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AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 25 No. 6
July 2025
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2025; 25(6): 26979-27000 https://doi.org/10.18697/ajfand.143.25575

Date	Submitted	Accepted	Published
	3rd December 2024	3rd May 2025	30th July 2025

AFLATOXIN B1 EXPOSURE AND ITS ASSOCIATION WITH NUTRITIONAL STATUS OF CHILDREN RECEIVING CEREAL-BASED SUPPLEMENTARY FOODS AT HEALTH CENTERS IN RWANDA

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Volume 25 No. 6
July 2025
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ISSN 1684 5374

ABSTRACT

In sub-Saharan Africa, as well as in various global tropical regions, cereal-based porridge serves as the primary dietary energy source, particularly for malnourished children who rely on these cereal-based porridge as supplementary nutritional provisions. Few studies on aflatoxin contamination in foods and feeds have been done in Rwanda; also, data on aflatoxin exposure estimate in children receiving cereal-based foods at the country's Health Center (HC) facilities are scanty. The present study aimed to investigate the association between aflatoxin B1 exposure and the nutritional status of children in Rwanda using an analytical retrospective study design. Children (n=216) aged 6–59 months were recruited from HCs in districts known to have high prevalence of chronic malnutrition. A case definition was established, wherein children with a recent diagnosis of malnutrition were included as cases. Two controls per case were considered. Urine samples were analyzed for AFM1 using enzyme-linked immunosorbent assay (ELISA). The association between AFM1 and the nutritional status of children was assessed using a multivariable logistic regression analysis in STATA 15. The results show that significantly more individuals in the case group (79.41%) had detectable AFM1 in urine compared to the number in the control group (59.57%) (p=0.002). The arithmetic means of AFM1 were approximately 1.24 ng/ml (±1.50) and 1.03 ng/ml (±3.11) for case and control groups, respectively (p=0.003). Occurrence of AFM1 in urine was significantly associated with the child's location (p=0.032). The only demographic variable associated with logarithmic AFM1 values was socioeconomic status, with category 3 (medium) exposure levels significantly higher than category 2 (poor) (p=0.031). In predicting malnutrition, children from category 1 (very poor) had significantly lower odds compared to those in category 2 (poor), while increasing AFM1 levels in urine significantly increased the odds of malnutrition. Therefore, we recommend the implementation of routine surveillance of aflatoxin levels in locally produced complementary foods, stricter enforcement of food safety regulations, and targeted public health interventions—such as educating caregivers on safe food storage and preparation practices—to reduce aflatoxin exposure among young children in high-risk areas of Rwanda.

Key words: Aflatoxin, AFM1, malnutrition, child, Rwanda, nutritional status, Ubudehe categories, supplementary foods

Citation: **Matabishi MA, Mutua F, Lindahl JF, Hahirwa I, Umugwaneza M, Musafili A and C Munyanshongore** Aflatoxin B1 exposure and its association with nutritional status of children receiving cereal-based supplementary foods at health centers in Rwanda. *Afr. J. Food Agric. Nutr. Dev.* 2025; **25(6):** 26979-27000. https://doi.org/10.18697/ajfand.143.25575

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INTRODUCTION

Aflatoxins (AFs) are a type of mycotoxins produced by certain fungi, found in agricultural products such as cereals and oil seeds from tropical and subtropical regions. These toxins are a health concern in developing countries where cereals and oil seeds are used as primary sources of food, especially in settings that rely on traditional farming and storage methods [1]. The techniques used to manage crops before and after harvesting influence the level of aflatoxin accumulation in food products [2, 3]. Even in modern agricultural and manufacturing practices, despite the meticulous adherence to Good Agriculture Practices (GAP) and Good Manufacturing Practices (GMP), complete eradication of aflatoxins in cereals and nuts is difficult [4].

In sub-Saharan Africa, maize and oil seeds are the main source of dietary energy and protein for the pediatric demographic, with a pronounced emphasis on malnourished children who receive these foods as supplementary nutritional provisions [5]. In addition to the nutrition these foods provide, children are at higher risk of aflatoxin exposure. The European Union has established the highest allowable level of AFB1 in foods intended for children, including foods for medical purposes at 0.1 µg/kg and other crops intended for human consumption at 2 µg/kg, respectively [6]. The East African Community standards organization has implemented a maximum threshold of 5 µg/kg for AFB1 in crops intended for human consumption [7]. A prior investigation, done within the same project, revealed the presence of AFB1 in supplementary foods meant for vulnerable and sick children in Rwanda, but with levels falling within the recommended standards [8]. Though regulatory bodies may set limits for certain foods, it is worth noting that no threshold is safe for children, especially when it comes to genotoxic and carcinogenic substances. Currently, the main strategy to reduce aflatoxin exposure is promoting food diversity. Dietary diversity reduces aflatoxin exposure by minimizing reliance on highly contaminated staple foods like maize and groundnuts, thereby lowering overall intake [9, 10]. However, this approach would be constrained by the absence (or the limited availability) of diverse food options for children in low and middle-income countries [11]. This highlights the need to address food safety challenges in this vulnerable group.

Aflatoxins exist in different forms, with aflatoxin B1 (AFB1) being the most toxic and carcinogenic [12]. When aflatoxin B1 is consumed in food, AFB1 undergoes a transformation process, producing hydroxylated metabolites like aflatoxin M1 (AFM1) found in urine, milk and blood of mammals. The presence of aflatoxin biomarkers in urine is indicative of acute exposure (i.e. exposure having occurred in the previous 72 hours), and some researchers suggest assessing metabolites to get reliable exposure estimates [13].

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Although some studies have detected AFB1 in food and feed in Rwanda, little has been done on the concentration of AFs biomarkers in children who receive cereal-based supplementary foods at HC level. This information is needed to better understand the extent of their exposure to AFB1. Prolonged exposure to AFs has been linked to potential health issues, including malnutrition, immunosuppression and cancer [14]. This study was designed to evaluate the excretion levels of AFM1 in urine of children receiving supplementary foods at HC level in Rwanda. Additionally, the study investigated the relationship between demographic factors, nutritional status, and AFM1 excretion. The findings are vital to develop interventions to improve nutrition and protect children from the harmful effects of AFB1 and other aflatoxins.

MATERIALS AND METHOD

Study sites

The research was carried out in four health districts (Kabutare, Ruhango, Kabgayi and Nyabihu) selected by convenience as mentioned in our previous study [8]. This project targeted children currently undergoing nutritional support, identified either, by their enrollment in the Stunting Prevention and Reduction Project (SPRP) in Rwanda—a program designed to help mothers and children especially those from socioeconomically disadvantaged households, during the crucial first 1000 days of a child's life—or due to a diagnosis of malnutrition within selected HCs.

Study design

An analytical retrospective study design was used to investigate the association between AFB1 exposure and nutritional status of children aged 6 to 59 months. Participants were recruited through a retrospective examination of their medical records, including anthropometric measurements. Data collection was conducted within the timeframe of June to December 2022. The study included participants (children with a recent diagnosis of any type of malnutrition) and a control group (children without a recent history of any illness, living within the same area of residence as the patients). The nutritional status of children was determined based on established criteria, with specific threshold values for each measurement. Height-for-age (used to identify stunting, an indicator of chronic malnutrition), weight-for-height, and mid-upper arm circumference (MUAC) (both used to identify wasting, an indicator of acute malnutrition) were assessed. Additionally, weight-for-age was evaluated to identify underweight, which reflects a combination of acute and chronic malnutrition when measurements fall below the World Health Organization (WHO) threshold [15]. These criteria and their respective thresholds are recognized measures for assessing various aspects of nutritional status. The influence of confounding factors including age, gender, location, and socioeconomic status was investigated.

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In this study, we consider the socio-economic context of participants utilizing the "Ubudehe" categories which is a Home-Grown Initiative (HGI) as defined by the Rwandan government through the Ministry of Local Government [16]. The Ubudehe categories, integrated into the Poverty Reduction Strategy (PRS), provide a framework for understanding the economic conditions of individuals and households participating in the SPRP in Rwanda. Therefore, this research did not require a separate survey to assess participants' socioeconomic status. Notably, households in Rwanda have been systematically categorized within the Ubudehe categories as part of the government's support infrastructure and only category 1 and 2 (the categories with lowest socioeconomic status) receive support from the government. In this study, Ubudehe Category 1 denotes the 'very poor,' Category 2 the 'poor,' and Category 3 the 'medium' socio-economic group [16, 17].

Sample size calculation

A sample size calculation was performed using OpenEpi software version 3 and the Fleiss with a continuity correction method was selected, considering a two-sided confidence level (1-Alpha) of 99 and a power of 90% to detect the hypothesized association between AFB1 exposure and the nutritional status of the children. The ratio of controls to cases was set at 2, and hypothetical proportions of controls and cases with exposure were assumed to be 42.4% and 71.8%, respectively, based on a similar study by Onyemelukwe *et al.* [18] on Nigerian children aged 7 to 60 months. The minimum detectable odds ratio was set at 3.46. Based on these parameters, the sample size was determined as follows: Cases: 67, Controls: 133. Therefore, a total of 200 participants were required for the study. A 10% contingency for non-respondent participants was added, resulting in a final adjusted sample size of 216 (70 cases and 146 controls)

Selection of cases

The case definition was children aged between 6 to 59 months with a recent history of any type of malnutrition and receiving supplementary foods for recovery at the HC level. To select patients that met our case definition, we reviewed medical records at the health center and with the help of community health workers (CHWs) and nutritionists, parents were invited for a meeting at the HC. Children who had experienced any type of malnutrition within the previous three months before data collection were identified and selected as cases for the study. The relevant data, including the type of malnutrition and its severity, were extracted from the medical records.

Selection of controls

Two controls were randomly chosen from each case's village. The selection process involved visiting the HC on the day of supplementary food distribution to children from the poorest households (i.e, from Ubudehe categories 1 & 2). Within this

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context, we identified two groups: children aged 6 to 23 months, who were the main beneficiaries of the SPRP, and children aged 24 to 59 months, who were no longer beneficiaries of the program directly, but their mothers were part of the program either due to pregnancy or lactation periods. It was assumed that the mothers might share the porridge with their older children who were no longer enrolled in the program. Consequently, a brief meeting was organized to explain the objectives of the ongoing study and to invite their participation. Children who had been sick in the preceding three months were excluded from the study.

Sample collection and analyses

Urine samples were collected at the health center using a 100 ml pediatric urine collector (MDS190505/Medline Industries, Northfield, Illinois). From each sample, 15 ml was transferred into a plastic tube and stored at -80°C at the Biotechnology Complex, University of Rwanda, Huye Campus, Southern Province. The samples were later sent to the Mycotoxin Center Laboratory, University of Rwanda, Nyarugenge Campus, Kigali City, for AFM1 measurement. As AFM1 is stable at low temperatures, samples were transported in a cool box with ice cubes without experiencing high variation of temperatures, since Kigali is only three and a half hours drive from Huye. AFM1 concentration in urine was evaluated using quantitative ELISA kits (Cat.No. 991AFLM01U-96/Helica Biosystems Inc./Santa Ana, California), according to the manufacturer's instructions.

Prior to laboratory analysis, urine samples and reagents were brought at room temperature. Five milliliters of urine sample were aliquoted into centrifuge tubes and centrifuged at 3000 rpm for 10 minutes. All standards (0.0, 0.15, 0.4, 0.8, 1.5, 4.0 ng/ml) and sample extracts were analyzed in duplicate micro-wells. Two hundred microliter of the aflatoxin-HRP conjugate was dispensed into each 96-well mixing plate and 100 µL of either aflatoxin M1 standard solution or sample extract was added to the appropriate mixing well containing conjugate and mixed. Content from each mixing well (100 µL) was transferred to a corresponding antibody-coated microtiter well and incubated for 15 min. PBS-Tween wash buffer was used to rinse the plate wells for five washes. A substrate reagent (100 µL) was added to each antibody-coated microtiter well and incubated for 5 min. A stop solution (100 µL) was added in the same sequence and at the same place as where the substrate reagent was added. The micro-wells were measured optically by a microplate reader (Thermo Scientific Multiskan FC, Thermo Fisher Scientific, Ratastie, Finland), with an absorbance filter of optical density 450 nm. A logit regression equation generated from standard ODs and corresponding standard concentrations was used to calculate the AFB1 concentration in sample extracts. The lowest quantifiable value of the standard curve, 0.15 ng/ml, was considered the limit of detection and quantification since the kit manual did not provide them.

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Statistical analysis

Statistical analyses were performed using STATA 15 software (StataCorp LLC, College Station, Texas). Descriptive statistics summarized the dataset, while inferential tests (t-test for means and chi-square for proportions) supported hypothesis testing. To normalize the data and reduce skewness, logarithmic transformation was utilized in statistical calculations, facilitating more accurate analysis. Additionally, a multivariable logistic regression explored the relationship between aflatoxin exposure and key predictor variables, including age, gender, socioeconomic category, and place of residence. Odds ratios with 95% confidence intervals were calculated for each predictor, with statistical significance set at $p < 0.05$.

Ethical considerations

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Approval to undertake the study was obtained from the Institutional Review Board of the College of Medicine and Health Sciences University of Rwanda (Approval number: IRB/CMHS No 252//2021). Permission was obtained from the respective health center administrations and ensured that the study purpose was properly communicated to health center staff and parents of study participants before sample collection as well as seeking their cooperation. Informed consent was obtained from the children's caregivers before enrollment, confirming their understanding of the study objectives, procedures, before their participation in the study. The parents/caregivers of the study participants consented to participate in the study by signing the consent form.

RESULTS AND DISCUSSION

Socio-demographic characteristics of study participants

A total of 216 children receiving supplementary food at HCs were recruited, aged 6 to 59 months. These included 70 children with various malnutrition conditions (mean age 24.64±13.40 months) and 146 healthy controls (mean age 23.34±11.7 months). Among the malnourished children, 51.43% were female, and data on socioeconomic characteristics revealed that 47.14% were from Ubudehe category 2 (poor). The nutritional status of participants in the case group showed that 67.11%, 27.14%, and 5.71% were underweight, wasted and stunted, respectively. In the control group, 55.48% were male, 58.90% were from Ubudehe category 2, and the nutritional status showed that 12.32 % were stunted and all of them were from the same location (Table 1).

This study identified some differences between the participants, although both case and control groups were predominantly from Ubudehe Category 2 (poor), as shown in Table 1. The nutritional status of children in the case group—characterized by

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Volume 25 No. 6
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underweight, wasting, and stunting—was consistent with their malnutrition diagnosis. Interestingly, stunting was also observed among children in the control group (Table 1). This was not entirely unexpected, as stunting can result from long-term nutritional deficits and may not indicate a recent or current health issue. Importantly, this does not interfere with our case definition, as our cases were selected based on a recent malnutrition diagnosis, defined as occurring within three months prior to data collection. The slightly higher percentage of females in the malnourished group suggested possible gender-related differences that may warrant further investigation. In addition, the presence of stunting within a specific geographical area among the control group (Table 1) indicated potential disparities in nutritional status linked to environmental factors. Although these findings provided important insights, a more comprehensive investigation of demographic variables is needed. Therefore, further research is necessary to explore these factors and their potential impact on nutritional outcomes.

AFM1 detection in the sampled population

A total of 209 out of 216 participants provided urine samples (Table 2). Sixty six percent of the samples had detectable AFM1 (≥ LOQ of 0.15 ng/ml) and included 79.41% of the cases (n=68) and 59.57% of the controls (n=141) (Figure 1). A chi-square test revealed a significant difference in these proportions (p = 0.002). The arithmetic means of AFM1 were 1.24 ng/ml (±1.50) and 1.03 ng/ml (±3.11) for case and control groups, respectively (Table 2). The Mann-Whitney U test showed significant difference in mean values between the groups (p = 0.003). The median for cases was 1.17 ng/ml, while it was 0.28 ng/ml for controls, and samples above 10 ng/ml were only found among controls, where the highest value of 28.79 was detected (Figure 2).

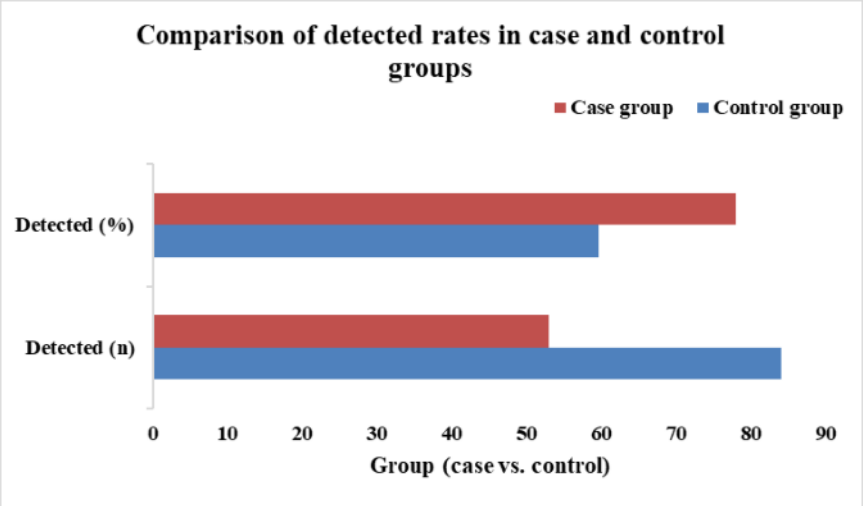

Figure 1: Proportion of samples with detectable AFM1 in case and control groups (χ^2= 9.29, p=0.002)

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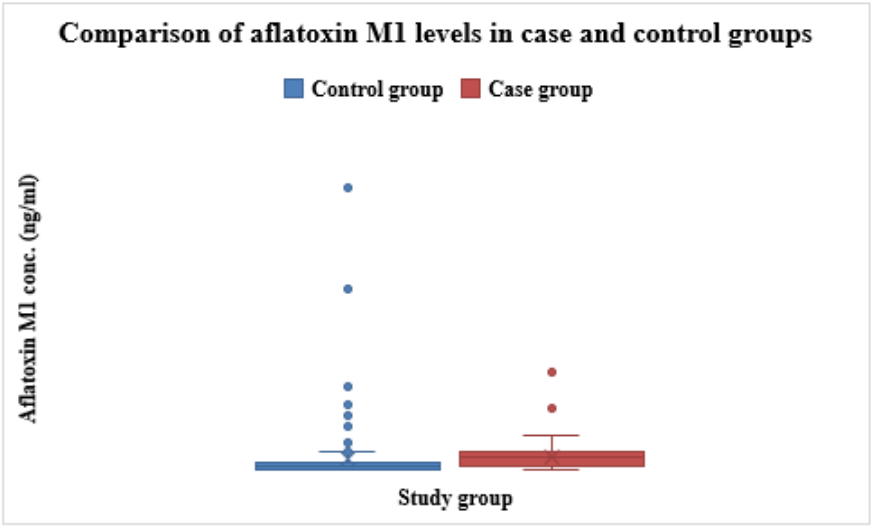

Figure 2: AFM1 concentration levels in case and control groups (t=-0.67, p=0.50)

This study assessed aflatoxin exposure by detecting urinary aflatoxin M1 using ELISA. The detection limits for AFM1 were not provided by the ELISA kit manufacturer. The Limit of Detection (LOD) and Quantification (LOQ) was operationally defined as 0.15 ng/mL, corresponding to the lowest standard point on the calibration curve. Although a blank was included in the assay to establish the baseline, representing 0 ng/mL of analyte, values between the blank and 0.15 ng/mL were not considered detectable in this study, as they fell below the validated quantifiable range of the assay. Therefore, values below 0.15 ng/mL were not considered in the aforementioned detectable proportion. However, for the calculation of means, all sample results, including detected and non-detected values, were considered to provide a comprehensive representation of AFM1 levels across the study population (Table 2).

The findings revealed that the majority of the children had detectable AFM1 with more children in the cases than in the control group (Figure 1). It may be that, unlike their healthy counterparts, malnourished children received the formulation as a therapeutic supplement and exhibited a higher level of adherence to their prescribed dietary regimen for their nutritional rehabilitation as they took the formulation daily. In contrast, although healthy children may take the porridge, they may have more flexibility in their dietary practices, potentially leading to variations in aflatoxin exposure from the HC supplementary foods. In addition, sick children were provided with cow's milk, which may further complicate the exposure dynamics. While cow's milk supplements the diet's nutrients, depending on feed quality, it may be an additional source of aflatoxin exposure. Furthermore, the compromised health status of malnourished children as stated in some studies [19, 20], could influence the metabolic pathways, potentially leading to differences in absorption and excretion

compared to their healthier counterparts. Onyemelukwe *et al.* compared AFM1 excretion in malnourished and control groups, and, like for the current study, found a statistical difference [18]. Therefore, the intricate interplay between the supplementary foods, cow's milk, and individual metabolic variations may contribute to the observed AFM1 discrepancy.

The mean concentration of AFM1 was slightly elevated in the case group with 1.24 ng/mL (±1.50) compared to the control group with 1.03 ng/mL (±3.11) (Table 2). These urinary AFM1 levels exceeded the anticipated values based on the Estimated Daily Intake (EDI) of AFB1, calculated at 44.5 ng/kg-bw/day derived from an exposure level of 2.7 ng/g from our previous study [8]. The above EDI calculation assumes an average body weight of 13 kg for a 2.5-year-old child [22], and a daily intake of 200 g of the formulation, provided as a weekly ration of 1.5 kg per child and the mentioned mean AFB1 concentration of 2.7ng/g. The expected AFM1 values range from (0.018 to 0.024 ng/mL) based on existing literature which estimates that approximately 1.5 to 2 % of ingested AFB1 is excreted as AFM1 [21]. Therefore, the obtained AFM1 mean values are about 50 times higher than the expected urinary concentrations. The elevated urinary AFM1 concentrations suggest potential exposure to AFB1 from additional environmental or dietary sources beyond the formulation provided at health center facilities in Rwanda. The current results indicate a higher frequency of AFM1 detection in urine compared to those reported in previous studies, as detailed below. A study which investigated aflatoxins exposure in children aged 24–48-months-old (n=50) and 12–30-months-old (n=50) from a high-risk region such as Guinea and a moderate-risk region such as Egypt, revealed urinary AFM1 detection in 64 of samples in Guinean children with detection (range 0.008–0.801 ng/ml) and 38 of samples in Egyptian children with detection (range 0.005–0.006 ng/ml) respectively [23]. Another study in Nigeria found AFM1 in 9 of urine samples of children aged 24–48-month-old (n=65) with detection range 0.032– 0.505 ng/ml [24]. In a different study conducted in Bangladesh, the frequency of AFM1 detection was 43.5 in infants (n=49) and 33.4 in young children (n=105). The average AFM1 levels for all infants were 0.0091 ng/ml ± 14.3 with a maximum of 0.0556 ng/ml, and for children, the average levels were 0.0088 ng/ml ± 12.9 with a maximum of 0.075.3 ng/ml [25]. However, these studies used either HPLC or LC-MS/MS methods which have a higher specificity compared to ELISA detection method [26].

Current results indicate a right-skewed distribution of AFM1 levels in both groups, suggesting very high exposure levels in some study participants. Notably, samples exceeding 10 ng/ml were only observed in the control group, with the highest value reaching 28.79 ng/ml (Figure 2). The mentioned flexibility could have contributed to these elevated AFM1 levels, as some participants in the control group might have

consumed products from sources other than the provided formulation. A prior study conducted as part of the same project as the current research revealed that some locally marked porridge ingredients were highly contaminated [8]. Therefore, the high exposure levels observed in the control group may be attributed to the consumption of these highly contaminated products.

Relationship between AFM1 and various predictors

The location of the child was significantly associated with occurrence of AFM1 in the urine. Children residing in Rango have a significantly higher likelihood of AFM1 detection compared to the reference location (Bigogwe). Specifically, the odds of AFM1 detection are approximately 2.56 times higher for children in Rango. The 95-confidence interval does not include 1, and the p-value is below 0.05, confirming the significance of this association (Table 3).

This study investigated the association between AFM1 detection and various demographic variables. Notably, there was a significant association between children's residential location and detectable urinary AFM1 levels in Rango (Table 3), a sector in Huye district. A previous study has shown that samples from open markets in Huye district have higher AFB1 contamination levels compared to other districts [8]. While elevated AFM1 in Rango aligns with these findings, the relationship may also be influenced by the greater sample size from Rango (Table 1), which likely impacted the observed outcomes. Differences in aflatoxin exposure based on location have been identified in other studies due to environmental factors such as temperature, humidity, and moisture variations [27, 28, 29] which can impact the growth of toxin producing mold and aflatoxin production on food items. Despite the diverse climatic conditions across the locations where study participants reside, all receive the same standardized supplementary foods—manufactured products distributed nationwide through health centers. Consequently, although minor variations may occur between batches, significant differences in the nutritional composition of the supplements across regions are unlikely.

Although other predictors such as age, gender were noticed to be non-significant, further investigation is necessary to understand the potential reason for these complexities. It is essential to acknowledge that, despite all children being exposed to similar levels of toxins, there are individual variations in toxin ingestion and metabolism [32]. It is worth noting that we collected urine samples from the children as random specimens obtained when they arrived at the health center. Other studies used early morning urine samples to capture temporal variations in toxin metabolism [33].

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Examining malnutrition rates across Ubudehe socioeconomic categories and AFM1 detection

There were significant ($p < 0.001$) differences between the Ubudehe socioeconomic categories, with 17.81% (13/73) children in category 1 (very poor) being malnourished, 42.86%) in category 2 (poor), and 100% in category 3 (medium) (Figure 3). Similarly, detection of AFM1 in urine was significantly associated with signs of malnutrition ($p=0.002$). Forty seven percent (47.10%; 65/138) of the children with detected AFM1 were either stunted, underweight or wasted compared to 23.94% of the 71 that had no detected AFM1 in urine (Figure 4).

In terms of predicting malnutrition, a multivariable model found that children in Ubudehe category 2 had significantly higher odds (OR: 3.304, p-value: 0.001) of being malnourished compared to those in Ubudehe 1. Additionally, increasing levels of AFM1 in the urine significantly increased the odds of malnutrition (OR: 1.264, P-value: 0.004) (Table 4). Similar results were found when detection of AFM1 was handled as a binary variable (Table 5).

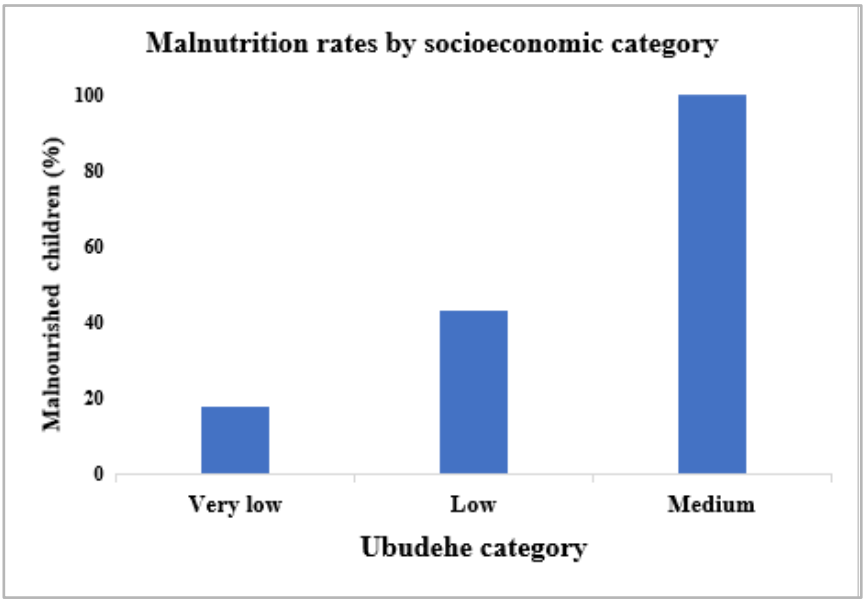

Figure 3: Proportion of malnourished children across Ubudehe categories ($p < 0.001$)

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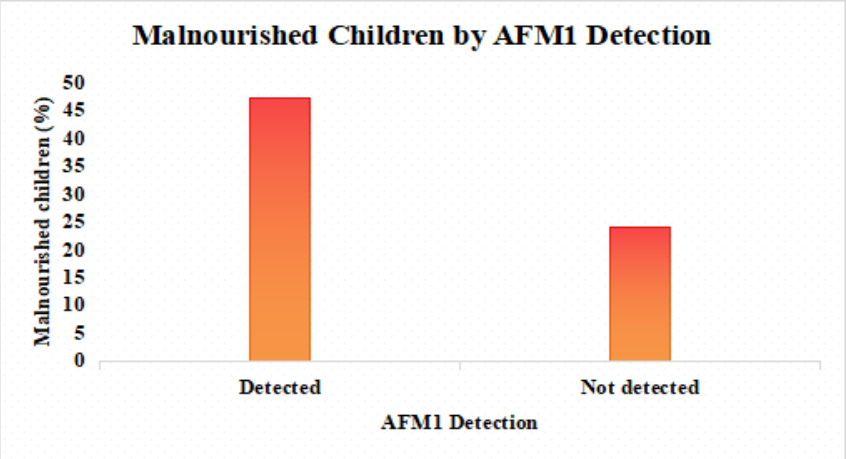

Figure 4: Proportion of malnourished children by AFM1 detection in urine (p=0.002)

Children in categories 1 and 2, representing the very poor and poor, both received the same level of government nutritional and health support, aimed either at preventing malnutrition or aiding recovery for those already affected. However, the malnutrition rate in category 2 (42.86%) is notably higher than in category 1 (17.81%). Despite similar levels of support, children in category 2 may face unique challenges that impact the effectiveness of these interventions. In contrast, category 3, the medium socioeconomic group, shows a 100% malnutrition rate and only receives support for recovery once children are already malnourished. This result was anticipated, as children in category 3 typically visit health facilities when sick, unlike categories 1 and 2, where support is also preventive. This finding underscored the critical role of preventive nutritional interventions, though it also revealed that, even with support, some children continue to experience malnutrition. This suggested that additional factors, whether environmental, socioeconomic, or linked to individual health conditions, may influence malnutrition risk, emphasizing the need for further research to identify and address these underlying determinants.

Current study identified a significant association between detectable AFM1 levels in urine and children's socioeconomic status as defined by Ubudehe categories (Table 5). The significant association between AFM1 detection and malnutrition indicates that aflatoxin exposure is a crucial factor contributing to nutritional status, especially among children in socioeconomic category 2. The multivariable model further confirms that children in category 2 have significantly higher odds of being malnourished compared to those in category 1. Additionally, increased AFM1 levels in urine are associated with a greater likelihood of malnutrition (Table 4). However, it is important to note that the association may be influenced by the sample size distribution, with category 2 having the majority of participants. This larger sample size in category 2 may have amplified the observed association between

socioeconomic status, AFM1 exposure, and malnutrition. A different study discovered that children from poor socioeconomic backgrounds are at a greater risk of exposure to AFs [30]. Furthermore, another study found higher AF exposure levels in households engaged in subsistence farming because they tend to store food for extended periods. This practice, however, contributes to higher AF exposure [31].

The study employed a well-structured case-control design, a robust method in epidemiological research, and included children from multiple health centers, which enhances the representativeness and potential applicability of the findings. The selection of study sites was based on convenience, which may have introduced some bias; however, including diverse geographic areas helped to improve the contextual relevance of the results. Exposure to aflatoxin was assessed using the ELISA method, a sensitive and specific assay for detecting AFM1 in urine, thereby strengthening the reliability of exposure measurement. While the use of random urine samples was practical in field conditions, it may not fully reflect peak toxin levels as early morning urine would. The study considered several background characteristics but did not capture all possible confounders, such as dietary diversity or household food security, which may have influenced the observed relationship between aflatoxin exposure and nutritional status.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

In conclusion, the present study revealed a high prevalence of AFM1 in the urine of children aged 6 to 59 months receiving supplementary foods from health centers in Rwanda, with significantly higher detection rates among malnourished children compared to their healthy counterparts. Although the mean AFM1 concentration was not statistically different between the groups, both case and control groups showed AFM1 levels far exceeding expected values, suggesting additional sources of aflatoxin exposure beyond the standardized health center formulations. Notably, children residing in Rango had significantly higher odds of AFM1 detection, indicating location-specific risk likely related to environmental and market-based factors. Furthermore, children in Ubudehe category 2 (poor) and those with higher urinary AFM1 levels were significantly more likely to be malnourished, underscoring a complex interaction between socioeconomic status, dietary exposure, and nutritional outcomes. Based on these findings, we recommend strengthening aflatoxin control efforts across the entire food chain, particularly focusing on locally sourced ingredients and market surveillance. Public health interventions should prioritize aflatoxin risk communication and training at the community level, and explore safer, quality-assured alternatives for supplementary foods. Additionally, targeted support for vulnerable socioeconomic groups and location-specific

monitoring could play a critical role in reducing aflatoxin exposure and improving child nutrition outcomes in Rwanda.

ACKNOWLEDGEMENTS

The authors acknowledge the mycotoxin laboratory/University of Rwanda/Nyarugenge campus for hosting the laboratory work. In addition, the authors wish to thank all health center staff, community health workers and parents of the children for their participation and also Dr Kizito Nishimwe for laboratory assistance.

Competing interests

The authors declare that the research was conducted without any commercial or financial relationships that might be construed as conflicts of interest.

Author Contributions

MM conceptualized and conducted the study under JL, MU and CM guidance and supervision. MM wrote the first draft of the manuscript under JL, FM, AM, and IH guidance. All authors read and approved the manuscript.

Funding

This study was supported by the Swedish International Development Cooperation Agency (Sida) through the Organization for Women in Science for the Developing World (OWSD), One Health Research, Education, and Outreach Center in Africa (OHRECA) and the donors of the ILRI/CGIAR system.

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SCHOLARLY, PEER REVIEWED
Volume 25 No. 6
July 2025
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Table 1: Socio-demographic characteristics of study participants

Variable	cases (n=70)	controls (n=146)	Total sample(n=216)
Age months			
Mean (SD) (Range)	24.64 (13.40) (9-58)	23.34 (11.70) (7-55)	23.99 (12.55) (7-58)
Gender n (%)			
Male	34 (48.57)	81(55.48)	115(53.24)
Female	36(51.43)	65(44.52)	101(46.76)
Ubudehe category n (%)			
1	13 (18.57)	60 (41.10)	73 (33.80)
2	33 (47.14)	86 (58.90)	119 (55.09)
3	24 (34.29)	0 (0.00)	24 (11.11)
Nutritional status n (%)			
Normal	0 (0.00)	128 (87.67)	128 (59.25)
Underweight	47 (67.14)	0 (0.00)	47 (21.75)
Stunted	4 (5.71)	18 (12.32)	22 (10.18)
Wasted	19 (27.14)	0 (0.00)	19 (8.79)
Study sites n (%)			
Rango	19 (27.14)	34 (23.28.)	53 (24.53)
Kivumu	17 (24.28)	32 (22.91)	49 (22.68)
Bigogwe	14 (20)	31 (21.23)	45 (20.83)
Ruhango	11 (15.71)	31 (21.23)	42 (19.44)
Kora	9 (12.85)	18 (12.32)	27 (12.5)

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Table 2: AFM1 detection in collected samples

	Sampled (n)	Detected n (%)	Mean AFM1±SD ng/ml	Median (Range) ng/ml
Cases	68	54 (79.41)	1.24 ± 1.50	1.17 (0.03 – 9.9)
controls	141	84 (59.57)	1.03±3.11	0.28 (0.01 - 28.79)
Total	**209**	**138 (66.03)**	**1.10± 2.69**	**(0.01 - 28.79)**

Table 3: Association between AFM1 detection and residence location of children

AFM1	Odds ratio	Std. Err.	95 Conf. Interval		p-value
Site			**Low**	**High**	
Bigogwe	Reference				
Kivumu	2.307692	1.022616	0.9682372	5.500144	0.059
Kora	1.666667	0.9218827	0.5636688	4.928032	0.356
Rango	2.564102	1.128175	1.082472	6.073711	0.032
Ruhango	1.111111	0.4829039	0.4740387	2.604361	0.808
Constant	1.2	0.363310	0.6629292	2.172178	0.547

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Table 4: Association between AFM1 levels, Ubudehe category 1 & 2, and malnutrition in children

	Odds Ratio	Std. Err.	95 Conf. Interval		p-value
log AFM1	1.264	0.104	1.076	1.486	0.004
Ubudehe* 2	3.304	0.113	0.146	0.627	0.001
Ubudehe 1	Reference				
Constant	0.954	0.219	0.607	1.498	0.838

*Ubudehe categories in Rwanda classify households based on economic status from 1 to 4, providing support to children from lowest categories 1 and 2, and to others primarily when sick

Table 5: Association between AFM1 detection, Ubudehe category 1 & 2, and odds of malnutrition

	Odds Ratio	Std. Err.	95 Conf. Interval		p-value
Detected AFM1	2.854676	1.067812	1.371384	5.942302	0.005
Ubudehe 2	3.297777	1.223365	1.593874	6.823206	0.001
Ubudehe 1	Reference				
Constant	0.1015489	0.0439591	0.0434712	0.2372185	0.000

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