# Factors associated with stunted growth in children under five years in Antananarivo, Madagascar and Bangui, Central African Republic

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## Table S1: Characteristics of the mothers

| | Bangui N=409 | Antananarivo N=424 | P-value |
|---|---|---|---|
| **Less than 15 years old at first pregnancy** | 71 (17.36%) | 46 (11%) | Pr < 0.0001 |
| Yes | 305 (75.5%) | 373 (88%) | |
| No | 33 (8%) | 5 (1%) | |
| Unknown | | | |
| **Prenatal consultations(s)** | | | Pr = 0.002 |
| Yes | 390 (97%) | 399 (94.5%) | |
| No | 7 (2%) | 23 (5.5%) | |
| Unknown | 4 (1%) | 0 (0%) | |
| **Place of giving birth** | | | Pr < 0.0001 |
| Hospital | 348 (85%) | 164 (38.5%) | |
| Home | 50 (12%) | 248 (58.5%) | |
| Other | 4 (1%) | 10 (2.5%) | |
| Unknown | 7 (2%) | 2 (0.5%) | |
| **Nutritional status of mother*** | | | Pr < 0.0001 |
| Normal weight | 234 (57%) | 256 (60.5%) | |
| Underweight | 56 (14%) | 63 (14.5%) | |
| Overweight | 66 (16%) | 91 (21.5%) | |
| Unknown | 53 (13%) | 14 (3.5%) | |
| **Mothers height (cm)** | 161.7 (161.0; 162.3) | 152.5 (151.9; 153.0) | Pr < 0.0001 |
| **Mothers education** | | | Pr < 0.0001 |
| None | 21 (5.5%) | 13 (3%) | |
| Primary | 151 (38%) | 203 (48.5%) | |
| Middle school | 168 (42.5%) | 180 (43%) | |
| High school or more | 55 (14%) | 23 (5.5%) | |
| **Mother is working** | | | Pr < 0.0001 |
| Yes | 307 (75%) | 266 (62.75%) | |
| No | 93 (23%) | 157 (37%) | |
| Unknown | 9 (2%) | 1 (0.25%) | |

* based on the BMI of the mother; pregnant mothers were classified based on the Ververs criteria[1]: Mothers with amenorrhea for less than 13 weeks: undernourished if BMI <= 20.5; mothers with amenorrhea for 13 weeks to less than 27 weeks: undernourished if BMI <= 21.5; mothers with at least 27 weeks of amenorrhea: undernourished if BMI <= 22.5. Mothers were not classified if they were pregnant and had a BMI larger than 25 (missing value in this case as difficult to assess if this is a sign of overnutrition or not)

## Table S2: Description of sanitary conditions

| | Bangui N=409 | Antananarivo N=424 | P-value |
|---|---|---|---|
| **No times mother washes hand/day*** | 2.61 (2.53; 2.71) | 4.16 (4.05; 2.53) | Pr < 0.0001 |
| **Soap in household** | | | Pr < 0.0001 |
| No or sometimes | 344 (84%) | 234 (55%) | |
| Always | 65 (16%) | 190 (45%) | |
| **Water for drinking is treated**** | 41 (10%) | 114 (27%) | Pr < 0.0001 |
| **Filtered drinking water** | 3 (0.5%) | 2 (0.5%) | Pr = 0.625 |
| **Chlorinated drinking water** | 36 (9%) | 34 (8%) | Pr = 0.684 |
| **Boiled drinking water** | 2 (0.5%) | 90 (21%) | Pr < 0.0001 |
| **Water used in kitchen is treated** | | | Pr = 0.016 |
| Yes | 29 (7%) | 51 (12%) | |
| No | 380 (93%) | 373 (88%) | |
| **Water for drinking/cooking is stored** | | | Pr = 0.005 |
| Yes | 6 (1.5%) | 21 (5%) | |
| No | 403(98.5%) | 403 (95%) | |
| **Contamination source less 5m from house***** | | | Pr < 0.0001 |
| Yes | 140 (34%) | 238 (56%) | |
| No | 253 (62%) | 83 (20%) | |
| Unknown | 16 (4%) | 103 (24%) | |
| **Disposal of wastewater** | | | Pr < 0.0001 |
| In the street | 213 (52%) | 29 (7%) | |
| Open well in the house/garden | 148 (36%) | 19 (4.5%) | |
| Open well outside the house/garden | 42 (10%) | 321 (75.5%) | |
| Other | 6 (2%) | 55 (13%) | |
| **Disposal of waste** | | | Pr < 0.0001 |
| Open well | 93 (23%) | 151 (35.5%) | |
| Closed well | 16 (4%) | 44 (10.5%) | |
| In the street | 168 (41%) | 57 (13.5%) | |
| Other | 132 (32%) | 172 (40.5%) | |

* self-reported by mother, not counted
**water in the same household can be treated by several methods
*** self-reported

## Table S3: Description of clinical features and comorbidities

| | Bangui N=409 | Antananarivo N=424 | P-value |
|---|---|---|---|
| **Blood taken** | | | Pr < 0.0001 |
| Yes | 363 (89%) | 413 (97.5%) | |
| No | 46 (11%) | 11 (2.5%) | |
| **Anemia (HB<11 g/l)*** | | | Pr < 0.0001 |
| Yes | 167 (47%) | 81 (20%) | |
| No | 190 (53%) | 332 (80%) | |
| **Ferritin low**** | | | Pr < 0.0001 |
| Normal (>=12 ng/ml) | 333 (91%) | 323 (78%) | |
| Low (< 12 ng/ml) | 32 (9%) | 89 (22%) | |
| **CRP level** | | | Pr < 0.0001 |
| Normal (<= 10 mg/l) | 284 (79%) | 375 (91%) | |
| High (>10 mg/l) | 74 (21%) | 36 (9%) | |
| **Urinary iodine levels (µg/l)** | No data | 101 (94.11; 107.88) | |
| **Vaccinations up to date** | | | Pr < 0.0001 |
| Yes | 2 (0.5%) | 302 (71%) | |
| Incomplete | 392 (96%) | 117 (28%) | |
| No vaccinations | 15 (3.5%) | 3 (1%) | |
| **Dermatitis** | | | Pr < 0.0001 |
| Yes | 57 (14%) | 27 (6%) | |
| No | 352 (86%) | 397 (94%) | |
| **Coughing** | | | Pr < 0.0001 |
| Yes | 211 (52%) | 156 (37%) | |
| No | 198 (48%) | 268 (63%) | |
| **Clogged nose** | | | Pr < 0.0001 |
| Yes | 52 (13%) | 222 (52%) | |
| No | 357 (87%) | 202 (48%) | |
| **Running nose** | | | Pr = 0.011 |
| Yes | 221 (54%) | 266 (63%) | |
| No | 188 (46%) | 158 (37%) | |
| **Caries** | | | |
| Yes | Not | 160 (38%) | |
| No | assessed | 264 (62%) | |
| **Previous episode of severe acute undernutrition** | | | Pr < 0.0001 |
| Yes | 34 (8.25%) | 10 (2%) | |
| No | 374 (91.5%) | 414 (98%) | |
| Unknown | 1 (0.25%) | 0 (0%) | |
| **Diarrhea at day of inclusion** | | | Pr = 0.001 |
| Yes | 0 (0%) | 11 (2.5%) | |
| No | 409 (100%) | 413 (97.5%) | |

* Hemoglobin levels for Madagascar are adapted for high altitude (WHO/NMH/NHD/MNM/11.1; http://www.who.int/vmnis/indicators/haemoglobin.pdf) ** Ferritin levels are adjusted for infection based on the CRP levels[2]

## Table S4: Risk factors independently associated with stunting in the merged dataset*

| | Non-stunted N= 426 | Stunted N= 323 | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|---|---|---|---|---|
| **Mothers education** | | | | |
| None | 12 (3%) | 20 (6%) | Ref | Ref |
| Primary | 172 (40.5%) | 160 (49.5%) | 0.56 (0.26; 1.18) | 0.76 (0.31; 1.84) |
| Middle school | 181 (42.5%) | 134 (1.5%) | 0.44 (0.21; 0.94) | 0.68 (0.28; 1.66) |
| High school or more | 61 (14%) | 9 (3%) | 0.09 (0.03; 0.24) | 0.15 (0.05; 0.45) |
| **Mother's height** | | | | |
| More than 150 cm | 343 (80.5%) | 220 (68%) | Ref | Ref |
| Less than 150 cm | 60 (14%) | 83 (26%) | 2.16 (1.49; 3.13) | 2.52 (1.58; 4.03) |
| Unknown | 23 (5.5%) | 20 (6%) | 1.36 (0.73; 2.53) | 1.47 (0.40; 5.42) |
| **Nutritional status of mother*** | | | | |
| Normal weight | 229 (54%) | 189 (58.8%) | Ref | Ref |
| Underweight | 48 (11%) | 49 (15%) | 1.24 (0.80; 1.92) | 1.01 (0.60; 1.69) |
| Overweight | 101 (24%) | 45 (14%) | 0.54 (0.36; 0.81) | 0.46 (0.28; 0.73) |
| Unknown | 48 (11%) | 40 (12.5%) | 1.01 (0.64; 1.60) | 0.81 (0.40; 1.66) |
| **Age at first pregnancy** | | | | |
| Less than 15 years | 51 (12%) | 58 (18%) | Ref | Ref |
| More than 15 years | 367 (86%) | 250 (77.5%) | 0.60 (0.40; 0.90) | 0.58 (0.36; 0.95) |
| Unknown | 8 (2%) | 15 (4.5%) | 1.65 (0.65; 4.21) | 5.80 (1.01; 33.47) |
| **Nutrition changed during pregnancy** | | | | |
| No | | | | |
| Yes | 340 (80%) | 277 (86%) | Ref | Ref |
| Unknown | 72 (17%) | 36 (11%) | 0.61 (0.40; 0.94) | 0.50 (0.30; 0.86) |
| | 14 (3%) | 10 (3%) | 0.88 (0.38; 2.00) | 0.11 (0.02; 0.86) |

| | | | | |
|---|---|---|---|---|
| **Partially breastfed ≥ 12 months** | | | | |
| **No** | 25 (6%) | 39 (12%) | Ref | Ref |
| **Yes** | 380 (89%) | 268 (83%) | 0.45 (0.27; 0.76) | 0.45 (0.24; 0.85) |
| **Unknown** | 21 (5%) | 16 (5%) | 0.49 (0.21; 1.11) | 0.30 (0.11; 0.82) |
| **Soap available in household** | | | | |
| Yes | 319 (75%) | 178 (55%) | Ref | Ref |
| No or only sometimes | 107 (25%) | 145 (45%) | 2.43 (1.78; 3.31) | 1.97 (1.35; 2.89) |
| **Anemia (Hb <11 g/l)** | | | | |
| No | 324 (76%) | 187 (58%) | Ref | Ref |
| Yes | 102 (24%) | 136 (42%) | 2.31 (1.69; 3.16) | 2.83 (1.89; 4.22) |
| **Birth weight **** | | | | |
| Average | 162 (38%) | 139 (43%) | Ref | Ref |
| Smaller than average | 46 (11%) | 83 (25.5%) | 2.10 (1.37; 3.22) | 2.32 (1.40; 3.85) |
| Bigger than average | 144 (34%) | 64 (20%) | 0.52 (0.36; 0.75) | 0.46 (0.29; 0.72) |
| Unknown | 74 (17%) | 37 (11.5%) | 0.58 (0.37; 0.92) | 0.58 (0.33; 1.04) |
| **Diarrhea at time of inclusion** | | | | |
| No | 425 (99.75%) | 313 (97%) | Ref | Ref |
| Yes | 1 (0.25%) | 10 (3%) | 13.58 (1.73; 106.62) | 12.13 (1.38; 106.38) |
| **Dermatitis at time of inclusion** | | | | |
| No | 393 (92%) | 282 (87%) | Ref | Ref |
| Yes | 33 (8%) | 41 (13%) | 1.73 (1.07; 2.81) | 1.92 (1.09; 3.38) |
| **Previous episode of severe acute undernutrition** | | | | |
| No | 421 (99%) | 292 (90%) | Ref | Ref |
| Yes | 5 (1%) | 31 (10%) | 8.94 (3.44; 23.26) | 5.96 (2.09; 17.04) |

* Analysis performed on 749 subjects; ** as perceived and remembered by mothers/ main caregivers; $adjusted for season of inclusion, age in years, gender and country of inclusion (matching) as well as the other independently associated variables.

## Extended Material and Methods

*Study design and study population*

The AFRIBIOTA study[3] is a case-control study for stunting in children aged 2-5 years in Bangui, CAR and Antananarivo, Madagascar. Inclusion criteria were HIV-negative children, neither suffering from acute malnutrition nor from any other severe disease, living in the 6$^{st}$, 7$^{st}$ or 8$^{th}$ district of Bangui, CAR or in two neighbourhoods of Antananarivo, Madagascar (Ankasina or

Andranomanalina Isotry). Children were recruited in the community by health workers and screened for eligibility during inclusion sessions at the local health district. Recruitment took place within these districts or neighbourhoods, and included children were admitted to the hospital for sample collection (blood, stool, duodenal and gastric aspirates) and anthropometric measurement. Sample tacking and interviewing took place centrally at the hospital. Stunting severity was defined based on the WHO 2006 standards, as described below. Stunted and control children were matched according to age (24-35 months, 36-47 months and 48-60 months), gender, neighbourhood and season of inclusion (dry or wet season). Recruitment took place between December 2016 and March 2018 in Antananarivo and between January 2017 and May 2018 in Bangui.

*Sample size estimation*

Sample size for AFRIBIOTA was calculated to respond to the primary objective, which was to find differences in the microbiota of stunted compared to non-stunted children. Analysis of factors associated with stunting was one of the study's secondary objectives. Assuming an alpha-error of 0.05%, a power of 80% and an expected 20% exposure in the cases, we needed at least 169 children per group. The initial total targeted sample size was 920 children. In each country, we intended to enrol stunted children (200/country) and children with no growth delay (260/country). The final sample size analysed in this study was of 836 children (Fig. 1).

*Anthropometric measurements*

Anthropometric variables included height, weight, head circumference and mid-upper arm circumference (MUAC). Height was measured on standing children, and weight with minimal clothing (underwear). All anthropometric measurements were taken twice and repeated, if the height or MUAC differed by more than 1 mm or the weight by more than 100g between the two measurements. The following variables were calculated using the WHO Anthro software (version 3.2.2, January 2011): height-for-age z-score (HAZ), weight-for-age z-score (WAZ), BMI-for-age z-score (zBMI), weight-for-height z-score. We used the following cut-offs defined by the WHO Growth Child standards 2006[4,5] : stunted: < -2 HAZ (moderately stunted: -3 ≤ HAZ < -2; severely stunted: HAZ < -3); acute malnutrition based on WHZ score: < -2 WHZ acutely malnourished).

*Data collection*

A standardized, paper questionnaire in French that was translated *ad hoc* to the local languages Sangho and Malagasy was used in both study sites and data was entered in double in an Access database. The questionnaire was given by trained data clerk (Bangui) or trained medical doctors (Antananarivo). Trained medical doctors performed all clinical examinations. The CRF included information about children's age, gender, family structure, socioeconomic status indicators (e.g. profession of parents, working

situation, household size, description of living conditions, household assets, means of transportation), sanitary indicators (e.g. drinking water, eating with fingers, access to sanitary facilities), data about the mother's pregnancy and child's and family nutrition and feeding practices (e.g. 24 hour recall, number of meals, breastfeeding). Each child was also examined by a medical doctor and comorbidities such as caries, non-severe respiratory complaints, mild diarrhoea and/or dermatitis were assessed. For each child, venous blood was further collected. Complete blood count, C-reactive protein (CRP) and ferritin levels were measured at the Institut Pasteur de Madagascar and the Institut Pasteur de Bangui within 4 hours after blood collection according to accredited methods. Ferritin levels were corrected for systemic inflammation[2] haemoglobin values were adjusted for altitude[6,7] and anaemia was defined as less than 110 g/l, according to WHO criteria[5,8]. A dietary diversity score (DDS) was calculated based on a 24 hour recall based on the seven good groups defined by WHO: (1) grains, roots and tubers; (2) legumes and nuts; (3) dairy products; (4) flesh foods (meats/fish/poultry); (5) eggs; (6) vitamin A-rich fruits and vegetables; and (7) other fruits and vegetables. According to WHO standards, a DDS of four is considered the minimum DDS for normal food diversity[9]. Mother's nutritional status was based on the Body Mass Index (BMI; weight (kilogram) divided by the square of body height (meter)). Non-pregnant mothers were classified by BMI categories as underweight if BMI was <18.5 kg/m², normal weight if BMI was ≥ 18.5kg/m² and ≤ 25kg/m², and overweight if BMI was >25kg/m². Pregnant mothers were classified according to the categories proposed by Ververs[1] : BMI <20.5 kg/m² if amenorrhea less than 13 weeks, BMI <21.5 kg/m² if amenorrhea between 13-27 weeks, and BMI <22.5 kg/m² if amenorrhea is 27 weeks or more. A wealth index was created based on the minimal set of assets, leading to a separation of subjects in three distinct groups in a principal component analysis (PCoA). The minimal set of assets included housing materials (floor and wall materials, ownership of a car, telephone, bike, motorcycle), access to specific utilities (electricity, bathroom, cooking location), and family size. Iteration tests were then performed to check whether the selected variables reflect the observed clusters. We defined three household wealth categories according to the cluster observed: poorest, middle and wealthiest category. The categories were defined as follows: lowest socioeconomic score: no telephone or house floor made of pounded earth; middle socioeconomic score: telephone, wooden or concrete house floor, no internal shower or internal kitchen; highest score: telephone, wooden or concrete house floor, either an internal shower or internal kitchen (separate room).

*Statistical analysis*

The statistical analysis was performed with Stata 13. Significance level was fixed for all analyses at 0.05 and all tests were performed bilaterally. Categorical variables were expressed as percentages; quantitative variables were expressed as a mean (± Standard Deviation) or median (interquartile range). The stunted vs. non-stunted groups were compared using Chi2 or Fisher Exact test for qualitative variables and the Student t test or the Mann-Whitney U test for quantitative variables. All variables were assessed in a bivariate analysis. Factors associated with stunting in bivariate analysis with a p-value of <0.2 were checked for

potential confounding factors and interactions and then included in a backward logistic regression. As there was not a perfect matching for age, gender and season of inclusion, these variables were forced in the multivariate model. Results are reported as adjusted OR with 95% CI, corrected for age in years, gender, season of inclusion and country of origin.

*Ethical considerations*

The AFRIBIOTA study protocol was approved by the Institutional Review Board of the Institut Pasteur (2016-06/IRB) and the national ethical review boards of Madagascar (55/MSANP/CE) and the Central African Republic (173/UB/FACSS/CSCVPER/16). Legal representatives of all child participants received oral and written information about the study and provided written consent for their children to participate in the study.

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