber	Inner spring	Other
c) Was the mattress covered with a blanket or sheet				Yes	No
d) What position was the baby placed when put to sleep?	Back		Stomach	Side	Other
Other –					
e) what was used to cover the baby: List items					
e) What position was the baby found dead?	Back		Stomach	Side	Other
Other –					
f) Has the baby been moved?				Yes	No
g) Face position when the baby was found dead			To the left	To the right	Face down
			Face up	Unknown	
h) Face and or chest squashed / wedged between any object(s) when the baby was found dead?			Yes	No	Unknown
If yes – details please –					
i) Was the nose and mouth of the baby covered by anything – eg blankets or anything else			Yes	No	Unknown
j) Were there other items in contact with the baby – eg pillow			Yes	No	Unknown
k) Did the baby use a Dummy (pacifier)?				Yes	No
l) Did the baby sleep in the same bed as the mother?				Yes	No
m) Did the baby sleep in her arms?				Yes	No
n) Did the baby sleep on her chest?				Yes	No
o) Did the baby sleep with the mother on a couch?				Yes	No
p) How many other people slept on the same bed as the baby at the time the baby died?					
q) Was anyone found on top of the baby while in the bed (Overlaying)?				Yes	No
r) Was the window where the baby slept on the day/night the baby died				Open	Closed
s) Did the mother or anyone in the house smoke while the baby slept on the night/day of death?					
t) When was the baby last fed?				Date	Time

4

u) Did the mother/caregiver use alcohol before going to bed with the baby on the night/day the baby was found dead? If yes, how much?			Yes	No
v) Did the mother/caregiver use drugs before going to bed with the baby on the night/day the baby was found dead? If yes, what drugs?			Yes	No
w) Did the mother/caregiver give the baby medication on the night/day of death? If yes, name of medication:			Yes	No
Section E About the baby				
1. Where was the baby born?	Hospital	Clinic	Home	Other
Name of hospital/clinic/other				
2. How was the baby born?			Normal vaginal delivery	Caesarian section
3. How much did the baby weigh at birth?				
4. Was the baby		Premature	Full term	Post dates (Overdue)
5. If the baby was premature, how premature was it?				
6. Did the baby receive Kangaroo care (KMC)			Yes	No
7. Did the mother carry the baby on her back?			Yes	No
8. Was the baby		Breast fed	Bottle/formula fed	Both breast and bottle fed
If formula, name of the milk –				
9. Was boiling water used to make the bottle?			Yes	No
10. What other food was use to feed the baby?				
11. Does the mother have the clinic card?			Yes	No
If yes – keep the card for the pathologist. If no – ask the mother to bring it to the facility				
12. Was the baby sick before it died?			Yes	No
If yes	<24h	>24h	> 2 weeks	Never
a) Did the baby have a cold/ runny nose?				
b) was the baby coughing?				
c) did the baby have diarrhea (runny tummy)?				

d) Was the baby unusually restless / irritable?				
e) Was the baby crying more than usual?				
f) Was there a difference /change in the appetite / feeding?				
g) Was the baby vomiting?				
h) Any fits / seizures?				
i) did the baby have a fever / showed increased sweating?				
j) Was the baby listless? (floppy)				
k) did the baby turn blue?				
13. Was the now deceased baby taken to	Hospital	clinic	doctor	Pharmacy
	Traditional healer	Other		
14. Did the baby come in contact with someone who is sick in the past two weeks?			Yes	No
If yes – who?				
15. Did the baby ever suddenly stopped breathing?		Yes	No	Unknown
16. When was the baby's last vaccination?				
18. Is the baby known to be allergic to anything?		Yes	No	Unknown
If yes, what?				
19. Did the family visit another country prior to the death of the baby?			Yes	No
If yes, give details				
20. Was the baby admitted to hospital in the past week before the death?			Yes	No
a) If yes, for how long and where:				
b) Why?				
c) Discharge date?				
d) Condition of baby after discharge:				
e) Medication after discharge from the hospital (names please)				
21. Was the baby taken to a traditional healer?			Yes	No
a) If yes, date when the baby was taken to the healer:				

b) What was given?				
c) Ask for the medication to be given to the pathologist.				
d) Condition of the baby after going to the healer?				
21. What did the baby wear when it died? (list clothing)				
Section F About the mother				
1. Is the mother			Married	Single
2. Is the mother employed?			Yes	No
3. Age of the mother?				
4. What standard of schooling did she achieve?				
5. Was she on contraception before she fell pregnant?			Yes	No
6. Did she take iron and vitamin tablets during her pregnancy?			Yes	No
7. Did she receive antenatal care?			Yes	No
8. Did the mother have diabetes in pregnancy?			Yes	No
9. Did the mother have high blood pressure in pregnancy?			Yes	No
10. Did the mother gain weight adequately in pregnancy?			Yes	No
11. Was she diagnosed with any illness during the pregnancy eg. HIV?			Yes	No
12. Was the mother on any medication during the pregnancy?			Yes	No
If yes, what medication:				
13. Were there any difficulties during the delivery?			Yes	No
If yes, what?				
14. Were there any problems with the baby after the delivery?			Yes	No
If yes, what?				

15. Was any specific instruction given about specific health care for the baby?			Yes	No
If yes, what?				
16. Was she depressed after the pregnancy?			Yes	No
17. Did she get any treatment?			Yes	No
18. How many babyren does she have?				
19. How old are they?				
20. Are they healthy?			Yes	No
21. Do any of the babyren have learning disability?			Yes	No
22. Do the living babyren have the same father as the deceased baby?			Yes	No
23. Does she look after the baby?			Yes	No
24. If not, who looks after the baby?				
25. Why is the mother unable to look after the baby?				
26. Did the mother smoke during the pregnancy?			Yes	No
If yes, how many per day?				
27. Did the mother drink during the pregnancy?			Yes	No
a) What did she drink?	Beer	Wine	Spirits	Other
b) how much did she drink?	Every day		Now and again	Weekends
1 glass	Every day		Now and again	Weekends
> 1 glass	Every day		Now and again	Weekends
A bottle of alcohol	Every day		Now and again	Weekends
> 1 bottle	Every day		Now and again	Weekends
28. Does she use drugs?			Yes	No
a) If yes, what drugs does she use?	Tik	Cocaine	Heroin	Mandrax	Other
b) How often does she use drugs?	Every day		Now and again	Weekends
29. Does the mother smoke after the pregnancy?			Yes	No
30. Does the mother know that smoking harms the unborn baby?			Yes	No
31. Does the husband/partner drinks?			Yes	No

32. Does the mother drink after the pregnancy?		Yes	No
33. Do the parents of the mother drink?		Yes	No
34. Does the mother know that alcohol harms the unborn baby?		Yes	No
35. Did the mother have a previous baby that died suddenly?		Yes	No
a) If yes, how many died?			
b) At what age?			
c) Was a PM done?		Yes	No
If yes, where was it done?			
36. Did the mother have a previous stillbirth?		Yes	No
Section G **Household environment**			
1. Place where the baby lives	House	Shack	Other
2. Number of bedrooms?			
3. Is the room in which the baby was found well ventilated?		Yes	No
4. Odour(s) present in the room the baby slept in?		Yes	No
5. Peeling paint in the room the baby slept in?		Yes	No
6. Fungal growth (mould) in the room the baby slept in?		Yes	No
7. Are there pets in the house?		Yes	No
If yes, type and number:			
8. Was the following in the room where the baby slept to heat the room?	Electric heater	"Galley"	Fire	Other
Describe other –				
9. Number of adults in the dwelling?				
10. Number of babyren in the dwelling?				
11. Total number of people in the dwelling?				
12. Estimated monthly income?				
13. Number of smokers in the dwelling?				
14. Are there mentally retarded/ challenged people in the dwelling?			Yes	No

COMMENTS TO PATHOLOGIST FROM THE FORENSIC OFFICER WHO ATTENDED THE SCENE AND INTERVIEWED DURING ID PROCESS:

ITEMS RETAINED AT THE SCENE OR FROM THE MOTHER DURING INTERVIEW

Date:

Signature / Thumbprint of deponent

I certify that the above statement was taken down by myself and that the deponent has acknowledged that he / she knows and understands the contents hereof.

Date _____ Time: _____

Place: _____

_____

Department of Health
Forensic Pathology Laboratory