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The Role of Streptococcus Pneumoniae Carriage on Wasting Among Vaccinated Gambian Infants: A Longitudinal Analysis of a Birth Cohort

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PREAMBLE

Declaration

I, Phindi Zwane (ZWNPHI006), 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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Date: 12 February 2022

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Dissertation Abstract

Wasting remains a serious public health problem, affecting 45 million children under five years old worldwide. A comprehensive understanding of the factors associated with child wasting is needed to inform interventions aimed at reducing and preventing wasting. Some studies have suggested that asymptomatic colonization of bacterial agents such as Streptococcus Pneumoniae (pneumococcus) may have negative impacts on child growth and nutritional status. The aim this study is to investigate the determinants of wasting and to assess the effect of pneumococcus carriage on wasting in children under the age of 2 years.

The rationale, aim and objectives of the current study, along with the methodology, data analysis plan and ethical considerations are outlined in the protocol presented in Part A. Part B presents a manuscript that outlines and discusses the findings of the study.

This study was a secondary analysis of data collected from 120 infants recruited at birth and followed up at regular intervals for two years. The data was collected in Rural Gambia between March 2013 and September 2015 as part of the Vaccination and Paediatric Microbiome Project.

Nutritional status was classified using weight-for-length (WLZ), length-for-age (LAZ) and weight-for-age (WAZ) z-scores for wasting, stunting and underweight respectively. Regression methods were used to evaluate the factors associated with both wasting, as a binary indicator, and weight-for-length z-scores among children aged 6-24 months.

Over the two years of follow-up, 66.7% (80/120) of children had at least one episode of wasting with 36.3% (29/80) experiencing repeated episodes. About 47.5% (57/120) became stunted and 41.7% (50/120) became underweight. Nearly all the children (97.5%) became pneumococcus carriers at least once in the two years of follow up, with 50% of children becoming carriers within the first month of life. A small proportion of the children (15.8%) were born carriers. Carriage was lowest in the dry season but began to increase in the wet season until February, where it peaked. The median duration of carriage was 269.1 days (IQR: 70-508 days)

Child age, mother's age at delivery, inflammation at birth, having at least one episode of wasting in the first six months of life, reporting a recent illness, and being observed in the rainy season had

significant effects on WLZ scores in both the adjusted and unadjusted models. Pneumococcus carriage only had a significant association with WLZ scores in the adjusted model [RR: -0.18 (95% CI: -0.35 – -0.01)], while low birthweight only had a significant association in the unadjusted model [RR: -0.78 (95% CI: -1.49 – -0.07)].

Mother's age at birth, inflammation at birth and reporting a recent illness was significantly associated with wasting as a binary indicator in both the adjusted and unadjusted models. Being observed in the rainy season decreased the odds of wasting, although, this association was only significant in the adjusted model [OR: 0.28 (95% CI: 0.10 – 0.75)]. Pneumococcus carriers had lower odds of wasting in the unadjusted model [OR: 0.29 (95% CI: -0.35 – 0.93)] and higher odds of wasting in the adjusted model [OR: 1.62 (95% CI: 0.70 – 3.74)]. Neither association was statistically significant.

This study found low birthweight, early episodes of wasting, inflammation at birth and recent illness to be associated with wasting in children between the ages of 6-24 months. Targeting maternal nutrition during pregnancy, neonatal care and infant feeding practises may help reduce wasting among children between 6-24 months of age by reducing low birthweight, undernutrition, illness, and inflammation in early infancy. Further research is needed to understand the role of pneumococcus on child undernutrition.

List of Abbreviations

AGP	Alpha (1)-Acid Glycoprotein
CRP	C-Reactive Protein
LAZ	Length-For-Age Z-Score
LBW	Low Birthweight
LMICs	Low- and middle-income countries
NP	Nasopharyngeal
NTDs	Neglected Tropical Diseases
OR	Odds Ratio
PCV	Pneumococcus Conjugate Vaccine
RR	Risk Ratio
RUTF	Ready-To-Use-Therapeutic Foods
SDGs	Sustainable Development Goals
sTfR	Soluble Transferrin Receptor
UIBC	Unsaturated Iron Binding Capacity
WAZ	Weigh-For-Age Z-Score
WHA	World Health Assembly
WHO	World Health Organization
WLZ	Weigh-For-Length Z-Score

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Part A: Research Protocol

1. Introduction

1.1. Background

Undernutrition is a serious public health problem associated with 45% of deaths among children under the age of five years globally(1). Stunting, wasting and underweight are the most used indicators of undernutrition in children. Stunting, often described as chronic undernutrition, is defined by a length-for-age Z-score (LAZ) below two standard deviations of the median of the World Health Organisation (WHO) reference population. Wasting is an acute form of undernutrition, characterised by a dangerously low body weight in relation to height resulting from muscle and fat tissue loss(1–4). A child is considered wasted when their weight-for-length score (WLZ) is less than two standard deviations below the median of the WHO reference population(1,5). Underweight is the composite of wasting and stunting and is defined by a weight-for-age Z-score (WAZ) less than two standard deviations below the median of the WHO reference population. Wasting is less prevalent than stunting, but it is considered a more server form of undernutrition and a much stronger predictor of child mortality (6). Children who are wasted have a weakened immune system and are therefore vulnerable to disease, having a higher risk of morbidity and mortality compared to children who are not wasted(1,7). Furthermore, children who have experienced one episode of wasting are at a higher risk of subsequent wasting and stunting (1). Although wasting is short-term and reversible with appropriate nutritional intervention, it can have long-term adverse effects on a child's physical and cognitive development(5,7,8).

In realisation of their goal to eliminate undernutrition in all forms by 2030, the United Nation's Sustainable Development Goals (SDGs) have incorporated the World Health Assembly's (WHA) targets to reduce and maintain the proportion of wasted children to below 5% in 2025 and to below 3% in 2030 (9,10). Since these goals were adopted in 2015, very little has changed. To date, an estimated 45.4 million (6.7%) children under the age of five years are wasted globally, and of those, 13.6 million are severely wasted (WLZ< -3) (11). Over a quarter of these children reside in Africa (11). To achieve the SDGs' targets on wasting a critical understanding of the risk factors associated with child wasting is needed to inform effective preventative interventions.

Risk factors known to be associated with child wasting include maternal health, nutrition and education, child diet, feeding practices, sex, infectious diseases, and environmental factors such as socioeconomic status, seasonality, and place of residence(5,7). Interestingly, infectious disease, in the context of undernutrition, is both a risk factor and a consequence of child wasting(12,13). Having a low body weight weakens the immune system and increases susceptibility to infection and having an infection increases energy demand and decreases appetite, leading to subsequent weight loss(7,12).

In addition to increased energy demands and suppressed appetite, pathogens of the enteric system increase the permeability of the intestines causing a disruption in the absorption of nutrients and frequently resulting in diarrhoea and weight loss(12). Infectious agents associated with undernutrition in children under the age of five years include plasmodium (malaria causing parasite), enteropathogens such as *H.pylori,* and *Streptococcus pneumoniae* (commonly called the pneumococcus) (14).

Pneumococcal infections are responsible for a significant proportion of the morbidity and mortality among under-fives globally (15). *Streptococcus pneumoniae* is a bacterium that causes serious infections including pneumonia, meningitis and septicaemia and it is implicated in a significant number of deaths among under-fives(16). The bacterium commonly inhabits the nasopharynx of young children and the elderly(17). Most children will be colonised by pneumococcus in early childhood but only a few will develop pneumococcal diseases (15). Pneumococcus colonization, although often asymptomatic, is considered an essential step in the development of pneumococcal disease and in the transmission of the pathogen across individuals(18).

The colonization of the nasopharynx has been targeted in the prevention of pneumococcal disease(19). In children, pneumococcal diseases have been significantly reduced by the administration of the pneumococcal conjugate vaccine (PCV) (20,21). The vaccine prevents pneumococcal disease by preventing colonization of specific disease-causing pneumococcus serotypes (19). PCV has been effective in reducing pneumococcal disease and the carriage (the colonization of the host) of vaccine-type serotypes but not in reducing overall pneumococcal carriage (16,19,20). This is because the vaccine only contains 13 of the 100 identified pneumococcus serotypes and upon reduction of vaccine type serotypes, non-vaccine type serotype colonizes the nasopharynx (18,19).

Initially, asymptomatic nasopharyngeal carriage was thought to be harmless (22). Recent studies, however, suggest that pneumococcal carriage may have adverse consequences on child health other than the development of pneumococcus disease (15,23,24). Of these studies, a few suggest that pneumococcal carriage may have adverse effects on child growth. A study conducted in South India, prior to the roll out of PCV, reported that significant reductions in growth and nutritional status at age six months is associated with pneumococcal carriage at age two months(15). Another study, also conducted in South India, reported a reduction in weight and length gain with increased duration of pneumococcal carriage among unvaccinated children(24).

The mechanism by which pneumococcus affects child growth remains unclear. Evidence from animal and in vitro studies suggests that pneumococcal carriage induces subclinical chronic inflammation in the nasopharynx, which is metabolically costly, increasing host energy demand (15,25–27). Pneumococcal carriage has also been associated with the release of proinflammatory cytokines, among other inflammatory mediators. These proinflammatory cytokines may have a direct effect on growth as they have been shown to be inversely associated with insulin-like growth factor-1, a vital hormone in child growth (15). Pneumococcal carriage, through eliciting an inflammatory response, may have adverse consequences on child growth.

The hypothesis that pneumococcal carriage influences child growth warrants further investigation, particularly in the West Africa sub-region where the prevalence of wasting in under-fives is 7.5%, higher than the global prevalence of 6.9%. (5,10,11). In The Gambia, undernutrition has not improved despite decades of unprecedented intensive interventions (3). As it stands, an estimated 7.2% of Gambian children are wasted (3,28). This is higher than the African prevalence (6.2%) and there are discrepancies in the prevalence of wasting between the rural and urban areas (11,28). Pneumococcal carriage is also highly common in this region with nearly all infants being colonised by three months of age, regardless of high PCV coverage (29).

Determining whether pneumococcal carriage affects child wasting has important implications for interventions aimed at reducing child wasting. If pneumococcal carriage has an adverse effect on child wasting, then interventions aimed at reducing pneumococcal disease should also aim to reduce pneumococcal carriage. Using intensively sampled longitudinal data on PCV vaccinated children, this study aims to investigate the determinants of wasting in Gambian children under the age of two years and to investigate the effect of pneumococcus carriage on wasting.

2. Study Aims and Objectives

2.1. Aim

The aim of this study is to investigate determinants of wasting and to assess the effect of pneumococcal carriage on wasting in Gambian children under the age two years.

2.2. Objectives

- To describe patterns of undernutrition in this population.
- To describe the patterns of pneumococcal carriage in this population

- To evaluate the factors associated with wasting including the effect of pneumococcal carriage on wasting.

3. Methodology

The proposed work is a secondary analysis of already collected data therefore, no participant recruitment or study procedures will be carried out. For completeness, details of the parent study design, measurements and methods are provided below.

3.1. Study design

The proposed study will be a secondary analysis of longitudinal data collected in rural Gambia as part of the Vaccination and Paediatric Microbiome Project. The parent study was a birth cohort aimed to determine the structure of the microbiome in children under the age of two years and to evaluate the impact of the Pneumococcal Conjugate Vaccine (PCV). A hundred and twenty mother-infant pairs were recruited at the birth of the infant and follow-up at regular intervals until their second birthday. Recruitment and data collection took place in the Western Region of The Gambia between March 2013 and September 2015.

The proposed analysis will use the collected anthropometric data to evaluate the outcomes of interest, namely wasting and WLZ scores, against pneumococcal carriage and other possible exposures such as maternal age at birth, breastfeeding practices, illness, inflammation, and seasonality. The use of longitudinal data has the potential to provide insight into the dynamic nature of wasting while allowing for the adjustment of dynamic confounders such as seasonality, weight, illness, inflammation, and pneumococcal carriage.

3.2. Characteristics of study population

The Western Region is made up of 55 villages spanning approximately 90km^2 of land with a population of roughly 60 000 people (30). This study area was selected as it provides an adequate representation of the rural areas in The Gambia.

The residents in this area are mostly subsistence farmers growing and selling maize, millets, and ground nuts (31). Food is least abundant during the rainy season, also termed the “hungry season”, between June and October (19,32). Following the “hungry season” is a long dry season where the prevalence of malnutrition, infectious disease, and pneumococcal carriage peaks (32,33).

The Gambia has an extensive immunization programme, with over 90% of infants having undergone routine vaccination by 12 months of age (28). More on the study population is detailed elsewhere (31).

3.3. Recruitment and enrolment

In the parent study, the research team was notified of all pregnancies and new births in each village. Mothers were then approached in the labour ward at Bwiam General Hospital and Sibanor Health Centre. Shortly after birth, and once informed parental consent was received, infants were enrolled into the study(34)

3.4. Research procedures and data collection methods

The data collection methods of the parent study have been detailed elsewhere (34,35). Briefly, 120 new-borns were recruited immediately after birth, then followed up monthly until 12 months, then at three-month intervals until 24 months of age. Blood, serum, nasopharyngeal (NP) swabs, and breast milk samples were collected according to the schedule in Table 1. Medical information was collected from infant health cards and anthropometric data was collected by trained field staff at each visit. Detailed socioeconomic, behavioural, nutritional status and clinical meta-data were collected at every follow-up visit using a structured questionnaire (Appendix B).

Table 1: Sample collection schedule in children and their mothers throughout the two years of follow up. The shaded areas represent the visit that the samples were collected

		birth	1m	2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m	15m	18m	21m	24m
child	blood	■		■			■							■		■		■
	serum	■		■			■							■		■		■
	NP swab	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
mother	blood	■																
	serum	■																
	NP swab	■		■		■	■	■		■		■		■	■	■	■	■
	Breast milk	■		■		■	■	■		■		■		■				

*NP= nasopharyngeal

Bacterial load of *Streptococcus pneumoniae* in the nasopharynx was determined though quantitative PCR. Iron and inflammation markers were measured from blood plasma using a fully automated biochemistry analyser (Cobas Integra 400 plus, UK).

3.5. Study measures

The first objective of this analysis is to describe the patterns of undernutrition, which manifest as wasting, stunting or being underweight. A child is classified as wasted, stunted or underweight if their weight-for-length (WLZ), length-for-age (LAZ) and weight-for-age (WAZ) score, is less than two standard deviations below the median of the WHO reference population, respectively(1,5). The overall aim is to determine the factors associated with wasting, including potential association with pneumococcus carriage. A child is classified as a pneumococcus carrier if their NP swab was positive for pneumococcal detection. Wasting is the undernutrition indicator of choice for this analysis as it is often short-term and reversible with appropriate interventions. Both continuous WLZ scores and wasting as a binary indicator will be used as outcomes in the regression analysis. Other variables to be used in this analysis are listed in Table 2.

Table 2: Variables to be used in this analysis.

Variable	Type	Categories
Maternal factors		
Age at delivery	Numerical- continuous	
Environmental factors		
Visit season	Categorical- binary	Rainy season, Dry Season
Season of birth	Categorical- binary	Rainy season, Dry Season
Infant factors		
Sex	Categorical- binary	Male, female
Birthweight	Numerical- continuous	
Low Birthweight	Categorical- binary	Low birthweight, Normal weight
Weight (kg)	Numerical- continuous	
Length (cm)	Numerical- continuous	
Feeding practices	Categorical- nominal	Exclusive breastfeeding, mixed feeding, no breastfeeding
Weight for age Z-score	Numerical- continuous	
Length for age Z-score	Numerical- continuous	
Weight for length Z-score	Numerical- continuous	
Wasted	Categorical- binary	Wasted, not wasted

Stunted	Categorical- binary	Stunted, not stunted
Underweight	Categorical- binary	Underweight, healthy weight
Inflammation markers		
Alpha-1-glycoprotein	Numerical- continuous	
C-reactive protein	Numerical- continuous	
Inflammation status	Categorical- binary	Inflammation, no inflammation
Pneumococcal carriage		
Pneumococcal carriage	Categorical- binary	Carriage, no carriage

3.6. Data safety and monitoring

The original data were entered in a secure Microsoft Access database. The data that will be used in this analysis will be provided by the data owners without any personal identifiers and stored on a password-protected computer that can only be accessed by the researcher.

3.7. Data analysis

The data provided by the data owners will be analysed using the statistical software R version 4.0.2. All infants without pneumococcus results at birth were excluded from parent study. Baseline characteristics will be summarised using either mean and standard deviation or median and inter-quartile range (IQR) for continuous variables depending on their distribution. Categorical variables will be summarised using frequencies and proportions. The age of first wasting, stunting or underweight episode was estimated using Kaplan-Meier estimators.

Factors associated with wasting and WLZ scores will be estimated using mixed effects models adjusted for known confounders such as maternal age, birthweight, and season of birth. Random effects, using an identifier for each child, will be fitted in the mixed effect models to account for correlation as a result of repeated measures within each child. A significance level of 5% will be used to guide the interpretation of all p-values. P values closer to zero but less than 0.05 will indicate stronger evidence of association compared to p values closer to 0.05.

4. Ethical considerations

The parent study was approved by the Joint MRC and Gambia Government Ethics Committee (Appendix C1). Prior to recruitment, the parents or caregivers of the participants provided written

informed consent. Ethical approval for this study will be sought from the University of Cape Town Faculty of Health Sciences Research Ethics Committee.

4.1. Risks

The proposed study poses no direct risks and minimal indirect risks to study participants, with the only potential risk being the loss of confidentiality. The data used in this analysis contains no personal identifiers and is kept in a password protected computer, minimizing the aforementioned risk.

4.2. Benefits

This study offers no direct benefit to any individual participant, however the knowledge gained will contribute to the existing information about wasting and may
indirectly benefit children in The Gambia and other low- and middle-income countries (LMICs).

4.3. Informed consent process

All parents or caregivers of infants participating in the parent study provided written informed consent.

4.4. Privacy and confidentiality

The data obtained from the parent study contains no identifying information.

5. Research Timetable

Table 3: Time frame from start of study to completion

	Jan - Mar 2021	**Apr - Jul 2021**	**Aug - Nov 2021**	**Dec 2021 - Feb 2022**
Data cleaning	✓			
Data analysis		✓	✓	
Draft Manuscript			✓	✓
Finalise this and submit for grading				✓

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Part B: Manuscript

Title of the article

The Role of Streptococcus Pneumoniae Carriage on Wasting Among Vaccinated Gambian Infants: A Longitudinal Analysis of a Birth Cohort

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Abstract

Background

Child wasting remains a public health problem in Africa and South Asia. Some studies have suggested that asymptomatic colonization of bacterial agents such as Streptococcus Pneumoniae (pneumococcus) may have negative impacts on child growth and nutritional status. The aim this study is to investigate the determinants of wasting in children and to assess the role of pneumococcus carriage on wasting.

Methods

This secondary analysis used longitudinal data collected from 120 infants recruited at birth and followed up at regular intervals for two years. The data was collected in Rural Gambia between March 2013 and September 2015 as part of the Vaccination and Paediatric Microbiome Project.

Nutritional status was classified using weight-for-length, length-for-age, and weight-for-age for wasting, stunting and underweight respectfully. Regression methods were used to evaluate the factors associated with both wasting, as a binary indicator, and weight-for-length among children aged 6-24 months.

Results

Over the two years of follow-up, 66.7% (80/120) of children had at least one episode of wasting with 36.3% (29/80) experiencing repeated episodes. About 47.5% (57/120) became stunted and 41.7% (50/120) became underweight.

Child age, mother's age at delivery, having at least one episode of wasting in the first six months of life, reporting a recent illness, and being observed in the rainy season had significant associations with WLZ scores in both the adjusted and unadjusted models. Pneumococcus carriage only had a significant association with WLZ scores in the adjusted model [RR: -0.18 (95% CI: -0.35 – -0.01)], while LBW only had a significant association in the unadjusted model [RR: -0.78 (95% CI: -1.49 – -0.07)].

Mother's age at birth, inflammation at birth and reporting a recent illness was significantly associated with wasting as a binary indicator in both the adjusted and unadjusted models. Being observed in the rainy season decreased the odds of wasting, although, this association was only significant in the adjusted model [OR: 0.28 (95% CI: 0.10 – 0.75)]. Pneumococcus carriers had lower odds of wasting in the unadjusted model [OR: 0.29 (95% CI: -0.35 – 0.93)] and higher odds of wasting in the adjusted model [OR: 1.62 (95% CI: 0.70 – 3.74)]. Neither association was statistically significant.

Conclusion

This study found low birthweight, early episodes of wasting, inflammation at birth and recent illness to be associated with wasting in children between the ages of 6-24 months. Targeting maternal nutrition and prenatal care may help combat undernutrition in infancy, which in turn may reduce wasting among children between 6-24 months of age. Further research is needed to understand the role of pneumococcus on child undernutrition.

Introduction

Undernutrition is a serious public health problem associated with 45% of deaths among children under the age of five years globally(1). Stunting, wasting and underweight are the most used indicators of undernutrition in children. Wasting is characterised by a dangerously low body weight in relation to height resulting from muscle and fat tissue loss(1,2). It affects 45.4 million children worldwide with 27% and 48% of those children residing in Africa and South Asia, respectively(3). A child is considered wasted when their weight-for-length z-score (WLZ) is less than two standard deviations below the median of the WHO reference population(1,4). Wasted children have a weakened immune system, leaving them vulnerable to infection and diseases(1,5). As a result, wasted children have an increased risk of morbidity and mortality. In addition, they have an increased risk of stunting, a chronic state of linear growth failure, characterised by a length-for-age z-score below negative two standard deviations below median on standard WHO growth reference chart(1,6). Wasting can also lead to long-term adverse effects on a child's physical and cognitive development(4,5,7).

Child wasting can often be treated with appropriate nutritional interventions. Currently, treatment methods target severely wasted children (WLZ less than negative 3 standard deviations on the WHO growth reference chart) between the ages of 6-59 months and involve supplementary feeding with energy-dense fortified pastes called ready-to-use-therapeutic foods (RUTF)(8,9). Treatment, although effective, does not reduce the risk of the reoccurrence of wasting or subsequent stunting(8). For that reason, preventative interventions would be preferred to treatment interventions, particularly for this age group. To inform these interventions, a critical understanding of factors associated with wasting is required.

Child wasting, just like most non-communicable diseases, has no single causal factor. It occurs as a result of an interplay among various factors including maternal health, nutrition and education, child diet, feeding practices, sex, infectious disease, and environmental factors such as socioeconomic status, seasonality, and place of residence(4,5). Risk factors such as maternal health and nutrition (particularly in pregnancy), child diet, feeding practices and infection are modifiable and can be targeted to reduce the burden of child wasting. Infectious diseases, in the context of wasting, are both a risk factor and a consequence for child wasting(10,11). Having a low body weight weakens the immune system and increases susceptibility to infection and having an infection increases energy demand and decreases appetite, leading to a reduction in body weight(5,11).

Regions burdened with a high prevalence of undernutrition also often have a high incident of infectious diseases(12). Streptococcus Pneumoniae (pneumococcus) is a gram-positive bacterium that is responsible for a significant proportion of the morbidity and mortality among children under five years across the globe(13,14). The bacterium causes diseases such as pneumonia, meningitis and septicaemia and commonly colonizes the nasopharynx of young children but does not always lead to disease(14). The nasopharyngeal colonization is considered an essential step in the development of pneumococcal disease and is targeted in the prevention of pneumococcal disease using the Pneumococcus Conjugate Vaccine (PCV)(15,16). The PCV reduces pneumococcal diseases by preventing the carriage (the colonization of the host) of specific disease-causing serotypes (16).

Asymptomatic nasopharyngeal carriage was initially thought to be harmless although emerging research suggests that pneumococcus carriage may have adverse consequences on child health other than the development of pneumococcal disease(14,17,18). A study conducted in South India reported that pneumococcal carriage at two months was associated with significant reductions in growth and nutritional status at six months(14). Another study, also in South India, reported that the duration of carriage was associated with a reduction in length and weight gain(18). Determining whether pneumococcal carriage influences child wasting has important implications for interventions aimed at reducing child wasting. If pneumococcal carriage has an adverse effect on child wasting, then interventions aimed at reducing pneumococcal diseases (such as the PCV) should also aim to reduce pneumococcal carriage.

This study uses intensively sampled longitudinal data among PCV vaccinated children in the Gambia to investigate the determinants of wasting in children under the age of 2 years, including the effect of pneumococcus carriage on wasting. The specific aims were to describe the patterns of wasting, stunting and underweight, to describe the patterns of pneumococcal carriage, and to evaluate the factors associated with wasting, including pneumococcal carriage in children under the age of two years from rural Gambia. The Gambia provides an ideal context for such research as child wasting remains high (6.2% of children in Rural Gambia were wasted in 2018), despite decades of unprecedented intensive interventions(19). Pneumococcal carriage is also highly common in this region with nearly all infants being colonised by three months of age, regardless of high PCV coverage (20).

Methods

Study design

This study is a secondary analysis of longitudinal data collected in rural Gambia as part of the Vaccination and Paediatric Microbiome Project. The parent study was aimed at determining the structure of the microbiome

in children under the age of two years and evaluating the impact of the PCV. This analysis used the collected anthropometric data to evaluate the outcomes of interest, namely wasting and WLZ score, against pneumococcal carriage and other possible exposures such as maternal age at birth, breastfeeding practices, illness, inflammation, and seasonality.

Study setting

The recruitment and data collection in the parent study took place in the Western Region of The Gambia between March 2013 and September 2015. The Western Region is made up of 55 villages spanning approximately 90km2 of land with a population of roughly 60 000 people(19,21,22). This study area was selected as it provides an adequate representation of the rural areas in The Gambia.

The residents in this area are mostly subsistence farmers growing and selling maize, millets, and ground nuts (23,24). Food is least abundant during the rainy season, also termed the "hunger season", between July and October as food stores from the previous harvest are almost exhausted(25,26). Following the "hungry season" is a long dry season where the infectious disease and pneumococcal carriage immediately peaks (21,27).

The Gambia has an extensive immunization programme, with over 90% of children having undergone routine vaccination by 12 months of age, including the PCV (28). More information on the study population is detailed elsewhere (23).

Data collection

The data collection methods of the parent study have been detailed elsewhere(24). Briefly, 120 new-borns were recruited immediately after birth, then followed up monthly until 12 months, and again at 15, 18, 21 and 24 months of age. Blood and serum samples were collected from children at birth and at month 2, 5, 12, 18 and 24. Nasopharyngeal (NP) swabs samples, anthropometric data, and medical information sourced from child health cards, were collected by trained field staff at each visit. Detailed socioeconomic, behavioural, nutritional status and clinical meta-data were collected at every follow-up visit using a standard questionnaire. Bacterial load of Streptococcus pneumoniae in the nasopharynx was determined though quantitative polymerase chain reaction. Iron and inflammation markers were measured from blood plasma using a fully automated biochemistry analyser (Cobas Integra 400 plus, UK).

Data measures

Child anthropometry was measured at birth and at each follow up visit. This included child weight, length, head and mid-upper arm circumference. Low birthweight (LBW) was defined as a weight at birth less than 2.5 kg. A weight of 2.5 kg or more was defined as normal birth weight(29). Weight, length, and age were used to calculate length-for-age, weight-for-length, and weight-for-age z-scores using the WHO standards(30). A child was classified as stunted, wasted or underweight if their length-for-age, weight-for-length and weight-for-age z-score was below two standard deviations of the median of the WHO reference population (Z-score <-2). Severe stunting and wasting were characterised by length-for-age and weight-for-length z-scores below three standard deviations of the median of the WHO reference population (Z-score <-3).

A child was classified as a pneumococcus carrier when their NP swab was positive for pneumococcal detection. Pneumococcus carriage onset was defined as the midpoint between the last negative culture and the first positive culture. The duration of carriage was calculated as the time from carriage onset to the midpoint between the last consecutive positive culture to the first subsequent negative culture. A positive culture detected after a negative culture would indicate a new episode of carriage. Similar definitions were used to calculate onset and duration of wasting, stunting and underweight.

Feeding categories were exclusive breastfeeding, mixed feeding (which was defined as breastfeeding supplemented with water and other liquids or solids), and no breastfeeding. Illness was defined as any nasal discharge, prolonged cough, ear discharge or difficulty breathing in the four weeks prior to the visit, by maternal report. Illness was also defined as any reported chest or ear infection, meningitis, sepsis, antibiotic use, or any other reported illness in the two weeks prior to the visit.

Markers of iron included Ferritin, transferrin, unsaturated iron binding capacity (UIBC), soluble transferrin receptor (sTfR) and hepcidin. Inflammation status was determined using alpha (1)-acid glycoprotein (AGP) and C-reactive protein (CRP). Inflammation was said to be present if a child's CRP was above 5mg/L and/or their AGP above 1mg/L (31,32). A CRP concentration 5mg/L or less and a AGP concentration of 1mg/L or less indicated the absence of inflammation.

Statistical analysis

This analysis was conducted using the statistical software R version 4.1.2. Baseline characteristics and time-varying characteristics were summarised using medians and interquartile ranges (IQR) or frequencies and proportions, where appropriate. Time to first pneumococcus carriage and time to first wasting, stunting or

underweight episode was estimated using Kaplan-Meier estimators. The duration of pneumococcus carriage, wasting, stunting and being underweight was regarded as the time from the midpoint between the last negative result and the first positive result, to the midpoint between the last positive result and the first negative result. Graphical methods, including LOESS smoothers, were used to illustrate the patterns of undernutrition and pneumococcal carriage over time.

Both wasting (as a binary indicator) and weight-for-length z-scores were outcomes of interest in this analysis. Generalised linear mixed models, with an identifier for each child fitted as a random intercept, were used to estimate the association between measured variables and wasting or WLZ scores. Only data points from 6 – 24 months of age were included in the regression analysis as growth patterns and factors that influence growth, in children under six months of age, differ from those older than six months(29). A significance level of 5% was used to guide the interpretation of all p-values.

Univariate models and random forests were used to select the variables that most influence WLZ scores and wasting. Variables with a statistically significant association on the outcomes, those that led to a relatively high mean decrease in accuracy (for wasting as outcome) or percentage increase in mean squared error (for WLZ scores as outcome), along with the variables; child age (in months), sex, and pneumococcus carriage were included in the multivariable models used to estimate the factors associate with WLZ scores and wasting. Both WLZ and wasting prevalence fluctuate over the calendar year. To adjust for this pattern, two seasonality factors (shown in equations (1) and (2)), derived from a harmonic regression model, were included in the multivariate model. Given that wasting is determined by WLZ scores, model selection was centred around WLZ scores, and variables used in the WLZ scores model were also used in the wasting model.

$$\text{Seasonal factor 1} = \cos(2\pi ti/12) \quad (1)$$

$$\text{Seasonal factor 2} = \sin(2\pi ti/12) \quad (2)$$

Where ti is the integer indicating the month of the year (1=Jan, … ,12=Dec)

Ethical Approval

The parent study was approved by the Joint MRCG and Gambia Government Ethics Committee. The current study was approved the University of Cape Town Faculty of Health Sciences Research Ethics Committee (HREC REF: 763/2020) (Appendix C2).

Results

Characteristics

A hundred and twenty children were enrolled and followed up from birth until two years of age. Sixty-seven (55.8%) were female and all children were full term and born to mothers with a median age of 30 years (IQR: 24, 33). Other child, maternal and environmental characteristics are summarised in Table 1. Table 2 summarises time-varying characteristics including breastfeeding patterns, nutritional status, illness and inflammation and pneumococcal carriage.

Nutritional Status

In the two years of follow up 57 (47.5%) children became stunted, 50 (41.7%) became underweight and 80 (66.7%) had at least one episode of wasting, with 29/80 (36.3%) experiencing repeated episodes of wasting. Of those that became stunted, 16/57 (28%) became severely stunted and of those that became wasted, 43/80 (53.8%) became severely wasted, with 18/43 (41.1%) experiencing recurrent episodes of severe wasting. Fifteen children experienced an episode of wasting and an episode of stunting concurrently at least once during the two years of follow up.

Twelve (13.2%) children were born wasted, 26 (24.8%) were born stunted and 4 (3.4%) were born underweight. The prevalence of wasting increased to 17.9% at 12 months and decreased to 6.4% at 24 months of age. The prevalence of stunting generally decreased in the first year of life and increased in the second year of life, with only 6% of the children being stunted at 12 months of age and 22% being stunted 24 months of age. The proportion of children who are underweight remained relatively low in the first year of life and began to increase in the second year of life, peaking at 22.4% among children who are 15 months old (Table 2).

Half of the children had experienced at least one episode of wasting by 10 months of age. Of those that became stunted, more than half became stunted by two months of age. The median duration of wasting was 55.75 days (IQR: 28 – 100.62 days), stunting 57 days (IQR: 28 – 110.1 days) and underweight 91.25 days (IQR: 35.62 – 109 days).

Length-for-age z-scores peaked shortly after birth and began declining around five months of age until around 18 months of age where the z-scores plateaued. Weight-for-age and weight-for-length z-scores began declining from six months until around 15 months of age, where these z-scores began to slightly increase. All three indices averaged below zero from birth until 24 months of age (Figure 1).

The average WLZ scores in this sample fluctuates throughout the year (figure 2, panel A). The lowest WLZ scores are seen in the dry seasons while an increase is seen in the wet season between July and September. This pattern is reflected in the figure showing the proportion of wasted children across a calendar year (Figure 2, panel B), where the proportion wasted children increases in the dry season (where WLZ scores are lowest) and decreases in the wet season (where WLZ scores increase).

Pneumococcus carriage.

One hundred and seventeen children became pneumococcus carriers at some point in the two years of follow up, with 50% of children becoming carriers by the 31st day of their lives 31 (95% CI: 30-54 days) and almost all the children becoming carriers by the 228th day of their lives. The median duration of carriage was 269.1 days (IQR: 70-508 days) and the proportion of pneumococcus carriage was highest around February and declined thereafter, until the beginning of the rainy season where it increased again. The trends of carriage prevalence across the calendar year are shown in supplementary Figure A1. Almost 16% of children were born colonised with pneumococcus. This number shot up to 84.5% by the third month of life. High prevalence of carriage was seen amongst all age groups with almost 90% of all children aged 12 months being carriers. Children aged 24 months had the lowest prevalence of carriage (68.8%).

Illness and inflammation

Over the two years of follow up, there were 829 reported cases of illnesses among 116 children. Of the reported illness cases, 96 were treated with antibiotics, suggesting an infectious (bacterial) origin. Majority of the children (92.5%) became ill at least once within the first year of life. No illness was reported at birth although roughly 16% of the children reported an illness at their third and sixth visit. The prevalence of illness remained high in the second year of life (Table 2). Not all cases of a recent illness were followed by inflammation and not all cases of inflammation were detected after a recent illness. A hundred and thirty-nine cases of recent illness reported at a visit had inflammation detected at that same visit. Forty-seven cases of reported illness had no detected inflammation and 169 cases of inflammation were detected with no reports of recent illness. It is worth noting that only 614 events had both illness and inflammation data since inflammation status was only assessed at birth and at 2, 5, 12, 18, and 24 months of age.

Factors associated with WLZ

Child age, mother's age at birth, low birth weight, inflammation at birth, having at least one episode of wasting in the first six months of life, reporting a recent illness, and being observed in the rainy season had

an inverse and statistically significant association with WLZ in the unadjusted model. Both seasonal factors one and two had positive and statistically significant associations with WLZ score in the unadjusted model. Pneumococcus carriage, being stunted at birth and being exclusively breastfed in the first six months of life were inversely associated with WLZ in the unadjusted model. In the unadjusted model, pneumococcus carriers, on average, had a WLZ score 0.02 less than infants who were not pneumococcus carriers [RR: -0.02 (95% CI: -0.18 – 0.13)]. Male children had a WLZ score 0.07 higher than female children [RR: 0.07 (95% CI: (-0.26 – 0.4)]. These associations, however, were not statistically significant (P-value > 0.05).

The inverse relationship between WLZ scores and child's age, mother's age at birth, inflammation at birth, having at least one episode of wasting in the first six months of life and reporting a recent illness persisted in the adjusted model. The relationship between WLZ and low birthweight and being observed in the rainy season did not persist in the adjusted model. In the adjusted model, infants with a low birthweight had a higher WLZ score compared to infants who did not have a low birthweight [OR: 0.05 (95% CI: -0.79 – 0.89)]. This association, however, was not statistically significant. Children observed in the rainy season had, on average, a WLZ score 0.33 higher than children observed in the dry season [RR: 0.33 (95% CI:0.11 – 0.55)]. This association was statistically significant with a p-value of 0.003. Both seasonal factors remain positively and significantly associated with WLZ score in the adjusted model. Pneumococcal carriage had a stronger and statistically significant association with WLZ in the adjusted model, with carriers having a WLZ score 0.18 lower than non-carriers [RR: -0.18 (95% CI: -0.35 – -0.01)]. The regression results with WLZ score as the outcome for the adjusted and unadjusted models are shown in Table 3.

Factors associated with wasting

Being male, a year increase in mother's age at birth, having a low birthweight, having inflammation at birth, being stunted at birth, having at least one episode of wasting in the first six months of life, being exclusively breastfed in the first six months of life, reporting a recent illness, being a colonised by pneumococcus [OR: 0.29 (95% CI: -0.35 – 0.93)] and being observed in the rainy season increased the odds of wasting in the unadjusted models. In the unadjusted model, a month's increase in child age decreased the odds of wasting by 1% [OR: -0.01 (95% OR: -0.05 – 0.03)]. However, this association is not statistically significant (P-value: 0.62).

Being male, a month's increase in child age, being stunted at birth, having at least one episode of wasting in the first six months of life, being exclusively breastfed in the first six months of life and being a pneumococcus carrier [OR: 1.62 (95% CI: 0.70 – 3.74)] increased the odds of wasting in the adjusted model. These associations, however, were not statistically significant. In the adjusted model, a year increase in maternal age at birth increased the odds of wasting by 9% [OR: 1.09 (95% CI: 1.02 – 1.17)]. Infants who had inflammation

at birth were more likely to be wasted [OR: 5.44 (95% CI: 1.09 – 27.22)], at least once between the ages of six and twenty-four months, compared to infants who had no inflammation at birth. Infants who reported a recent illness were also at increased odds of being wasted [OR: 2.46 (95% CI: 1.39 – 4.45)]. Infants who were observed in the rainy season were less likely to be wasted [OR: 0.28 (95% CI: 0.10 – 0.75)]. The associations between wasting and mother's age at birth, inflammation at birth, reporting a recent illness and being observed in the rainy season were statistically significant. The regression results with wasting as the outcome for the adjusted and unadjusted models are shown in Table 4.

Both seasonal factors one and two had a statistically significant relationship with wasting in both the adjusted and unadjusted models. However, the relationship between wasting and seasonal factor one and two was inverse in the unadjusted model and positive in the adjusted model.

Discussion

Child wasting remains endemic to low-and-middle-income-countries (LMICs) such as the Gambia despite extensive intervention (19). This analysis used intensively sampled data from a birth cohort in rural Gambia to determine the factors associated with wasting, including assessing the effect of pneumococcus carriage on child wasting. We began by describing the patterns of undernutrition that manifested as stunting, wasting and underweight in this sample. We then proceeded to investigate the factors associated with wasting and weight-for-length z-scores using generalised linear mixed models.

This study showed that the prevalence of wasting, stunting and being underweight and their respective indices (WLZ, LAZ, WAZ) varied with age. WLZ, LAZ and WAZ scores were highest in the first six months of life and gradually decreased thereafter until around 18 months of age. After 18 months, the median WLZ and WAZ scores increased but never reaching the magnitude they were at birth or in the first six months. LAZ scores remained low even after 18 months. The prevalence of wasting, stunting and being underweight was, on average, lower in the first year of life than in the second year of life, peaking at around 15 months of age. The prevalence of wasting and being underweight began to decrease after 15 months of age while the prevalence of stunting remained high (Supplementary Figure A2).

Prior research on rural Gambian children under the age of two years reported similar growth patterns to the ones observed in this sample. Nabwera and colleagues(19) reported that children in rural Gambia were born small, with LAZ, WAZ and WLZ scores highest in the first six months and gradually declining thereafter until the second year of life where the z-scores plateau. Another research team, analysing the same data as

Nabwera and colleagues reported trends of stunting and wasting by age similar to the ones reported in this study(26). The gradual decline in z-score or increase in undernutrition prevalence with age, could be a result of decreased breastfeeding with inadequate complementary feeding practices or the ability of older children to interact with other children and the environment which increases exposure to infectious agents through contaminated food and water often as a result of unhygienic environments(4).

The results of this study suggest that WLZ scores and wasting have multiple overlapping determinants. This study found that increasing child age (in months), increasing maternal age at delivery, low birthweight, wasting in the first six months of life, reporting a recent illness, and pneumococcus carriage are factors significantly associated with a lower WLZ score. The odds of wasting significantly increased with inflammation at birth, reporting a recent illness and a year increase in maternal age. Male children had lower WLZ scores and higher odds of wasting than female children. Several other studies reported male children as being more prone to wasting compared to female children(4,34–36). This association can be attributed to the belief that male children require more calories for growth and development compared to female children as they tend to be more active(34,36). In addition, male children are more likely to be influenced by environmental stressors than female children(35). The physiological basis for this discrepancy is still unknown.

Low birthweight (<2.5kg) has been reported to increase the risk of undernutrition in children(4,37,38). Low birthweight reflects intrauterine growth restrictions. Children who are born with a low birthweight are essentially born undernourished and even with appropriate nutrient intake, they may not fully recover and are likely to be undernourished in their early life(38). This phenomenon could explain why, in this analysis, children who are born with a low birthweight and those that are born stunted have lower WLZ scores and higher odds of wasting. Being wasted in the first six months of life also increased the odds of subsequent wasting and lowered WLZ scores. This observation is consistent with results reported by Mertens and colleagues(1).

Exclusive breastfeeding in the first six months of life is known to enhance child immunity, protecting against infectious diseases such as respiratory or diarrhoeal diseases that could lead to wasting. However, it is important to restrict exclusive breastfeeding to the first six months of life as breast milk alone provides inadequate nutrition for children six months or older(39). The increased odds of wasting seen in children who have been exclusively breastfed in the first six months in this sample, could be due to children who were exclusively breastfed in the first six months continuing exclusive breastfeeding beyond six months. It is also

plausible that children who were exclusively breastfed were better nourished in the first six months and experienced a dramatic decrease in WLZ score when introduced to complementary feeding.

Childhood illness, particularly respiratory and diarrhoeal illness, often result in weight loss because of the body's increased energy demand to fight the illness, the reduction of appetite and poor nutrient absorption(34,39). Illness often also induces an inflammatory response which results in the release of proinflammatory cytokines, among other inflammatory mediators(14). These proinflammatory cytokines may have a direct effect on child growth as they are inversely associated with insulin-like growth factor-1, a vital hormone in child growth(14). The current study found that children reporting a recent illness and children who had inflammation at birth had lower WLZ scores and higher odds of wasting. The association of wasting or lower WLZ scores with reporting a recent illness have been reported in other studies(4,34,37,39). To our knowledge, no studies have reported an association between our outcomes and having inflammation at birth. Our results, however, are not implausible as increased energy demand at birth, coupled with unmet nutritional requirements could result in early wasting that increases a child's risk of subsequent wasting episodes.

The Gambian climate is characterised by two distinct seasons: the rainy season between July and October and the dry season from November to June. Previous studies have reported growth deficits during the rainy season (1,19,40). This reduction in growth is often attributed to the high incidence of infectious diseases such as malaria and diarrhoea in the rainy season and the shortage of food during this time as food stores from the last harvest are limited. The current study, however, reported an improvement in z-scores and undernutrition in the rainy season followed by a decrease in z-scores and an increase in undernutrition prevalence in the few months immediately after the rainy season (Supplementary Figure A3 and Figure A4). Our regression analysis also suggested that children observed in the rainy season had higher WLZ scores and had lower odds of being wasted than children observed in the dry season. This observation was unexpected and may be due to chance or the increase in uptake of existing interventions during the rainy season.

This study found that pneumococcus carriers had lower WLZ scores, and higher odds of wasting compared to children who were not carriers, although only the relationship with carriage and WLZ scores was significant. These findings contrasted those reported in earlier studies. A study done in Vellore India reported no association with carriage and WLZ or wasting, although they did report reduced weight and height gain and lower WAZ and LAZ with increased duration of carriage(41). A subsequent study in Tamil Nadu, also in India, reported no association with wasting at six months of age with carriage at two months, four months or both

two and four months of age. However, carriage at two months was associated with a reduction in mean weight, mean height, WAZ and LAZ at six months of age(14).

The Vellore study assessed children every three weeks from birth to the first year of life, while the Tamil Nadu study only assessed children at two, four and six months of age. The discrepancies between the results of this study and the two aforementioned studies could be attributed to the difference in age groups observed and timing of observations. This study included data of children between the ages of 6 and 24 months, excluding the first six months of life as growth during that period is most influenced by in utero conditions(40). The current analysis did not distinguish between pneumococcal carriage and pneumococcal disease. As a result, the association between pneumococcus carriage and wasting or WLZ could be overestimated as poor weight gain in carriers could be attributed to the development of pneumococcal disease. This, however, is unlikely as all children in this sample were vaccinated with PCV. PCV has been shown to drastically decrease invasive pneumococcal disease, particularly in children between 2-24 months of age (42). In addition, the confounding effect of disease was adjusted for through the illness variable.

The relationship between carriage and wasting or WLZ scores warrants further investigation in settings such as the Gambia where pneumococcus carriage remains highly prevalent despite of wide PCV coverage. In this sample, 7 out of 10 children were colonised by 2 months of age and the proportion colonised remained alarmingly high throughout the 2 years of follow-up. Pneumococcal carriage also demonstrated seasonal patterns with the prevalence of carriage peaking in the dry season. The patterns of carriage were consistent with those reported Bojang and colleagues in a different sample of children in rural Gambia(21).

This study had several strengths and limitations. One of the biggest strengths was the use of longitudinal data, allowing for the assessment of duration and recurrent episodes of wasting. In addition, longitudinal data allows for the adjustment of dynamic exposures such as seasonality. An important limitation was the lack of data on socioeconomic status, parental education, and maternal nutritional status at birth. Several studies investigating the determinants of undernutrition in Sub-Saharan Africa reported maternal or paternal education, maternal BMI and household socioeconomic status as factors associated with wasting(4,37). Maternal BMI is a proxy for maternal nutritional status which can provide useful information regarding the child nutritional status in utero. Parental education and socioeconomic status are often correlated with child nutritional status as more effluent parents (often because of better education) can provide their child with sufficient nutrition and can often afford health care. This limitation is not likely to bias our estimates as socioeconomic status of people living in this part of rural Gambia is assumed to be uniform and previous

research demonstrated that WHZ does not differ across economic status category(43). Furthermore, only 4/120 mothers in this cohort were employed. In addition, primary health care in the study region was free. Universal access to primary health care has the potential to mitigate bias resulting from socioeconomic inequalities.

Another limitation is this study's inability to account for environmental factors such as neglected tropical diseases (schistosomiasis and soil-transmitted helminthiases), natural disasters (floods) and political conflict that is endemic and common in this region(44). Failure to account for the above can potentially inflate the association observed with our outcomes and other variables.

This study did not assess the temporal relationship between wasting and pneumococcus carriage or recent illness. It is well documented that the relationship between undernutrition and illness is synergistic, and each one can often lead to the other. Assessing the temporal relationship would have allowed us to determine whether pneumococcus carriage led to wasting or wasted children were more susceptible to carriage.

Conclusion

The relationship between carriage and wasting or WLZ scores warrants further investigation in settings such as the Gambia where pneumococcus carriage remains highly prevalent despite of wide PCV coverage. Other than pneumococcus carriage, this study found low birthweight, early episodes wasting, inflammation at birth and recent illness to be associated with wasting in children between the ages of 6-24 months. Targeting maternal nutrition during pregnancy, prenatal and neonatal care and infant feeding practises may help reduce wasting among children between 6-24 months of age by reducing low birthweight, episodes of wasting, illness and inflammation in early infancy.

Abstract

Background

Child wasting remains a public health problem in Africa and South Asia. Some studies have suggested that asymptomatic colonization of bacterial agents such as Streptococcus Pneumoniae (pneumococcus) may have negative impacts on child growth and nutritional status. The aim this study is to investigate the determinants of wasting in children and to assess the role of pneumococcus carriage on wasting.

Methods

This secondary analysis used longitudinal data collected from 120 infants recruited at birth and followed up at regular intervals for two years. The data was collected in Rural Gambia between March 2013 and September 2015 as part of the Vaccination and Paediatric Microbiome Project.

Nutritional status was classified using weight-for-length, length-for-age, and weight-for-age for wasting, stunting and underweight respectfully. Regression methods were used to evaluate the factors associated with both wasting, as a binary indicator, and weight-for-length among children aged 6-24 months.

Results

Over the two years of follow-up, 66.7% (80/120) of children had at least one episode of wasting with 36.3% (29/80) experiencing repeated episodes. About 47.5% (57/120) became stunted and 41.7% (50/120) became underweight.

Child age, mother's age at delivery, having at least one episode of wasting in the first six months of life, reporting a recent illness, and being observed in the rainy season had significant associations with WLZ scores in both the adjusted and unadjusted models. Pneumococcus carriage only had a significant association with WLZ scores in the adjusted model [RR: -0.18 (95% CI: -0.35 – -0.01)], while LBW only had a significant association in the unadjusted model [RR: -0.78 (95% CI: -1.49 – -0.07)].

Mother's age at birth, inflammation at birth and reporting a recent illness was significantly associated with wasting as a binary indicator in both the adjusted and unadjusted models. Being observed in the rainy season decreased the odds of wasting, although, this association was only significant in the adjusted model [OR: 0.28 (95% CI: 0.10 – 0.75)]. Pneumococcus carriers had lower odds of wasting in the unadjusted model [OR: 0.29 (95% CI: -0.35 – 0.93)] and higher odds of wasting in the adjusted model [OR: 1.62 (95% CI: 0.70 – 3.74)]. Neither association was statistically significant.

Conclusion

This study found low birthweight, early episodes of wasting, inflammation at birth and recent illness to be associated with wasting in children between the ages of 6-24 months. Targeting maternal nutrition and prenatal care may help combat undernutrition in infancy, which in turn may reduce wasting among children between 6-24 months of age. Further research is needed to understand the role of pneumococcus on child undernutrition.

Introduction

Undernutrition is a serious public health problem associated with 45% of deaths among children under the age of five years globally(1). Stunting, wasting and underweight are the most used indicators of undernutrition in children. Wasting is characterised by a dangerously low body weight in relation to height resulting from muscle and fat tissue loss(1,2). It affects 45.4 million children worldwide with 27% and 48% of those children residing in Africa and South Asia, respectively(3). A child is considered wasted when their

weight-for-length z-score (WLZ) is less than two standard deviations below the median of the WHO reference population(1,4). Wasted children have a weakened immune system, leaving them vulnerable to infection and diseases(1,5). As a result, wasted children have an increased risk of morbidity and mortality. In addition, they have an increased risk of stunting, a chronic state of linear growth failure, characterised by a length-for-age z-score below negative two standard deviations below median on standard WHO growth reference chart(1,6). Wasting can also lead to long-term adverse effects on a child's physical and cognitive development(4,5,7).

Child wasting can often be treated with appropriate nutritional interventions. Currently, treatment methods target severely wasted children (WLZ less than negative 3 standard deviations on the WHO growth reference chart) between the ages of 6-59 months and involve supplementary feeding with energy-dense fortified pastes called ready-to-use-therapeutic foods (RUTF)(8,9). Treatment, although effective, does not reduce the risk of the reoccurrence of wasting or subsequent stunting(8). For that reason, preventative interventions would be preferred to treatment interventions, particularly for this age group. To inform these interventions, a critical understanding of factors associated with wasting is required.

Child wasting, just like most non-communicable diseases, has no single causal factor. It occurs as a result of an interplay among various factors including maternal health, nutrition and education, child diet, feeding practices, sex, infectious disease, and environmental factors such as socioeconomic status, seasonality, and place of residence(4,5). Risk factors such as maternal health and nutrition (particularly in pregnancy), child diet, feeding practices and infection are modifiable and can be targeted to reduce the burden of child wasting. Infectious diseases, in the context of wasting, are both a risk factor and a consequence for child wasting(10,11). Having a low body weight weakens the immune system and increases susceptibility to infection and having an infection increases energy demand and decreases appetite, leading to a reduction in body weight(5,11).

Regions burdened with a high prevalence of undernutrition also often have a high incident of infectious diseases(12). Streptococcus Pneumoniae (pneumococcus) is a gram-positive bacterium that is responsible for a significant proportion of the morbidity and mortality among children under five years across the globe(13,14). The bacterium causes diseases such as pneumonia, meningitis and septicaemia and commonly colonizes the nasopharynx of young children but does not always lead to disease(14). The nasopharyngeal colonization is considered an essential step in the development of pneumococcal disease and is targeted in the prevention of pneumococcal disease using the Pneumococcus Conjugate Vaccine (PCV)(15,16). The PCV

reduces pneumococcal diseases by preventing the carriage (the colonization of the host) of specific disease-causing serotypes (16).

Asymptomatic nasopharyngeal carriage was initially thought to be harmless although emerging research suggests that pneumococcus carriage may have adverse consequences on child health other than the development of pneumococcal disease(14,17,18). A study conducted in South India reported that pneumococcal carriage at two months was associated with significant reductions in growth and nutritional status at six months(14). Another study, also in South India, reported that the duration of carriage was associated with a reduction in length and weight gain(18). Determining whether pneumococcal carriage influences child wasting has important implications for interventions aimed at reducing child wasting. If pneumococcal carriage has an adverse effect on child wasting, then interventions aimed at reducing pneumococcal diseases (such as the PCV) should also aim to reduce pneumococcal carriage.

This study uses intensively sampled longitudinal data among PCV vaccinated children in the Gambia to investigate the determinants of wasting in children under the age of 2 years, including the effect of pneumococcus carriage on wasting. The specific aims were to describe the patterns of wasting, stunting and underweight, to describe the patterns of pneumococcal carriage, and to evaluate the factors associated with wasting, including pneumococcal carriage in children under the age of two years from rural Gambia. The Gambia provides an ideal context for such research as child wasting remains high (6.2% of children in Rural Gambia were wasted in 2018), despite decades of unprecedented intensive interventions(19). Pneumococcal carriage is also highly common in this region with nearly all infants being colonised by three months of age, regardless of high PCV coverage (20).

Methods

Study design

This study is a secondary analysis of longitudinal data collected in rural Gambia as part of the Vaccination and Paediatric Microbiome Project. The parent study was aimed at determining the structure of the microbiome in children under the age of two years and evaluating the impact of the PCV. This analysis used the collected anthropometric data to evaluate the outcomes of interest, namely wasting and WLZ score, against pneumococcal carriage and other possible exposures such as maternal age at birth, breastfeeding practices, illness, inflammation, and seasonality.

Study setting

The recruitment and data collection in the parent study took place in the Western Region of The Gambia between March 2013 and September 2015. The Western Region is made up of 55 villages spanning approximately 90km2 of land with a population of roughly 60 000 people(19,21,22). This study area was selected as it provides an adequate representation of the rural areas in The Gambia.

The residents in this area are mostly subsistence farmers growing and selling maize, millets, and ground nuts (23,24). Food is least abundant during the rainy season, also termed the "hunger season", between July and October as food stores from the previous harvest are almost exhausted(25,26). Following the "hungry season" is a long dry season where the infectious disease and pneumococcal carriage immediately peaks (21,27).

The Gambia has an extensive immunization programme, with over 90% of children having undergone routine vaccination by 12 months of age, including the PCV (28). More information on the study population is detailed elsewhere (23).

Data collection

The data collection methods of the parent study have been detailed elsewhere(24). Briefly, 120 new-borns were recruited immediately after birth, then followed up monthly until 12 months, and again at 15, 18, 21 and 24 months of age. Blood and serum samples were collected from children at birth and at month 2, 5, 12, 18 and 24. Nasopharyngeal (NP) swabs samples, anthropometric data, and medical information sourced from child health cards, were collected by trained field staff at each visit. Detailed socioeconomic, behavioural, nutritional status and clinical meta-data were collected at every follow-up visit using a standard questionnaire. Bacterial load of Streptococcus pneumoniae in the nasopharynx was determined though quantitative polymerase chain reaction. Iron and inflammation markers were measured from blood plasma using a fully automated biochemistry analyser (Cobas Integra 400 plus, UK).

Data measures

Child anthropometry was measured at birth and at each follow up visit. This included child weight, length, head and mid-upper arm circumference. Low birthweight (LBW) was defined as a weight at birth less than 2.5 kg. A weight of 2.5 kg or more was defined as normal birth weight(29). Weight, length, and age were used to calculate length-for-age, weight-for-length, and weight-for-age z-scores using the WHO standards(30). A child was classified as stunted, wasted or underweight if their length-for-age, weight-for-length and weight-for-age z-score was below two standard deviations of the median of the WHO reference population (Z-score <-2).

Severe stunting and wasting were characterised by length-for-age and weight-for-length z-scores below three standard deviations of the median of the WHO reference population (Z-score <-3).

A child was classified as a pneumococcus carrier when their NP swab was positive for pneumococcal detection. Pneumococcus carriage onset was defined as the midpoint between the last negative culture and the first positive culture. The duration of carriage was calculated as the time from carriage onset to the midpoint between the last consecutive positive culture to the first subsequent negative culture. A positive culture detected after a negative culture would indicate a new episode of carriage. Similar definitions were used to calculate onset and duration of wasting, stunting and underweight.

Feeding categories were exclusive breastfeeding, mixed feeding (which was defined as breastfeeding supplemented with water and other liquids or solids), and no breastfeeding. Illness was defined as any nasal discharge, prolonged cough, ear discharge or difficulty breathing in the four weeks prior to the visit, by maternal report. Illness was also defined as any reported chest or ear infection, meningitis, sepsis, antibiotic use, or any other reported illness in the two weeks prior to the visit.

Markers of iron included Ferritin, transferrin, unsaturated iron binding capacity (UIBC), soluble transferrin receptor (sTfR) and hepcidin. Inflammation status was determined using alpha (1)-acid glycoprotein (AGP) and C-reactive protein (CRP). Inflammation was said to be present if a child's CRP was above 5mg/L and/or their AGP above 1mg/L (31,32). A CRP concentration 5mg/L or less and a AGP concentration of 1mg/L or less indicated the absence of inflammation.

Statistical analysis

This analysis was conducted using the statistical software R version 4.1.2. Baseline characteristics and time-varying characteristics were summarised using medians and interquartile ranges (IQR) or frequencies and proportions, where appropriate. Time to first pneumococcus carriage and time to first wasting, stunting or underweight episode was estimated using Kaplan-Meier estimators. The duration of pneumococcus carriage, wasting, stunting and being underweight was regarded as the time from the midpoint between the last negative result and the first positive result, to the midpoint between the last positive result and the first negative result. Graphical methods, including LOESS smoothers, were used to illustrate the patterns of undernutrition and pneumococcal carriage over time.

Both wasting (as a binary indicator) and weight-for-length z-scores were outcomes of interest in this analysis. Generalised linear mixed models, with an identifier for each child fitted as a random intercept, were used to estimate the association between measured variables and wasting or WLZ scores. Only data points from 6 – 24 months of age were included in the regression analysis as growth patterns and factors that influence growth, in children under six months of age, differ from those older than six months(29). A significance level of 5% was used to guide the interpretation of all p-values.

Univariate models and random forests were used to select the variables that most influence WLZ scores and wasting. Variables with a statistically significant association on the outcomes, those that led to a relatively high mean decrease in accuracy (for wasting as outcome) or percentage increase in mean squared error (for WLZ scores as outcome), along with the variables; child age (in months), sex, and pneumococcus carriage were included in the multivariable models used to estimate the factors associate with WLZ scores and wasting. Both WLZ and wasting prevalence fluctuate over the calendar year. To adjust for this pattern, two seasonality factors (shown in equations (1) and (2)), derived from a harmonic regression model, were included in the multivariate model. Given that wasting is determined by WLZ scores, model selection was centred around WLZ scores, and variables used in the WLZ scores model were also used in the wasting model.

$$\text{Seasonal factor 1} = \cos(2\pi ti/12) \quad (1)$$

$$\text{Seasonal factor 2} = \sin(2\pi ti/12) \quad (2)$$

Where ti is the integer indicating the month of the year (1=Jan, … ,12=Dec)

Ethical Approval

The parent study was approved by the Joint MRCG and Gambia Government Ethics Committee. The current study was approved the University of Cape Town Faculty of Health Sciences Research Ethics Committee (HREC REF: 763/2020) (Appendix C2).

Results

Characteristics

A hundred and twenty children were enrolled and followed up from birth until two years of age. Sixty-seven (55.8%) were female and all children were full term and born to mothers with a median age of 30 years (IQR: 24, 33). Other child, maternal and environmental characteristics are summarised in Table 1. Table 2 summarises time-varying characteristics including breastfeeding patterns, nutritional status, illness and inflammation and pneumococcal carriage.

Table 1: Characteristics of children included in this analysis summarised as *median (IQR) or n (%)*

Characteristic	Overall (N=120)
Mother's age at delivery	30 (24.0, 33)
Birthweight (kg)	3.2 (2.9, 3.4)
Length at birth	48 (46, 50)
Weight for age Z score (WAZ) at birth	-0.3 (-0.8, 0.3)
Length for age Z score (LAZ) at birth	-0.6 (-1.7, 0.5)
Weight for length Z score (WLZ) at birth	0.2 (-1.3, 1.1)
Number of siblings with the same mother	3 (1, 4)
Sex	
- Female	67 (55.8%)
- Male	53 (44.2%)
Low birthweight	
- No	114 (95%)
- Yes	6 (5%)
Stunted in first 6 months	
- No	82 (68.3%)
- Yes	38 (31.7%)
Wasted in first 6 months	
- No	68 (56.7%)
- Yes	52 (43.3%)
Exclusively breastfed for 6 months	
- No	19 (15.8%)
- Yes	101 (84.2%)
Mother employed	
- No	115 (96.6%)
- Yes	4 (3.4%)
Room flooring	
- Modern	68 (58.1%)
- Earth/Sand/Dung	49 (41.9%)

Nutritional Status

In the two years of follow up 57 (47.5%) children became stunted, 50 (41.7%) became underweight and 80 (66.7%) had at least one episode of wasting, with 29/80 (36.3%) experiencing repeated episodes of wasting. Of those that became stunted, 16/57 (28%) became severely stunted and of those that became wasted, 43/80 (53.8%) became severely wasted, with 18/43 (41.1%) experiencing recurrent episodes of severe wasting. Fifteen children experienced an episode of wasting and an episode of stunting concurrently at least once during the two years of follow up.

Twelve (13.2%) children were born wasted, 26 (24.8%) were born stunted and 4 (3.4%) were born underweight. The prevalence of wasting increased to 17.9% at 12 months and decreased to 6.4% at 24 months of age. The prevalence of stunting generally decreased in the first year of life and increased in the second year of life, with only 6% of the children being stunted at 12 months of age and 22% being stunted 24 months of age. The proportion of children who are underweight remained relatively low in the first year of life and began to increase in the second year of life, peaking at 22.4% among children who are 15 months old (Table 2).

Half of the children had experienced at least one episode of wasting by 10 months of age. Of those that became stunted, more than half became stunted by two months of age. The median duration of wasting was 55.75 days (IQR: 28 – 100.62 days), stunting 57 days (IQR: 28 – 110.1 days) and underweight 91.25 days (IQR: 35.62 – 109 days).

Length-for-age z-scores peaked shortly after birth and began declining around five months of age until around 18 months of age where the z-scores plateaued. Weight-for-age and weight-for-length z-scores began declining from six months until around 15 months of age, where these z-scores began to slightly increase. All three indices averaged below zero from birth until 24 months of age (Figure 1).

The average WLZ scores in this sample fluctuates throughout the year (figure 2, panel A). The lowest WLZ scores are seen in the dry seasons while an increase is seen in the wet season between July and September. This pattern is reflected in the figure showing the proportion of wasted children across a calendar year (Figure 2, panel B), where the proportion wasted children increases in the dry season (where WLZ scores are lowest) and decreases in the wet season (where WLZ scores increase).

Table 2: Average z-scores (median (IQR)) and the proportions (n (%)) of children who had undernutrition, a recent illness, inflammation and who are carriers at birth, at 3, 6, 12, 15, 18 and 24 months of age.

Characteristics	Birth (N=120)	3 months old (N=110)	6 months old (N=102)	12 months old (N=106)	15 months old (N=108)	18 months old (N=106)	24 months old (N=109)
Weight for length Z score (WLZ)	0.2 (-1.3, 1.1)	-0.4 (-1.3, 0.4)	-0.2 (-1.0, 0.6)	-0.6 (-1.6, 0.1)	-1.0 (-1.8, -0.5)	-0.5 (-1.4, -0.0)	-0.6 (-1.2, 0.0)
Length for age Z score (LAZ)	-0.6 (-1.7, 0.5)	0.1 (-0.6, 1.0)	-0.2 (-1.3, 0.5)	-0.7 (-1.1, 0.0)	-1.0 (-1.7, -0.4)	-1.3 (-2.0, -0.7)	-1.4 (-1.9, -1.0)
Weight for age Z score (WAZ)	-0.3 (-0.8, 0.3)	-0.1 (-0.9, 0.4)	-0.4 (-1.0, 0.3)	-0.7 (-1.6, -0.1)	-1.2 (-1.9, -0.8)	-0.9 (-1.8, -0.4)	-1.1 (-1.7, -0.8)
Wasted							
- No	79 (86.8%)	98 (89.1%)	96 (95.0%)	87 (82.1%)	83 (77.6%)	92 (86.8%)	102 (93.6%)
- Yes	12 (13.2%)	12 (10.9%)	5 (5.0%)	19 (17.9%)	24 (22.4%)	14 (13.2%)	7 (6.4%)
Stunted							
- No	79 (75.2%)	105 (95.5%)	93 (92.1%)	100 (94.3%)	85 (79.4%)	85 (80.2%)	85 (78.0%)
- Yes	26 (24.8%)	5 (4.5%)	8 (7.9%)	6 (5.7%)	22 (20.6%)	21 (19.8%)	24 (22.0%)
Underweight							
- No	113 (96.6%)	101 (91.8%)	92 (91.1%)	92 (86.8%)	83 (77.6%)	88 (83.0%)	96 (88.1%)
- Yes	4 (3.4%)	9 (8.2%)	9 (8.9%)	14 (13.2%)	24 (22.4%)	18 (17.0%)	13 (11.9%)
Illness							
-No	105 (100.0%)	89 (84.0%)	83 (83.8%)	60 (56.6%)	35 (36.1%)	38 (38.8%)	48 (44.9%)
-Yes	0 (0.0%)	17 (16.0%)	16 (16.2%)	46 (43.4%)	62 (63.9%)	60 (61.2%)	59 (55.1%)
***Inflammation**							
-No	108 (94.7%)	- -	- -	26 (25.2%)	- -	36 (34.0%)	42 (38.9%)
-Yes	6 (5.3%)	- -	- -	77 (74.8%)	- -	70 (66.0%)	66 (61.1%)
Carrier of pneumococcus							
- No	101 (84.2%)	17 (15.5%)	13 (12.7%)	11 (10.4%)	21 (19.4%)	21 (19.8%)	34 (31.2%)
- Yes	19 (15.8%)	93 (84.5%)	89 (87.3%)	95 (89.6%)	87 (80.6%)	85 (80.2%)	75 (68.8%)

*Inflammation markers were not assessed at month 3, 6 and 15 months of age.

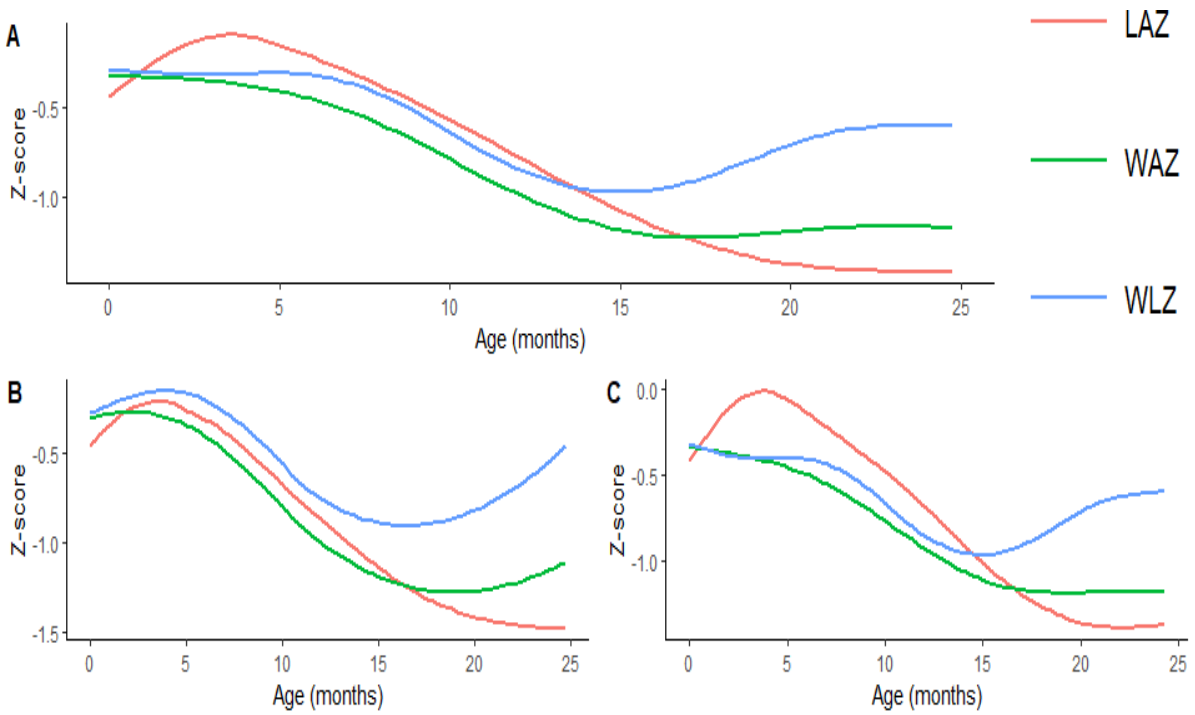

Figure 1: Growth patterns of Gambian children under two years old illustrated using LOESS smoothers. The red, green and blue line indicate the average length-for-age (LAZ), weight-for-age (WAZ) and weight-for-length (WLZ) z-scores in (A) the whole cohort, (B) male children and (C) female children.

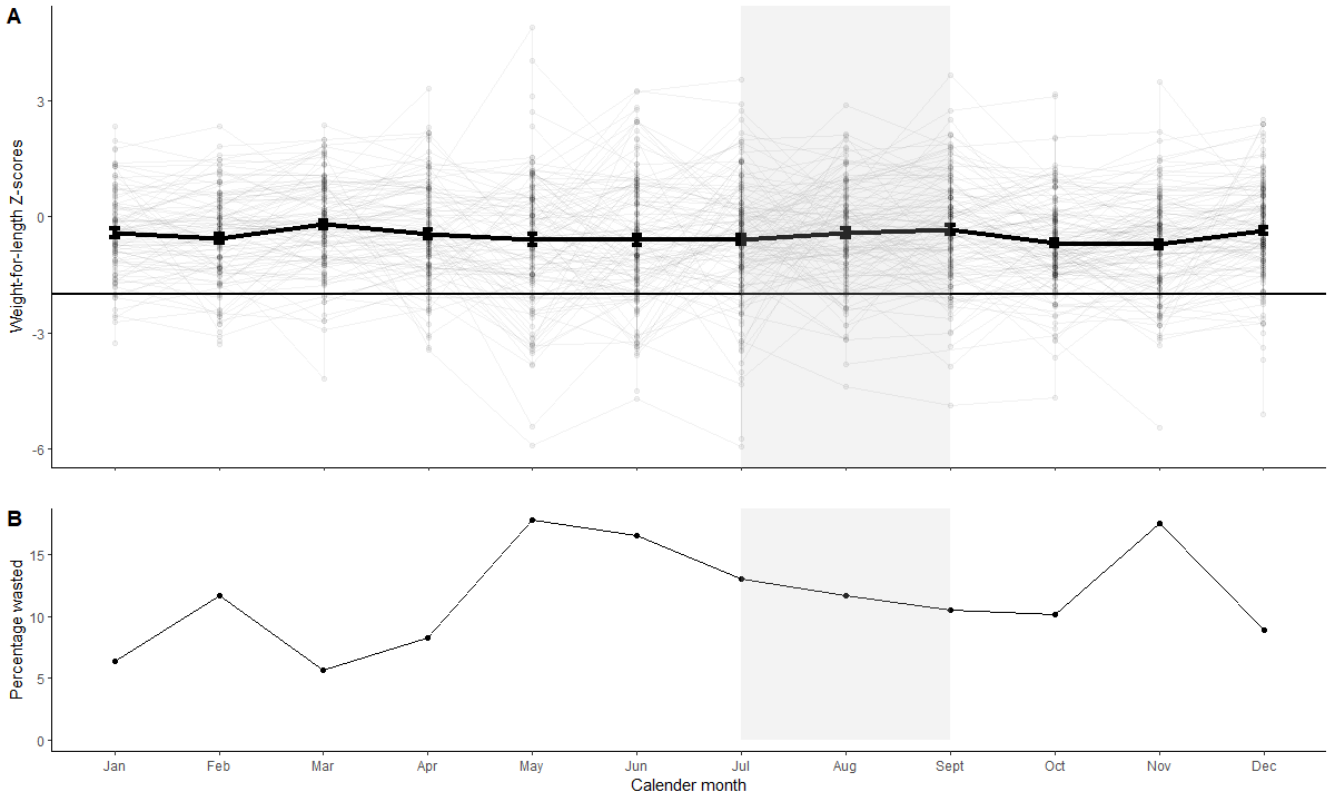

Figure 2: Seasonality of WLZ and wasting. Panel A presents individual WLZ trajectories (grey lines), the average WLZ scores (black lines) and the negative two threshold for wasting (horizontal solid line). Panel B presents the proportion of wasted children across the calendar year. The grey shaded region indicates the rainy season.

Pneumococcus carriage.

One hundred and seventeen children became pneumococcus carriers at some point in the two years of follow up, with 50% of children becoming carriers by the 31st day of their lives 31 (95% CI: 30-54 days) and almost all the children becoming carriers by the 228th day of their lives. The median duration of carriage was 269.1 days (IQR: 70-508 days) and the proportion of pneumococcus carriage was highest around February and declined thereafter, until the beginning of the rainy season where it increased again. The trends of carriage prevalence across the calendar year are shown in supplementary Figure A1. Almost 16% of children were born colonised with pneumococcus. This number shot up to 84.5% by the third month of life. High prevalence of carriage was seen amongst all age groups with almost 90% of all children aged 12 months being carriers. Children aged 24 months had the lowest prevalence of carriage (68.8%).

Illness and inflammation

Over the two years of follow up, there were 829 reported cases of illnesses among 116 children. Of the reported illness cases, 96 were treated with antibiotics, suggesting an infectious (bacterial) origin. Majority of the children (92.5%) became ill at least once within the first year of life. No illness was reported at birth although roughly 16% of the children reported an illness at their third and sixth visit. The prevalence of illness remained high in the second year of life (Table 2). Not all cases of a recent illness were followed by inflammation and not all cases of inflammation were detected after a recent illness. A hundred and thirty-nine cases of recent illness reported at a visit had inflammation detected at that same visit. Forty-seven cases of reported illness had no detected inflammation and 169 cases of inflammation were detected with no reports of recent illness. It is worth noting that only 614 events had both illness and inflammation data since inflammation status was only assessed at birth and at 2, 5, 12, 18, and 24 months of age.

Factors associated with WLZ

Child age, mother's age at birth, low birth weight, inflammation at birth, having at least one episode of wasting in the first six months of life, reporting a recent illness, and being observed in the rainy season had an inverse and statistically significant association with WLZ in the unadjusted model. Both seasonal factors one and two had positive and statistically significant associations with WLZ score in the unadjusted model. Pneumococcus carriage, being stunted at birth and being exclusively breastfed in the first six months of life were inversely associated with WLZ in the unadjusted model. In the unadjusted model, pneumococcus carriers, on average, had a WLZ score 0.02 less than infants

who were not pneumococcus carriers [RR: -0.02 (95% CI: -0.18 – 0.13)]. Male children had a WLZ score 0.07 higher than female children [RR: 0.07 (95% CI: (-0.26 – 0.4)]. These associations, however, were not statistically significant (P-value > 0.05).

Table 3: Linear mixed model regression results with WLZ scores as the outcome for the adjusted and unadjusted models

Weight for length Z score (WLZ)				
	Unadjusted		Adjusted	
Variables	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Sex				
Male	0.07 (-0.26 – 0.4)	0.67	-0.03 (-0.37 – 0.30)	0.85
Female	Reference			
Child age	-0.02 (-0.03 – -0.01)	**<0.001**	-0.01 (-0.02 – -0.00)	**0.04**
Mother's age at birth	-0.04 (-0.06 – -0.01)	**0.003**	-0.04 (-0.06 – -0.01)	**0.004**
Low birthweight				
Yes	-0.78 (-1.49 – -0.07)	**0.03**	0.05 (-0.79 – 0.89)	0.91
No	Reference			
Inflammation at birth				
Yes	-0.91 (-1.6 – -0.22)	**0.01**	-0.97 (-1.70 – -0.23)	**0.01**
No	Reference			
Child stunted at birth				
Yes	-0.39 (-0.79 – 0.01)	0.06	-0.28 (-0.69 – 0.13)	0.18
No	Reference			
Wasted in first 6 months				
Yes	-0.44 (-0.76 – -0.12)	**0.01**	-0.40 (-0.74 – -0.05)	**0.03**
No	Reference			
***EBF in first 6 months**				
Yes	-0.11 (-0.56 – 0.33)	0.62	-0.12 (-0.60 – 0.36)	0.64
No	Reference			
Recent illness				
Yes	-0.36 (-0.47 – -0.25)	**<0.001**	-0.25 (-0.37 – -0.12)	**<0.001**
No	Reference			
Pneumococcus Carriage				
Yes	-0.02 (-0.18 – 0.13)	0.76	-0.18 (-0.35 – -0.01)	**0.04**
No	Reference			
Visit Season				
Rainy	-0.26 (-0.41 – -0.11)	**<0.001**	0.33 (0.11 – 0.55)	**0.003**
Dry	Reference			
Seasonal factor 1	0.25 (0.17 – 0.33)	**<0.001**	0.38 (0.27 – 0.49)	**<0.001**
Seasonal factor 2	0.21 (0.13 – 0.29)	**<0.001**	0.21 (0.12 – 0.30)	**<0.001**

*EBF = Exclusive Breastfeeding

The inverse relationship between WLZ scores and child's age, mother's age at birth, inflammation at birth, having at least one episode of wasting in the first six months of life and reporting a recent illness persisted in the adjusted model. The relationship between WLZ and low birthweight and being observed in the rainy season did not persist in the adjusted model. In the adjusted model, infants with a low birthweight had a higher WLZ score compared to infants who did not have a low birthweight [OR: 0.05 (95% CI: -0.79 – 0.89)]. This association, however, was not statistically significant. Children observed in the rainy season had, on average, a WLZ score 0.33 higher than children observed in the dry season [RR: 0.33 (95% CI:0.11 – 0.55)]. This association was statistically significant with a p-value of 0.003. Both seasonal factors remain positively and significantly associated with WLZ score in the adjusted model. Pneumococcal carriage had a stronger and statistically significant association with WLZ in the adjusted model, with carriers having a WLZ score 0.18 lower than non-carriers [RR: -0.18 (95% CI: -0.35 – -0.01)]. The regression results with WLZ score as the outcome for the adjusted and unadjusted models are shown in Table 3.

Factors associated with wasting

Being male, a year increase in mother's age at birth, having a low birthweight, having inflammation at birth, being stunted at birth, having at least one episode of wasting in the first six months of life, being exclusively breastfed in the first six months of life, reporting a recent illness, being a colonised by pneumococcus [OR: 0.29 (95% CI: -0.35 – 0.93)] and being observed in the rainy season increased the odds of wasting in the unadjusted models. In the unadjusted model, a month's increase in child age decreased the odds of wasting by 1% [OR: -0.01 (95% OR: -0.05 – 0.03)]. However, this association is not statistically significant (P-value: 0.62).

Being male, a month's increase in child age, being stunted at birth, having at least one episode of wasting in the first six months of life, being exclusively breastfed in the first six months of life and being a pneumococcus carrier [OR: 1.62 (95% CI: 0.70 – 3.74)] increased the odds of wasting in the adjusted model. These associations, however, were not statistically significant. In the adjusted model, a year increase in maternal age at birth increased the odds of wasting by 9% [OR: 1.09 (95% CI: 1.02 – 1.17)]. Infants who had inflammation at birth were more likely to be wasted [OR: 5.44 (95% CI: 1.09 – 27.22)], at least once between the ages of six and twenty-four months, compared to infants who had no inflammation at birth. Infants who reported a recent illness were also at increased odds of being wasted [OR: 2.46 (95% CI: 1.39 – 4.45)]. Infants who were observed in the rainy season were less likely to be wasted [OR: 0.28 (95% CI: 0.10 – 0.75)]. The associations between wasting and mother's age at

birth, inflammation at birth, reporting a recent illness and being observed in the rainy season were statistically significant. The regression results with wasting as the outcome for the adjusted and unadjusted models are shown in Table 4.

Both seasonal factors one and two had a statistically significant relationship with wasting in both the adjusted and unadjusted models. However, the relationship between wasting and seasonal factor one and two was inverse in the unadjusted model and positive in the adjusted model.

Table 4: General linear mixed model (using logit link) regression results with wasting as the outcome for the adjusted and unadjusted models

	Wasted			
	Unadjusted		Adjusted	
Variables	OR (95% CI)	P-value	OR (95% CI)	P-value
Sex				
Male	0.25 (-0.61 – 1.1)	0.57	1.94 (0.82 – 4.58)	0.13
Female		Reference		
Child age	-0.01 (-0.05 – 0.03)	0.62	0.99 (0.94 – 1.04)	0.58
Mother's age at birth	0.07 (0.01 – 0.13)	**0.03**	1.09 (1.02 – 1.17)	**0.008**
Low birthweight				
Yes	1.53 (-0.08 – 3.14)	0.06	1.93 (0.30 – 12.37)	0.49
No		Reference		
Inflammation at birth				
Yes	1.57 (-0.02 – 3.16)	**0.05**	5.44 (1.09 – 27.22)	**0.04**
No		Reference		
Child stunted at birth				
Yes	0.71 (-0.28 – 1.71)	0.16	1.18 (0.41 – 3.45)	0.76
No		Reference		
Wasted in first 6 months				
Yes	0.58 (-0.26 – 1.42)	0.18	1.49 (0.60 – 3.72)	0.39
No		Reference		
***EBF in first 6 months**				
Yes	0.52 (-0.74 – 1.78)	0.41	1.40 (0.35 – 5.52)	0.63
No		Reference		
Recent illness				
Yes	0.93 (0.45 – 1.41)	**<0.001**	2.46 (1.36 – 4.45)	**0.003**
No		Reference		
Pneumococcus Carriage				
Yes	0.29 (-0.35 – 0.93)	0.37	1.62 (0.70 – 3.74)	0.261
No		Reference		
Visit Season				
Rainy	0.13 (-0.48 – 0.74)	0.68	0.28 (0.10 – 0.75)	**0.011**
Dry		Reference		
Seasonal factor 1	-0.4 (-0.72 – -0.07)	**0.02**	0.43 (0.25 – 0.73)	**0.002**
Seasonal factor 2	-0.39 (-0.73 – -0.05)	**0.02**	0.49 (0.32 – 0.75)	**0.001**

*EBF = Exclusive Breastfeeding

Discussion

Child wasting remains endemic to low-and-middle-income-countries (LMICs) such as the Gambia despite extensive intervention (19). This analysis used intensively sampled data from a birth cohort in rural Gambia to determine the factors associated with wasting, including assessing the effect of pneumococcus carriage on child wasting. We began by describing the patterns of undernutrition that manifested as stunting, wasting and underweight in this sample. We then proceeded to investigate the factors associated with wasting and weight-for-length z-scores using generalised linear mixed models.

This study showed that the prevalence of wasting, stunting and being underweight and their respective indices (WLZ, LAZ, WAZ) varied with age. WLZ, LAZ and WAZ scores were highest in the first six months of life and gradually decreased thereafter until around 18 months of age. After 18 months, the median WLZ and WAZ scores increased but never reaching the magnitude they were at birth or in the first six months. LAZ scores remained low even after 18 months. The prevalence of wasting, stunting and being underweight was, on average, lower in the first year of life than in the second year of life, peaking at around 15 months of age. The prevalence of wasting and being underweight began to decrease after 15 months of age while the prevalence of stunting remained high (Supplementary Figure A2).

Prior research on rural Gambian children under the age of two years reported similar growth patterns to the ones observed in this sample. Nabwera and colleagues(19) reported that children in rural Gambia were born small, with LAZ, WAZ and WLZ scores highest in the first six months and gradually declining thereafter until the second year of life where the z-scores plateau. Another research team, analysing the same data as Nabwera and colleagues reported trends of stunting and wasting by age similar to the ones reported in this study(26). The gradual decline in z-score or increase in undernutrition prevalence with age, could be a result of decreased breastfeeding with inadequate complementary feeding practices or the ability of older children to interact with other children and the environment which increases exposure to infectious agents through contaminated food and water often as a result of unhygienic environments(4).

The results of this study suggest that WLZ scores and wasting have multiple overlapping determinants. This study found that increasing child age (in months), increasing maternal age at delivery, low

birthweight, wasting in the first six months of life, reporting a recent illness, and pneumococcus carriage are factors significantly associated with a lower WLZ score. The odds of wasting significantly increased with inflammation at birth, reporting a recent illness and a year increase in maternal age. Male children had lower WLZ scores and higher odds of wasting than female children. Several other studies reported male children as being more prone to wasting compared to female children(4,34–36). This association can be attributed to the belief that male children require more calories for growth and development compared to female children as they tend to be more active(34,36). In addition, male children are more likely to be influenced by environmental stressors than female children(35). The physiological basis for this discrepancy is still unknown.

Low birthweight (<2.5kg) has been reported to increase the risk of undernutrition in children(4,37,38). Low birthweight reflects intrauterine growth restrictions. Children who are born with a low birthweight are essentially born undernourished and even with appropriate nutrient intake, they may not fully recover and are likely to be undernourished in their early life(38). This phenomenon could explain why, in this analysis, children who are born with a low birthweight and those that are born stunted have lower WLZ scores and higher odds of wasting. Being wasted in the first six months of life also increased the odds of subsequent wasting and lowered WLZ scores. This observation is consistent with results reported by Mertens and colleagues(1).

Exclusive breastfeeding in the first six months of life is known to enhance child immunity, protecting against infectious diseases such as respiratory or diarrhoeal diseases that could lead to wasting. However, it is important to restrict exclusive breastfeeding to the first six months of life as breast milk alone provides inadequate nutrition for children six months or older(39). The increased odds of wasting seen in children who have been exclusively breastfed in the first six months in this sample, could be due to children who were exclusively breastfed in the first six months continuing exclusive breastfeeding beyond six months. It is also plausible that children who were exclusively breastfed were better nourished in the first six months and experienced a dramatic decrease in WLZ score when introduced to complementary feeding.

Childhood illness, particularly respiratory and diarrhoeal illness, often result in weight loss because of the body's increased energy demand to fight the illness, the reduction of appetite and poor nutrient absorption(34,39). Illness often also induces an inflammatory response which results in the release of proinflammatory cytokines, among other inflammatory mediators(14). These proinflammatory

cytokines may have a direct effect on child growth as they are inversely associated with insulin-like growth factor-1, a vital hormone in child growth(14). The current study found that children reporting a recent illness and children who had inflammation at birth had lower WLZ scores and higher odds of wasting. The association of wasting or lower WLZ scores with reporting a recent illness have been reported in other studies(4,34,37,39). To our knowledge, no studies have reported an association between our outcomes and having inflammation at birth. Our results, however, are not implausible as increased energy demand at birth, coupled with unmet nutritional requirements could result in early wasting that increases a child's risk of subsequent wasting episodes.

The Gambian climate is characterised by two distinct seasons: the rainy season between July and October and the dry season from November to June. Previous studies have reported growth deficits during the rainy season (1,19,40). This reduction in growth is often attributed to the high incidence of infectious diseases such as malaria and diarrhoea in the rainy season and the shortage of food during this time as food stores from the last harvest are limited. The current study, however, reported an improvement in z-scores and undernutrition in the rainy season followed by a decrease in z-scores and an increase in undernutrition prevalence in the few months immediately after the rainy season (Supplementary Figure A3 and Figure A4). Our regression analysis also suggested that children observed in the rainy season had higher WLZ scores and had lower odds of being wasted than children observed in the dry season. This observation was unexpected and may be due to chance or the increase in uptake of existing interventions during the rainy season.

This study found that pneumococcus carriers had lower WLZ scores, and higher odds of wasting compared to children who were not carriers, although only the relationship with carriage and WLZ scores was significant. These findings contrasted those reported in earlier studies. A study done in Vellore India reported no association with carriage and WLZ or wasting, although they did report reduced weight and height gain and lower WAZ and LAZ with increased duration of carriage(41). A subsequent study in Tamil Nadu, also in India, reported no association with wasting at six months of age with carriage at two months, four months or both two and four months of age. However, carriage at two months was associated with a reduction in mean weight, mean height, WAZ and LAZ at six months of age(14).

The Vellore study assessed children every three weeks from birth to the first year of life, while the Tamil Nadu study only assessed children at two, four and six months of age. The discrepancies

between the results of this study and the two aforementioned studies could be attributed to the difference in age groups observed and timing of observations. This study included data of children between the ages of 6 and 24 months, excluding the first six months of life as growth during that period is most influenced by in utero conditions(40). The current analysis did not distinguish between pneumococcal carriage and pneumococcal disease. As a result, the association between pneumococcus carriage and wasting or WLZ could be overestimated as poor weight gain in carriers could be attributed to the development of pneumococcal disease. This, however, is unlikely as all children in this sample were vaccinated with PCV. PCV has been shown to drastically decrease invasive pneumococcal disease, particularly in children between 2-24 months of age (42). In addition, the confounding effect of disease was adjusted for through the illness variable.

The relationship between carriage and wasting or WLZ scores warrants further investigation in settings such as the Gambia where pneumococcus carriage remains highly prevalent despite of wide PCV coverage. In this sample, 7 out of 10 children were colonised by 2 months of age and the proportion colonised remained alarmingly high throughout the 2 years of follow-up. Pneumococcal carriage also demonstrated seasonal patterns with the prevalence of carriage peaking in the dry season. The patterns of carriage were consistent with those reported Bojang and colleagues in a different sample of children in rural Gambia(21).

This study had several strengths and limitations. One of the biggest strengths was the use of longitudinal data, allowing for the assessment of duration and recurrent episodes of wasting. In addition, longitudinal data allows for the adjustment of dynamic exposures such as seasonality. An important limitation was the lack of data on socioeconomic status, parental education, and maternal nutritional status at birth. Several studies investigating the determinants of undernutrition in Sub-Saharan Africa reported maternal or paternal education, maternal BMI and household socioeconomic status as factors associated with wasting(4,37). Maternal BMI is a proxy for maternal nutritional status which can provide useful information regarding the child nutritional status in utero. Parental education and socioeconomic status are often correlated with child nutritional status as more effluent parents (often because of better education) can provide their child with sufficient nutrition and can often afford health care. This limitation is not likely to bias our estimates as socioeconomic status of people living in this part of rural Gambia is assumed to be uniform and previous research demonstrated that WHZ does not differ across economic status category(43). Furthermore, only 4/120 mothers in this cohort were employed. In addition, primary health care in the study region was free.

Universal access to primary health care has the potential to mitigate bias resulting from socioeconomic inequalities.

Another limitation is this study's inability to account for environmental factors such as neglected tropical diseases (schistosomiasis and soil-transmitted helminthiases), natural disasters (floods) and political conflict that is endemic and common in this region(44). Failure to account for the above can potentially inflate the association observed with our outcomes and other variables.

This study did not assess the temporal relationship between wasting and pneumococcus carriage or recent illness. It is well documented that the relationship between undernutrition and illness is synergistic, and each one can often lead to the other. Assessing the temporal relationship would have allowed us to determine whether pneumococcus carriage led to wasting or wasted children were more susceptible to carriage.

Conclusion

The relationship between carriage and wasting or WLZ scores warrants further investigation in settings such as the Gambia where pneumococcus carriage remains highly prevalent despite of wide PCV coverage. Other than pneumococcus carriage, this study found low birthweight, early episodes wasting, inflammation at birth and recent illness to be associated with wasting in children between the ages of 6-24 months. Targeting maternal nutrition during pregnancy, prenatal and neonatal care and infant feeding practises may help reduce wasting among children between 6-24 months of age by reducing low birthweight, episodes of wasting, illness and inflammation in early infancy.

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Appendices

A. Supplementary figures included in the manuscript

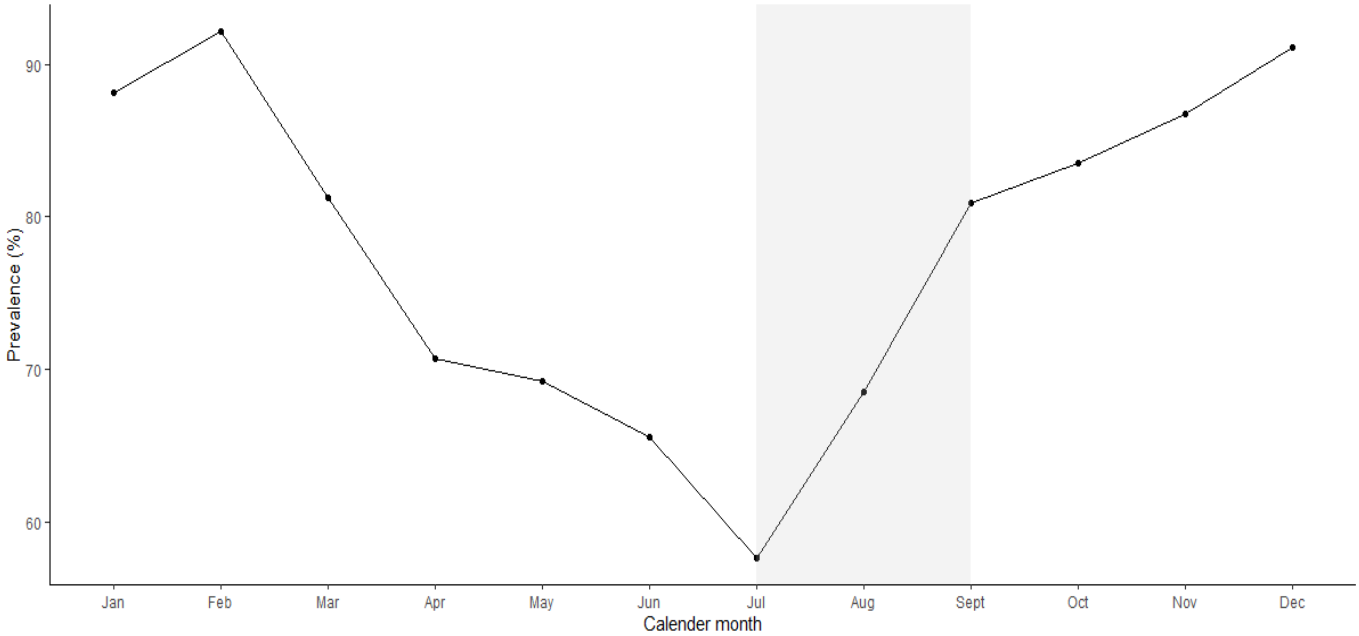

Figure A1: Trends of pneumococcus prevalence across calendar year.

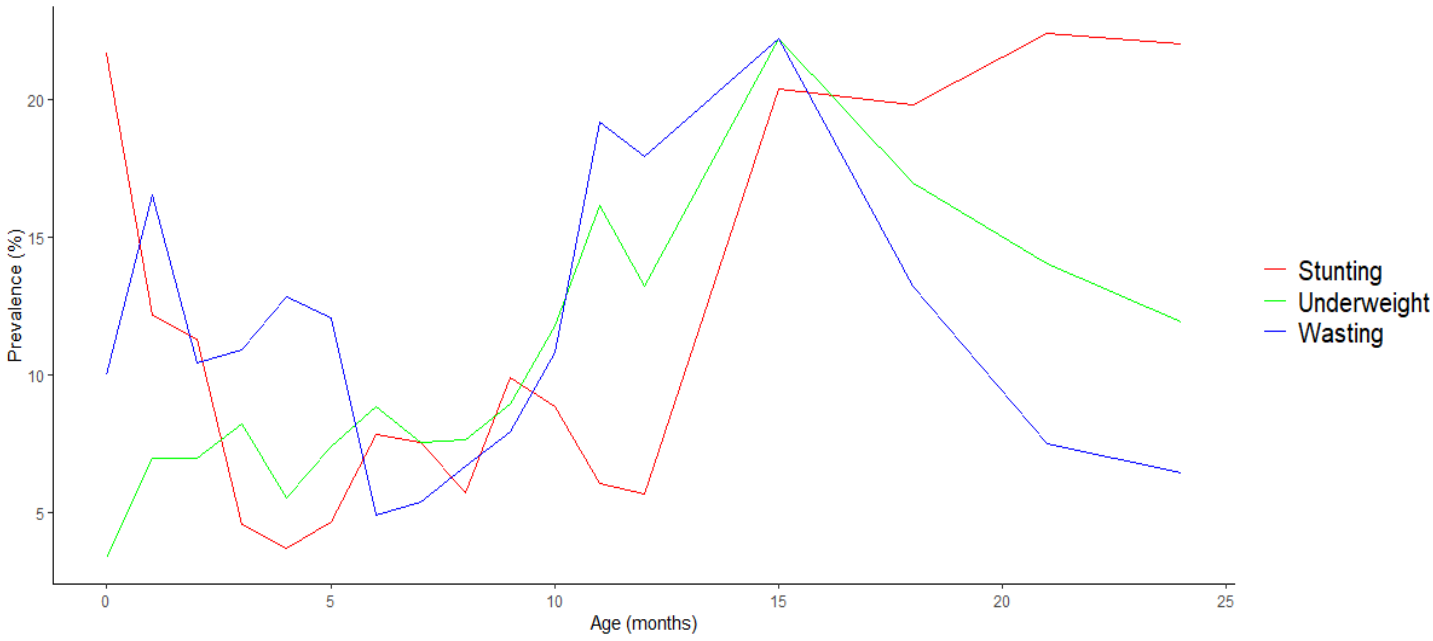

Figure A2: Wasting, stunting and underweight prevalence over the first two years of life.

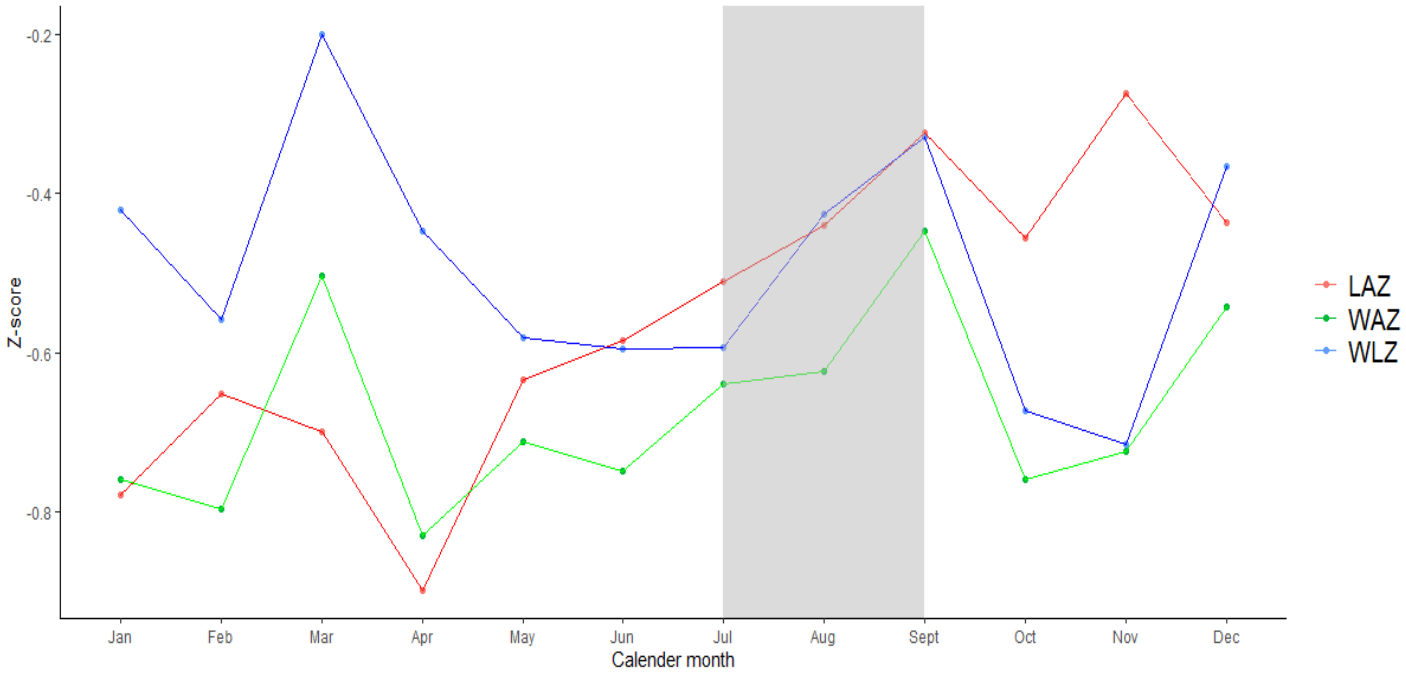

Figure A3: Average LAZ, WAZ and WLZ scores across the calendar year

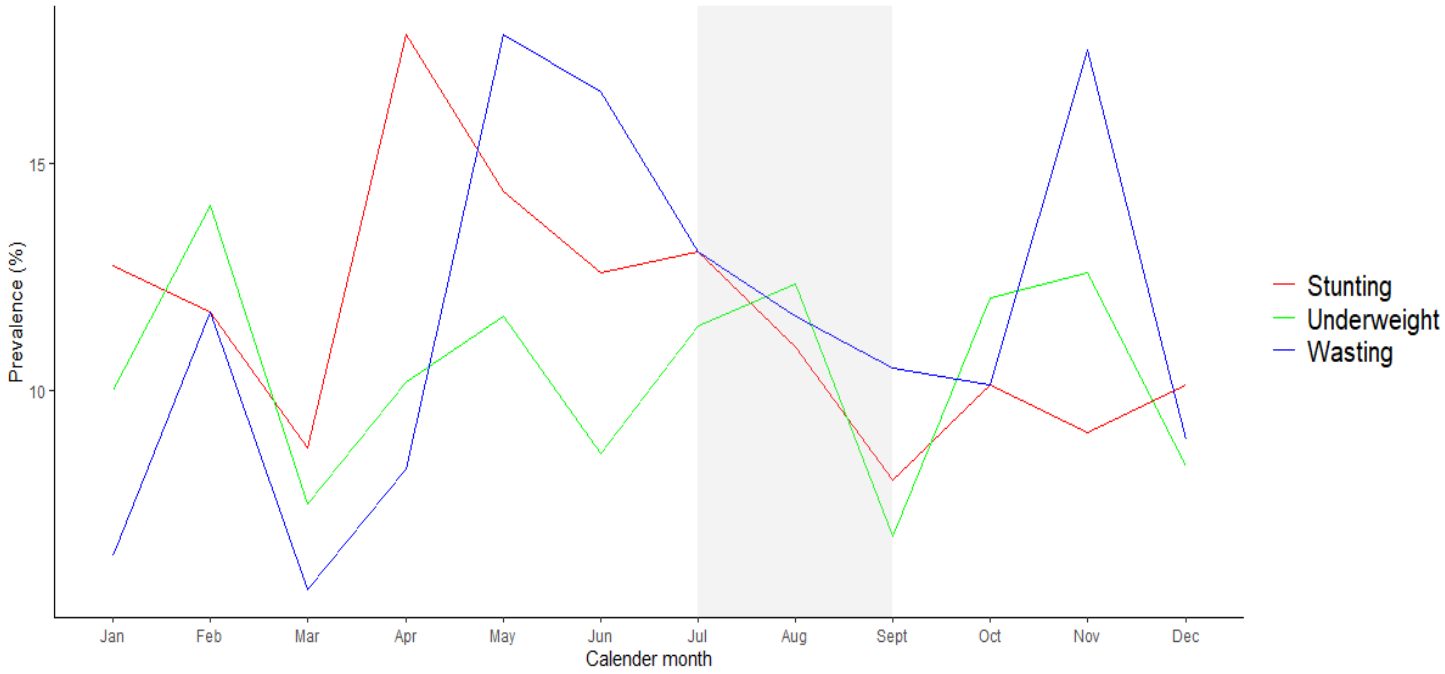

Figure A4: Prevalence of stunting, underweight and wasting across the calendar year

B. Data Collection Tool used in Parent Study

REGISTRATION FORM FOR NEWBORNS

Version 2.0 – 17/11/2012

Vaccination and the Pediatric Microbiome (VPM) (SCC 1286), Protocol [*0001*]

Village Name: ……..

Infant ID: |__|__||__|__||__|__||__|

Name of Compound Head: First …… Last ……..

Date of Birth: |__|__|/|__|__|/|__|__|__|__| Place of Birth: |_| *(1=Hospital, 2=Health centre, 3=Home/TBA)*

Type of Birth: |__| *(1= Vaginal, 2=Caesarean section)* Age of mother: |_|_|

Term of Baby: |_| *(1=Full term, 2=Premature)*

No. of older siblings (same mother): |__|__| Sex of Baby: |__| *(M=Male, F=Female)*

Ethnicity |__| *(Mandinka, 2=Jola, 3=Fula, 4=Wollof, 5=Sarahule, 6=Senegalese, 7=Other)*

Is the mother of the child vaccinated with PCV7? Yes…….. No……

If yes, date of vaccination……

Is the mother of the child vaccinated with MenC? Yes…….. No……

If yes, date of vaccination……

Are any of the child's siblings vaccinated with PCV7? Yes…….. No……

If yes, please fill in the table below.(Nurse should check health card or MRC card)

SIBLING NO. *Start with youngest*	MRC SVT ID	Date of Birth	DATE DOSE 1	DATE DOSE 2	DATE DOSE 3
1					
2					
3					
4					
5					
6					
7					
8					

Did the mother receive antibiotics two weeks prior to delivery? Yes…….. No…… Unknown……..
If yes, which antibiotics? |__|__|
(01=Co-trimoxazole (Septrin), 02=Amoxycillin, 03=Penicillin, 04=Ampicillin, 05=Chloramphenicol, 06=Erythromycin, 07=Gentamicin, 08=Cloxacillin, 10=Tetracycline, 11=Nitrofurantoin, 12=other

What type of flooring is in the room where the child lives |__|
1 = Earth/Sand, 2 = Dung, 3 = Wood planks, 4 = Palm/Bamboo, 5 = Vinyl or asphalt strips, 6 = Ceramic tile, 7 = Cement, 8 = Carpet, 9 = Other

What is the main water source?
1=piped, 2=well, 3=borehole, 4= spring, 5=river or stream, 6=rainwater, 7=prepackaged water, 8=other

What is the main source of fuel for the household? |__|
1=Charcoal, 2=Wood, 3=Kerosene, 3=Electricity, 4=Other

How many people sleep on the same bed as the child? |_|_|
How many people sleep in the same room as the child? |_|_|
How many people live in the same house as the child? |_|_|
How many people less than five years old live in the same house as the child |__|__|
Father's Name: First |__|__|__|__|__|__|__|__| Last |_|_|_|_|_|_|_|_|

Child's Birth Weight: |__|__|.|__| (Kg)

What is the ethnic group of this child |__|
(1=Mandinka, 2=Jola, 3=Fula, 4=Wollof, 5=Sarahule, 6=Senegalese, 7=Other Specify.....)

Mother's Name: First ……. Last ……..

Name of Reporter: First.....Last

. . . . . .

Date of Report |___|___|/|___|___|/|___|___|___|___|

REGISTRATION FORM FOR HOUSEHOLD CONTACTS

Version 1.0 – 17/11/2012Vaccination and the Pediatric Microbiome (VPM) (SCC 1286), Protocol[*0001*]

Village Name: …….. Infant ID: |__|__||__|__||__|__||__|

Name of Compound Head: First …….. Last ……..

Date of Birth: |__|__|/|__|__|/|__|__|__|__| Date of interview|__|__|/|__|__|/|__|__|__|__|

Relationship to subject |__|
1=sibling, 2=cousin, 3=uncle/aunt, 4=father, 5=relative, 6=other

Sex: |__| *(M=Male, F=Female)*

Ethnicity |_| *(Mandinka, 2=Jola, 3=Fula, 4=Wollof, 5=Sarahule, 6=Senegalese, 7=Other)*

For the following questions, the nurse should check the health card or MRC card.

Is subject vaccinated with PCV7? Yes…….. No…… Unknown……..

If yes, date of vaccination……..

Is the mother of the subject vaccinated with MenC? Yes…….. No……

If yes, date of vaccination……..

What type of flooring is in the room where the subject lives |__|
1 = Earth/Sand, 2 = Dung, 3 = Wood planks, 4 = Palm/Bamboo, 5 = Vinyl or asphalt strips, 6 = Ceramic tile, 7 = Cement, 8 = Carpet, 9 = Other

What is the main water source for the subject?
1=piped, 2=well, 3=borehole, 4= spring, 5=river or stream, 6=rainwater, 7=prepackaged water, 8=other

What is the main source of fuel for the subject? |__|
1=Charcoal, 2=Wood, 3=Kerosene, 3=Electricity, 4=Other

How close is subject to the child

1=sleep on the same bed, 2=sleep in the same roon, 3=sleep in the same house, 4= live in the same compound

How many people sleep on the same bed as the subject? |_|_|
How many people sleep in the same room as the subject? |_|_|
How many people live in the same house as the subject? |_|_|
How many people less than five years old live in the same house as the subject |_|_|
Father's Name: First |__|__|__|__|__|__|__|__| Last |_|_|_|_|_|_|_|_|

What is the ethnic group of this subject |__|
(1=Mandinka, 2=Jola, 3=Fula, 4=Wollof, 5=Sarahule, 6=Senegalese, 7=Other Specify .....)

Name: First …….. Last ……

RISK FACTOR FORM FOR NEWBORNS

Version 2.0 – 17/11/2012

Vaccination and the Pediatric Microbiome (VPM) (SCC 1286), Protocol [*0001*]

1101 Study ID **1102 Sample serial ID: [ ]**

1103 Date of interview: |__|__|/|__|__|/|__|__|__|__|

1103B INTERVIEWER'S ID

1104 Has the child been vaccinated since last visit Yes..... No.....

If yes go to question 114a, if no, then go to question 1105.

1104a PCV	Dates:
BCG	Dates:
Hepatitis B	Dates:
Pentavalent	Dates:
Measles	Dates:
Yellow fever	Dates:
Polio	Dates:
Other (please specify)	Dates

1105 Weight |__|__|__| Kg 1105a Height |__|__|__| cm 1105b Axillary temperature |_|_|°C

1106c Head circumference |_|_|_| cm

1105c Respiratory rate |__|__|__| rate per minute

1106 Has the child had nasal discharge in the last four weeks? Yes..... No.....

1107 Has the child had a cough lasting more than four weeks? Yes..... No.....

1107b Has the child had any ear discharge visible in the last four weeks? Yes..... No.....

1108 Has the child had difficulty breathing in the last four weeks Yes..... No.....

Please check child's health card for the following questions

1109 Has the child visited a health center in the last two weeks? Yes..... No.....

1109a Was the child diagnosed with a chest infection? Yes..... No.....

1109b Was the child diagnosed with an ear infection? *Yes.....* *No.....*

1109c Was the child diagnoses with meningitis or sepsis *Yes.....* *No.....*

(0=No, 1=Yes , 8=Not applicable, 9=Not known)

1110b Does the subject know the reason for the antibiotics being prescribed? *(Check health card)*|__|

(0=Chest infections, 1=Ear and Upper Respiratory Tract infections, 2=Diarrhea, 3=Meningitis, 4=Malaria, 5 Other, 9=Not known)

If yes to any of the above questions please provide any additional details you can (were cultures taken etc)

.....

1110 Has the subject had any antibiotics in the last two weeks? *(Check health card)*| __|

1111 If yes to question 1110, what were the antibiotics from most recent prescription within the last two weeks?

First antibiotic Second antibiotic

| | | | | |

(1118) (1119)

(01=Co-trimoxazole (Septrin), 02=Amoxycillin, 03=Penicillin, 04=Ampicillin, 05=Chloramphenicol, 06=Erythromycin, 07=Gentamicin, 08=Cloxacillin, 10=Tetracycline, 11=Nitrofurantoin, 12=other, 88=not applicable 99=Not known) OTHER:

Has the child had any other illnesses in the last four weeks (e.g. diarrhea and vomiting, high temperature, skin complaints)? Yes……. NO…… (Please provide details)

.....

1112 Relation of interviewee to this child |__|

(1=Mother, 2=Father, 3=Grandmother, 4=other blood relatives, 5= other adult)

1113 Is the subject breastfed? |__| (0=No, 1=Mixed, 2=Breast milk with water, 3=Exclusive breast feeding)

1114 Is the subject in daycare? Yes……. No……..

1115 Is the child exposed to smoke during cooking? Yes……. No……..

1115a What is the main fuel source? |__|

1= Charcoal

2= Wood

3= Kerosene

4= Electricity

5= Other

1115b Specify Other……

1116 Is the child exposed to cigarette smoke (household contact)? Yes……. No……..

1116a Does the smoker smoke when the child is in the same room? Yes……. No……..

1117 Do any animals live or come to the compound where child lives? Yes……. No……..

Do any of the following animals live or come to the compound where the child lives?

1117 a) Dog Yes……. No…….
b) Cat Yes……. No…….
c) Fowl Yes……. No…….
d) Cow Yes……. No…….
e) Goat Yes……. No…….
f) Sheep Yes……. No…….
g) Rodents Yes……. No…….

1118 What is the main water source for the child (drinking and other uses)? |__|

1) Piped water Yes……. No……..
2) Well Yes……. No……..
3) Borehole Yes……. No…….
4) Spring Yes……. No…….
5) River or stream Yes……. No…….
6) Rainwater Yes……. No…….
7) Pre-packaged water Yes……. No…….
8) Other

1118a Specify other .....

1118b Is the child's drinking/feeding water treated? Yes…… No…….

(NB: Water treatment includes chlorination, boiling, sun exposure, filtering, alum or other chemical additives)

1119 Is the child mobile i.e. crawling or walking? Yes…… No……

1120 Is the child teething? Yes…… No……

1121 Does the child appear undernourished? (*Nurse should assess*) Yes…… No……

1122 Is the mother of the child currently employed? Yes…… No……

1123 Has the child travelled out of this village in the last two weeks? |__| (0=No, 1=Yes)

If yes, where did you go? |__|

(1=Other Foni village, 2=Basse, 3=FF, 4=SK, 5=FK, 6=BJL, 7=BK, 8=Senegal village, 10=Senegal town, 11=Othercountry, specify 9=Not known)

1024 Was nasopharyngeal swab taken? Yes…… No…….

1025 Was a blood sample taken? Yes……. No…….

1026 Was a nasal secretion sample taken? Yes……. No……

1027 If no to question 1120, why? |__| (Attach consent form)

(1=consent was not given, 2=child was not at home, 3=Other, (specify.....)

Please stick the NPS, breastmilk and blood sample label id stickers below:

RISK FACTOR FORM FOR HOUSEHOLD CONTACTS

Version 2.0 – 17/11/2012

Vaccination and the Pediatric Microbiome (VPM) (SCC 1286), Protocol [*0001*]

1101 Study ID **1102 Sample serial ID: [ ]**

1103 Date of interview: |__|__|/|__|__|/|__|__|__|__| 1103b Interviewer's ID code |__|__|__|

1104 Has the subject been vaccinated since last visit Yes..... No..... N/A.....

If yes go to question 114a, if n or N/A, then go to question 1105.

1104a PCV Dates:

BCG Dates:

Hepatitis B Dates:

Pentavalent Dates:

Measles Dates:

Polio Dates:

Yellow Fever Dates:

1105 Does the subject (household contact) sleep on the same bed as the child? Yes..... No.....

1105a If no to question 1105, does the subject sleep in the same room as the child? Yes..... No.....

1105b If no to question 1105a, does the subject live in the same house as the child? Yes..... No.....

1106 Has the subject had nasal discharge in the last four weeks? Yes..... No.....

1107 Has the subject had a cough lasting more than four weeks? Yes..... No.....

1107b Has the subject had any ear discharge visible in the last four weeks? **(Nurse should check)** Yes..... No.....

1108 Has the subject had difficulty breathing in the last four weeks Yes..... No.....

Please check subject's health card for the following questions

1109 Has the subject visited a health centre in the last four weeks? Yes..... No.....

1109a Was the subject diagnosed with a chest infection? Yes..... No.....

1109b Was the subject diagnosed with an ear infection? Yes..... No.....

1110 Has the subject had any antibiotics in the last four weeks? *(Check health card)* Yes..... No.....

1110b Does the subject know the reason for the antibiotics being prescribed? *(Check health card)* |__|

(0=Chest infections, 1=Ear and Upper Respiratory Tract infections, 2=Diarrhea, 3=Meningitis, 4=Malaria, 5 Other, 9=Not known

1111 If yes to question 1110, what were the antibiotics from most recent prescription within the last four weeks?

First antibiotic | | | (1118)

Second antibiotic | | | (1119)

(01=Co-trimoxazole (Septrin), 02=Amoxycillin, 03=Penicillin, 04=Ampicillin, 05=Chloramphenicol, 06=Erythromycin, 07=Gentamicin, 08=Cloxacillin, 10=Tetracycline, 11=Nitrofurantoin, 12=other, specify _____ _____ 88=not applicable 99=Not known)

1112 Relation of interviewee to this subject |__|

(1=Mother, 2=Father, 3=Grandmother, 4=other blood relatives, 5= other adult)

1113 Is the subject breastfed? |__| (0=No, 1=Mixed, 2=Breast milk with water, 3=Exclusive breast feeding)

1114 Is the subject in daycare or school? Yes……. No…….

1115 Is the subject exposed to smoke during cooking? Yes……. No…….

1115a What is the main fuel source?

a) Charcoal
b) Wood
c) Kerosene
d) Electricity
e) Other

1115b Specify Other……

1116 Is the subject exposed to cigarette smoke (household contact)? Yes……. No…….

1116a Does the household member smoke in the same room as the subject? Yes……. No…….

1117 Do any animals live or come to the compound where subject lives? Yes……. No…….

Do any of the following animals live or come to the compound where the subject lives?

1117 a) Dog Yes……. No…….
b) Cat Yes……. No…….
c) Fowl Yes……. No…….
d) Cow Yes……. No…….
e) Goat Yes……. No…….
f) Sheep Yes……. No…….
g) Rodents Yes……. No…….

1118 What is the main water source for the subject (drinking and other uses)? |__|

9) Piped water Yes……. No…….
10) Well Yes……. No……..
11) Borehole Yes……. No…….
12) Spring Yes……. No…….
13) River or stream Yes……. No…….
14) Rainwater Yes……. No…….
15) Pre-packaged water Yes……. No…….
16) Other

1118a Specify other .....

1118b Is the subject's drinking/feeding water treated? Yes…… No…….

(NB: Water treatment includes chlorination, boiling, sun exposure, filtering, alum or other chemical additives)1119 Is the subject employed? Yes…… No……

1120 Is the subject a farmer? Yes…… No……

1121 Does the subject appear undernourished? (*Children only, nurse should assess*) Yes…… No…… N/A…….

1122 Is the mother of the subject currently employed? *(Children only)* Yes…… No……

1123 Has the subject travelled out of this village in the last two weeks? |__| (0=No, 1=Yes)

If yes, where did he/she go? |__|

(1=Other Foni village, 2=Basse, 3=FF, 4=SK, 5=FK, 6=BJL, 7=BK, 8=Senegal village, 10=Senegal town, 11=Othercountry, specify 9=Not known)

1024 Was nasopharyngeal swab taken? Yes…… No…….

1025 Was a blood sample taken? Yes…….. No……..

1026 If no to question 1120, why? |__| (Attach consent form)

(1=consent was not given, 2=subject was not at home, 3=Other, (specify .....)

Please stick the NPS sample label id sticker

C. Ethics Approval Documents

C1. Ethical Approval for the parent study

The Gambia Government/MRC Joint
ETHICS COMMITTEE

C/o MRC Unit: The Gambia, Fajara
P. O. Box 273, Banjul
The Gambia, West Africa
Fax: +220 – 4495919 or 4496 513
Tel: +220 – 4495442-6 Ext. 2308

31 December 2012

Dr Martin Antonio
Vaccinology Research Theme
MRC Unit, The Gambia
Fajara

Dear Dr Antonio

SCC 1315v2, Vaccination and the Paediatric Microbiome (vpm) Project

Thanks you for submitting your proposal dated 19 November 2012 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on the 21 December 2012.

The Committee is pleased to approve your request to collect additional samples and for PBMC storage.

With best wishes

Yours sincerely

Mr Malcolm Clarke
Chairman, Gambia Government/MRC Joint Ethics Committee

CC: Dr Brenda Kwambana

Additional documents submitted for review:-

- Consent Form (newborns and mothers), Version 2.0 – 17 November 2012
- Consent form (household contacts), Version 2.0 – 17 November 2012
- Participant information sheet (*Household Contact*), Version 2.0 – 17 November 2012
- Information sheet (parental/guardian), Version 2.0 – 17 November 2012

C2: Ethics approval for current study

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

20 November 2020

HREC REF: 763/2020

A/Prof M Lesosky
Division of Epidemiology & Biostatistics
Public Health & Family Medicine, Falmouth Building-FHS
Email: maia.lesosky@uct.ac.za
Student: Zwnphi006@myuct.ac.za

Dear A/Prof Lesosky

PROJECT TITLE: THE ROLE OF STREPTOCOCCUS PNEUMONIAE CARRIAGE ON WASTING AMONG VACCINATED GAMBIAN INFANTS: A LONGITUDINAL ANALYSIS OF A BIRTH COHORT-MASTERS CANDIDATE-MISS PHINDI ZWANE

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 November 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: Miss Phindi Zwane 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.

HREC/REF-763/2020sa

Yours sincerely

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-763/2020sa

D. Manuscript preparation for the International Journal of Epidemiology

Detailed instructions for original articles

Overall guidelines*	
Words:	Around 3000, excluding Abstract, Key Messages and References
Tables and figures:	Up to 8 in total
References:	Up to 50
Title:	Short and specific; subtitles may be used
Abstract:	250 words in four sections: Background, Methods (include study period and sample size), Results (include main results with estimates and confidence intervals) and Conclusions
Key Messages:	A summary of the main messages of the paper in 3–5 succinct bullet points, each a complete sentence

* Aspects of your paper may exceed these guidelines, but if they do, please explain in your covering letter why this is necessary.

Original articles may be research papers, methods papers, systematic reviews or meta-analyses.

Manuscript preparation

Papers must be submitted in good, grammatical English. If English is not your first language, you may wish to have your paper edited by an English-language editor before submitting.

Initial presentation

Please check the specific instructions for the type of article you are submitting for full details of word limits and required article structure.

The main document of an article should be submitted as an editable Word document (not as a pdf file*) and designated ‘Main document’ when uploaded.

The main document may contain all tables and figures at submission, or these may be provided separately as ‘Table’ and ‘Figure’ files (note that you must be able to supply high-resolution versions of your figures if your paper is later accepted).

Headings and titles of tables and figures should be presented in sentence case (i.e., capitalise only the first word and any proper nouns).

Manuscripts should be double spaced with margins of 2.5cm.

The main document should include a title page with author names and affiliations.

The word count should be included on the title page.

All pages should be numbered.

Footnotes are not permissible in the text of a paper.

UK, not US English, should be used, and jargon should be avoided.

References should be formatted according to *IJE* style.

Supplementary material for online-only publication only should be submitted separately. It may comprise one or more files and each should be designated 'Supplementary file (for online publication only)' when uploaded.

* If your manuscript was created with LaTeX, please submit it as a PDF file initially. Either word processor or original LaTeX files will need to be supplied should your article proceed to acceptance.

Presentation for accepted papers

If accepted, papers must be presented as several files as follows:

The main document of an article should be submitted as an editable word processor document (not as a pdf file) and should contain the text (including title page, abstract and references) only.* This file should be designated 'Main document' when uploaded.

Tables should be provided as a separate editable word processor document (not as a pdf file) and designated 'Table' when uploaded.

Each figure should be provided as a separate high-resolution file in an image format (such as tiff, eps or jpg) and designated 'Figure' when uploaded.

Supplementary material for online publication only may comprise one or more files and each should be designated 'Supplementary file (for online publication only)' when uploaded.

* If your manuscript was created with LaTeX, you must provide either word processor or original LaTeX files if your article proceeds to acceptance.

General instructions

The following general instructions for manuscript preparation should be followed in conjunction with the specific requirements for the type of article being submitted.

Quick links to:
Title; Author list; How to list consortia and working groups; Availability of data and materials; Abbreviations/acronyms; Numbers and units; Describing significance; Special notes for statistical papers; Tables; Figures; Footnotes to tables and figures; Copyright permission; Declarations; Ethics approval; Author contributions; Data availability; Supplementary data; Funding; Acknowledgements; Conflict of interest; References; Citing preprints; Citing data sources; Appendices; Supplementary material

Title

Titles should be short and specific. Subtitles may be used to amplify the main title.

Author list

The *IJE* follows the recommendations of the International Committee of Medical Journal Editors (ICMJE). To comply with ICMJE recommendations, all the authors must meet *all* of the following four conditions:

substantial contribution to conception and design, acquisition of data, or analysis and interpretation of data;

drafting the article or revising it critically for important intellectual content;

final approval of the version to be published; and

agreement to be accountable for all aspects of the work thereby ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All authors must be added to the author section within ScholarOne on submission of a paper. The corresponding author should ensure that the details added to ScholarOne for each author are correct. If a co-author is already listed in the system, but with incorrect or out-of-date details, the corresponding author should advise the relevant co-author(s) to correct these errors in their user accounts.

The contribution of each author and the identity of the author who will act as guarantor for the paper should be provided in the 'Author contributions' section (detailed instructions below).

The affiliations of each author must be given. If an author's present affiliation is different from that under which the work was done, both should be given.

The name of the corresponding author should be marked with an asterisk (*) and both a postal and an email address should be provided in the "*Corresponding author" under the affiliations list. Only one corresponding author is permitted.

The *IJE* allows authors to appear as joint first or senior authors or as equal contributors. Such authors should be marked with a superscript symbol (e.g. †) and a footnote describing their contribution (e.g. '†Joint first authors') should appear under the details for the corresponding author.

Note that the *IJE* will not publish studies that use data, infrastructure or personnel from any low- or middle-income country unless at least one researcher included in the author list is a national of that country.

Back to General instructions

How to list consortia and working groups

If a consortium or working group is involved, all members of the group who meet the full criteria and requirements for authorship, as described above, may be listed individually as authors. Alternatively, the official group or consortium name may appear at the end of the byline but must be accompanied by at least one named author who meets full authorship criteria. A single named author in the byline must be designated the Corresponding author and presented as described above. Affiliation details must be provided under the byline for named authors.

Remaining members of the group should be listed in a section headed "Notes" at the end of the text. For example:

> Notes
>
> The ABC Consortium: John B Smith (affiliation), Richard B Jones (affiliation), etc.

PubMed (but not PubMed Central) will list as collaborators the names of individual group members appearing in the Notes section when the group or consortium name is included in the author byline. PubMed will not list affiliations or other information from the Notes section. Please see the relevant PubMed rules for further information.

A consortium or working group may alternatively designate one or more individuals who meet full authorship criteria as authors writing "on behalf of" the group. In this case, the official group or consortium name should be included in the main author list after "on behalf of", and remaining group members may be listed at the end of the text under 'Notes" as shown above. Note that PubMed Central will include a group or consortium name following "on behalf of" in the author byline, but PubMed will omit it.

If named authors are writing “on behalf of” a group or consortium, the names of individual group members appearing in the Notes section will not be listed as collaborators in either PubMed or PubMed Central.

Back to General instructions

Availability of data and materials

Where ethically feasible, the *International Journal of Epidemiology* strongly encourages authors to make all data and software code on which the conclusions of the paper rely available to readers. Authors are required to include a Data Availability Statement in their article.

We suggest that data be presented in the main manuscript or additional supplementary material, or deposited in a public repository whenever possible. Information on general repositories for all data types, and a list of recommended repositories by subject area, is available on the Research Data Policy page.

Back to General instructions

Abbreviations/acronyms

The use of abbreviations should be kept to a minimum.

Non-standard abbreviations should be avoided.

All abbreviations should be spelled out in full with the abbreviation in parentheses the first time they appear in:

the Abstract;

the Key Messages or Key Features;

the text;

tables and figures (usually as footnotes); and

any supplementary material.

Thereafter the abbreviation should be used.

Abbreviations should be avoided in titles, table and figure titles, headings and sub-headings.

Any abbreviation that appears in a table or figure must be spelled out in full in a footnote to that table or figure. This applies also to Supplementary tables and figures.

Numbers and units

In the text, numbers from one to nine should be spelled out and numbers above nine given as Arabic numerals.

Numbers followed by a unit should always be written as numerals.

Numerals should not be used to start a sentence.

Numerals up to 9999 should not be separated by spaces or commas, while those from 10 000 on should use a space as a separator.

Per cent should be written as % throughout.

All measures should be reported in SI units, followed, in the text, by traditional units in parentheses. There are two exceptions: blood pressure should be expressed in mmHg and haemoglobin as g/dL.

Where age grouping is appropriate, ages should be grouped as mid-decade to mid-decade (i.e. in five-year age groups such as 35–44 or 35–39, 40–44, etc, but not in full decades such as 20–29, 30–39 or other groupings).

Back to General instructions

Describing significance

In the *IJE*, we actively discourage the use of the term 'statistically significant' or just 'significant' and such statements in method sections as 'findings at P<0.05 were considered significant'. Please provide effect estimates with confidence intervals and exact P values, and refrain from using the term 'significant' in either the results or discussion sections of papers.

Our justification of this position is given in: Sterne J, Davey-Smith G. Sifting the evidence — What's wrong with significance tests? BMJ 2001; 322: 226-231. See also: Wasserstein RL, Lazar NA. The ASA's statement on P-values: context, process, and purpose. The American Statistician 2016: DOI: 10.1080/00031305.2016.1154108.

Special notes for statistical papers

The correct preparation of statistical manuscripts is particularly important, and the precise nature and position of each symbol must be clear. In general, distinction should be made between:

upper case and lower case letters

ordinary and bold-faced letters

certain Greek letters and similar Roman letters

subscripts, superscripts and 'ordinary' symbols.

Please use a P (upper case, italics) to indicate a P value. Please use n (lower case, italics) to indicate number of subjects.

Statistical symbols are automatically set in italics and need not be underlined except to prevent ambiguity (e.g. when an isolated letter, such as a, occurs in the text). Symbols should not be used to start a sentence.

Back to General instructions

Tables

Tables should be:

able to be interpreted independently of the text, with meaningful titles, legends and adequate footnotes.

submitted in editable Word or Excel* format (not pdf);

numbered consecutively in Arabic numerals;

presented in a separate file or files from that of the main text;

able to be interpreted independently of the text, with meaningful titles and adequate footnotes.

All abbreviations appearing in tables should be spelled out in the footnote.

Each table should be cited at an appropriate place in the text.

To ensure accurate reproduction of tables, please use separate table rows for each line of data (including sub-headings). Do not use paragraph returns to separate data items in tables.

Vertical rules should not be used in tables.

* If tables are submitted in Excel format, please carefully check the pdf proof of the article before submitting the paper to ensure that tables (particularly any in landscape format) are not split across pages, as these are difficult for the editors and reviewers to follow.

Back to General instructions

Figures

Figures should be able to be interpreted independently of the text, with meaningful titles, legends and adequate footnotes.

All abbreviations appearing in figures should be spelled out in the footnote.

Each figure should be submitted separately from the text in an image format such as eps or high-resolution tiff or jpg files (a minimum resolution of 300dpi).

As figures are published in black and white in the print version of the Journal, please use distinctly different shades, different line types (dashed etc), bar/column types (hatched etc) and different symbols to allow the figure to be interpreted when printed in black and white.

Please ensure that your electronic image files print clearly at a legible size and are of high quality for publication (We recommend a minimum of 150mm wide at 300dpi for a single-column figure in the print Journal, 300mm wide at 300dpi for full page width figures).

For graphs, please provide axis labels of a legible size; note that graphics programs often produce large figures with tiny labels which will become illegible when the entire figure is resized for publication.

Figures and illustrations should be numbered consecutively in Arabic numerals.

Each figure should be cited at an appropriate place in the text).

Please state your preferred option (i.e. agreement to pay £350/figure for colour in both print and online, or black and white in print and colour online only at no charge) in your cover letter when submitting your article so the editors can determine whether the supplied images are satisfactory.

Footnotes to tables and figures

Please use superscript a, b, c, d, etc. as footnote symbols.

Back to General instructions

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Back to General instructions

Declarations

The following declarations, in the following order, should appear at the end of the text and before the References.

Ethics approval

Published research based on human subjects must include a declaration in relation to ethics approval. Please place, under the subheading “Ethics approval” at the end of your main text immediately before the Funding section, a statement that:

provides the name of the local ethics committee that approved the study and the approval number; OR

confirms that such approval is not needed and includes the reason why this is the case; AND/OR

states how the study conforms to recognised standards such as the Declaration of Helsinki.

Further information on publication ethics.

Author contributions

The contribution of each author and the identity of the author who will act as guarantor for the paper should be provided under the heading ‘Author contributions’. The Author contributions statement should be added to the declarations for up to 25 authors.

If there are more than 25 authors, the Author contributions statement should be provided as a Supplementary file designated ‘for online publication only’ and the text under the Author contributions heading in the paper should read: “Author contributions are available as a Supplementary file at *IJE* online.”

The Author contributions statement should be detailed. For example, “AB designed the Study. BC directed the study’s implementation. CD designed the analytical strategy and helped to interpret the findings. DE conducted the literature review and helped to prepare the Introduction and Methods sections of the text. EF drafted the Discussion.”

Data availability

The inclusion of a Data Availability statement is a requirement for articles published in the *International Journal of Epidemiology*. Data Availability statements provide a standardized format for readers to understand the availability of data underlying the research results described in the article. The statement may refer to original data generated in the course of the study or to third-party data analyzed in the article. The statement should describe and provide means of access, where possible, by linking to the data or providing the required unique identifier.

You can find more information and example Data Availability statements on the Research Data Policy page.

Supplementary data

Please add “Supplementary data are available at *IJE* online” under the heading “Supplementary data”.

Funding

The following rules should be followed:

Any funding used while completing the submitted work should be detailed in a separate Funding section, placed before any Acknowledgements section at the end of the article. If no funding was received, add 'Funding: None'.

The sentence on funding should begin: 'This work was supported by …'.

The full official name of the funding agency should be given; e.g. 'the National Cancer Institute at the National Institutes of Health' or simply 'National Institutes of Health', not 'NCI' (one of the 27 sub-institutions) or 'NCI at NIH'. See the full Research Information Network-approved list for details of UK funding agencies. It is important to have this information correct because institution names are used to tag NIH-funded articles so that they are deposited at PubMed Central (PMC). Note that papers are only deposited in PMC once they have been published in an issue of the journal and not after they been published on Advance Access.

Grant numbers should be complete and accurate and provided in brackets as follows: '[grant number ABX CDXXXXXX]'.

Multiple grant numbers should be separated by a comma as follows: '[grant numbers ABX CDXXXXXX, EFX GHXXXXXX]'.

Funding agencies should be separated by semicolons (plus 'and' before the last funding agency).

Where individual authors 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]'.

Example:

This work was supported by the National Institutes of Health [P50 CA098252 and CA118790 to R.B] and the Alcohol & Education Research Council [HFY GR667789].

Crossref Funding Data Registry: To meet funding requirements, authors are required to name their funding sources, or state if there are none, during the submission process. For further information on this process or to find out more about the CHORUS initiative, please see the CHORUS website.

Acknowledgements

This section is optional. It should include consultants or minor contributors involved in the work. It is good practice to ensure that any person named in the Acknowledgements has given consent to be named.

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Cracknell R. The Ageing Population. House of Commons Library Research. 2010. http://www.parliament.uk/documents/commons/lib/research/key_issues/Key-Issues-The-ageing-population2007.pdf (5 February 2019, date last accessed).

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