Undernutrition and antibody response to measles, tetanus and Haemophilus Influenzae type b (Hib) vaccination in pre-school south African children: The VHEMBE birth cohort study

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Abstract

Background: Under-vaccination is undoubtedly driving recent worldwide measles outbreaks, but undernutrition may also be playing a role in low- and middle-income countries. Studies have shown reduced immune response to vaccines in undernourished children but few have followed children beyond infancy, when they are more likely to be exposed to infectious diseases.

Methods: In the Venda Health Examination of Mothers, Babies and the Environment (VHEMBE) South African birth cohort study, we examined the relationship between undernutrition, as measured by stunting and other growth measures, and vaccine-specific serum antibody level to three different vaccine types: measles, tetanus and *Haemophilus influenzae* type b (Hib). We included 621 fully-vaccinated children with anthropometric measurements at ages 1, 2, and 3.5 years and antibody levels at 3.5 and 5 years.

Results: At 5 years of age, 90.4% of fully-vaccinated children were protected against measles, 66.7% against tetanus, and 56.1% against Hib. Children who were stunted or had any indicator of diminished growth at 3.5 years averaged a 24.1% (95% CI = -44.2, 0.6) or a 27.2% (95% CI = -45.1, −1.3) lower antibody titer for measles, respectively, relative to those with normal growth. In addition, girls, but not boys, with any indicator of diminished growth at 3.5 years averaged a 36.8% (−59.3, −7.0) lower antibody titer for tetanus. We found no association between undernutrition and Hib antibody titers.

Conclusions: Early life undernutrition may be associated with lower induction or persistence of antibody responses to certain vaccines. Addressing child undernutrition may improve vaccine efficacy and reduce the burden of vaccine-preventable diseases.

Highlights

- Infectious diseases are a major source of childhood morbidity/mortality worldwide.
- Undernutrition may affect the immune response to certain vaccines.
- The VHEMBE study examined relationships between undernutrition and antibody levels.
- Undernourished vaccinated children had lower measles antibody levels at 3.5/5 years.
- Undernutrition was associated with lower tetanus antibodies in girls but not boys

1 Introduction

In March 2024, the United States Center for Disease Control and Prevention (CDC) issued an emergency health advisory to clinicians and public health authorities of a global increase in measles cases. [1] An estimated 136,200 measles deaths occurred globally in 2022, primarily in those under the age of five. [2] Low vaccination rates could explain this increase, given that only 74% of children worldwide received two doses of the measles vaccine. [3] However, there have not been parallel increases in other vaccine-preventable illnesses such as tetanus and *Haemophilus influenzae* type b (Hib). The World Health Organization estimated in 2022 that only 84% of children received all 3 doses of diphtheria-tetanus-pertussis (DTP3) vaccine and 76% received all 3 doses of the Hib vaccine. [3] Although low vaccination rates and 'missed doses' during the recent SARS-COV-2 pandemic [4] clearly contributed to the measles outbreaks, some children who are vaccinated may not mount an adequate immune response (i.e., primary vaccine failure) or may lose immunity more rapidly post-vaccination (i.e., secondary vaccine failure); undernutrition has been hypothesized to play a role in such occurrences. [5]

Approximately 22.3% or 148 million children under 5 years worldwide in 2022 are stunted, with the highest burden in Asia and sub-Saharan Africa. [6] Stunting and other markers of undernutrition can be associated with essential nutrient deficiencies that are crucial to development and can have both short- and long-term effects on physical health, emotional development, and cognition and can have economic costs. [7] Undernourished children are also more susceptible to infections and illnesses, which can further impede growth and development. [8] Even if an undernourished child can initially mount an adequate immune response to a vaccine, they may not maintain long-term protection. This can be especially problematic for vaccine schedules requiring multiple doses over time. Most previous studies of undernutrition and vaccine response have examined immunologic response soon after vaccination, which may not reveal waning of immunity. [5,9]

In the present study, we examine the relationship between undernutrition, as assessed by stunting and other anthropometric measures, and IgG antibody titers specific to three different types of vaccines: measles, a live-attenuated virus vaccine; tetanus, an adjuvanted protein vaccine; and Hib, an aluminum-salt adjuvanted, conjugated polysaccharide vaccine. We examined not only the early response to these vaccines in fully immunized children but also followed the evolution of these responses up to 5 years of age in a low-income rural South African population.

2 Methods

2.1 Study population

We used data from the Venda Health Examination of Mothers, Babies and the Environment (VHEMBE) birth cohort. The VHEMBE cohort has previously been described. [10] Briefly, pregnant women in early stages of labor (contractions more than five minutes apart) were recruited at Tshilidzini Hospital in the Vhembe district of Limpopo Province, South Africa, between August 2012 and December 2013. Eligibility criteria included: being at least 18 years of age, speaking TshiVenda as the main language at home, having no diagnosis of malaria during pregnancy, living within 20 km of the hospital with no plans to relocate, and giving birth to a live singleton. Among 1649 women who were screened, 920 were deemed eligible, and 752 completed a baseline questionnaire and provided a blood sample.

We obtained written informed consent from mothers around delivery or mothers or primary caregivers, thereafter. The Institutional Review Boards at the University of California, Berkeley; McGill University; the University of Pretoria; the Limpopo Department of Health; and the Ethics Committee of Tshilidzini Hospital approved the study.

2.2 Study procedures

Shortly after delivery, mothers were interviewed by trained bilingual (TshiVenda/English) interviewers. A home visit was conducted at approximately one week postpartum. All interviews and home visits were completed in TshiVenda. [10] Information was gathered on demographic characteristics, medical and reproductive history, household characteristics including income and food security, smoking and alcohol use during pregnancy, dietary intake, and the child's home environment (such as home visit assessment of the home's physical characteristics, pesticide containers around the home, materials used for cooking and heating, or the presence of bugs or other pests in the living areas). Maternal HIV status was based on self-report of diagnosis or use of antiretroviral drugs reported on medical records. The method of birth (vaginal or Cesarean) was abstracted from medical records. Mothers or primary caregivers were further interviewed at subsequent visits, which occurred when the children were approximately 1, 2, 3.5, and 5 years of age. Mothers/caregivers were queried in detail, including about breastfeeding habits and the child's medical history and dietary habits. Food security in the household was assessed using the six-item United States Department of Agriculture (USDA) Food Security Questionnaire, [11] and household poverty using Statistics South Africa thresholds. [12] An index of household wealth was constructed from data on household assets and living conditions. [13]

Registered nurses abstracted information on the child's health from the “Road to Health”, a government-provided booklet containing information on all the child's clinic visits, including child health, growth measurements, information from the child's medical records in the event of any hospital admissions, and immunizations. The South Africa routine vaccination schedule includes two doses of measles vaccine at 9 and 18 months and four doses of tetanus and Hib vaccines at 6, 10 and 14 weeks and 18 months. In addition, booster vaccines may be administered to children if an outbreak occurs. Blood samples were collected via venipuncture at the 3.5- and 5-year visits.

Visit completion numbers include 700 at 1 year, 685 at 2 years, 667 at 3.5 years, and 640 at 5 years. For the present analyses, we include the 621 children who were fully vaccinated and

had antibody levels for any of the 3 vaccines measured in samples collected at either the 3.5 or 5-year visit (for measles $n = 615$ total, $n = 602$ at 3.5 years and $n = 571$ at 5 years; for tetanus and Hib $n = 573$ total, $n = 557$ at 3.5 years and $n = 531$ at 5 years).

2.3 Anthropometric measurements

Child weight was measured by trained study staff at 1 and 2 years using a pediatric digital scale (Tanita BD-590; Tokyo, Japan), and at 3.5 and 5 years using a standard digital scale (Tanita HD-351; Tokyo, Japan). Child length was measured at the 1-year visit using a portable infantometer (Seca 417, Chino, CA, USA) and height, thereafter, using a stadiometer (Seca 213). Triplicate height measures were averaged for each time point. Age- and sex-standardized z-scores for weight-for-age, height/length-for-age, and weight-for-height/length were determined based on WHO growth standards. [14] Children were categorized as adequately nourished (z-scores ≥ -2 SD), moderately undernourished ($-3 \leq z < -2$), and severely undernourished ($z < -3$ SD) for each of these measures, except for BMI, which was categorized based on z-score percentiles: underweight (<5th percentile), normal weight (5th–95th percentile), overweight (85th–95th percentile), and obese (>95th percentile).

2.4 Vaccine-specific IgG antibody measurement

We used enzyme-linked immunosorbent assay (ELISA) to measure IgG antibody titers elicited by the measles, tetanus, and Hib vaccines. All assays were performed at the Research Institute of the McGill University Health Centre. Briefly, sera were diluted 1:100 in sample diluent and distributed as singlets (100 μL diluted serum/well) in 96-well plates precoated with the vaccine antigens for 1 h (tetanus toxoid: GWB-FCBEAB; Genway Biotech Inc., San Diego, CA; measles antigen: GWB-984A72; Genway Biotech Inc., San Diego, CA; or Hib polyribosylribitolphosphate: RE56351; IBL International, Hamburg, Germany; fulfilled by Affinity Diagnostics, North York, ON). Antigen-specific IgG was detected using a secondary horseradish-peroxidase conjugated anti-human IgG antibody as per the manufacturers' instructions. Following 3,3′,5,5′-tetramethylbenzidine substrate reaction, absorbance was measured at 450 nm (EL800 microplate reader: BioTek Instruments Inc., Winooski, VT). For quality control, 10% of the samples were run in duplicate. Antibody concentrations were derived from standard curves using serially-diluted (1:5 in sample diluent) international standards that were included on each plate in duplicate (plus two blanks) (measles (97/648), tetanus (TE-3), and Hib (12/306) standards were obtained from NIBSC; Blanche Lane, Ridge, Herts, UK). The lower limit of detection for tetanus, measles, and Hib ELISAs were 0.05 IU/mL, 0.05 IU/mL, and 0.06 μg/mL, respectively, based on the lowest standard dilution and blank wells.

2.5 Statistical analysis

Dependent variable definition. Because very few children were considered severely undernourished (≤1% at each visit), the "moderately undernourished" ($-3 \leq z < -2$) and "severely undernourished" ($z < -3$) categories for length/height for age, weight-for-age, and weight-for-length were combined, creating a binary measure of "adequately nourished" vs "undernourished" for each of these indicators. Stunting was defined as having a length/height for age z-score < -2. We also created a composite measure of "any undernutrition indicator," combining the binary indicators for length/height for age, weight-for-age, and weight-for-length, as well as including underweight based on BMI percentile.

Independent variable definition . Antibody titers were modeled in two ways: as a binary outcome with thresholds based on standards of protection (250 mIU/mL for measles, [15] 0.1 IU/mL for tetanus, [16] and 1.0 μg/mL for Hib [16]), and as a continuous measurement. Antibody titers were right-skewed and were log10 transformed to approximate a normal distribution. Estimates were back-transformed using the formula $(10^{\beta}-1)*100$ and represent a percent difference in antibody titers between those who were undernourished (vs not).

Modeling. We applied analysis of variance and Pearson's correlations for bivariate analyses. We estimated associations between markers of undernutrition and vaccine-specific antibody titers based on Marginal Structural Models (MSMs) for repeated outcomes with stabilized Inverse Probability of Treatment (IPTW) and Inverse Probability of Censoring (IPCW) weights to adjust for potential confounding and selection due to loss to follow-up, respectively. MSMs generate estimates with a causal interpretation under the three identifiability assumptions of exchangeability, positivity, and consistency and rely on a correct specification of the models used to generate the weights. [17] IPTWs were computed as the ratio of the marginal probability of the independent variables (i.e. undernutrition indicators) divided by the probability of these same variables, conditional on confounders as assessed based on multivariable logistic regression. While standard regression generates conditional estimates that are generally interpreted as associations "holding other variables constant", applying these weights allowed us to construct a pseudo-population in which measured confounders are equally distributed across levels of exposure, as they would be in a randomized-controlled trial. Estimates thus compare the expected IgG levels under scenarios in which the entire population is malnourished vs none of the population. Confounders were identified based on Directed Acyclic Graphs and included: maternal age (continuous), education (<12th grade, 12th grade, >12th grade), marital status (married or living as married, not married or living as married), alcohol consumption (any, none), smoking (ever, never), exposure to environmental tobacco smoke (ever, never), and HIV status (positive, negative) during pregnancy; household poverty (above or below the food poverty threshold) and wealth index (continuous); method of delivery (vaginal delivery, c-section) and child sex (male, female). Because vaccine-induced IgG titers typically wane over time in the absence of booster vaccinations or natural exposures, the IPTW models also included time between the last vaccine and the time of blood collection, given as a 3-knot restricted cubic spline. In addition, we included variables identifying children who received a booster vaccine against measles or tetanus before age 3.5 and between age 3.5 and 5 years (yes, no). IPCWs were estimated as the ratio of the marginal probability of remaining uncensored to the probability of remaining uncensored, conditional on the variables listed above. Final weights were computed as the product of IPTWs and IPCWs. Estimates of association were computed-based on modified Poisson regression. We used interval-censored regression models for continuous outcomes to account antibody measures falling outside of the lower or higher standards. We bootstrapped the entire procedure 1000 times to obtained percentile-based 95% confidence intervals and *p* -values.

To assess whether associations between markers of undernutrition and antibody titers differed between boys and girls, we ran MSMs that included sex and a cross-product of sex and undernutrition. For these models, IPTWs were computed as the ratio of the observed exposure conditional on sex divided by the observed exposure conditional on confounders. [18]

All analyses were performed using Stata 17.0.

Table 1. Demographics and sample characteristics of all fully-vaccinated 5-year-old children by protection status based on antibody titers to measles, tetanus, and Hib, VHEMBE cohort, South Africa.

		Measles protection at 5 years [a]		Tetanus protection at 5 years [b]		Hib protection at 5 years [b]	
	All participants N (%) or M ± SD	**Protected N (%) or M ± SD**	**Not protected N (%) or M ± SD**	**Protected N (%) or M ± SD**	**Not protected N (%) or M ± SD**	**Protected N (%) or M ± SD**	**Not protected N (%) or M ± SD**
All participants	621 (100.0)	516 (90.4)	55 (9.6)	354 (66.7)	177 (33.3)	298 (56.1)	233 (43.9)
Maternal Characteristics							
Age at delivery	26.4 ± 6.3	26.3 ± 6.2	27.9 ± 7.2	25.9 ± 5.9*	27.3 ± 6.6*	26.4 ± 6.1	26.3 ± 6.3
Education							
<12th grade	344 (55.4)	283 (54.8)	33 (60.0)	191 (54.0)	97 (54.8)	162 (54.4)	126 (54.1)
Grade 12	187 (30.1)	161 (31.2)	13 (23.6)	108 (30.5)	57 (32.2)	92 (30.9)	73 (31.3)
> High school	90 (14.5)	72 (14.0)	9 (16.4)	55 (15.5)	23 (13.0)	44 (14.5)	34 (14.6)
Parity							
0	271 (43.6)	221 (42.8)	23 (41.8)	159 (44.9) †	71 (40.1) †	129 (43.3)	101 (43.4)
1	169 (27.2)	145 (28.1)	13 (23.6)	105 (29.7)	44 (24.9)	82 (27.5)	67 (28.8)
≥2	181 (29.2)	150 (29.1)	19 (34.6)	90 (25.4)	62 (35.0)	87 (29.2)	65 (27.9)
Alcohol use during pregnancy							
Yes	32 (5.2)	27 (5.2)	2 (3.6)	20 (5.7)	6 (3.4)	16 (5.4)	10 (4.3)
No	589 (94.9)	489 (94.8)	53 (96.4)	334 (94.4)	171 (96.6)	282 (94.6)	223 (95.7)
Smoked during pregnancy							
Yes	1 (0.2)	1 (0.2)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.4)
No	620 (99.8)	515 (99.8)	55 (100.0)	353 (99.7)	177 (100.0)	298 (100.0)	232 (99.6)
Around smokers during pregnancy							
Yes	231 (37.2)	198 (38.4)	17 (30.9)	123 (34.8) †	77 (43.5) †	117 (39.3)	83 (35.6)
No	390 (62.8)	318 (61.6)	38 (69.1)	231 (65.3)	100 (56.5)	181 (60.7)	150 (64.4)
HIV status							
Positive	72 (11.6)	57 (11.1)	9 (16.4)	33 (9.3)	23 (13.0)	26 (8.7)	30 (12.9)
Negative	549 (88.4)	459 (89.0)	46 (83.6)	321 (90.7)	154 (87.0)	272 (91.3)	203 (87.1)
Child Characteristics							
Age at 3.5-year visit (months)	42.9 ± 1.2	42.9 ± 1.1	42.9 ± 0.9	42.9 ± 1.1	43.0 ± 1.0	42.9 ± 1.0	42.9 ± 1.1
Age at 5-year visit (months)	62.0 ± 1.9	62.0 ± 2.0	61.7 ± 0.7	61.9 ± 1.9	62.1 ± 1.8	61.9 ± 1.4 †	62.1 ± 2.4 †
Sex							
Male	316 (50.9)	267 (51.7)	24 (43.6)	181 (51.1)	86 (48.6)	142 (47.7)	125 (53.7)
Female	305 (49.1)	249 (48.3)	31 (56.4)	173 (48.9)	91 (51.4)	156 (52.4)	108 (46.4)

Preterm delivery (<37 weeks)							
Yes	75 (12.1)	61 (11.8)	6 (10.9)	40 (11.3)	21 (11.9)	35 (11.7)	26 (11.2)
No	546 (87.9)	455 (88.2)	49 (89.1)	314 (88.7)	156 (88.1)	263 (88.3)	207 (88.8)
Low birth weight (<2500 g)							
Yes	47 (7.6)	37 (7.2)	5 (9.1)	26 (7.3)	14 (7.9)	24 (8.1)	16 (6.9)
No	574 (92.4)	479 (92.8)	50 (90.9)	328 (92.7)	163 (92.1)	274 (92.0)	217 (93.1)
Method of delivery							
Vaginal	472 (76.0)	393 (76.2)	41 (74.6)	263 (74.3)	199 (78.5)	224 (75.2)	178 (76.4)
C-section	149 (24.0)	123 (23.8)	14 (25.5)	91 (25.7)	38 (21.5)	74 (24.8)	55 (23.6)
Duration of breastfeeding							
≤18 months	357 (57.9)	299 (58.0)	32 (58.2)	204 (57.6)	106 (59.9)	167 (56.0)	143 (61.4)
>18 months	260 (42.1)	217 (42.1)	23 (41.8)	150 (42.4)	71 (40.1)	131 (44.0)	90 (38.6)
Time last dose and 3.5-year visit (months)		23.2 ± 4.1	23.1 ± 3.8	22.9 ± 4.2	23.4 ± 3.5	23.5 ± 3.4*	22.6 ± 4.4*
Time last dose and 5-year visit (months)		29.1 ± 15.7	29.7 ± 16.0	41.7 ± 4.8 †	42.5 ± 3.9 †	42.3 ± 3.3	41.8 ± 5.0
Household characteristics							
Below the food poverty level at 2 years (R441/monthly per capita)							
Yes	255 (41.9)	220 (43.4) †	16 (29.6) †	146 (41.7)	78 (45.4)	124 (42.5)	100 (43.5)
No	354 (58.1)	287 (56.6)	38 (70.4)	204 (58.3)	94 (54.7)	168 (57.5)	130 (56.5)
Below the food poverty level at 3.5 years (R498/monthly per capita)							
Yes	237 (39.1)	203 (40.1)	16 (29.1)	137 (39.6)	67 (38.3)	110 (37.5)	94 (41.2)
No	369 (60.9)	303 (59.9)	39 (70.9)	209 (60.4)	108 (61.7)	183 (62.5)	134 (58.8)

[a] Based on 565 participants in the study sample with information about measles antibodies from the 5-year visit.

[b] Based on 528 participants in the study sample with information about tetanus and Hib antibodies from the 5-year visit.

† $p < 0.1$; * $p < 0.05$.

3 Results

Mothers averaged 26.4 years at delivery; 55.4% had less than a high school education and 43.6% were primiparous (see Table 1). Seventy-two women or 11.6% were HIV positive. Overall 12.1% of the infants were born preterm (<37 weeks gestation) and 7.6% were of low birth weight (<2500 g); 42.1% of infants were breastfed for longer than 18 months. At 2 and 3.5 years, 41.9% and 39.1%, respectively, of the children were living below the food poverty level.

As shown in Table 2 , 14.4% at 1 year, 30.1% at 2 years, 12.5% at 3.5 years, and 7.2% at 5 years of age were moderately or severely stunted. When we consider all indicators of undernutrition, 19.5% at 1 year, 31.4% at 2 years, 15.2% at 3.5 years, and 10.5% at 5 years were moderately or severely undernourished. Table 3 shows the breakdown and mean and standard deviation for each of these indicators by age. Supplemental Table 1 shows that boys were more likely to be stunted and to have low weight-for-age than girls.

Table 2. Growth indicators at ages 1, 2, 3.5 and 5 years for fully-vaccinated VHEMBE children, South Africa.

	1 year N (%) or Mean ± SD	**2 years N (%) or Mean ± SD**	**3.5 years N (%) or Mean ± SD**	**5 years N (%) or Mean ± SD**
Length/height-for-age				
Normal (z ≥ -2SD)	525 (85.6)	432 (69.9)	540 (87.5)	556 (92.8)
Moderately undernourished (−3 ≤ z < −2 SD)	71 (11.6)	148 (24.0)	70 (11.4)	42 (7.0)
Severely undernourished (z < -3SD)	17 (2.8)	38 (6.2)	7 (1.1)	1 (0.2)
Weight-for-age				
Normal (z ≥ -2SD)	561 (91.2)	582 (94.3)	597 (96.3)	581 (97.2)
Moderately undernourished (−3 ≤ z < −2 SD)	48 (7.8)	31 (5.0)	19 (3.1)	16 (2.7)
Severely undernourished (z < −3)	6 (1.0)	4 (0.7)	4 (0.7)	1 (0.2)
Weight-for-length/height				
Normal (z ≥ −2)	587 (95.8)	607 (98.4)	606 (98.2)	NA
Moderately undernourished (−3 ≤ z < −2 SD)	21 (3.4)	9 (1.5)	11 (1.8)	
Severely undernourished (z < −3 SD)	5 (0.8)	1 (0.2)	0 (0.0)	
BMI for age				
Underweight (<5th percentile)	38 (6.2)	12 (1.9)	19 (3.1)	25 (4.2)
Normal weight (5th–95th percentile)	440 (71.8)	414 (67.1)	500 (81.0)	515 (86.1)
Overweight (85th–95th percentile)	69 (11.3)	101 (16.4)	71 (11.5)	41 (6.9)
Obese (>95th percentile)	66 (10.8)	90 (14.6)	27 (4.4)	17 (2.8)
Any undernutrition indicator [a]				
No	495 (80.5)	424 (68.6)	526 (84.8)	536 (89.5)
Yes	120 (19.5)	194 (31.4)	94 (15.2)	63 (10.5)
Growth z-scores				
Length/height-for-age	−0.86 ± 1.09	−1.48 ± 0.98	−0.98 ± 0.88	−0.63 ± 0.90
Weight-for-age	−0.41 ± 1.22	−0.55 ± 0.98	−0.57 ± 0.87	−0.46 ± 0.87
Weight-for-length/height	0.01 ± 1.24	0.19 ± 1.02	0.01 ± 0.95	NA
BMI for age	0.13 ± 1.24	0.53 ± 1.04	0.08 ± 0.95	−0.12 ± 0.90

[a] Includes participants moderately (z-score between −2 and − 3 SD) or severely (z-score < -3SD) for height/length for age, weight for age, or weight for height/length, OR who were considered underweight according to BMI (BMI for age < 5th percentile).

Table 3. Measles, tetanus and Hib antibody titer levels at ages 3.5 and 5 years in VHEMBE children, South Africa.

	N	% > LOQ	% protected	GM ± GSD	Min	10%	25%	50%	75%	90%	Max
Measles (IU/mL)											
3.5 years	598	99.5	91.9	1.78 ± 2.48	<LOQ	0.49	1.26	2.32	3.24	4.24	7.94
5 years	568	99.5	90.4	1.90 ± 3.20	<LOQ	0.45	0.97	1.94	3.78	7.55	> 22.53
Tetanus (IU/mL)											
3.5 years	557	88.7	79.5	0.24 ± 3.39	<LOQ	<LOQ	0.12	0.24	0.50	1.24	> 5.00
5 years	531	83.8	66.7	0.18 ± 3.42	<LOQ	<LOQ	0.08	0.18	0.42	0.80	> 5.00
Hib (µg/mL)											
3.5 years	557	97.9	73.8	2.38 ± 4.65	<LOQ	0.38	0.92	2.24	6.70	> 27.6	> 27.6
5 years	531	98.1	56.1	1.32 ± 3.77	<LOQ	0.28	0.63	1.13	2.97	8.32	> 27.6

LOQs: 0.06 IU/mL for measles, 0.06 IU/mL for tetanus, and 0.08 µg/mL for Hib.
Antibody cut-off values for protection: 0.25 IU/mL for measles, 0.10 IU/mL for tetanus, and 1.0 µg/mL for Hib.
Several values were above the highest standard value and could not be quantified exactly: 16 values for measles at 5 years, 3 values for tetanus at 3.5 years, 1 value for tetanus at 5 years, 68 values for Hib at 3.5 years, and 14 values for Hib at 5 years.
Abbreviations: LOQ: Limit of quantification; GM: geometric mean; GSD: geometric standard deviation.

These fully-vaccinated children were inoculated at a median age of 9.5 (IQR = 9.1–10.2) and 18.5 (IQR = 18.2–19.6) months against measles, and 1.4 (IQR = 1.4–2.3), 2.4 (IQR = 2.3–3.3), 3.5 (IQR = 3.3–4.3) and 18.6 (IQR = 18.1–19.9) months against tetanus and Hib. Seven children received an additional measles vaccination (booster) before age 3.5 years and 240 children received a measles booster between ages 3.5 and 5 years. Only 1 received a tetanus booster before 3.5 years and 1 between 3.5 and 5 years; none received Hib boosters. By 3.5 years, 23 of the children had contracted measles and despite full vaccination, 8 additional children had contracted measles between the 3.5- and 5-year visits, demonstrating the continued circulation of this virus during the study period. We note that all 8 children who contracted measles between the 3.5- and 5-year visits had antibody titers above the "protection" threshold of 0.25 IU/mL.

As shown in Table 1 and 3 , 9.6%, 33.3%, and 43.9% of the children by 5 years had antibody levels below the level of protection for measles, tetanus, and Hib, respectively. The geometric mean titers (geometric standard deviation) for measles antibodies were 1.78 (2.48) IU/mL at 3.5 years and 1.90 (3.20) IU/mL at 5 years; for tetanus antibodies were 0.24 (3.39) IU/mL at 3.5 years and 0.18 (3.42) IU/mL at 5 years; and for Hib antibodies were 2.38 (4.65) μg/mL at 3.5 years and 1.32 (3.77) μg/mL at 5 years. Children with sub-protective measles antibody titers at age 5 years had older mothers and were more likely to live in households above the food poverty level at delivery and at ages 2 and 3.5 years than children who were protected. Children with sub-protective tetanus antibody titers at age 5 had older mothers and were somewhat more likely to live with smokers than children who were protected. The time interval since their last vaccination was longer in children with low anti-tetanus or anti-Hib antibodies compared to those with protective antibody titers, confirming the waning of vaccine-induced responses over time.

Neither stunting nor any undernutrition indicator at 1 or 2 years was related to the risk of being unprotected for measles, tetanus, or Hib (Table 4). However, stunting and any undernutrition indicator at 3.5 years was associated with lower antibody titers at 3.5/5 years. Specifically, children who had any indicator of undernutrition had a 27.2% (95% CI = -45.1, −1.3) lower geometric mean measles antibody titers than those who did not. We also found marginal associations between being moderately or severely stunted and lower measles titers (−24.1%, 95% CI = -44.2, 0.6). We observed a similar result for stunting or any indicator of undernutrition at 3.5 years and tetanus geometric mean titers (−19.6%, 95% CI = -40.7, 9.3; and − 19.5%, 95% CI = -38.7, 7.7, respectively), although those associations did not reach statistical significance. Stunting or any indicator of undernutrition at any age was not related to Hib antibody titers.

Table 4. Association between stunting and any undernutrition indicator at 1, 2, or 3.5 years and risk of being unprotected and difference in antibody titers to measles, tetanus and Hib at 3.5 and 5 years of age in VHEMBE children, South Africa.

Infection	Exposure	Age of visit	Risk of being unprotected RR (95% CI) [a]	Difference in antibody levels % (95%CI) [a]
Measles	Stunting	1 year	0.79 (0.31, 1.50)	−0.2% (−20.7, 23.5)
		2 years	1.27 (0.73, 2.12)	−8.3% (−23.8, 10.9)
		3.5 years	1.07 (0.36, 2.06)	−24.1% (−44.2, 0.6)#
	Any undernutrition indicator	1 year	0.99 (0.46, 1.76)	−3.2% (−22.0, 16.8)
		2 years	1.22 (0.71, 1.99)	−6.3% (−22.0, 12.9)
		3.5 years	1.25 (0.54, 2.22)	−27.2% (−45.1, −1.3) *
Tetanus	Stunting	1 year	0.80 (0.51, 1.13)	−1.6% (−26.3, 30.1)
		2 years	1.02 (0.76, 1.31)	−6.4% (−24.5, 15.2)
		3.5 years	1.18 (0.74, 1.61)	−19.6% (−40.7, 9.3)
	Any undernutrition indicator	1 year	0.82 (0.58, 1.11)	4.1% (−18.2, 31.0)
		2 years	1.03 (0.77, 1.31)	−5.9% (−23.8, 16.6)
		3.5 years	1.09 (0.73, 1.52)	−19.5% (−38.7, 7.7)
Hib	Stunting	1 year	1.10 (0.85, 1.37)	−6.7% (−33.4, 29.4)
		2 years	1.08 (0.85, 1.33)	−4.6% (−29.9, 29.1)
		3.5 years	0.87 (0.58, 1.21)	16.1% (−24.6, 76.8)
	Any undernutrition	1 year	0.99 (0.76, 1.26)	6.6% (−21.8, 41.6)
		2 years	1.05 (0.84, 1.30)	−0.8% (−25.5, 32.3)
		3.5 years	0.83 (0.56, 1.10)	18.5% (−15.4, 73.7)

* $p < 0.05$; # $p < 0.1$.

[a] Controlling for maternal age, education, marital status, alcohol consumption, smoking, exposure to environmental tobacco smoke and HIV status during pregnancy; household poverty and wealth index; method of delivery and child sex. Models with measles and tetanus outcomes additionally controlled for whether the children received booster doses of the vaccine.

The above associations for measles and Hib geometric mean titers did not differ by sex. However, girls had lower tetanus geometric mean titers than boys (boys' 0.27, girls' 0.21, $p = 0.02$). Girls but not boys who were stunted or had any indicator of undernutrition (compared to those without these indicators) at 3.5 years (Supplemental Table 2) had an increased risk of being unprotected against tetanus and to have lower geometric mean titers: Girls:- 38.7% (95% CI = -59.6, −8.0); Boys: 2.7% (95% CI = -31.3, 58.1); interaction p -value = 0.10 for stunting and Girls: −36.8% (95% CI = -59.3, −7.0); Boys: 0.5% (95% CI = -32.7, 54.8); interaction p-value = 0.13 for any indicator of undernutrition. A similar pattern was observed in associations between tetanus geometric mean titers and either stunting or any indicator of undernutrition at 2 years (Girls: −24.9% (95% CI = -45.0, −0.05), Boys: 14.3% (95% CI = -13.2, 53.3); interaction p-value = 0.03).

4 Discussion

Most deaths worldwide due to childhood infectious diseases, including vaccine-preventable infections such as measles, tetanus, and Hib, occur in children younger than 5-years old. Although the global incidence of measles declined steadily between 2000 and 2016, [2] this trend began to reverse in 2018 and measles has made a strong comeback with significant outbreaks in many regions of the world. [1] In a large part, these outbreaks may be due to under vaccination exacerbated by missed doses during the pandemic [4]. In addition, some children do not mount adequate antibody levels despite being fully vaccinated. We found that among fully vaccinated children, 90.4%, 67.7%, and 56.1% of the children by 5 years had antibody levels at the protective level for measles, tetanus, and Hib, respectively. Similar levels of protection for measles were observed in a study of immigrants to the United States where nearly 90% at age 5 had protective antibody levels. [19] However, we found higher percent with protective antibody levels for tetanus than the 40–50% noted for children in studies from Democratic Republic of the Congo [20] and from Nigeria but a lower percent for Hib than children in a study from Gambia (70%). [21,22] Our study suggests that even among preschool-age children who are fully vaccinated, being undernourished may play a role in the lower measles immunity by school age. In addition, fully-vaccinated undernourished girls but not boys may have lower immunity for tetanus. We did not find that undernutrition impacted Hib immunity.

Previous studies have shown lower seroconversion rates after measles vaccination in children with various indicators of undernutrition, including stunting (based on length/height-for-age), [23] wasting (weight-for-age), [9] underweight, and in children with Marasmus and/or Kwashiokor. [24] However, some early studies failed to show an association, possibly due to small sample size or using assays that were less sensitive than ELISA (e.g., the hemagglutination-inhibition assay). [9,25] Few previous studies have followed children after the full measles vaccination series to assess possible decrements in immunity into the school years. [26] That 31 children contracted measles in our study before age 5 despite being vaccinated supports the hypothesis that vaccine-induced immunity in this population was suboptimal.

Although few studies have examined the possible influence of undernutrition on tetanus immunity, some have reported lower titers in undernourished children, [26] although sometimes less profound than for measles, [24] while others have not found any association. [27] Since we observed an association of undernutrition and tetanus immunity only for girls, studies that did not stratify by sex may have obfuscated their findings. No cases of tetanus were reported in our study population during follow-up despite the relatively high level of vulnerability suggested by our data; however, even in the pre-vaccine era the annual incidence of tetanus in African countries was low–in the range of 2/100,000. [28]

Very few studies have examined the relationship of undernutrition in children with Hib antibody titers and to our knowledge, only one study in Peruvian children has reported lower Hib antibody titers (and the proportion protected) associated with underweight. [26]

The disparity in the impact of undernutrition on the vaccine-induced antibody titers among the three types of vaccines may be explained in part by the different mechanisms that underlie the ability of these vaccines to stimulate antibody responses. As an attenuated but living virus, the measles vaccine is expected to elicit both antibodies and strong cell-mediated immunity. [29] In addition, since measles virus was still circulating in this community during the study, some

children received booster vaccines and others may have been exposed to the natural virus. This could explain in part the observation of a small increase in mean measles virus titers between 3.5 and 5 years of age while antibody levels for tetanus and Hib fell. The toxoid protein in the tetanus vaccine is a T cell independent antigen that can induce an antibody response by itself in even young children. The toxoid also contains multiple T cell epitopes, one of which can interact with a wide range of MHC II molecules. [30,31] In contrast, the Hib capsular polysaccharide is a T cell dependent antigen in young children that requires the presence of a conjugated protein 'hapten' to induce the T cell help needed to elicit antibodies. [32] In both of these latter vaccines, aluminum-salt adjuvants are included specifically to enhance antibody responses. [33]

The current study had limitations. We examined the relationship of gross measures of undernutrition but did not study specific nutrient deficiencies, such as of Vitamin D, zinc, and ferritin; [34] such an investigation may shed light on potential mechanisms and possible interventions. Given differences in diet across populations, our findings may not be generalizable. Also, given the timing of our blood collection, we cannot determine whether undernutrition is associated with the initial vaccine response (i.e., primary vaccine failure, low response) and/or the durability of response (i.e., secondary vaccine failure, waning immunity). Waning immunity may have implications for health even into adulthood, including for child-bearing women and their babies. Another shortcoming of this study is that, although our sample size was relatively large, it was inadequate to consider the contribution of pre-existing health conditions (e.g., HIV) on reduced immune response to vaccines. Although we gathered information on food security, we unexpectedly found that children with protected immunity against measles were more likely to be food insecure. It is possible that the maternal food security questionnaire measure developed by the USDA [11] did not accurately represent the food intake of these South African families.

This study has several strengths, including a relatively large sample size, longer length of follow-up post-vaccination in fully-vaccinated children, and examination of immune response to three distinctly different types of vaccine. In general, there are very few serosurveys in any country that have determined decay in serologic response in young school age children and beyond. [35]

After the provision of adequate nutrition and clean water, vaccination is the most important measure to prevent disease in children. The present study suggests that undernutrition will add to the risk of disease posed by suspended vaccination programs as noted during the pandemic. Given our finding on measles seroprotection, immunity to other live-attenuated virus vaccines, e.g., yellow fever, BCG, rotavirus, mumps, rubella, varicella, may also be attenuated with undernutrition, further increasing the risk of these diseases. Furthermore, with the potential emergence of new infectious diseases with climate change, it is imperative that children are adequately prepared to mount strong immune responses to new vaccines. Addressing undernutrition through interventions, such as supplementary feeding programs, may help to improve the immune health in undernourished children.

5 Conclusions

Our data strongly reinforce the limited existing literature showing that child undernutrition can have a negative impact on the efficacy of vaccines by compromising the immune system's ability to mount effective responses to vaccines and/or by altering the durability of these responses. The extent of the impact can vary depending on many factors including the specific

vaccine, the specific nutritional intake of the child, and other health-related factors. Importantly, our data suggest that some of these negative impacts may be sex-specific. Addressing child undernutrition through nutrition-focused interventions may be critical for improving vaccine efficacy and reducing the burden of vaccine-preventable diseases.

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CRediT authorship contribution statement

Brenda Eskenazi: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition. **Stephen Rauch:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Basant Elsiwi:** Writing – review & editing, Formal analysis. **Riana Bornman:** Writing – review & editing, Supervision, Investigation. **Muvhulawa Obida:** Writing – review & editing, Supervision, Project administration, Investigation, Data curation. **Angela Brewer:** Writing – review & editing, Resources, Formal analysis, Data curation. **Brian J. Ward:** Writing – review & editing, Writing – original draft, Resources, Formal analysis, Data curation, Conceptualization. **Jonathan Chevrier:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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