INCIDENCE AND RISK FACTORS FOR INFLUENZA LOWER RESPIRATORY TRACT INFECTION IN A SOUTH AFRICAN BIRTH COHORT

by

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DECLARATION

I, Nchimunya Hapeela (HPLNCH001), hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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DISSERTATION ABSTRACT

Background: Lower respiratory tract infection (LRTI) is a significant cause of morbidity and mortality in children under five years, particularly in low- and middle-income countries (LMICs). However, data on the incidence and risk factors for influenza-associated LRTI in young children in these settings are limited. This study prospectively investigated the incidence of and risk factors for influenza-associated LRTI from birth through early childhood, in a South African birth cohort, the Drakenstein Child Health Study (DCHS).

Methods: Children enrolled in the DCHS between May 2012 to September 2015 were followed from birth up to 5 years of age. Data on socio-economic status, maternal and infant characteristics, and LRTI episodes were collected. Quantitative real-time polymerase chain reaction (qPCR) was used to identify respiratory organisms, including the influenza virus associated with each LRTI episode. Influenza-associated LRTI was defined as the presence of clinical LRTI symptoms with a laboratory-confirmed influenza infection (qPCR cycle threshold <40 for at least one influenza target gene). Generalized estimating equations (GEE) models were used to assess associations between potential risk factors and influenza-associated LRTI.

Results: Among 1143 live births, 51% (586/1143) were male, 17% (189/1143) were born premature, and 22% (248/1143) were HIV-exposed uninfected, while two were living with HIV. The population experienced high levels of socio-economic disadvantages, with 61% (695/1143) of mothers having an education below secondary level, and 86% (982/1143) of households earning less than 5,000 South African rands per month. Maternal smoking and alcohol use during pregnancy were reported by 28% (323/1142) and 13% (137/1067) of mothers, respectively. None of the mothers received influenza vaccination during pregnancy, and no child was vaccinated against influenza through the study, although coverage for other routine childhood vaccines exceeded 98%.

Overall, 47% (521/1108) of children experienced LRTI episodes (incidence rate of 22 episodes per 100 child-year, [95% CI 21, 23]). Among 472 children with qPCR results, 70 influenza-associated LRTI episodes occurred in 64 children (14%); incidence rate: 1.4 episodes per 100 child-year, [95% CI:1.1, 1.8] per 100 child-years. Of these 64 children with influenza-associated LRTI, 13% (9/70) required hospitalization, and one influenza-associated LRTI death was recorded among the hospitalized.

Risk factors for all-cause LRTI included lower maternal education, maternal HIV, prenatal alcohol use, preterm birth, male sex, and winter birth. After adjusting for other factors, influenza-associated LRTI was significantly associated with older age (odds ratio for a one-month increase in age: 1.02, [95% CI: 1.00-1.04]) and winter season.

Conclusion: Influenza remains a significant cause of LRTI, particularly among children affected by socio-economic disadvantages and maternal HIV exposure, and during the winter season. Expanding maternal and early childhood influenza vaccination programs, alongside broader public health interventions addressing underlying social vulnerabilities, could meaningfully reduce influenza-associated LRTI and improve child health outcomes.

Keywords: Childhood influenza, lower respiratory tract infection; birth cohort; child health; risk factors; incidence

ORGANISATION OF DISSERTATION

The dissertation is divided into three sections: Part A, B and C.

Part A is the research protocol outlining the study's overview.

Part B is the manuscript. It gives the introduction, methodological details, results, and discussion of findings, ending with a conclusion.

Part C is the appendices section, which includes data collection tools, ethics documentation and selected journal submission guidelines.

The style of referencing for the whole dissertation is BMC Infectious Diseases

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

- AOR – Adjusted Odds Ratio
- ARI – Acute Respiratory Infection
- BCG – Bacillus Calmette-Guérin (Tuberculosis Vaccine)
- CDC – Centre for Disease Control and Prevention
- CI – Confidence Interval
- DCHS – Drakenstein Child Health Study
- DTP – Diphtheria, Tetanus, and Pertussis Vaccine
- EPI-SA – Expanded Programme on Immunization in South Africa
- ELISA – Enzyme-Linked Immunosorbent Assay
- HEU –-– HIV-exposed, uninfected children
- CLHIV – Children Living with HIV
- FluVax – Influenza Vaccination
- GEE – Generalized Estimating Equation
- H1N1 – Hemagglutinin Type 1 and Neuraminidase Type 1 (Swine Flu)
- H3N2 – Hemagglutinin Type 3 and Neuraminidase Type 2 (Seasonal Flu)
- Hib – Haemophilus influenzae type b Vaccine
- HIV – Human Immunodeficiency Virus
- HREC – Human Research Ethics Committee
- IA-LRTI – Influenza-Associated Lower Respiratory Tract Infection
- ILI – Influenza-Like Illness
- IAP – Indoor Air Pollution
- LCWI – Lower Chest Wall Indrawing
- LMIC - Low to middle-income countries

- LRTI – Lower Respiratory Tract Infections
- NP – Nasopharyngeal
- OR – Odds Ratio
- PCV – Pneumococcal Conjugate Vaccine
- PCV13 – 13-Valent Pneumococcal Conjugate Vaccine
- VTP – Vertical Transmission Prevention
- p-Value – Probability Value
- qRT-PCR – Quantitative Real-Time Polymerase Chain Reaction
- RTHC – Road to Health Card
- RR – Risk Ratio
- SABC – South African Birth Cohort
- SARI – Severe Acute Respiratory Infection
- SES – Socioeconomic Status
- SD – Standard Deviation
- STGG – Skim milk-tryptone-glucose-glycerol
- TB – Tuberculosis
- WAZ - Weight-for-age Z-score
- WHO – World Health Organization
- FTDResp33 - Fast-Track Diagnostics Respiratory Pathogens 33

PART A: STUDY PROTOCOL

1.0 Background

1.1 Introduction

Seasonal influenza is a common cause of lower respiratory tract infections (LRTIs) in young children [1]. It is responsible for a substantial burden of severe respiratory illness and hospitalization of children younger than six months [1, 2]. Although influenza is a self-limiting disease, it can be associated with severe morbidity and mortality in healthy children and those with underlying comorbidities [3, 4]. It is a contagious acute respiratory illness caused by influenza A, B or C viruses. Currently, there is no vaccine or antiviral treatment approved for children under the age of six months.

Secondary bacterial infections are a major determinant of disease progression and outcome in influenza-associated LRTIs. Influenza virus infection disrupts the respiratory epithelium, impairs mucociliary clearance, and alters innate immune defences, thereby facilitating bacterial adherence and invasion by common respiratory pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae* [5]. These viral–bacterial interactions synergistically exacerbate disease severity, prolong hospitalization, and increase mortality. Evidence from Zar *et al.* [6] indicates that infants younger than six months are particularly vulnerable, exhibiting a higher incidence of secondary bacterial infections compared with older children.

Moreover, contemporary clinical studies estimate that secondary bacterial infections account for 25–50% of deaths in severe influenza cases, emphasizing the critical need to strengthen preventive interventions, enhance diagnostic capacity, and improve antimicrobial management, especially among infants and young children in low- and middle-income countries where healthcare resources are limited. Influenza viruses are genetically dynamic and

evolve in unpredictable ways [7, 8]. Due to antigenic shift, seasonal influenza epidemics can be caused by new viral strains that are antigenically distinct from previously circulating virus strains to which a population has immunity. These emergent strains, to which there is little, or no existing immunity have the potential to cause pandemics as was the case with 2009 pandemic influenza A (H1N1) virus [9]. The burden of hospitalizations and deaths due to influenza can therefore vary substantially from year to year depending on the transmission and virulence characteristics of the circulating strain(s). This further argues for continued surveillance of influenza and associated risk factors.

Three large, randomized trials in Mali [10], Nepal [11] and South Africa [12] have evaluated the efficacy and safety of maternal immunization to prevent maternal and infant influenza disease. The results showed that maternal influenza vaccination provided partial protection against confirmed influenza in women and in infants who were not exposed to HIV. The efficacy of vaccination against any influenza illness ranged between 31-70% in pregnant mothers and between 30-40% in their infants [10, 13]. More studies are being done to guide policy decisions on the implementation of maternal immunization programs in low- and middle-income countries (LMIC) [13-15].

Although the influenza vaccine is recommended by the World Health Organisation (WHO) for pregnant women and children, only a few LMICs have national policies in place. Where present, vaccine uptake in most LMICs, including South Africa, is low [1]. Identifying risk factors associated with seasonal influenza and influenza-associated lower respiratory tract infections is pivotal for guiding targeted prevention strategies among vulnerable populations. Such insights can inform evidence-based policy decisions, including the prioritization of high-risk groups for vaccination and other preventive interventions in resource-limited settings. Furthermore, incidence data derived from these analyses can be leveraged to advocate for expanded access to, and equitable coverage of, influenza prevention and control measures.

1.2 Seasonal Influenza - a Global Problem

Acute lower respiratory infections (ALRI) are still the leading cause of global child mortality [16]. Viral infection, particularly influenzae virus, are strongly associated with ALRI, in low-resource settings [17]. In 2018, an estimation of 109.5 million influenza virus episodes (uncertainty range [UR] 63.1 million - 190.6 million) were recorded among children under 5 years globally [1]. Of these, 10.1 million were influenza-virus-associated ALRI cases (UR: 6.8 million - 15.1 million); 870,000 influenza-virus-associated ALRI hospital admissions (UR: 543 000-1 415 000) occurred, resulting in 15,300 in-hospital deaths (UR: 5,800-43 43,800). A total of up to 34,800 (UR: 13,200-97,200) influenza-virus-associated ALRI deaths were recorded, and about 82% of in-hospital deaths occurred in LMICs [1]. Influenza accounted for 7% of ALRI cases, 5% of ALRI hospital admissions, and 4% of ALRI deaths in children under 5 years. Furthermore 23% of 870,000 influenza-associated ALRI hospitalizations and 36% of the 15,300 influenza-associated ALRI deaths were infants under 6 months [1].

1.2.1 Seasonal Influenza- the South African Context

In South Africa, it is estimated that nearly 10,000 seasonal influenza-associated deaths and 40,000 episodes of influenza-associated hospitalizations occur annually [18, 19]. During influenza season (between May and September), nearly 14% of inpatients with LRTI and 25% of patients with influenza-like illness test positive for influenza (by PCR testing). In addition, a recent study has shown that children younger than 5 years (<6 months [adjusted odds ratio (aOR), 37.6; 95% confidence interval (CI), 8.3–106.4], 6–11 months [aOR, 31.9; 95% CI, 9.1–111.8], 12–23 months [aOR, 22.1; 95% CI, 6.9–70.9], 24–59 months [aOR, 7.1; 95% CI, 2.4–21.5]) had an increased risk of influenza-associated hospitalization than those older than 5 years [20]. Data from the Drakenstein Child Health Study (DCHS) demonstrated that the detection of influenza in respiratory samples was strongly associated with lower respiratory

tract infection (LRTI) (odds ratio [OR] 4.13; 95% confidence interval [CI] 2.06–8.26), underscoring the substantial contribution of influenza to the LRTI burden in early childhood and highlighting the need for targeted preventive strategies within similar high-risk populations) [6]. Beyond its association with severe morbidity and mortality, seasonal influenza also imposes a considerable burden on healthcare services through increased emergency department and outpatient medical visits. [19].

1.2.2 Risk Factors

Immunization is the most effective means of preventing influenza. However, there are no current vaccines licensed for children less than 6 months of age [21]. This is a particularly vulnerable age group, and a comprehensive assessment of potential risk factors remains unclear. Despite maternal immunization, which is poorly done, available data indicate that several children have no protective antibody levels at birth and that in most cases the duration of protection is limited to the first 8 weeks of the postnatal period [14]. Therefore, infants remain unprotected for an extended period before vaccines can be administered [22].

To understand risk factors associated with childhood influenza, recent researchers have increased their focus on how risk factors may impact children's respiratory health [23-30]. Several maternal risk factors, such as age, human immunodeficiency virus (HIV), poverty, household smoking, indoor air pollution (IAP) and lack of maternal influenza vaccination, have been linked with influenza-associated LRTI [14, 20, 24, 25, 31]. In addition to maternal risk factors, several child risk factors have been reported to be associated with influenza-associated LRTI, including: age under 2 years, prematurity, low birth weight, presence of chronic underlying medical condition or an immature immune system [23-25, 27, 31, 32].

Data on risk factors for influenza disease in infants are limited, especially in LMIC. Such data are important for preventative strategies to reduce the burden of childhood influenza. Much of

the currently available evidence is derived from studies conducted in high-income countries [33]. Therefore, there is an increasing need for the identification of potential risk factors that increase the risk of influenza and/or influenza-associated LRTI in LMIC settings. South Africa has a high incidence of HIV infection (4.95 per 1,000 persons aged 15–49 years) and tuberculosis (TB) (~615 per 100,000 population). The country's intersecting HIV and TB epidemics continue to represent one of the most formidable public health challenges globally, contributing substantially to respiratory morbidity and mortality. Within this context, understanding additional infectious contributors to respiratory disease is critical. The proposed research, therefore seeks to elucidate the risk factors associated with influenza-associated lower respiratory tract infection (LRTI) in infants and young children participating in the South African birth cohort, the Drakenstein Child Health Study (DCHS), thereby addressing an important knowledge gap in high-burden, resource-limited settings.

2.0 Rationale and Study Objectives

2.1 Research Question

What is the incidence and risk factors for influenza lower respiratory tract infection in a South African birth cohort?

2.2 Problem Statement

South Africa has high levels of poverty, environmental tobacco smoke (ETS) pollution exposure and low levels of maternal influenza immunization. These underlying factors play a significant role in the presentation of influenza and its morbidity [34, 35]. Even though a policy to provide free influenza vaccine to high risk groups such as children from 6 months to 5 years, adults older than 65 years, pregnant women, health care workers and anyone with underlying medical conditions has been in existence for years, coverage in the public immunization

program is low; coverage is approximately 5% in all risk groups except pregnant women (14%) [36].

2.3 Justification

Childhood influenza and its associated risk factors are of paramount public health concern, particularly in LMICs. Despite the substantial burden of influenza-related morbidity and mortality among infants in LMICs, there remains a significant dearth of comprehensive epidemiological data and research on the incidence and specific risk factors contributing to this health challenge. The existing body of literature primarily originates from high-income settings, limiting its applicability to the unique socio-economic, healthcare, and environmental conditions prevalent in LMICs. Considering this significant research gap, there is an urgent need for studies that are contextually tailored to LMICs, offering insights into the factors that drive infant influenza incidence, severity, and transmission dynamics. By addressing this knowledge gap, researchers can contribute to the development of targeted, evidence-based interventions that are essential for reducing the impact of infant influenza in these resource-constrained settings. This study will focus on examining the associations between childhood seasonal influenza and birth, family and other risk factors in the first five years of life. The outcome of this study will not only improve our knowledge about risk factors associated with seasonal influenza, but it may have important implications for preventative strategies to help reduce the burden of childhood influenza a disease frequently under-evaluated and potentially other respiratory illnesses, such as LRTI.

2.4 Study Aim

To investigate the incidence and risk factors for influenza lower respiratory tract infection in a South African birth cohort.

2.5 Study Objectives

1. To identify the incidence of influenza-associated LRTI in infancy.

2. To determine outcomes, including death/discharge from hospital, associated with influenza-associated LRTI.

3. To investigate antenatal and postnatal risk factors associated with influenza-associated LRTI.

3.0 Methodology

3.1 Setting

This study is a secondary analysis of data collected in a well-established South African birth cohort study (the "Drakenstein Child Health Study," DCHS). The primary aim of the DCHS was to investigate the early life determinants of child health, including aetiology, progression and risk factors for childhood LRTI [29]. The Drakenstein municipality is a peri-urban sub-district within the Cape Winelands region, Western Cape, South Africa, with a population of approximately 200,000[29]. The study location is a stable, semi-urban, low socio-economic status community. More than 90% of the population access health care in the public sector, including antenatal and child health services [37]. There is a well-established vertical transmission prevention (VTP) program with a current rate of approximately 1.4 -1.5% [38, 39]. Antiretroviral therapy for children and adults is provided free of charge through public clinics. In addition, there is a high coverage of immunization. Haemophilus influenzae type B (HiB) and 13-valent pneumococcal conjugate vaccine (PCV-13) have been included in the national immunization program. At the time of the study (2012-2015), South Africa's national influenza vaccination policy recommended but did not routinely implement influenza

vaccination for pregnant women and children aged 6 months – 5 years in the public sector. As such, there were little/no influenza vaccinations for infants in our study. In addition, there is also low coverage with maternal influenza vaccination in pregnancy (only one mother reported to have been vaccinated in our study). Like many other LMICs, the area has a high incidence of childhood diseases, including LRTI with a high burden of environmental tobacco smoke exposure, or other poverty-related exposures [6, 29, 40].

3.2 Study Population

This study includes data collected from participants enrolled in the DCHS. Inclusion criteria were pregnant women older than 18 years (20-28 weeks gestation) residing in the Drakenstein region, attending one of the two local primary health clinics serving distinct populations – (TC Newman serving a mixed ancestry population and Mbekweni serving a black African population). To be included in the study, the participants had to intend to remain in the area for at least 1 year. Inclusion criteria for this longitudinal study were broad to ensure generalizability of results. Exclusion criteria included women who were planning to move out of the area, those under 18 years of age, those without informed consent, and those who did not attend study clinics for antenatal care. Enrolment occurred over a 3-year period from March 2012 to March 2015, so this study will include all data collected from infants born during the period from March 2012 to July 2015.

3.3 Study Measures and Follow-up

Antenatal and postnatal visits were at primary healthcare clinics, while birth, 6-week and annual study visits occurred at Paarl Hospital. Infants were followed at the two clinics in the area, Mbekweni and TC Newman, where passive and active surveillance were done. Mother-infant pairs attended numerous visits during this period; at enrolment, mothers were able to

choose to participate in usual study follow-up (main cohort) or an intensive cohort, in which 2 weekly follow-ups with nasopharyngeal sampling were done in children throughout the first year of life[14]. Study visits were synchronised with the national programme where feasible at 6, 10 and 14 weeks, and 6, 9, 18, 30, 36, 42, 54 and 60 months. During study visits, maternal physical and socio-demographic risk factors were investigated antenatally and postnatally [29, 41].

Children were given vaccinations as per the national vaccination schedules. BCG was given at birth; 13-valent pneumococcal conjugate vaccine (PCV13) at 6 weeks, 14 weeks and 9 months); hexavalent vaccine (including acellular pertussis and Hib) was administered at 6, 10, and 14 weeks and 18 months; and the measles vaccine at 9 and 18 months.

Presence of LRTI was defined by WHO criteria [42], which includes a cough or difficulty breathing with age-appropriate tachypnoea or lower chest wall indrawing [43]. Measurement of LRTI included ambulatory and hospitalized cases [6]. In addition, a strong surveillance system was established using healthcare workers, cell phones and active surveillance at health facilities. Strong community engagement was undertaken by trained health workers. Cohort retention was promoted by using several strategies such as automated study visit reminders, a close working relationship with clinical staff, a cell phone system always enabling two-way communication with study participants and regular follow-up synchronised with routine visits.

3.4 Sample Collection and Testing

An intensive subset of approximately 75% of the cohort had fortnightly nasopharyngeal (NP) swabs collected in the first year. At each study visit, NP swabs were collected and transferred into nucleic acid preservation medium (PrimeStore MTM ™]), before being transported on ice to the laboratory and frozen at -80 °C for batch processing. NP swabs were also collected during scheduled clinic visits at 6, 12, 18, 24, 36, 48, and 60 months of age and during any

episode of LRTI. For each LRTI, 2 NPs (FLOQSwabs™, Copan Diagnostics, CA) were obtained, followed by an induced sputum (IS) specimen [44]. The IS specimen was transported to the laboratory on ice and split into two aliquots. The first NP was immediately transferred into nucleic acid preservation medium (PrimeStore®), Longhorn Vaccines & Diagnostics, Texas), the second was placed into 1 ml of skim milk-tryptone-glucose-glycerol (STGG) transport medium. The swab in STGG was cultured for bacteria and total nucleic acid was extracted from the swab in Primestore using mechanical lysis on a TissueLyzer LT (Qiagen, Germany) followed by extraction with the QIAsymphony® Virus/Bacteria mini kit (Qiagen, Germany). Quantitative multiplex real-time PCR (qPCR) was done using Fast-Track Diagnostics Respiratory Pathogens 33 (FTDResp33) (Fast-track Diagnostics, Luxembourg), to identify up to 33 potential organisms including respiratory viruses (influenza A, B, C; parainfluenza 1, 2, 3, 4; coronaviruses NL63, 229E, OC43, HKU1; human metapneumoviruses A, B, rhinovirus, respiratory syncytial viruses A, B, adenovirus, enterovirus, parechovirus, bocavirus, cytomegalovirus), fungi (P. jirovecii) and bacteria (*M. pneumoniae, C. pneumoniae; S. pneumoniae, H. influenzae type b; S. aureus; M. catarrhalis; B. pertussis; K. pneumoniae; Legionella species; Salmonella species; H. influenzae). K. pneumoniae* and *Legionella spp*. Standard curves were derived using plasmid standards supplied by the manufacturer for each organism. A blood culture for bacteria was obtained in hospitalized cases.

4.0 Research Procedure

4.1 Data Collection

The primary outcome of this study is laboratory confirmed influenza -associated LRTI in the first year of life identified through routine follow-up visits as well as active surveillance for respiratory symptoms conducted over 5 influenza seasons. All risk factors and outcome data in the DCHS are collected longitudinally. This study will make use of metadata previously

collected as part of the DCHS. Subsequent to validation, data will be exported to STATA software (Stata Corporation, College Station, TX) for analysis. Patient identifiers will be removed, and participants will be allocated unique study IDs generated by the randomization procedure.

4.2 Key Data Required

4.2.1 Risk Factors

4.2.1.1 Maternal and Environmental Risk Factors

Maternal and environmental risk factors were measured longitudinally, including HIV exposure, and maternal self-reported smoking behaviour pre- and postnatally. Season of birth was recorded and included to adjust for seasonality, as certain seasons (winter and autumn) are linked with LRTI episodes [43]. Alcohol consumption was self-reported. In addition, the mother's HIV status was also recorded.

4.2.1.2 Child Characteristics Risk Factors

Childbirth characteristics, including gestational age and birth weight, were collected by study staff using a validated questionnaire as previously described [45]. Birth weight/height standardised z-scores were calculated using the updated Fenton newborn growth charts, which account for prematurity [46, 47]. Biological risk factors such as malnutrition and underlying conditions were recorded. The weight of each child, as measured on admission, was used to evaluate nutritional status using WHO weight for age Z scores (WAZ). Mild under-nutrition was defined as ≤ -1 WAZ > -2, moderate under-nutrition ≤ -2 WAZ > -3 and severe under-nutrition WAZ ≤ -3 [48]. Each child was screened for HIV exposure and/or infection. HIV infection was determined by an ELISA test (Architect HIV Ag/Ab Combo, Abbott Diagnostics, Wiesbaden). The diagnosis of HIV infection was made if both the ELISA and an HIV PCR test

(COBAS AmpliPrep/COBAS TaqMan HIV-1, Roche Molecular Diagnostics, Pleasanton, CA) were positive in children younger than 18 months. Further, the vaccination status of each child was verified using the national standardized immunization handheld record, the Road to Health Card (RTHC).

4.2.1.3 Socio-Demographic Factors

At baseline, socio-economic status (SES) was collected and comprised of four socio-economic variables based on a composite validated score including level of maternal education, employment status, household income, and asset ownership. The standardized scores were divided into quartiles labelled 'low', 'low-moderate', 'moderate-high' and 'high' [43]. Other socio-demographic factors, including type of housing, access to amenities such as tap water, electricity, and toilet facilities, were obtained.

4.2.2 LRTI

Data on LRTI episodes and any severe hospitalised episodes were collected by trained study staff (nurses) during active LRTI surveillance and were assessed in real time [43]. These nurses were trained in respiratory examination of children and were frequently re-trained. LRTI was defined according to the World Health Organization (WHO) criteria which included a cough or difficulty breathing with age-appropriate tachypnoea or lower chest wall indrawing [43]. Nasopharyngeal swabs (NPs) were collected at scheduled study visits, as well as at any LRTI episode. The history of the presence and duration of recent respiratory symptoms, as well as presence and number of other household members was also recorded. The severity of illness was graded by WHO categorisation including any general danger sign in children older than 2 months or age specific tachypnoea, lower chest wall indrawing or general danger in infants less than 2 months [43]. Hospitalisation or hypoxia (O2 saturation < 92%) will also be used as measures of severity.

5.0 Data Analysis

Initial exploratory statistics will be performed using STATA software (Stata Corporation, College Station, TX). Simple descriptive statistics will be used to describe the study population, summarizing continuous data as means (standard deviations) or median (Interquartile range, IQR) as appropriate. Categorical data will be presented as frequencies and proportions. Mann-Whitney rank sum and Kruskal-Wallis tests will be used to test for differences between continuous variables in relation to categorical variables. Pearson Chi-square ($\chi 2$) test or Fisher Exact tests will be used to test for statistical differences between categorical variables with a two-tailed cut-off significance set at $p < 0.05$.

To appropriately account for the multiple episodes of Lower Respiratory Tract Infection (LRTI) experienced by some children during the study period, Generalized Estimating Equation (GEE) models will be employed. In addition, mixed-effect models will be used to investigate the impact of identified risk factors on seasonal influenza over time.

6.0 Presentation and Dissemination of the Results

A dissertation will be submitted to the University of Cape Town according to the structural guidelines for an M. Phil dissertation as provided by the university. The results will also be published in an open-source journal. The article will be available on PubMed Central without any restrictions. Data used to generate figures in the publication will be deposited in relevant repositories for access by the scientific community. The results and data will be useful to both scientists working in the field of maternal and child health and influenza surveillance. Moreover, we will translate the research findings for publication in magazines for the general community outside of science. This will broaden the impact of our research and ensure that our findings are understood by the public and the people who contributed samples for the study.

Furthermore, we will undertake public engagements in township communities around the Drakenstein area, where most of the study participants come from to share the research findings and educate them on risk factors associated with seasonal influenza. We will also attend and present our data at both local and international conferences. This will allow us to disseminate our research findings and build networks for collaboration.

7.0 Feasibility and Ethical Considerations

This proposed study will use secondary data and as such it is feasible as minimal resources are required. The DCHS was approved by the Health Sciences, Human Research Ethics Committee (HREC) of the University of Cape Town (401/2009) and by the Western Cape Health Committee. Approval for this sub-study will be sought from the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town. Mothers provided written informed consent at enrolment and annually thereafter. The informed consent forms were in English, Afrikaans and isiXhosa. All Consent was done with the assistance of trained study staff in a participant's preferred language. Participants were informed of the aim of the study, objectives, potential harms and benefits, if any. They were also informed that they can withdraw from the study at any time and that doing so will not have any impact on their health care and management.

The anonymity and confidentiality of all the study subjects will be guaranteed using alphanumeric codes in the data set. This ensures that all participant identifiers are removed from the data collected. All collected information is stored in locked filling systems at the site and with the data management team. Computers used for this study are all password protected; access is only granted to authorized study staff members. The study will be conducted in accordance with the principles laid down in the Declaration of the Helsinki.

8.0 Conclusions

There are limited data on potential risk factors that increase the risk of influenza-associated LRTI from low- and middle-income countries (LMIC). The high burden of HIV infection and other comorbidities is likely to play a significant role in driving influenza disease progression and associated complications. In this observational study, we will investigate, in detail, the association of risk factors with influenza-associated LRTI and the severity of disease in infants enrolled in a longitudinal birth cohort study in the Drakenstein sub-district, Cape Town, South Africa.

9.0 References

1. Lafond KE, Nair H, Rasooly MH, Valente F, Booy R, Rahman M, Kitsutani P, Yu H, Guzman G, Coulibaly D *et al*: **Global Role and Burden of Influenza in Pediatric Respiratory Hospitalizations, 1982-2012: A Systematic Analysis**. *PLoS Med* 2016, **13**(3):e1001977.
2. Marchisio P, Baggi E, Bianchini S, Principi N, Esposito S: **Clinical and socioeconomic impact of pediatric seasonal and pandemic influenza**. *Human vaccines & immunotherapeutics* 2012, **8**:17-20.
3. Shang M, Blanton L, Brammer L, Olsen SJ, Fry AM: **Influenza-Associated Pediatric Deaths in the United States, 2010-2016**. *Pediatrics* 2018, **141**(4).
4. Nunes M, Madhi S: **Influenza vaccination during pregnancy for prevention of influenza confirmed illness in the infants: A systematic review and meta-analysis**. *Human Vaccines & Immunotherapeutics* 2017, **14**:00-00.
5. Jochems SP, Marcon F, Carniel BF, Holloway M, Mitsi E, Smith E, Gritzfeld JF, Solórzano C, Reiné J, Pojar S *et al*: **Inflammation induced by influenza virus impairs**

human innate immune control of pneumococcus. *Nature Immunology* 2018, **19**(12):1299-1308.

6. Zar HJ, Barnett W, Stadler A, Gardner-Lubbe S, Myer L, Nicol MP: **Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: a nested case-control study of the Drakenstein Child Health Study**. *The Lancet Respiratory Medicine* 2016, **4**(6):463-472.

7. Wang X, Li Y, O'Brien KL, Madhi SA, Widdowson M-A, Byass P, Omer SB, Abbas Q, Ali A, Amu A *et al*: **Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study**. *Lancet Glob Health 2020; 8(4):e497-e510* 2020, **8**(4).

8. Wang Q-y, Yuan L, Lin J-y, Zhuo Z-q, Wang Y-m, Li S-s, Zhang M, Wu X-d: **Clinical characteristics of severe influenza virus-associated pneumonia complicated with bacterial infection in children: a retrospective analysis**. *BMC Infectious Diseases* 2023, **23**(1):545.

9. Archer BN, Timothy GA, Cohen C, Tempia S, Huma M, Blumberg L, Naidoo D, Cengimbo A, Schoub BD: **Introduction of 2009 pandemic influenza A virus subtype H1N1 into South Africa: clinical presentation, epidemiology, and transmissibility of the first 100 cases**. *J Infect Dis* 2012, **206 Suppl 1**(Suppl 1):S148-153.

10. Tapia MD, Sow SO, Tamboura B, Tégueté I, Pasetti MF, Kodio M, Onwuchekwa U, Tennant SM, Blackwelder WC, Coulibaly F *et al*: **Maternal immunisation with trivalent inactivated influenza vaccine for prevention of influenza in infants in Mali: a prospective, active-controlled, observer-blind, randomised phase 4 trial**. *Lancet Infect Dis* 2016, **16**(9):1026-1035.

11. Steinhoff MC, Katz J, Englund JA, Khatry SK, Shrestha L, Kuypers J, Stewart L, Mullany LC, Chu HY, LeClerq SC *et al*: **Year-round influenza immunisation during**

pregnancy in Nepal: a phase 4, randomised, placebo-controlled trial. *Lancet Infect Dis* 2017, **17**(9):981-989.

12. Madhi SA, Cutland CL, Kuwanda L, Weinberg A, Hugo A, Jones S, Adrian PV, van Niekerk N, Treurnicht F, Ortiz JR *et al*: **Influenza vaccination of pregnant women and protection of their infants**. *N Engl J Med* 2014, **371**(10):918-931.
13. Fell DB, Azziz-Baumgartner E, Baker MG, Batra M, Beauté J, Beutels P, Bhat N, Bhutta ZA, Cohen C, De Mucio B *et al*: **Influenza epidemiology and immunization during pregnancy: Final report of a World Health Organization working group**. *Vaccine 35(43):5738-5750* 2017, **35**(43).
14. Ortiz JR, Neuzil KM: **Influenza Immunization in Low- and Middle-Income Countries: Preparing for Next-Generation Influenza Vaccines**. *The Journal of Infectious Diseases* 2019, **219**(Supplement_1):S97-S106.
15. UNICEF: **Levels and Trends in Child Mortality - United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME), Report 2019**. In.; 2019.
16. WHO: **World Health Organization Pneumonia in Children**. In., 11 November 2022 edn; 2022.
17. PERCH: **Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study**. *Lancet* 2019, **394**(10200):757-779.
18. Solanki G, Cornell M, Lalloo R: **Uptake and cost of influenza vaccines in a private health insured South African population**. *S Afr J Infect Dis* 2018, **33**(5).
19. O'Brien MA, Uyeki TM, Shay DK, Thompson WW, Kleinman K, McAdam A, Yu XJ, Platt R, Lieu TA: **Incidence of outpatient visits and hospitalizations related to influenza in infants and young children**. *Pediatrics* 2004, **113**(3 Pt 1):585-593.

20. Tempia S, Walaza S, Moyes J, Cohen AL, von Mollendorf C, Treurnicht FK, Venter M, Pretorius M, Hellferscee O, Mtshali S *et al*: **Risk Factors for Influenza-Associated Severe Acute Respiratory Illness Hospitalization in South Africa, 2012-2015**. *Open Forum Infect Dis* 2017, **4**(1):ofw262.

21. Nunes MC, Aqil AR, Omer SB, Madhi SA: **The Effects of Influenza Vaccination during Pregnancy on Birth Outcomes: A Systematic Review and Meta-Analysis**. *Am J Perinatol* 2016, **33**(11):1104-1114.

22. Principi N, Esposito S: **Protection of children against influenza: Emerging problems**. *Hum Vaccin Immunother* 2018, **14**(3):750-757.

23. Lakhan N, Clarke M, Mathew SM, Marshall H: **Retrospective review of factors associated with severe hospitalised community-acquired influenza in a tertiary paediatric hospital in South Australia**. *Influenza Other Respir Viruses* 2016, **10**(6):479-485.

24. Harerimana JM, Nyirazinyoye L, Thomson DR, Ntaganira J: **Social, economic and environmental risk factors for acute lower respiratory infections among children under five years of age in Rwanda**. *Arch Public Health* 2016, **74**:19.

25. Dharan NJ, Sokolow LZ, Cheng PY, Gargiullo P, Gershman K, Lynfield R, Morin C, Thomas A, Meek J, Farley MM *et al*: **Child, household, and caregiver characteristics associated with hospitalization for influenza among children 6-59 months of age: an emerging infections program study**. *Pediatr Infect Dis J* 2014, **33**(6):e141-150.

26. Brittain K, Myer L, Koen N, Koopowitz S, Donald KA, Barnett W, Zar HJ, Stein DJ: **Risk Factors for Antenatal Depression and Associations with Infant Birth Outcomes: Results From a South African Birth Cohort Study**. *Paediatr Perinat Epidemiol* 2015, **29**(6):505-514.

27. Abadom TR, Smith AD, Tempia S, Madhi SA, Cohen C, Cohen AL: **Risk factors associated with hospitalisation for influenza-associated severe acute respiratory illness in South Africa: A case-population study**. *Vaccine* 2016, **34**(46):5649-5655.

28. le Roux DM, Nicol MP, Myer L, Vanker A, Stadler JAM, von Delft E, Zar HJ: **Lower Respiratory Tract Infections in Children in a Well-vaccinated South African Birth Cohort: Spectrum of Disease and Risk Factors**. *Clin Infect Dis* 2019, **69**(9):1588-1596.

29. Zar HJ, Barnett W, Myer L, Stein DJ, Nicol MP: **Investigating the early-life determinants of illness in Africa: the Drakenstein Child Health Study**. *Thorax* 2015, **70**(6):592-594.

30. MacGinty RP, Lesosky M, Barnett W, Stein DJ, Zar HJ: **Associations between maternal mental health and early child wheezing in a South African birth cohort**. *Pediatr Pulmonol* 2018, **53**(6):741-754.

31. Ma HY, Wu JL, Lu CY, Chen JM, Lee PI, Chang LY, Huang LM: **Risk factors associated with severe influenza virus infections in hospitalized children during the 2013 to 2014 season**. *J Microbiol Immunol Infect* 2016, **49**(3):387-393.

32. Principi N, Esposito S: **Severe influenza in children: incidence and risk factors**. *Expert Rev Anti Infect Ther* 2016, **14**(10):961-968.

33. Fischer WA, 2nd, Gong M, Bhagwanjee S, Sevransky J: **Global burden of influenza as a cause of cardiopulmonary morbidity and mortality**. *Glob Heart* 2014, **9**(3):325-336.

34. Cohen C, Moyes J, Tempia S, Groom M, Walaza S, Pretorius M, Dawood H, Chhagan M, Haffejee S, Variava E *et al*: **Severe influenza-associated respiratory infection in high HIV prevalence setting, South Africa, 2009-2011**. *Emerg Infect Dis* 2013, **19**(11):1766-1774.

35. Walaza S, Cohen C, Nanoo A, Cohen AL, McAnerney J, von Mollendorf C, Moyes J, Tempia S: **Excess Mortality Associated with Influenza among Tuberculosis Deaths in South Africa, 1999-2009**. *PLoS One* 2015, **10**(6):e0129173.

36. McMorrow ML, Tempia S, Walaza S, Treurnicht FK, Ramkrishna W, Azziz-Baumgartner E, Madhi SA, Cohen C: **Prioritization of risk groups for influenza vaccination in resource limited settings - A case study from South Africa**. *Vaccine* 2019, **37**(1):25-33.

37. WC: *Regional Development Profile Cape Winelands District, Cape Town: Provincial Treasury* 2013.

38. Health: **P. G. o. t. W. C. D. o. The Western Cape consolidated guidelines for HIV treatment: Prevention of mother-to-child transmission of HIV (PMTCT), children, adolescents and adults. 2015 (Amended Version). In: Department of Health HASTHD, editor**. 2015.

39. van der Merwe TA, van Zyl GU, Lombard CJ, Theron GB: **Intrapartum human immunodeficiency virus transmission rate in a central hospital in the Western Cape province after universal antiretroviral therapy roll-out**. *S Afr J Infect Dis* 2020, **35**(1):192.

40. Stein DJ, Koen N, Donald KA, Adnams CM, Koopowitz S, Lund C, Marais A, Myers B, Roos A, Sorsdahl K *et al*: **Investigating the psychosocial determinants of child health in Africa: The Drakenstein Child Health Study**. *J Neurosci Methods* 2015, **252**:27-35.

41. Donald KA, Hoogenhout M, du Plooy CP, Wedderburn CJ, Nhapi RT, Barnett W, Hoffman N, Malcolm-Smith S, Zar HJ, Stein DJ: **Drakenstein Child Health Study (DCHS): investigating determinants of early child development and cognition**. *BMJ Paediatr Open* 2018, **2**(1):e000282.

42. Wolter N, Cohen C, Tempia S, Madhi SA, Venter M, Moyes J, Walaza S, Malope Kgokong B, Groome M, du Plessis M *et al*: **HIV and influenza virus infections are associated with increased blood pneumococcal load: a prospective, hospital-based observational study in South Africa, 2009-2011**. *J Infect Dis* 2014, **209**(1):56-65.

43. le Roux DM, Myer L, Nicol MP, Zar HJ: **Incidence of childhood pneumonia: facility-based surveillance estimate compared to measured incidence in a South African birth cohort study**. *BMJ Open* 2015, **5**(12):e009111.

44. Planting NS, Visser GL, Nicol MP, Workman L, Isaacs W, Zar HJ: **Safety and efficacy of induced sputum in young children hospitalised with suspected pulmonary tuberculosis**. *Int J Tuberc Lung Dis* 2014, **18**(1):8-12.

45. Walaza S, Tempia S, Dawood H, Variava E, Moyes J, Cohen AL, Wolter N, Groome M, von Mollendorf C, Kahn K *et al*: **Influenza virus infection is associated with increased risk of death amongst patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010–2011**. *BMC Infectious Diseases* 2015, **15**(1):26.

46. Fenton TR, Nasser R, Eliasziw M, Kim JH, Bilan D, Sauve R: **Validating the weight gain of preterm infants between the reference growth curve of the fetus and the term infant**. *BMC Pediatr* 2013, **13**:92.

47. Fenton TR, Kim JH: **A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants**. *BMC Pediatrics* 2013, **13**(1):59.

48. WHO: **Child growth standards**. 2015.

PART B: MANUSCRIPT

Incidence and Risk Factors for Influenza lower respiratory tract infection in a South African Birth Cohort

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ABSTRACT

Background: Lower respiratory tract infection (LRTI) is a significant cause of morbidity and mortality in children under five years, particularly in low- and middle-income countries (LMICs). However, data on the incidence and risk factors for influenza-associated LRTI in young children in these settings are limited. This study prospectively investigated the incidence and risk factors of influenza-associated LRTI from birth through early childhood within the South African Drakenstein Child Health Study (DCHS) birth cohort.

Methods: Children enrolled in the DCHS between May 2012 and September 2015 were followed from birth through to 5 years. Data on socio-economic status, maternal and infant characteristics, and LRTI episodes were collected. Respiratory pathogens, including influenza viruses, were identified using the quantitative real-time polymerase chain reaction (qPCR). Influenza-associated LRTI was defined as the presence of clinical signs of LRTI in a participant with a laboratory-confirmed influenza infection, indicated by a qPCR cycle threshold of <40 for at least one influenza target gene. Generalized estimating equations (GEE) models were used to assess associations between potential risk factors and influenza-associated LRTI in children.

Results: Amongst 1143 live births, 51% (586/1143) were male, 17% (189/1143) were born premature, 22% (248/1143) were HIV-exposed uninfected, and two were living with HIV. The population was of poor socio-economic conditions, with 61% (695/1143) of mothers having education below secondary level, and 86% (982/1143) of households earning less than ZAR5,000 per month. Maternal smoking and alcohol use during pregnancy were reported by 28% (323/1142) and 13% (137/1067) of mothers, respectively. None of the mothers received influenza vaccination during pregnancy, nor did any child receive influenza vaccine through the study, although immunization coverage for other childhood vaccines was high (>98%).

Overall, 521 children experienced 1108 LRTI episodes (incidence 22 episodes per 100 child-year, [95% CI 21, 23]). Among 472 children with qPCR results, 70 influenza-associated LRTI episodes occurred in 14% (64/472) of the children (incidence 1.4 episodes per 100 child-year, [95% CI:1.1, 1.8] per 100 child-years). Of the 64 children with influenza-associated LRTI, 13% (9/70) of influenza-associated LRTI episodes resulted in hospitalisation, and one influenza-associated LRTI-related death occurred among hospitalized children.

Risk factors for all-cause LRTI included lower maternal education, maternal HIV, prenatal alcohol use, preterm birth, male sex, and winter birth. Compared to non-influenza LRTI and after adjusting for other factors, influenza-associated LRTI was significantly associated with older age (odds ratio [OR] for a one-month increase in age: 1.02, [95% CI: 1.00-1.04]) and winter season, OR: 7.83 [95% CI: 2.34-26.14], $p = 0.001$.

Conclusion: Influenza remains a significant cause of LRTI, particularly among children affected by socio-economic disadvantages and maternal HIV exposure, and during the winter season. Strengthening maternal and childhood influenza vaccination programs could help reduce the incidence of LRTI and mitigate this burden.

Keywords: Childhood influenza, lower respiratory tract infection; birth cohort; child health; risk factors; incidence

1.0 Introduction

Childhood influenza is a significant cause of acute lower respiratory tract infection (LRTI) in young children. In 2018, 109.5 million episodes of influenza-associated LRTI occurred among children under 5 years, causing around 870,000 hospital admissions and up to 34,000 deaths. Notably, 82% of in-hospital deaths occurred in low- and middle-income countries (LMICs) [1], underscoring the disproportionate burden of influenza-related morbidity and mortality among young children in LMICs.

Although influenza vaccination is recommended by the World Health Organization (WHO) for pregnant women and children older than 6 months, few LMICs have incorporated this into their national Expanded Programme on Immunization (EPI). Randomized trials conducted in Mali, Nepal, and South Africa showed the efficacy of vaccination against any influenza illness of 31-70% in pregnant mothers and 30-40% in their infants [2, 3]. Despite the benefit of vaccination, the uptake of the influenza vaccine remains low in most LMICs, including South Africa [1].

South Africa's health surveillance systems have estimated that approximately 10 million South Africans (20% of the population) experience influenza-associated illness annually, of which 98.7% are mild, 1.2% severe, and 0.1% are fatal [4]. The highest rates of severe non-fatal influenza-associated illness occurred in infants aged 6, with an incidence of 1,550 per 100,000 population year, and a mortality rate of 80 per 100,000 population year. Additionally, children aged 6-59 months experienced the highest rates of mild influenza-associated illness, with an incidence of 23,983 per 100,000 population year [4, 5, 6]. However, these studies were limited, as they were largely cross-sectional and focused on hospitalized cases [7, 8]. This study prospectively investigated the incidence and risk factors for influenza-associated LRTI from birth through early childhood within a South African birth cohort, providing insight into an LMIC context.

2.0 Methods

2.1 Study Design and Participants

We conducted a prospective study of LRTI and influenza-associated LTRI in children enrolled in the Drakenstein Child Health Study (DCHS), a South African birth cohort, from birth through 5 years [9, 10]. The methodology for DCHS has been described [11]. Briefly, pregnant women older than 18 years, in their 2nd trimester of pregnancy and residing in the Drakenstein region, attending one of the two local primary health care facilities were included. Enrolment occurred from 29 May 2012 to 03 September 2015.

Infants were prospectively followed from birth through at least 5 years for LRTI and aetiological testing. Additional study visits were synchronised with routine health or immunization visits. Longitudinal measurements of risk factors for all-cause LRTI, encompassing environmental exposures, socioeconomic determinants and maternal factors, were prospectively collected.

2.2 Risk Factors Examined

Socio-economic status (SES) measures, including level of maternal education, employment status, household income, and household density, were assessed [9, 10]. Self-reported smoking and alcohol use were recorded during pregnancy and postnatally [11]. Maternal HIV status was ascertained during pregnancy as per the Western Cape prevention of vertical transmission (VTP) guidelines [12]. HIV-exposed children were tested for HIV by polymerase chain reaction (PCR) at 6 weeks, enzyme-linked immunosorbent assay (ELISA) or rapid antibody testing at 9 months, and rapid antibody testing at 18 months, as per guide- lines [12]. HIV-exposed children who tested negative for HIV were classified as HIV-exposed, uninfected. Children born to mothers without HIV were considered HIV-unexposed [9, 10].

Gestational age was determined through 2nd trimester antenatal ultrasound. Birth characteristics, including gestational age and birth weight, were collected by study staff, as previously described [13, 14]. Birth weight/height standardised z-scores were calculated using the WHO Child Growth Standards for infants born full-term (≥37 weeks) and the updated Fenton newborn growth charts for infants born preterm [9]. Prematurity was defined as birth <37 weeks' gestation, and late preterm as infants born between more than 34 weeks and up to 37 weeks of gestation (34 weeks and 0 days to 36 weeks and 6 days).

Children were vaccinated as per the national EPI. BCG was given at birth; 13-valent pneumococcal conjugate vaccine (PCV-13) at 6 weeks, 14 weeks, and 9 months; hexavalent vaccine (including acellular pertussis and *Haemophilus influenzae* type B conjugate vaccine (Hib) was administered at 6, 10, 14 weeks and 18 months; and measles vaccine at 9 and 18 months. Vaccination status of each child was verified using the national standardized immunization hand-held record, the Road to Health Card (RTHC). At the time of the study (2012-2015), South Africa's national influenza vaccination policy recommended but did not routinely implement influenza vaccination for pregnant women and children aged 6 months – 5 years in the public sector. As such, there is little/no influenza vaccinations recorded in our study.

2.3 LRTI Assessment

Data on ambulatory or hospitalised LRTI episodes were collected by trained study staff using active surveillance as previously described [9, 10]. LRTI was defined according to WHO criteria, which included a cough or difficulty in breathing with age-appropriate tachypnoea or lower chest wall indrawing (LCWI) [14]. Severe LRTI was defined by the presence of tachypnoea >60 breathes/minute or LCWI, in an infant less than 2 months or cyanosed, unable to drink, seizures, or decreased level of consciousness in children of any age. [9, 10]

Nasopharyngeal (NP) swabs were collected at scheduled study visits, as well as at any LRTI episode, to determine LRTI aetiology. The history and duration of recent respiratory symptoms were also recorded. Additionally, the admission weight of each child was used to assess nutritional status, with WHO weight-for-age Z scores (WAZ).

2.4 Laboratory Procedures

NP swabs (FLOQSwabs™, Copan Diagnostics, CA) were collected at each study visit and at LRTI. NP swabs were suspended in PrimeStore® Molecular Transport medium (Longhorn Vaccines & Diagnostics, MD, USA), transported on ice and subsequently stored at -80°C. Total nucleic acids were extracted using mechanical lysis on a TissueLyzer LT (Qiagen, Germany) followed by purification on the QIAsymphony® SP Virus/Bacteria mini kit (Qiagen, Germany) as previously described [15]. Nucleic acid extracts were tested using a quantitative real-time polymerase chain reaction (qPCR) (Fast-Track Diagnostics, Luxembourg)[7] to identify up to 33 potential pathogens including respiratory viruses (influenza A/B/C; parainfluenza 1/2/3/4; coronaviruses NL63, 229E, OC43, HKU1; human metapneumoviruses A/B, rhinovirus, respiratory syncytial viruses A/B, adenovirus, enterovirus, parechovirus, bocavirus, cytomegalovirus), bacteria (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae; Streptococcus pneumoniae, Haemophilus influenzae type b; Staphylococcus aureus; Moraxella catarrhalis; Bordetella pertussis; Klebsiella pneumoniae; Legionella species; Salmonella species; H. influenzae species*) and fungi (*Pneumocystis jirovecii*). Specimens were considered positive if they had a well-defined amplification curve that crossed the cycle threshold (C*q*) within 40 cycles. Assay performance was monitored by the positive, negative and internal amplification controls that were provided with the kit. Standard curves were derived using plasmid standards supplied by the manufacturer. We applied a predefined threshold (>6.9 $\log_{10}$ copies/mL) for defining high density *S. pneumoniae* that best differentiates case-control status, as previously described [13].

The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee (HREC) of the University of Cape Town (401/2009) and (608/2020) as well as the Western Cape Provincial Child Health Research Committee. Mothers provided written informed consent at enrolment and annually thereafter in their language of preference (English, Afrikaans, or isiXhosa).

2.5 Statistical Analysis

Simple descriptive statistics were used to describe the study population, summarising continuous data as means (with standard deviations, SD) or medians (with interquartile ranges, IQR) as appropriate, using STATA software (Stata Corporation, College Station, TX). Categorical data were presented as frequencies and proportions. Mann-Whitney rank sum or Kruskal-Wallis tests were used to test for differences in continuous variables. Pearson Chi-square ($\chi 2$) test or Fisher's Exact tests were used to test for statistical differences between categorical variables with a two-tailed cut-off significance set at $p<0.05$. Generalised estimating equations (GEE) models were used to explore factors associated with influenza-associated LRTI versus non-influenza LRTI, with results presented as odds ratios (OR) with 95% confidence intervals (CI). Potential confounders, including age, were identified *a priori* and controlled for in adjusted models.

3.0 Results

3.1 Demographics and Clinical Characteristics

Of 1137 mothers included (Figure 1), there were 1143 live births (4 sets of twins and 1 set of triplets) between 29 May 2012 to 03 September 2015. Overall, 61% (695/1137) of mothers had attained an educational level below secondary, the parental employment rate was 49% (563/1143), and the monthly income was less than South African rand (ZAR) 5,000 per month

for 86% of households. Self-reported maternal smoking and alcohol use occurred in 28% (323/1142) and 13% (137/1067), respectively. No maternal vaccination against influenza was given during pregnancy, and only one mother received influenza vaccination postnatally.

Of 1143 children, 51% (586/1143) were male, 17% (189/1143) were prematurely born (<37 weeks' gestation), of which 68% (128/189) were late preterm. Further, 22% (248/1143) of the children were HIV-exposed, and only 2 were HIV-infected. Immunization coverage for all expanded programmes on immunization vaccines was high (>98%), including for Haemophilus influenzae type B (Hib) and pneumococcal 13-valent conjugated vaccine (PCV-13). However, no child received the influenza vaccine during follow-up. A total of 981 children were enrolled and followed up until 5 years of age (Figure 1).

Over the 5-year period from May 2012 to September 2015, the 1143 children enrolled contributed to 5052 child-years of follow-up period. We recorded 1108 LRTI episodes, with an incidence of 22 episodes per 100 child-years [95% CI: 21, 23]. Among 472 children with qPCR results, 70 influenza-associated LRTI episodes occurred in 14% (64/472, [6 children had two episodes each]) of the children, resulting in an incidence of 1.4 episodes per 100 child-years, [95% CI: 1.1, 1.8] (Table 1).

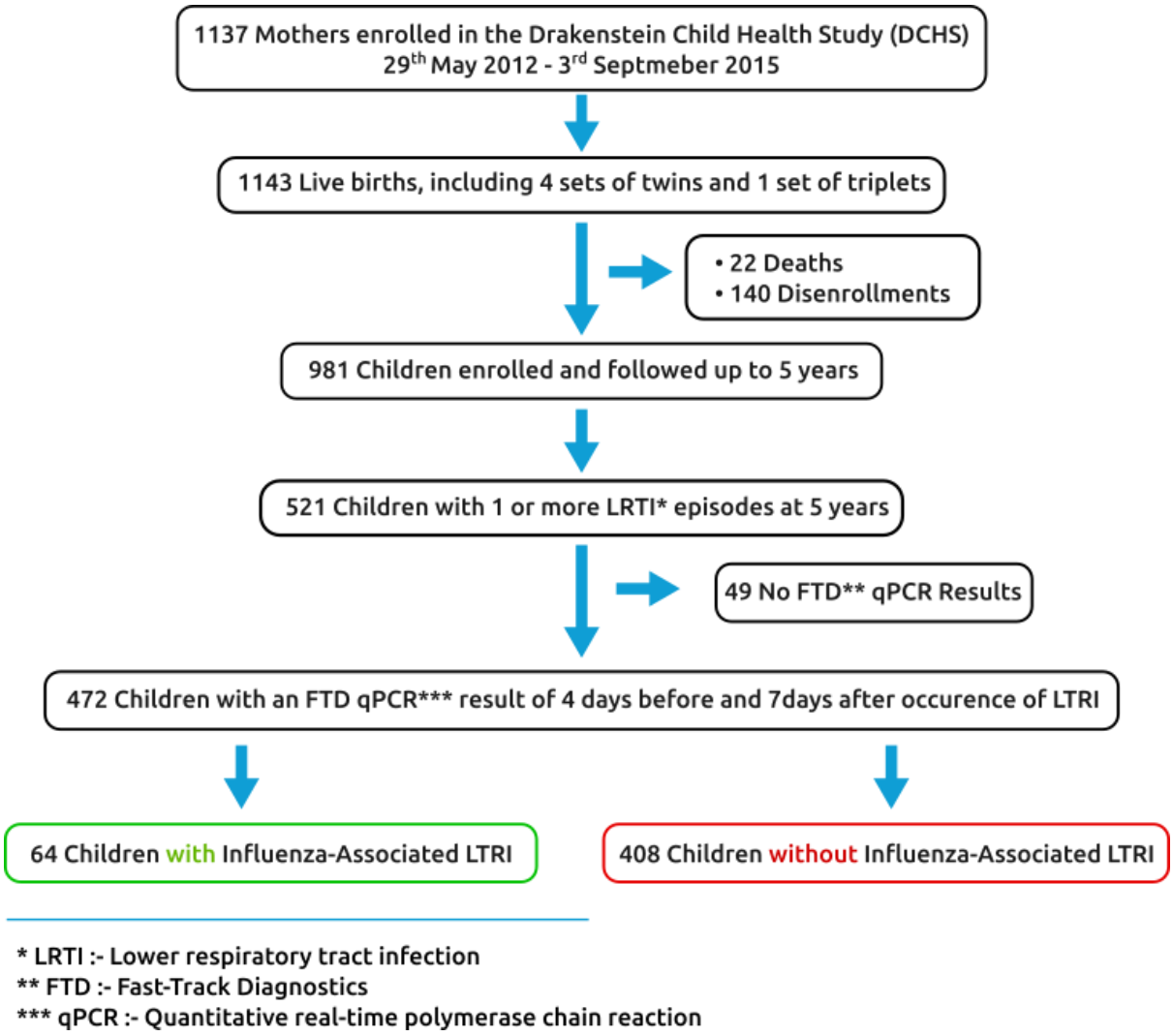

Figure 1 :Study LTRI flow diagram

3.2 Risk Factors for LRTI

LRTI was associated with lower level of maternal education, maternal HIV, alcohol use, preterm delivery, male child and birth month, season (winter) (Table 1).

Table 1 : Maternal, household, and Infant characteristics associated with LRTI				
	All children (N=1143)	**Children with LRTI episode(s) (N=521)**	**Children with no LRTI episodes (n=622)**	***p*-value**
Maternal characteristics				
Median (IQR) Maternal age	25.8 [22.0,30.8]	26.3 [22.0, 31.2]	25.4 [22.0, 30.2]	0.131
Less than secondary *Secondary/any tertiary*	695 (61%) 448 (39%)	338 (65%) 183 (35%)	357 (57%) 265 (43%)	**0.010**

Maternal self-reported prenatal alcohol use (n=1067)	137 (13%)	80 (16%)	57 (10%)	**0.005**
Maternal self-reported prenatal smoking (n=1142)	323 (28%)	162 (31%)	161 (26%)	**0.049**
Maternal self-reported postnatal smoking (n=1080)	381 (35%)	194 (38%)	187 (33%)	0.097
Influenza vaccination	1 (<1%)	1 (<1%)	0 (0%)	-
Parent employed	580 (51%)	255 (49%)	325 (52%)	0.265
Household income per month (South African Rand; n=1142))				
<R1000/m	386 (34%)	173 (33%)	213 (34%)	
R1000-5000/m	596 (52%)	282 (54%)	314 (51%)	
>R5000/m	160 (14%)	66 (13%)	94 (15%)	0.364
Housing type: Informal shack (n=1139)	419 (37%)	197 (38%)	222 (36%)	0.481
Overcrowding (n=1141)				
≤3 household members	381 (33%)	167 (32%)	214 (34%)	
4-5 household members	387 (34%)	176 (34%)	211 (34%)	
≥6 household members	373 (33%)	177 (34%)	196 (32%)	0.607
Delivery mode: Caesarean section (n=1136)	230 (20%)	104 (20%)	126 (20%)	0.873
Infant characteristics				
Male	586 (51%)	300 (58%)	286 (46%)	**<0.001**
Preterm (<37 weeks gestation)	189 (17%)	107 (21%)	82 (13%)	**0.001**
Weight-for-age z-score at birth (n=1138)	-0.25 [-0.93, 0.40]	-0.34 [-1.0, 0.33]	-0.23 [-0.87, 0.47]	**0.020**
HIV-exposed uninfected	248 (22%)	133 (26%)	115 (18%)	**0.004**
Birth season				
Summer	287 (25%)	129 (25%)	158 (25%)	
Autumn	281 (25%)	152 (29%)	129 (21%)	
Winter	306 (27%)	127 (24%)	179 (29%)	
Spring	269 (24%)	113 (22%)	156 (25%)	**0.009**
Months of breastfeeding (n=1067)	6.0 [1.7, 18.0]	6.0 [1.6, 18.1]	6.0 [1.8, 18.0]	0.898
Months of exclusive breastfeeding (n=1066)	1.5 [0.7, 3.2]	1.4 [0.7, 3.2]	1.6 [0.7, 3.2]	0.543
Vaccination coverage				
6 weeks (n=1059)	1055 (>99%)	507 (>99%)	548 (>99%)	1.000
10 weeks (n=1046)	1041 (>99%)	507 (>99%)	534 (99%)	1.000
14 weeks (n=1034)	1028 (99%)	503 (>99%)	525 (99%)	0.687
9 months (n=1010)	993 (98%)	490 (99%)	503 (98%)	0.504
18 months (n=914)	819 (90%)	394 (89%)	425 (90%)	0.522
Influenza vaccination – child	0 (0%)	0 (0%)	0 (0%)	-

Data presented as n (%) or median [inter-quartile range; IQR].

Table 2 : Comparison of Maternal, household, and infant characteristics in children with influenza or non-influenza LRTI

	All children with LRTI episode(s) (N=472)	**Influenza-associated LRTI (N=64)**	**Non-Influenza LRTI (N=408)**	***p*-value**
Maternal characteristics				
Maternal age (years)	26.3 [22.0, 31.4]	26.6 [23.0, 31.7]	26.3 [21.7, 31.2]	0.330

Maternal education				
Less than secondary	301 (64%)	35 (55%)	266 (65%)	
Secondary/any tertiary	172 (36%)	29 (45%)	142 (35%)	0.104
Maternal self-reported prenatal alcohol use (n=457)	74 (16%)	11 (18%)	63 (16%)	0.722
Maternal self-reported prenatal smoking (n=471)	143 (30%)	16 (25%)	127 (31%)	0.316
Maternal self-reported postnatal smoking (n=467)	177 (38%)	21 (33%)	156 (39%)	0.366
Influenza vaccination – mother	1 (<1%)	0 (0%)	1 (<1%)	-
Housing type: Informal shack (n=471)	181 (38%)	28 (44%)	153 (38%)	0.346
Overcrowding (n=471)				
≤3 household members	150 (32%)	22 (34%)	128 (31%)	
4-5 household members	160 (34%)	23 (36%)	139 (34%)	
≥6 household members	159 (34%)	19 (30%)	140 (34%)	0.755
Parent employed	233 (49%)	35 (55%)	198 (49%)	0.360
Household income per month (South African Rand)				
<R1000/m	153 (33%)	24 (38%)	131 (32%)	
R1000-5000/m	254 (54%)	35 (55%)	219 (54%)	
>R5000/m	63 (13%)	5 (8%)	58 (14%)	0.333
Infant characteristics				
Male	271 (57%)	32 (50%)	239 (59%)	0.197
Delivery mode: Caesarean section (n=471)	95 (20%)	12 (19%)	83 (20%)	0.811
Preterm (<37 weeks' gestation)	98 (21%)	15 (23%)	83 (20%)	0.570
Weight-for-age z-score at birth (n=471)	-0.33 [-1.00, 0.35]	-0.23 [-1.26, 0.26]	-0.36 [-0.98, 0.38]	0.855
HIV-exposed	118 (25%)	22 (34%)	96 (24%)	0.062
Months of breastfeeding (n=460)	6.0 [1.5, 18.0]	6.0 [2.0, 13.7]	6.0 [1.4, 18.0]	0.637
Months of exclusive breastfeeding (n=459)	1.4 [0.7, 3.1]	1.6 [0.9, 3.3]	1.4 [0.7, 3.0]	0.669
Vaccination coverage				
6 weeks (n=463)	461 (>99%)	63 (98%)	398 (>99%)	0.258
10 weeks (n=463)	461 (>99%)	63 (98%)	398 (>99%)	0.258
14 weeks (n=460)	458 (>99%)	63 (98%)	395 (>99%)	0.259
9 months (n=451)	444 (98%)	62 (97%)	382 (99%)	0.260
18 months (n=403)	356 (88%)	50 (83%)	306 (89%)	0.191
Influenza vaccination – child	0 (0%)	0 (0%)	0 (0%)	-

Data presented as n (%) or median [inter-quartile range; IQR]; EBF: exclusive breastfeeding.

3.3 Clinical Features of Influenza- LTRI

Children with influenza-associated LRTI were older than those with non-influenza LRTI (Table 3). Among children with influenza-associated LRTI, 13% (9/70) were hospitalised, and there

was a single death; severity was similar to those with non-influenza LRTI (Table 3). There were no clinical features that distinguished influenza-associated LRTI from non-influenza LRTI except wheezing, which was less common in influenza-associated illness, summarized in Table 3

Table 3 : Clinical features of Influenza-associated LRTI compared to non-Influenza LRTI				
Variable	**All LRTI episodes**	**Influenza-associated LRTI [1] episodes**	**Non-Influenza LRTI episodes**	***p*-value [2]**
Number of episodes	963	70	893	
Age in months at LRTI	9.3 [3.8, 19.3]	14.1 [8.5, 19.1]	8.9 [3.7, 19.5]	**0.007**
Weight-for-age z-score at LRTI (n=957)	-0.08 [-0.98, 0.82]	-0.03 [-0.89, 0.81]	-0.08 [-1.00, 0.82]	0.752
HIV exposed uninfected	257 (27%)	25 (36%)	232 (26%)	**0.076**
Season of LRTI				
Summer	134 (14%)	3 (4%)	131 (15%)	
Autumn	276 (29%)	14 (20%)	262 (29%)	
Winter	316 (33%)	44 (63%)	272 (30%)	
Spring	237 (25%)	9 (13%)	228 (26%)	<0.001
Symptoms and signs				
Fever (n=934)	634 (68%)	54 (77%)	580 (67%)	0.147
Cough (n=962)	915 (95%)	70 (100%)	845 (95%)	-
Rhinorrhoea (n=956)	604 (63%)	51 (73%)	553 (62%)	0.362
Heart rate (n=914)	142 [132, 160]	136 [132, 157]	143 [132, 160]	0.252
Respiratory Rate (n=928)	53 [46,60]	48 [44,56]	54[46,60]	0.038
Lower chest wall indrawing (n=935)	626 (67%)	41 (63%)	585 (67%)	0.489
Chest auscultation abnormal (n=939)	540 (58%)	32 (48%)	508 (58%)	0.148
Wheezing (n=962)	400 (42%)	20 (29%)	380 (43%)	**0.047**
Oxygen saturation (n=910)	98 [95, 99]	98 [96, 99]	98 [95, 99]	0.491
Severity and outcomes				
Oxygen given	120 (12%)	4 (6%)	116 (13%)	0.268
Hospitalized	219 (23%)	9 (13%)	210 (24%)	0.127
Discharge status, among children hospitalized (n=219)	211 (96%)	8 (89%)	203 (97%)	0.289
Discharged home Transferred to another health facility	4 (2%)	0 (0%)	4 (2%)	
Died	4 (2%)	1 (11%)	3 (1%)	
Data presented as n (%) or median [inter-quartile range; IQR]; [1] 70 Influenza-associated LRTI among 64 children; [2] P-values from generalised estimating equation (GEE) models, adjusted for age at episode.				

3.4 Risk Factors Associated with Influenza-Associated LTRI

Generalised estimating equation (GEE) models, adjusted for all covariates, indicated that influenza-associated LRTI was significantly associated with the winter season (aOR: 7.06; 95% CI [2.15, 23.13], $p = 0.001$) (Table 4).

Table 4 : Multivariate analysis or risk factors associated with Influenza-associated LRTI versus non-Influenza LRTI

Maternal characteristics	**Unadjusted OR [95%CI][1]**	**P-value**	**Adjusted OR [95% CI][2]**	***p*-value**
Self-reported prenatal smoking	0.69 [0.39, 1.23]	0.206	0.65 [0.26, 1.63]	0.357
Self-reported postnatal smoking	0.71 [0.42, 1.22]	0.215	0.89 [0.39, 2.05]	0.790
Household characteristics				
Parent employed	1.38 [0.84, 2.27]	0.203	1.33 [0.72, 2.45]	0.356
Household income per month (ref: >R5000/m)				
R1000-5000/m	2.02 [0.75, 5.39]	0.163	2.08 [0.78, 5.55]	0.142
<R1000/m	2.14 [0.82, 5.58]	0.122	2.41 [0.83, 7.03]	0.106
Child characteristics				
Age at LRTI (months)	1.02 [1.00, 1.04]	**0.043**	1.02 [1.00, 1.04]	0.085
Sex: Male (vs female)	0.61 [0.38, 1.01]	0.052	0.65 [0.39, 1.09]	0.105
Born preterm	0.87 [0.48, 1.58]	0.644	0.89 [0.48, 1.65]	0.709
Birth weight-for-age z-score	0.93 [0.74, 1.18]	0.565	0.96 [0.74, 1.23]	0.735
HIV-exposed	1.58 [0.94, 2.65]	0.084	1.49 [0.86, 2.58]	0.155
Season of birth (ref: summer)				
Autumn	0.50 [0.24, 1.03]	0.062	0.57 [0.27, 1.19]	0.134
Winter	0.96 [0.49, 1.89]	0.905	0.92 [0.46, 1.87]	0.825
Spring	1.00 [0.51, 1.97]	0.993	1.21 [0.60, 2.46]	0.593
Duration of exclusive breastfeeding (months)	0.97 [0.85, 1.11]	0.669	0.99 [0.87, 1.12]	0.829
Season of LRTI (ref: summer)				
Autumn	2.33 [0.66, 8.24]	0.190	2.05 [0.56, 7.47]	0.276
Winter	7.06 [2.15, 23.13]	**0.001**	7.83 [2.34, 26.14]	**0.001**
Spring	1.72 [0.46, 6.47]	0.421	1.94 [0.51, 7.39]	0.331

[1] Odds ratios (OR) from generalised estimating equation (GEE) models; [2] Adjusted for all covariates shown.

3.5 Respiratory Organisms Associated with Influenza-Associated LTRI

Table 5 summarises the associations between other organisms detected in NP swabs at the time of influenza-associated LRTI. Children with influenza-associated LRTI had a reduced likelihood of having rhinovirus (aOR: 0.07, [95% CI: 0.02-0.23]), RSV A/B (aOR: 0.26, [95%

CI: 0.09-0.72]), parainfluenza virus 1/2/3/4 (aOR: 0.13, [95% CI: 0.02-0.97]), or *S. aureus* (aOR: 0.29, [95% CI: 0.09-0.99]). Co-infections with *M. catarrhalis* and *S. pneumoniae* were noted, although not significant.

Table 5 : Associations between other microbes and Influenza-associated LRTI versus non-Influenza LRTI during the first 5 years of life, among 963 LRTI episodes

	Adjusted OR [95% CI] [1]	***p*-value**
Viruses		
Cytomegalovirus	0.79 [0.48, 1.30]	0.353
Rhinovirus	0.10 [0.04, 0.27]	<**0.001**
RSV A/B	0.25 [0.09, 0.71]	**0.009**
Adenovirus	0.81 [0.39, 1.66]	0.562
Parainfluenza 1/2/3/4	0.13 [0.02, 0.96]	**0.046**
Bacteria		
M. catarrhalis	1.18 [0.63, 2.21]	0.603
S. pneumoniae	1.32 [0.78, 2.22]	0.299
S. pneumoniae >10^6.95 copies	0.80 [0.40, 1.59]	0.518
H. influenzae	0.81 [0.50, 1.31]	0.390
S. aureus	0.29 [0.09, 0.97]	**0.045**
K. pneumoniae	0.69 [0.25, 1.92]	0.477

[1]Odds ratios (OR) from generalised estimating equation (GEE) models, adjusted for age at episode.

4.0 Discussion

This birth cohort study provides a comprehensive longitudinal analysis of the incidence and risk factors associated with influenza-associated LRTI through the first 5 years of life in an LMIC context. The cohort comprised 1143 live-born children. There were 70 episodes of influenza-associated LRTI recorded in 64 children (1.4 cases per 100 child-years). Of these LRTI episodes 13% (9/70) were hospitalised, and 11% (1/9) of influenza-associated LRTI-related deaths were recorded. The incidence of influenza-associated LRTI reported in our setting is higher than in a Nicaraguan cohort (1.0 cases [0.8–1.2] per 100 person-years) [16]. The difference could be attributed to the lack of influenza vaccination for pregnant women and children over the age of 6 months of age in our setting as seen on Table 2, compared to the Nicaraguan cohort which had a coverage of over 71% in pregnant women [17]. In the absence of maternally transferred antibodies or vaccine-induced immunity during early infancy, young

children remain susceptible to circulating infectious agents [18]. The reported incidence in our setting is likely to be underestimated, as we did not include episodes without FTD Resp33 qPCR results, 13% (145/1108).

The incidence of LRTI appears to be higher among HIV-exposed children. Data on the epidemiology of influenza in children living with HIV remain limited, particularly in sub-Saharan Africa, where HIV prevalence is high. [19, 20]. In a South African cohort of children under five years of age, influenza virus was detected in 7% (613/8,394) of enrolled participants, 358 of whom were children living with HIV. The incidence of influenza-associated acute lower respiratory tract infection (LRTI) was four to eight times higher among children living with HIV (186–228 per 100,000) than among HIV-unexposed uninfected children (26–54 per 100,000) (*Cohen et al., 2015*). In our cohort, although few participants were children living with HIV, a significantly higher proportion of children with LRTI episodes (26%, n = 133) were HIV-exposed uninfected than those without LRTI episodes (18%). This increased susceptibility among HIV-exposed uninfected children may reflect the impact of maternal immune compromise during pregnancy. [19].

Besides the factors regarding vaccination and HIV exposure, several other risk factors for influenza-associated LRTI were identified in this birth cohort including maternal education and prenatal smoking. In our study, a higher proportion of mothers with nothing beyond secondary education (61%, n= 695) had their children experiencing LRTI episodes than mothers with secondary/tertiary education. Our analysis was similar to studies conducted by Amugsi *et al* [18] which showed that maternal decision-making based on educational level was associated with childhood morbidity and also showed the impact of socioeconomic disadvantage on disease susceptibility [18]. In our study, we observed a higher frequency of LRTI episodes in children whose mothers smoked during pregnancy due to suspected issues such as preterm birth, low birth weight, and potential harm to the baby's developing lungs and brain [21, 22].

Smoking during pregnancy has consistently shown harmful effects on both pregnant women and their unborn children [23-25]

Among infant-specific factors, preterm birth (<37 weeks gestation) significantly elevated LRTI risk (p=0.001), consistent with the known vulnerability of preterm infants to respiratory infections due to immature immune systems and underdeveloped lungs [26, 27]. In addition, preterm infants are more likely to have a low birth weight, and this has been associated with an increased risk of LRTIs, including hospitalisation due to pneumonia [28, 29]. Our study found a significantly higher risk of LRTI episodes in low-birth-weight children, consistent with a case study by Nascimento et al. [30] who attributes this to reduced immune response and weakened pulmonary function in children with low birth weight [30, 31]. Our results also showed that influenza-associated LTRI was significantly more common during the winter months ($p < 0.001$), coinciding with other studies on the reported high rates of hospitalisation due to influenza-associated LTRI and other seasonal respiratory outbreaks [32]. This finding has the effect of creating and reinforcing public health policies that emphasise targeted preventive measures during peak influenza seasons.

While our study benefited from a prospective design, a large and well-characterized cohort, and rigorous longitudinal follow-up, certain limitations should be acknowledged. The low influenza vaccination rate in the study and in South Africa limits the generalizability of our findings to settings with higher vaccination coverage. Furthermore, despite adjusting for various potential confounders, residual confounding cannot be entirely ruled out. Future studies should explore the potential interactions between different pathogens in co-infections and investigate the interplay between host immune response and environmental factors in modulating the severity and progression of LRTI in children, which reflects the cumulative impact of various risk factors, including nutritional and non-nutritional prenatal or postnatal exposures [33].

5.0 References

1. Wang X, Li Y, O'Brien KL, Madhi SA, Widdowson M-A, Byass P, Omer SB, Abbas Q, Ali A, Amu A *et al*: **Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study**. *Lancet Glob Health 2020; 8(4):e497-e510* 2020, **8**(4).
2. Fell DB, Azziz-Baumgartner E, Baker MG, Batra M, Beauté J, Beutels P, Bhat N, Bhutta ZA, Cohen C, De Mucio B *et al*: **Influenza epidemiology and immunization during pregnancy: Final report of a World Health Organization working group**. *Vaccine 35(43):5738-5750* 2017, **35**(43).
3. Ortiz JR, Neuzil KM: **Influenza Immunization in Low- and Middle-Income Countries: Preparing for Next-Generation Influenza Vaccines**. *The Journal of Infectious Diseases* 2019, **219**(Supplement_1):S97-S106.
4. Tempia S, Walaza S, Moyes J, McMorrow ML, Cohen AL, Edoka I, Fraser H, Treurnicht FK, Hellferscee O, Wolter N: **Influenza disease burden among potential target risk groups for immunization in South Africa, 2013–2015**. *Vaccine* 2020, **38**(27):4288-4297.
5. Walaza S, Cohen C, Nanoo A, Cohen AL, McAnerney J, von Mollendorf C, Moyes J, Tempia S: **Excess Mortality Associated with Influenza among Tuberculosis Deaths in South Africa, 1999-2009**. *PLoS One* 2015, **10**(6):e0129173.
6. Cohen C, Walaza S, Moyes J, Groome M, Tempia S, Pretorius M, Hellferscee O, Dawood H, Chhagan M, Naby F: **Epidemiology of viral-associated acute lower respiratory tract infection among children< 5 years of age in a high HIV prevalence setting, South Africa, 2009–2012**. *The Pediatric infectious disease journal* 2015, **34**(1):66-72.

7. Solanki G, Cornell M, Lalloo R: **Uptake and cost of influenza vaccines in a private health insured South African population**. *S Afr J Infect Dis* 2018, **33**(5).

8. O'Brien MA, Uyeki TM, Shay DK, Thompson WW, Kleinman K, McAdam A, Yu XJ, Platt R, Lieu TA: **Incidence of outpatient visits and hospitalizations related to influenza in infants and young children**. *Pediatrics* 2004, **113**(3 Pt 1):585-593.

9. Fenton TR, Nasser R, Eliasziw M, Kim JH, Bilan D, Sauve R: **Validating the weight gain of preterm infants between the reference growth curve of the fetus and the term infant**. *BMC Pediatr* 2013, **13**:92.

10. Fenton TR, Kim JH: **A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants**. *BMC Pediatrics* 2013, **13**(1):59.

11. Zar HJ, Barnett W, Myer L, Stein DJ, Nicol MP: **Investigating the early-life determinants of illness in Africa: the Drakenstein Child Health Study**. *Thorax* 2015, **70**(6):592-594.

12. Health PGotWCDo: **The Western Cape consolidated guidelines for HIV treatment: Prevention of mother-to-child transmission of HIV (PMTCT), children, adolescents and adults**. *2015 (Amended Version) In: Department of Health HASTHD, editor* 2015.

13. Walaza S, Tempia S, Dawood H, Variava E, Moyes J, Cohen AL, Wolter N, Groome M, von Mollendorf C, Kahn K *et al*: **Influenza virus infection is associated with increased risk of death amongst patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010–2011**. *BMC Infectious Diseases* 2015, **15**(1):26.

14. Roux DMl, Myer L, Nicol MP, Zar HJ: **Incidence of childhood pneumonia: facility-based surveillance estimate compared to measured incidence in a South African birth cohort study**. *BMJ Open* 2015, **5**(12):e009111.

15. Dube FS, Kaba M, Robberts FJ, Ah Tow L, Lubbe S, Zar HJ, Nicol MP: **Respiratory microbes present in the nasopharynx of children hospitalised with suspected**

pulmonary tuberculosis in Cape Town, South Africa. *BMC Infect Dis* 2016, **16**(1):597.

16. Maier HE, Kuan G, Gresh L, Chowell G, Bakker K, Lopez R, Sanchez N, Lopez B, Schiller A, Ojeda S *et al*: **The Nicaraguan Pediatric Influenza Cohort Study, 2011-2019: Influenza Incidence, Seasonality, and Transmission**. *Clin Infect Dis* 2023, **76**(3):e1094-e1103.

17. Arriola CS, Vasconez N, Thompson M, Mirza S, Moen AC, Bresee J, Talavera I, Ropero AM: **Factors associated with a successful expansion of influenza vaccination among pregnant women in Nicaragua**. *Vaccine* 2016, **34**(8):1086-1090.

18. Amugsi DA, Aborigo RA, Oduro AR, Asoala V, Awine T, Amenga-Etego L: **Socio-demographic and environmental determinants of infectious disease morbidity in children under 5 years in Ghana**. *Global health action* 2015, **8**(1):29349.

19. Cohen C, Moyes J, Tempia S, Groom M, Walaza S, Pretorius M, Dawood H, Chhagan M, Haffejee S, Variava E *et al*: **Severe influenza-associated respiratory infection in high HIV prevalence setting, South Africa, 2009-2011**. *Emerg Infect Dis* 2013, **19**(11):1766-1774.

20. Madhi SA, Schoub B, Simmank K, Blackburn N, Klugman KP: **Increased burden of respiratory viral associated severe lower respiratory tract infections in children infected with human immunodeficiency virus type-1**. *The Journal of Pediatrics* 2000, **137**(1):78-84.

21. Stick SM, Burton PR, Gurrin L, Sly P, LeSouef P: **Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants**. *The Lancet* 1996, **348**(9034):1060-1064.

22. Horta BL, Victora CG, Menezes AM, Halpern R, Barros FC: **Low birthweight, preterm births and intrauterine growth retardation in relation to maternal smoking**. *Paediatric and perinatal epidemiology* 1997, **11**(2):140-151.

23. Butler NR, Goldstein H: **Smoking in Pregnancy and Subsequent Child Development**. *British Medical Journal* 1973, **4**(5892):573-575.

24. KĀLLÉN K: **The impact of maternal smoking during pregnancy on delivery outcome**. *European Journal of Public Health* 2001, **11**(3):329-333.

25. Hardy J, Mellits ED: **DOES MATERNAL SMOKING DURING PREGNANCY HAVE A LONG-TERM EFFECT ON THE CHILD ?** *The Lancet* 1972, **300**(7791):1332-1336.

26. Collins A, Weitkamp JH, Wynn JL: **Why are preterm newborns at increased risk of infection?** *Arch Dis Child Fetal Neonatal Ed* 2018, **103**(4):F391-f394.

27. Lawn JE, Davidge R, Paul VK, Xylander Sv, de Graft Johnson J, Costello A, Kinney MV, Segre J, Molyneux L: **Born too soon: care for the preterm baby**. *Reproductive health* 2013, **10**(1):1-19.

28. Coles CL, Fraser D, Givon-Lavi N, Greenberg D, Gorodischer R, Bar-Ziv J, Dagan R: **Nutritional Status and Diarrheal Illness as Independent Risk Factors for Alveolar Pneumonia**. *American Journal of Epidemiology* 2005, **162**(10):999-1007.

29. Suwanjutha S, Ruangkanchanasetr S, Chantarojanasiri T, Hotrakitya S: **Risk factors associated with morbidity and mortality of pneumonia in Thai children under 5 years**. *Southeast Asian J Trop Med Public Health* 1994, **25**(1):60-66.

30. Nascimento L, Marcitelli R, Agostinho F, Gimenes C: **Hierarchical approach to determining risk factors for pneumonia in children**. *Jornal Brasileiro de Pneumologia* 2004, **30**:445-451.

31. Wilcox AJ: **On the importance--and the unimportance--of birthweight**. *Int J Epidemiol* 2001, **30**(6):1233-1241.

32. Ahrens JO, Morrow BM, Argent AC: **Influenza A(H1N1)pdm09 in critically ill children admitted to a paediatric intensive care unit, South Africa**. *Southern African Journal of Critical Care (Online)* 2015, **31**:4-7.

33. Roth DE, Caulfield LE, Ezzati M, Black RE: **Acute lower respiratory infections in childhood: opportunities for reducing the global burden through nutritional interventions**. *Bull World Health Organ* 2008, **86**(5):356-364.

PART C: APPENDICES

APPENDIX 1: HUMAN RESEARCH ETHICS COMMITTEE (HREC) APPROVAL LETTER

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

06 October 2020

HREC REF: 608/2020

Mr R MacGinity
Department of Paediatrics & Child Health
Unit B11 (REACH) Red Cross War Memorial Children's Hospital
Rondebosch
Email: rae.macginity@uct.ac.za
Student: nchimunya.hapeela@myuct.ac.za

Dear Mr MacGinity

PROJECT TITLE: RISK FACTORS ASSOCIATED WITH CHILDHOOD SEASONAL INFLUENZA IN A LIMITED RESOURCE SETTING" LINKED TO DRAKENSTEIN CHILD HEALTH STUDY (DCHS)- MPHIL CANDIDATE- NCHIMUNYA HAPEELA-SUB-STUDY LINKED TO 401/2009

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

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

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

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

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

The HREC acknowledge that the student: Nchimunya Hapeela will also be involved in this study.

Please quote the HREC REF in all your correspondence.

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

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

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/REF:608/2020sa

APPENDIX 2: HUMAN RESEARCH ETHICS COMMITTEE (HREC) ANNUAL RENEWAL

HUMAN RESEARCH ETHICS COMMITTEE
20 OCT 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA · UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.10.2023
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee	Signed by candidate	Date Signed	23/10/2022

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	19 Oct 2022		
HREC REF Number	608/2020	Current Ethics Approval was granted until	30 Oct 2022
Protocol title	Risk Factors Associated With Childhood Seasonal Influenza In A Limited Resource Setting		
Protocol number (if applicable)	NA		
Are there any sub-studies linked to this study?	☐ Yes	✓ No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note**: A separate FHS016 must be submitted for each sub-study.			

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Principal Investigator	Mr. Rae MacGinty
Department / Office Internal Mail Address	Faculty of Health Sciences Department of Paediatrics and Child Health Child Health Unit University of Cape Town

1.1 Does this protocol receive US Federal funding?	☐ Yes	✓ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	***Description***	***New fee (Vat Incl.)***	***tick ✓***
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	✓
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. **Invoice billing – Directly to Sponsor**

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	
Contact person	
Telephone number	
Email Address	
2. **Internal Journal Billing:**	
Fund Number:	
Cost Centre Number:	
Account Holder Name:	
Division of Account Holder:	

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open Enrolment
☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
✓	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	1232
Number of participants enrolled, since last HREC Progress report (continuing review)	0

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	605

6. Cumulative summary of participants

Total number of participants who provided consent	1232
Number of participants determined to be ineligible (i.e. after screening)	1471
Number of participants currently active on the study	980
Number of participants completed study (without events leading to withdrawal)	0
Number of participants withdrawn at participants' request (i.e. changed their mind)	33
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	0
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	239
Unable to contact: 37 Relocated: 82 Pregnancy losses/Child died: 67 Mother/guardian unable to attend clinic: 33 Other:20 Total:239	
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	0

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
Risk Factors Associated With Childhood Seasonal Influenza In A Limited Resource Setting is a sub study of the Drakenstein Child Health study (HREC 401/2009). Substantial progress has been made towards achieving the objectives of the study. Data collection and curation is complete. Initial exploratory statistical analysis and regression analysis is almost finalised for inclusion into the final thesis write up.

8. Protocol violations and exceptions (tick ✓ all that apply)

✓	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

✓	No Prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	✓ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

☐ Yes	☐ No	✓ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	✓ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	✓ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
☐	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ Yes	☐ No	☐ Not Applicable – N/A	
If yes, please complete the following:			
Insurer's name:			
Policy no.		*Coverage Period:	
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	✓ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI	Signed by candidate	Date	11 October 2022

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

v

APPENDIX 3: MATERNAL PHYSICAL EXAMINATION FORM

CRF0X: Maternal Physical Examination Form

Visit: ☐ ANC2 Mother Participant ID: _ _ _ _ /_ / _ / _ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

1	Are you currently well?	☐Yes ☐No
2	If No, what is the problem/s? *Tick all that apply.*	☐High blood pressure ☐Heart problem ☐Depression ☐HIV ☐Urine Infection ☐Asthma ☐TB ☐Emphysema ☐Chronic Bronchitis ☐Pneumonia ☐Other (specify):

PREGNANCY COMPLICATIONS		
Have you been diagnosed with any of the following during this pregnancy? *Circle answer.*		Yes/No
3	Diabetes	Y / N
4	Hyperemesis (excessive vomiting)	Y / N
5	Eclampsia	Y / N
6	Pre-eclampsia	Y / N
7	Pelvic inflammatory disease	Y / N
8	High blood pressure	Y / N
9	Asthma	Y / N
10	Other	Y / N
11	Specify:	

RESPIRATORY SYMPTOMS		
Do you currently have any of the following symptoms? *Circle answer.*		
	Respiratory Symptoms	Yes/No
12	Wheezing	Y / N
13	Tightness in your chest	Y / N
14	Shortness of breath	Y / N
15	Coughing	Y / N
16	Phlegm on the chest	Y / N
17	Are you taking any supplements or medicines currently? *Record in medication chart.*	Y / N

CRF0X: Maternal Physical Examination Form

Visit: ☐ ANC2 Mother Participant ID: _ _ _ _ /_ / _ / _ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

PHYSICAL EXAMINATION			
18	Weight		(kg)
19	Height		(cm)
20	Height of fundus (SF measurement)		(cm)
21	Blood pressure		
22	Heart Rate		per minute
23	Respiratory Rate		per minute
24	Witnessed cough	☐Yes ☐No	
25	Audible wheeze	☐Yes ☐No	
	Record if any of the following are present:		Yes/No
	26	Clubbing	Y / N
	27	Pallor	Y / N
	28	Oedema	Y / N
	29	Jaundice	Y / N
30	Chest auscultation: Is chest auscultation normal?	☐Yes (if yes, skip to Q32) ☐No (complete Q31)	
	Findings *Circle answer.*	Left Side Yes/No	Right Side Yes/No
31	Wheeze	Y / N	Y / N
32	Have you been diagnosed with any of the following today? *Tick all that apply.*	☐HIV ☐High blood pressure ☐TB ☐Diabetes ☐Pneumonia ☐Infection ☐Asthma (wheezing) ☐ Other (specify): _____	
33	Was any medication started today? *If yes, record in medication chart*	☐ARVs ☐Supplements/multivitamins ☐Antibiotics ☐Anti-hypertensive/blood pressure meds ☐Iron ☐TB Meds ☐Other (specify):_____	
34	Were any of the following investigations done today? *If yes, complete on maternal results form.*	☐FBC ☐Urine Dipstick ☐U & E ☐Haemoglobin Glucose Test ☐Random Glucose Level ☐Fasting Glucose Level ☐24 Hour Urine Creatinine Collection ☐Urine MC&S ☐Other (specify): _____	
CRF Completed by: _____ Date: _ _ / _ _ _ / _ _ _ _ DD MMM YYYY			

CRF0X: Food Security Questionnaire

Visit: ☐ ANC2 Mother Participant ID: _ _ _ _ / _ /_ /_ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

FOOD SECURITY QUESTIONNAIRE		
1	In the last 3 months, were any meals made smaller for the children in your home because there wasn't enough money for food?	☐ Yes ☐ No
2	In the last 3 months, did any children in the household ever skip the following meals because there wasn't enough money for food? *Please tick all that apply.*	☐ Breakfast ☐ Lunch ☐ Supper ☐ No meals skipped (go to Q4)
3	If yes, how often did this happen in the past three months?	☐ Every month ☐ During one month ☐ During two months
4	In the last 3 months, were any children in the household ever hungry but you just couldn't afford more food?	☐ Yes ☐ No
5	In the last 3 months, did any children in the household ever not eat for a whole day because there wasn't enough money for food?	☐ Yes ☐ No

CRF Completed by: _____ Date: _ _ / _ _ _ / _ _ _ _
DD MMM YYYY

CRF0X: Adult Antenatal Food Frequency Questionnaire

Visit: ☐ ANC2 Mother Participant ID: _ _ _ _ /_ /_ /_ _ Date: _ _ / _ _ _ / _ _ _
DD / MMM / YYYY

ADULT FOOD FREQUENCY QUESTIONNAIRE					
How often do you eat the following foods? *Tick best answer choice.*					
	Food item	**Never**	**Daily**	**Weekly**	**Monthly**
1	Starch (potato/rice/pasta/bread/maize)				
2	Crisps/chips (lays, sppokies)				
3	Red meat				
4	Organ meat (liver/kidney/other)				
5	Fish (fresh/canned)				
6	Poultry (chicken/turkey)				
7	Eggs				
8	Processed meat (ham/polony/salami)				
9	Legumes (beans/peas/lentils)				
10	Alcohol				
11	Coffee				
12	Dairy (milk/yoghurt/cheese)				
13	Vegetables (fresh/frozen/canned)				
14	Fruit (fresh/frozen/canned)				
15	Cold drinks (diet/sweetened)				
16	Sweets/Chocolates/Pastries (cakes/doughnuts)				
17	Fried Foods				
18	Fruit juice				
19	Butter/brick margarine				
20	Soft/tub margarine				

CRF Completed by: _____ Date: _ _ / _ _ _/ _ _ _ _
DD MMM YYYY

APPENDIX 4: ENVIRONMENTAL ANTENATAL HOME VISIT

CRF0X: Environmental Measures Drop-off/Pick-up

Visit: ☐ Antenatal ☐ Postnatal

Mother Participant ID: __ __ __ __ / __ / __ / __ __
Child Participant ID: __ __ __ __ / __ / __ / __ __

Environmental Home Measurement		
1	Address of home:	
2	For person receiving field worker, what is their relationship to study child?	☐Mother ☐Father ☐Uncle ☐Aunt ☐Grandmother ☐Grandfather ☐Neighbour ☐Family friend ☐Other (specify): _____
3	Location of measures: N.B. Should be 1) left in the same place 2) at breathing level 3) where most persons living in home spend the most time 4) out of the reach of children 5) affixed to something to avoid disturbance 6) where the air is free flowing over devices.	☐Major living area ☐Other (specify reason):_____ _____
4	Height of radiello, VOC & Elf Pump measures (distance from the floor, if not on stand):	Approximate height in meters, if not on stand: Meters: _____
5	Height of dust samplers (distance from the floor): *Should be placed at 1.5 meters from floor.*	☐< 1 meter high ☐approximately 1 meter high ☐ 1> 2 meters high ☐> 2 meters high
6	Where was dust sampler placed? *Tick all that apply. It should not be placed near window or door, if possible.*	☐Main living room ☐Child's bedroom/where the child will sleep. ☐Main living room/child's bedroom (same space) ☐Near window/door

Measurement Drop off

	Device		Reference Number		Date		Time *(24 hour clock)* Hr	Min
	Radiello	7.1	__ __ __ __ __	7.2	__ __/ __ __ __/ __ __ __ __ DD / MMM / YYYY	7.3	__ __	__ __
	VOC	8.1	Mi __ __ __ __ __ __	8.2	__ __/ __ __ __/ __ __ __ __ DD / MMM / YYYY	8.3	__ __	__ __
	Elf Pump	9.1 9.2 9.3 9.4	Ref: __ __ __ __ __ __ Pump #: _____ Flow rate: _____ Minutes at start:	9.5	__ __/ __ __ __/ __ __ __ __ DD / MMM / YYYY	9.6	__ __	__ __
	CO Monitor	10.1	Serial #:	10.2	__ __/ __ __ __/ __ __ __ __ DD / MMM / YYYY	10.3	__ __	__ __

11	Checklist for drop off: All must be ticked once completed.	☐Adult in home told not to touch/move measures ☐Instructions left with adult living in home ☐Date & time of pick up specified
12	Date & time of pick up for Elf pump	Date: Time:

CRF0X: Environmental Measures Drop-off/Pick-up

Visit: ☐ Antenatal ☐ Postnatal

Mother Participant ID: __ __ __ __ / __ / __ / __ __
Child Participant ID: __ __ __ __ / __ / __ / __ __

Measurement Pick Up									
13	Are measures located in same place where it was dropped off?			☐Yes (if yes, skip to Q15) ☐No					
14	Please comment on how measures were moved: *Tick all that apply.*			☐Measures not moved ☐Radiello moved by persons in home ☐VOC moved by persons in home ☐Elf Pump moved by persons in home ☐Dust sampler (main living room) moved by persons in home ☐Dust sampler (child's bedroom) moved by persons in home ☐Found in a different location but in the same room ☐Found in a different room ☐Protective covering removed from radiello ☐Protective covering removed from VOC ☐Protective covering removed from Elf Pump ☐Other (specify): _____					
	Device	**Reference Number**			**Date**		**Time***(24 hour clock)*		
							Hr		**Min**
	Radiello	15.1	__ __ __ __ __	15.2	__ __ / __ __ __ / __ __ __ __ DD / MMM / YYYY		15.3	__ __	__ __
	VOC	16.1	Mi __ __ __ __ __ __	16.2	__ __ / __ __ __ / __ __ __ __ DD / MMM / YYYY		16.3	__ __	__ __
	Elf Pump	17.1 17.2 17.3	Ref: __ __ __ __ __ __ Pump #: _____ Minutes at finish:	17.4	__ __ / __ __ __ / __ __ __ __ DD / MMM / YYYY		17.5	__ __	__ __
	CO Monitors	18.1	Serial #	18.2	__ __ / __ __ __ / __ __ __ __ DD / MMM / YYYY		18.3	__ __	__ __

Results						
	Device		Average		Peak	
	19.1	CO Monitors	19.2		19.3	

CRF Completed by: _____ Date: _ _ / _ _ _/ _ _ _ _
DD MMM YYYY

CRF0X: Environmental Antenatal Home Visit

Mother Participant ID: _ _ _ _ _ / _ _ / _ _ / _ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

Home Environment		
1	Time at start of home visit: *(24 hour clock)*	__ __ : __ __
2	Is this the home of the child in this study?	☐Yes↓ ☐No (do not continue)
3	For the person receiving field worker, what is their relationship to the study child?	☐Mother ☐Father ☐Uncle ☐Aunt ☐Grandmother ☐Grandfather ☐Neighbour ☐Family friend ☐Other (specify): _____
4	What type of home is this?	☐ House ☐ Informal construction/shack ☐ Flat ☐ Other (specify): _____
5	What are the <u>walls</u> in the home primarily made of? *Tick one.*	☐Bricks ☐Tin / iron sheeting ☐Mud/traditional ☐Cement/concrete ☐Wood ☐Plaster ☐Stone ☐Other (specify): _____
6	What is the <u>roof</u> in the home primarily made of? *Tick one.*	☐Thatch ☐Tin/iron sheeting/metal/corrugated ☐Cement/concrete ☐Wood ☐Tiled ☐Other (specify): _____
7	How many rooms are there in the home including the bathroom?	Number:

8	How many of these rooms are used for sleeping?	Number:
9	Are there pets at the home?	☐Yes↓ ☐No (if no, skip to Q15)
	If yes, which of the following and how many? *If none, please specify "0"*	**A** **Pet** **B Number** 10 Cat/s 11 Dog/s 12 Bird/s 13 Other 14 (specify)
	Are any of the following animals kept on the property? *If none, please specify "0"*	**A** **Animal** **B Number** 15 Cow/s 16 Goat/s 17 Chicken/s 18 Other 19 (specify)
20	What kind of toilet facilities does the home have?	☐Flush toilet (connected to sewage) ☐Pit toilet ☐No facility/bush/field ☐Other (specify): _____
21	Is this toilet a communal toilet?	☐Yes ☐No
22	Which of the following items, in working order, does the household have? *Tick all that apply.*	☐A Fridge/freezer ☐A Polisher or vacuum cleaner ☐A television ☐A Hi-Fi or Music Center (radio excluded) ☐A washing machine ☐A video cassette recorder/DVD player ☐None
23	Which of the following are present in the home? *Tick all that apply.*	☐Running Water ☐Domestic Servant ☐At least One Car/truck ☐A built-in Kitchen Sink ☐A working Telephone (this includes a cell phone) ☐Cupboard ☐Radio ☐Bicycle ☐Motorcycle/ Scooter

CRF0X: Environmental Antenatal Home Visit

Mother Participant ID: _ _ _ _ _ / _ _ / _ _ / _ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

24	Is there electricity in the home?	☐Yes ☐No
25	What is the <u>main</u> source of drinking water in household for most of the year (more than 6 months)?	☐Pond, river or stream ☐Natural spring ☐Rainwater ☐Borehole ☐Public tap ☐Piped into yard / plot ☐Piped into dwelling ☐Other (specify): _____
26	How often do trucks pass along the street where you live?	☐Never ☐A few times a month ☐ A few times a week ☐Frequently throughout the day
27	How far is the home from a street where traffic passes continuously?	☐ Less than 50 meters ☐ 50-100 meters ☐ 100-200 meters ☐ 200-500 meters ☐ more than 500
28	Are there any large power lines, radars, T.V. or radio wave receivers directly over the plot where the home is?	☐Yes ☐No
29	Are any of the following kept in the home? *Tick all that apply.*	☐ Doom ☐ Rat-ex ☐ Raid ☐ Mortein Target ☐ Other spray pesticides ☐ Liquid pesticides ☐None

Mother Participant ID: _ _ _ _ _ / _ _ / _ _ / _ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

Cooking Environment		
30	What type of kitchen is in the home?	☐ Inside without partitions/open plan ☐ Separate room inside home ☐ Outside Kitchen
31	Is there a stove in the home?	☐Yes↓ ☐No (if no, skip to Q 33)
32	If yes, what type of stove is present? *Tick all that apply.*	☐ Wood burning stove ☐ Gas Stove ☐ Paraffin Stove ☐ Electric Stove ☐ Microwave Stove ☐ Coal Stove ☐ Other (specify)
33	If paraffin is used, where and how is it stored? *Tick all that apply.*	☐ Stored inside home ☐ Stored outside home ☐ Stored in closed/sealed container ☐ Stored in open container ☐ Accessible to child, describe the reason for assessment: _____ ☐Unknown ☐No paraffin
34	Have there been any improvements, renovations or extensions to the home in the last 12 months?	☐Yes↓ ☐No (if no, skip to Q36)
35	If yes, please indicate which of the following were changed.	☐ Added/renovated a living room ☐ Added/renovated a bedroom ☐ Upgraded windows ☐ Upgraded roof ☐Added/renovated bathroom ☐Added/renovated kitchen ☐ Other (specify): _____

CRF0X: Environmental Antenatal Home Visit

Mother Participant ID: _ _ _ _ _ / _ _ / _ _ / _ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

	Kitchen (room where cooking is done)		Major Living Area			Bedroom (of mother)		Bathroom
Is there any evidence of the following on ceilings and/ or roof?	36.1 36.2 36.3	☐ Mould or mildew ☐ Peeling paint ☐ Water Stains ☐ None	36.4 36.5 36.6	☐ Mould or mildew ☐ Peeling paint ☐ Water Stains ☐ None	36.7 36.8 36.9	☐ Mould or mildew ☐ Peeling paint ☐ Water Stains ☐ None	36.10 36.11 36.12	☐ Mould or mildew ☐ Peeling paint ☐ Water Stains ☐ None
Is there visible mould or mildew on the wall?	37.1	☐Yes ☐No	37.2	☐Yes ☐No	37.3	☐Yes ☐No	37.4	☐Yes ☐No
How many openable windows are in this room?	38.1	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ More than 3	38.2	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ More than 3	38.3	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ More than 3	38.4	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ More than 3
Were the windows opened during the course of this visit?	39.1	☐ Yes ☐ No	39.2	☐ Yes ☐ No	39.3	☐ Yes ☐ No	39.4	☐ Yes ☐ No
Is there any form of ventilation in the room? *Tick appropriate type.*	40.1 40.2 40.3 40.4 40.5 40.6 40.7	☐ Fan ☐ Chimney ☐Hole in ceiling or roof ☐Pipe or duct to exterior ☐ None ☐Other (describe): _____	40.8 40.9 40.10 40.11 40.12 40.13 40.14	☐ Fan ☐ Chimney ☐Hole in ceiling or roof ☐Pipe or duct to exterior ☐ None ☐Other (describe): _____	40.15 40.16 40.17 40.18 40.19 40.20 40.21	☐ Fan ☐ Chimney ☐Hole in ceiling or roof ☐Pipe or duct to exterior ☐ None ☐Other (describe): _____	40.22 40.23 40.24 40.25 40.26 40.27 40.28	☐ Fan ☐ Chimney ☐Hole in ceiling or roof ☐Pipe or duct to exterior ☐ None ☐Other (describe): _____
What type of floor covering is in the room? *Tick the appropriate box.*	41.1 41.2	☐ Wood or tile ☐ Cement ☐ Linoleum ☐ Carpeted/Rugs ☐Other (describe): _____	41.3 41.4	☐ Wood or tile ☐ Cement ☐ Linoleum ☐ Carpeted/Rugs ☐Other (describe): _____	41.5 45.6	☐ Wood or tile ☐ Cement ☐ Linoleum ☐ Carpeted/Rugs ☐Other (describe): _____	41.7 41.8	☐ Wood or tile ☐ Cement ☐ Linoleum ☐ Carpeted/Rugs ☐Other (describe): _____
Is there visible mould or mildew on the floor or floor covering?	42.1	☐ Yes ☐ No	42.2	☐ Yes ☐ No	42.3	☐ Yes ☐ No	42.4	☐ Yes ☐ No
Is there any form of heating in the room? *Tick appropriate type.*	43.1 43.2 43.3 43.4 43.5 43.6 43.7	☐Wood ☐Gas ☐Paraffin ☐Electric ☐Coal ☐None ☐Other (specify) e.g. dung, grass: _____	43.8 43.9 43.10 43.11 43.12 43.13 43.14	☐Wood ☐Gas ☐Paraffin ☐Electric ☐Coal ☐None ☐Other (specify) e.g. dung, grass: _____	43.15 43.16 43.17 43.18 43.19 43.20 43.21	☐Wood ☐Gas ☐Paraffin ☐Electric ☐Coal ☐None ☐Other (specify) e.g. dung, grass: _____	43.22 43.23 43.24 43.25 43.26 43.27 43.28	☐Wood ☐Gas ☐Paraffin ☐Electric ☐Coal ☐None ☐Other (specify) e.g. dung, grass: _____
Is there any chimney, vent or extraction device equipped to the heating device? (If YES, describe)	44.1	☐None	44.2	☐None	44.3	☐None	44.4	☐None

Mother Participant ID: _ _ _ _ / _ / _ / _ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

Home Measurements. Complete the measurement indicated for each of the rooms listed. *N.B. If the bathroom is communal or external to the home, write N/A. If kitchen and major living room are not separated by walls, indicate measurements underneath most appropriate designation and mark "shared" underneath other column.*					
		Kitchen (room where cooking is done)	**Major Living Area**	**Bathroom**	**Bedroom (mother's)**
45	Length of the room (meters)				
46	Width of the room (meters)				
47	Height of the (meters)				

48	Do you ever make open fires in your home (in a barrel or can) for heating, social or cooking purposes?	☐ Yes ☐ No (skip to Q51)
49	If yes, how many times per week on average?	☐ Once per week ☐ Twice per week ☐ 3-5 times per week ☐ Most days/nights (more than 5 times)
50	What materials do you use to make the fire?	☐ Paper ☐ Wood ☐ Coal ☐ Paraffin ☐ Other (specify): _____
51	Do you ever burn any of the following items in your home? *This should include any items giving off smoke that have not been captured elsewhere on the CRF.*	☐ Herbs ☐ Plants ☐ Incense (wierook) ☐ Imphepho ☐ Other (specify): _____
52	If yes, on average, how often are these items burned inside your home?	☐ Weekly ☐ Monthly ☐ A few times each year

CRF Completed by: _____ Date: _ _ / _ _ _ / _ _ _ _ Time Visit Completed: _ _: _ _
DD M MM YYYY

APPENDIX 5: BIRTH FORM

Confidential

Birth Form

Child PID	_____
Date CRF completed	_____
Pregnancy complications	
Q1. Has the mother been diagnosed with any of the following complications during this pregnancy?	☐ Diabetes ☐ Hyperemesis (excessive vomiting) ☐ Eclampsia ☐ Pre-eclampsia ☐ Pelvic inflammatory disease ☐ High blood pressure ☐ Asthma ☐ HIV ☐ TB ☐ Other
Q10. Please specify other	_____
BIRTH	
Q12. Was birth live at delivery?	◯ Yes ◯ No
Q13. What is the reason for stillbirth?	☐ Congenital abnormaility ☐ Cord around neck ☐ Infection ☐ Other ((If baby was stillborn: Continue wit this CRF; mark NA for Q38, 41 and 44-58))
Q13. Please specify other reason	_____
Q14. How many babies did the mother give birth to?	☐ One ☐ Two ☐ Three ☐ More than three ((If more than one, complete multiple birth form for each additional baby. Complete this form for first child.))
Q15. Did the mother have an anaesthetic during labour?	◯ No ◯ Yes ◯ Unknown
Q16. If yes, tick all that apply?	☐ Epidural ☐ Spinal ☐ General anaesthetic ☐ Gas (nitrous oxide) ☐ Local anaesthetic ☐ Other

Q16. Please specify other	_____
Q17. Did the mother have any analgesics during labour?	◯ Yes ◯ No ◯ Don't know
Q18. If yes, please tick all that apply?	◯ Morphine ◯ Other
Q18. Please specify other	_____
Q19. Did the mother have any oxytocin like substances during labour?	◯ No ◯ Yes ◯ Unknown

	Induce/ augment labour, strengthen contractions	Postpartum syntometrine with delivery of placenta	Other
If oxytocin like substanceswere given, what was the indication?	◯	◯	◯

if Other, specify	_____
Q20. Is the mother HIV infected?	◯ No ◯ Yes ◯ Unknown
Q21. Is the mother on ARVs?	◯ No ◯ Yes ◯ Unknown
Q22. Did the mother receive any antiretrovirals during labour?	◯ No ◯ Yes ◯ Unknown
Q23. If yes, tick the appropriate ARV	◯ AZT/NVP ◯ HAART
Q24. Does the mother currently have TB?	◯ No ◯ Yes ◯ Unknown
Q25. If yes, is she taking TB treatment?	◯ Yes ◯ No
Q26. Method of delivery -	☐ Normal vaginal ☐ Vaginal forceps ☐ Vaginal vacuum ☐ Assisted breech ☐ Elective Caesarean section ☐ Emergency Caesarean section

Q27. If Caesarean section, what was the reason?	☐ Meconium stained liquor ☐ Fetal distress ☐ Placenta pravia ☐ placental abruption ☐ Cord prolapse ☐ Eclampsia ☐ Failure to progess ☐ CPD (cephalophelic disproportion) ☐ Breech ☐ other
Q27. Please specify other reason	_____
Q28. Was the time of the membrane rupture recorded?	◯ Yes ◯ No
Q29. If yes, time of membrane rupture	_____
Q28. Was the date of the membrane rupture recorded?	◯ Yes ◯ No
Q30. If yes, date of membrane rupture	_____
Q31. Time of delivery	_____
Date of delivery/birth	_____
Q33. Did the mother have amnionitis?	◯ No ◯ Yes ◯ Unknown
Q34. Did the mother have placental bleeding?	◯ No ◯ Yes ◯ Unknown
Q35. Was mother given any supplements or medication during hospital stay or at discharge?	◯ No ◯ Yes ◯ Unknown
Q36. Gestational age at delivery - Full term	◯ Full term (37 weeks) ◯ Preterm
If preterm, what is the gestational age (weeks)?	_____
Q37. What is the sex of the baby?	◯ Female ◯ Male
Q38. Apgars - 1 min	_____

Q38. Apgars - 5 min	_____
Q38. Apgars - 10 min	_____
Q39. Record length of baby	_____ (cm)
Q39. Record weight of baby	_____ (kg)
Q39. Record head circumference of baby	_____ (cm)
Q40. Were there any abnormalities present?	☐ Fractured clavicle ☐ Cephalhaematoma ☐ Gross deformities ☐ Other ☐ No abnormalities (tick all that apply)
Q40. Please specify Other	_____
Q41. After delivery was the baby given any of the following? - Vitamin K	☐ Vitamin K ☐ Intubation ☐ Bag and mask ventilation ☐ Free flow oxygen ☐ Oxygen mask ☐ Eye ointment ☐ Suctioned ☐ Other
Q41. Please specify other	_____
Q42. At delivery, was there meconium stained liquor?	◯ No ◯ Yes ◯ Not recorded
Q43. If yes, grade:	_____
Q44. Where did the baby get admitted immediately after birth?	◯ Roomed with mother ◯ Nursery ◯ Transferred to Tygerberg NICU ◯ Discharged home ◯ Don't know

Q45. If admitted to nursery or neonatal unit, what was the reason why? - Pneumonia or breathing problems	☐ Pneumonia or breathing problems ☐ Diarrhoea ☐ Poor weight gain ☐ Seizures ☐ Fever ☐ Urine infection ☐ Meningitis ☐ Septicemia ☐ Accident ☐ Other (Tick all that apply. If admitted for pneumonia / hypoxia / breathing problems, also complete Pneumonia CRF)
Q45. Please specify other	_____
Q46. Did baby have respiratory problems after birth?	◯ Yes ◯ No
Q47. If yes, what were the problems? - Grunting	☐ Grunting ☐ Fast breathing (RR>60) ☐ Baby needed oxygen ☐ Coughing ☐ Recession ☐ Cyanosis ☐ Apnoea ☐ Other (tick all that apply)
Q47. Please specify other	_____
Q48. Was the baby jaundiced before discharge?	◯ Yes ◯ No
Q49. If yes, what therapy did baby receive?	☐ Phototherapy ☐ Transferred to Tygerberg for therapy ☐ Other therapy ☐ None
Q49. Please specify other therapy	_____
Q50. Did the baby have an abnormal blood sugar?	◯ No ◯ Yes ◯ Unknown
Q51. If yes, tick appropriate:	◯ High blood sugar (>7 mmol/L ◯ Low blood sugar (< 3 mmol/L)
Q52. If yes, what was the treatment? -	◯ Feeds ◯ IV fluids containing glucose ◯ Oral glucose ◯ Unknown

Q53. Was the baby given any medication before discharge?	◯ No ◯ Yes ◯ Unknown (If yes, record on medication chart)
Q54. Was the baby given any supplements before discharge?	◯ No ◯ Yes ◯ Unknown (If yes, record on medication chart)
Q55. Was breastfeeding initiated prior to discharge?	◯ No ◯ Yes ◯ Not recorded
Q56. Date of discharge	_____
Q57. Weight at discharge	_____ (kg)
Q58. What was the outcome of the baby following birth? -	◯ Discharged home ◯ Dead (complete autopsy CRF) ◯ Other
Please specify other	_____
What was the placental weight?	_____
What was the hosptial number given to the baby?	_____

APPENDIX 6: SOCIO-ECONOMIC STATUS FORM

Confidential

Ses

Child PID	_____
IMPORTANT NOTICE: Any caregiver, not just the biological mother, may complete this questionnaire as long as they meet the following criteria: 1 - Live in the same house as the study child. 2 - Care for the child for 3 or more days in a week.	
CRF completion date	_____
What is the relationship of the respondent to study child?	◯ Mother ◯ Father ◯ Uncle ◯ Aunt ◯ Grandmother ◯ Grandfather ◯ Neighbour ◯ Family friend ◯ Other
How is this questionnaire being completed?	◯ In person ◯ Telephonically
Is this the old version or new version? New version should be ticked after July 2015.	◯ Old version ◯ New version
Q1. How many children (under 18 years) do you have?	_____
Q2. How many people normally live in your household, including yourself? (Include people that live there for more that 6 months of the year)	_____
Q3.1. How many of these are adults over 18 years?	◯ No adults ◯ 1-3 Adults ◯ More than 3 adults
Q3.2. If more than 3 adults, please specify number	_____ ([sesq3_1] = '3')
Q4.1. How many of these adults over 18 years are men?	_____
Q4.2. How many of these adults over 18 years are women?	_____
Q5. How many children aged 5 to 18 years normally live in your household (live there for more than 6 months of the year)?	_____

Q6.1. How many of these children aged 5 to 18 years are girls?	_____
Q6.2. How many of these children aged 5 to 18 years are boys?	_____
Q7. How many children younger than 5 years normally live in your household?	_____
Q8.1. How many of these children younger than 5 years are girls?	_____
Q8.2. How many of these children younger than 5 years are boys?	_____
Q9.1. What is your relationship to each adult or child with you at home?	☐ Your spouse/partner ☐ Your son or daughter ☐ Your son-in-law or daughter-in-law ☐ Your grandchild ☐ Your parent ☐ Your parent-in-law ☐ Your brother or sister ☐ Your nephew or niece ☐ Your adopted/foster/step-child ☐ Other ☐ Not related (tick all that apply)
Q9.11. If other, specify	_____
Q10.1. What is your race?	◯ Black ◯ White ◯ Coloured ◯ Indian/Asian ◯ Other
Q10.2. If other, specify	_____
Q11.1. What language do you speak at home?	◯ English ◯ Afrikaans ◯ isiXhosa ◯ Other
Q11.2. If other, specify	_____
Q12.1. What is your religion?	◯ Muslim ◯ Christian ◯ Jewish ◯ None ◯ Other
Q12.2. If other, specify	_____

Q13.1. Where were you born?	◯ In Paarl ◯ Outside of Paarl, in the Western Cape ◯ Outside of the Western Cape, in South Africa ◯ Outside of South Africa
Q13.2. If born outside of the Western Cape but in South Africa, please specify where	_____
Q13.3. If born outside of South Africa, please specify where	_____
Q14. How far did you get in school?	◯ No education ◯ Completed Grade 1 (Sub A) to Grade 5 (Standard 3) ◯ Completed Grade 6 (Standard 4) to Grade 7 (Standard 5) ◯ Completed Grade 8 (Standard 6) to Grade 11 (Standard 9) ie High school without matriculating ◯ Completed Grade 12 (Standard 10) ie high school with matriculating ◯ Part of university/ college/ post-matric education ◯ Completed university/ college/ post-matric education
Q15.1. What is your current employment status?	◯ Working now ◯ Self-employed ◯ Looking for work: Unemployed ◯ Temporarily Laid off ◯ Homemaker ◯ Student ◯ Illness/sickness ◯ Disabled ◯ Other
Q15.2. If other, specify	_____
Q16. Do you receive any social assistance in the form of a government grant?	◯ Yes ◯ No
Q17. If yes, what kind of grant do you receive?	☐ Childcare grant ☐ Disability grant ☐ Care dependency grant (tick all that apply)
Q18. What is your own average income per month (i.e. average over the past 6 months)?	◯ Less than R1000 per month ◯ R1000-R5000 per month ◯ R5000-R10 000 per month ◯ More than R10 000 per month ◯ Unknown
Q19. What is your average household income per month (i.e. average over the past 6 months)?	◯ Less than R1000 per month ◯ R1000-R5000 per month ◯ R5000-R10 000 per month ◯ R10 000-R15 000 per month ◯ More than R15 000 per month ◯ Unknown (Please tick - your best estimate is fine)

Q20.1. What is your marital status?	◯ Single (never married) ◯ Not married, but in a marriage like relationship (ie. Living together) ◯ Married ◯ Divorced ◯ Widowed
Q20.2. How many months have you been in a marriage/marriage like relationship?	_____
Q21. How many times in your life have you been married and/or lived together with a partner (include current partner if living together)?	_____
Q22. If you are married or in a marriage-like relationship, using a scale from 1 to 10 where 1 means "the worst possible marriage/relationship" and 10 means "the best", how would you rate your current marriage/relationship?	◯ 1 (worst) ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 (best)
Q23. How far did your spouse/partner get in school?	◯ No education ◯ Completed Grade 1 (Sub A) to Grade 5 (Standard 3) ◯ Completed Grade 6 (Standard 4) to Grade 7 (Standard 5) ◯ Completed Grade 8 (Standard 6) to Grade 11 (Standard 9) ie High school without matriculating ◯ Completed Grade 12 (Standard 10) ie high school with matriculating ◯ Part of university/ college/ post-matric education ◯ Completed university/ college/ post-matric education ◯ Unknown
Q24.1. What is your spouse/partner's current employment status?	◯ Working now ◯ Self-employed ◯ Looking for work: Unemployed ◯ Temporarily Laid off ◯ Homemaker ◯ Student ◯ Illness/sickness ◯ Disabled ◯ Other
Q24.2. If other please specify	_____
Q25. Does your spouse/partner receive assistance in the form of a government grant?	◯ Yes ◯ No
Q26.1. If yes, what kind of grand does he receive?	☐ Childcare grant ☐ Disability grant ☐ Care dependency grant (tick all that apply)

Q27. If receives childcare grant, for how many children does he get the grant?	_____
Q28. What is your spouse/partner's average income per month (i.e. average of the past 6 months)?	◯ Less than R1000 per month ◯ R1000-R5000 per month ◯ R5000-R10 000 per month ◯ More than R10 000 per month ◯ Don't know (Please tick - your best estimate is fine)
Q29. Which of the following best describes the area in which you live?	◯ Urban ◯ Rural ◯ Township
Q30. How many years have you lived at the current address?	_____ (YEARS)
Q31.1. Which of the following best describes your home (type of dwelling)?	◯ Shack ◯ Wendy House or backyard dwelling ◯ House ◯ Flat ◯ Refugee centre/homeless shelter ◯ Other
Q31.2. If other please specify	_____
Q32. How many people share your bedroom (including participant)?	_____
Q33.1. Which of the following do you have in your home? - Electricity	☐ Electricity ☐ Tap or running water ☐ Domestic servant ☐ A flush toilet inside ☐ A built-in kitchen sink ☐ An electric stove or hotplate ☐ A working telephone (this includes a cell phone) ☐ At least one motor car or truck ☐ A motorcycle or scooter ☐ A bicycle ☐ None (tick all that apply)
Q34.1. Is the house where you stay owned, rented or an informal settlement plot?	◯ Own ◯ Rent ◯ Neither, informal settlement plot ◯ Other
Q34.2. If other please specify	_____
Q35. Do you own any land other than the land where your house it?	◯ Yes ◯ No

Q36.1. Do you personally do any of the following?	☐ Shop at supermarkets ☐ Use any financial services (such as bank account, ATM card or credit card) ☐ Have an account at a retail store (eg. Pep, Jet etc) (tick all that apply)
Q37. Complete this section if you feel important information about the participant or their situation may influence the information provided in this CRF. Please talk to a Team Leader (before the participant leaves) if an urgent matter needs to be addressed.	_____

APPENDIX 7: NASOPHARYNGEAL RESULTS FORM

Confidential

Nasopharyngeal Results

Barcode	_____
PID	_____
Transcribed by	◯ Sam ◯ Fadheela ◯ Charmaine ◯ Other ◯ Dilshaad
If other, specify who	_____
A.1 Date of collection	_____
A.3 Date performed	_____
A.3a Growth?	◯ No growth ◯ Growth
A4. Staphylococcus aureus	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+
If < 20, specify # col	_____
A5. Haemophilus influenzae	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+
If < 20, specify # col	_____
A6. Streptococcus pneumoniae	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+
If < 20, specify # col	_____
A7. Moraxella species	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+

If < 20, specify # col	_____
A7. Moraxella species: Colony stored?	◯ Yes ◯ No
A8. Gram Negative bacilli	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+
If < 20, specify # col	_____
How many 'other' organisms were isolated?	◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ 4
A9. Other	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+
If other, specify isolate	_____
If < 20, specify # col	_____
A9. Other: Colony stored?	◯ Yes ◯ No
A10. Other	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+
If other, specify isolate	_____
If < 20, specify # col	_____
A10. Other: Colony stored?	◯ Yes ◯ No
A11. Other	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+
If other, specify isolate	_____

If < 20, specify # col	_____
A11. Other: Colony stored?	◯ Yes ◯ No
A12. Other	◯ < 20 ◯ 1+ ◯ 2+ ◯ 3+
If other, specify isolate	_____
If < 20, specify # col	_____
A12. Other: Colony stored?	◯ Yes ◯ No
Quality assurance done by:	◯ Sam ◯ Fadheela ◯ Charmaine ◯ Other ◯ Dilshaad
If other, specify who	_____
Date of quality assurance	_____

APPENDIX 8: JOURNAL SUBMISSION GUIDELINES

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Manuscript Format and Structure

Please refer to a recent issue of *Clinical Infectious Diseases* for guidance on style and layout of articles. Also refer to the Article type section for guidance on relevant information for each article type.

Initial Manuscript Submission: "Format Free" Submission

CID has a streamlined submission process designed to avoid unnecessary author work.

enjoy reading and reviewing a manuscript formatted in this way? Some basic guidelines are provided below. **You will still need to comply with the requirements and limitations of the selected article type, such as word count or maximum references.**

Basic Formatting Guide

- On the first (title) page please include manuscript title, author names and affiliations, and the address (including email) of the corresponding author. Major Articles, Reviews, and Viewpoints should also include a 40-word summary of the article's main point.
- Please list all author contributions in the Acknowledgment section.
- Provide a short abstract, as the second page of the manuscript, avoiding abbreviations and reference citations. The abstract should follow the article type requirements, e.g. a structured abstract of no more than 250 words for major articles.
- Define non-standard abbreviations at the first occurrence.
- Include line and page numbers.
- Number tables and figures consecutively by appearance, and provide a legend for each. Avoid overcrowding in tables and unnecessary clutter in figures. The initial submission can include figures and tables in the manuscript file, if desired. Upon request, please be prepared to provide high-resolution figures (300dpi or more) separately, in a common image format (e.g. eps or tif).
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- References can be formatted in any readable style at submission. Authors are responsible for the accuracy of the

- In the acknowledgement section, before the references, please include details of funding sources and grant numbers at the end of the text. Use author initials to indicate which authors were in receipt of grants. Also include any potential conflicts of interest.
- If applicable, in the acknowledgement section, please include a statement of data availability at the end of the text. For example: *Data available at doi:10.5061/dryad.XXXX; Data available at GEO Series accession number GSE123456 and Proteome Xchange database PXD1234567; Data available in supplementary material; Data not publicly available*. Where datasets have a DOI, please cite the data in the text, and place the citation in the reference section.
- Please include at submission all files containing supplementary material cited in the text.
- As part of the upload process, include a list of at least 5-6 potential unbiased reviewers, with their e-mail addresses and a brief description for each of his/her qualifications as a reviewer.

After initial review, you may be asked to supply editable files that match journal formatting requirements, and high-resolution figures.

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Manuscript submissions are preferred as a single file.

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Authors should use inclusive and person-first language in manuscripts. Describe people as having a condition or disease, experiencing a circumstance, or doing something specific rather than the condition, disease, circumstance, or activity being part of their identity. For example, they should use "people with obesity," "person with HIV," "person who injects

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6. Critical Care and Sepsis
7. Education and Training
8. Emerging Infections
9. Endovascular Infections
10. Equity, Diversity, Inclusion and Social Justice
11. Fungal Infections
12. Global Health and Neglected Tropical Diseases
13. Hepatitis
14. HIV/AIDS
15. Immunocompromised Hosts
16. Infection Control and Hospital Epidemiology
17. Infections in Older Adults
18. Medical Microbiology
19. Mycobacterial Infections
20. Parasitic Infections

24. Surgical Infectious Diseases
25. Vaccines
26. Vector-borne Diseases
27. Viral Infections

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All manuscripts, including Correspondence, must have a title page.

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Guide to Creating Graphical Abstracts via Tidbit to Accompany Journal Articles

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Authors are encouraged to follow the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals*. They should strive for a concise article without excessive detail (word limits are specified under Categories of Articles). All but the shortest articles should have subheadings.

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The following rules should be followed:

- The sentence should begin: "This work was supported by ..."
- The full official funding agency name should be given, ie "the National Cancer Institute at the National Institutes of Health" or simply "National Institutes of Health" not "NCI" (one of the 27 subinstitutions) or "NCI at NIH." Please see here for a full RIN-approved list of UK funding agencies

- Multiple grant numbers should be separated by a comma as follows: "[grant numbers ABX CDXXXXXX, EFX GHXXXXXX]"
- Agencies should be separated by a semi-colon (plus "and" before the last funding agency)
- Where individuals need to be specified for certain sources of funding the following text should be added after the relevant agency or grant number "to [author initials]."

An example is given here: "This work was supported by the National Institutes of Health [P50 CA098252 and CA118790 to R.B.S.R.] and the Alcohol & Education Research Council [HFY GR667789]."

Crossref Funding Data Registry and CHORUS

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- Gorecki DC, Monaco AP, Derry JMJ, Walker AP, Barnard, EA, Barnard, PJ. Expression of four alternative dystrophin transcripts in brain regions regulated by different promoters. Hum Mol Genet, **1995** ; 155: 505-511.
- Francis V, Bastin M. (2000) Gene targeting in rat embryo fibroblasts promoted by the polyomavirus large T antigen. Nucleic Acids Res [in press].
- Maniatis T, Fritsch EF, Sambrook J. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, **1982** .
- Huynh TV, Young RA, Davis RW. DNA Cloning. In: Glover DM. DNA Cloning - A Practical Approach. Vol 1. Oxford, UK: IRL Press; **1988** :49-78.
- Public Health Service Task Force. Recommendations for the use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. Available at: http://www.aidsinfo.nih.org. Accessed 24 April 2002.
- Lyon DJ, Cheng AFB, Norrby SR. Mechanisms of cefotaxime resistance in blood culture isolates of Enterobacter high prevalence of extended-spectrum β-lactamases [abstract C43]. In: Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco). Washington, DC: American Society for Microbiology, 1995:47.
- Pre-print example: Bar DZ, Atkatsh K, Tavarez U, Erdos MR, Gruenbaum Y, Collins FS. Biotinylation by antibody recognition- A novel method for proximity labeling. BioRxiv 069187 [Preprint]. August 11, 2016 [cited 2017 Jan 12]. Available from: https://doi.org/10.1101/069187.

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