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Research Article

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Abstract

Aflatoxins M1 contamination of milk affect the general population, and with particular attention to children who frequently consume milk as part of complementary food. This study determined AFM1 contamination of cow's milk and estimated the risk of exposure to these toxins through consumption of cow's milk among children (6 to 36 month) in Magadu ward of Morogoro region in Tanzania.

A total of 165 mother-baby pairs were recruited and interviewed on child feeding practices with focus to feeding of cow's milk in the past 24-hours. Alongside interview, 100 raw cows' milk samples were collected from subsampled respondent households and were analyzed for AFM1 using Enzyme Linked Immunosorbent Assay (ELISA).

The results showed that, about 35% of the surveyed children consumed cows' milk in form of plain milk, incorporated in porridge and/or tea. Amount consumed varied from 62.5-500 ml with median of 125 (125, 250) ml at a frequency of 1 to 2 times a day. All raw cows' milk (100%) samples (n=100) were found contaminated with AFM1 at concentration ranging from 0.052 to 9.310 µg/L, median 2.076 µg/L (1.27, 2.48). All samples were contaminated by AFM1 at levels above the limits of 0.05 µg/L of raw milk set by Tanzania Bureau of Standard and the European Union, while 97% exceeded 0.5 µg/L set by the US Food and Drug Authority. Exposure to AFM1 due to consumption of cow's milk ranged from 0.0024- 0.077 µg/kg bw per day with a median of 0.019 (0.0016, 0.026) µg/kg bw per day, while the Margin of Exposure (MOE) ranged from 5.19- 166.76, median 20.68 (15.33, 25.40) implying high risk of public health concern. This study recommends that, advocacy for consumption of cows' milk to combat undernutrition in children should consider a holistic approach that takes into account the safety aspect of the milk.

Introduction

Aflatoxins (AFs) are the most dangerous type of mycotoxins of public health concern, which affect large number of people worldwide (Bokhari et al. 2017). They are produced as secondary metabolites of fungi *Aspergillus flavus* and *Aspergillus parasiticus* that contaminate a wide range of agricultural products, including maize, groundnuts, wheat, rice, millet, sorghum, beans including soya beans, peanuts, and spices (Magembe et al. 2016; Obade et al. 2015); causing an economic burden of about 25% of food crops loss each year (WHO 2018; Roshdy et al. 2020). The problem is especially pronounced in countries with weather conditions of temperature and humidity which favor growth of fungi, both in field and storage (Roshdy et al. 2020).

AFs exist in four main forms: aflatoxins B1 (AFB1), aflatoxins B2 (AFB2), aflatoxins G1 (AFG1), and aflatoxins G2 (AFG2). Of the forms, AFB1 is the most prevalent and poisonous, and is classified as a group I human carcinogen by the International Agency for Research on Cancer (IARC 1993, 2012). In addition to causing cancer, AFs are known to cause kidney failures, immunosuppression, stunted growth in children and low birth weight in infants whose mother were exposed to these toxins (Obade et al. 2015). Turner et al. (2007) reported that, AFs can cross placental barrier causing *in utero* exposure. Immunosuppression can further cause susceptibility to infections and thus affect the overall child health (Gong et al. 2016).

Consumption of food and feeds contaminated with AFs results to human and animal respectively, exposure to these toxins (Kumar et al. 2021). When consumed by humans or animals, AFB1 is metabolized in the liver to form numerous metabolites including the highly toxic aflatoxin M1 (AFM1); which is also classified as a human carcinogen (IARC 2012). Consumption of AFs' contaminated crops is the main route of dietary exposure to these toxins among human populations (Boni et al. 2021; Magembe et al. 2016). Obade et al. (2015) reported that, about 4.5 million people in developing countries, including Tanzania have been chronically exposed to AFs through food consumption. Similarly, consumption of cow's or breast milk from an animal or human previously exposed to AFB1 can cause exposure to AFM1 (Anthony et al. 2016; Akeberegn et al. 2018). Studies reported prevalence of AFs contamination in cows feed (65%, n = 20) in Singida region (Mohamed et al. 2016) and (up to 88%, n = 80) in selected locations of Northern, Central and Lake zones (Kitigwa et al. 2022) of Tanzania. In the same studies, prevalence of AFM1 in cow's milk was 84% (n = 37) in Singida and 30% (n = 141) in the selected locations of the three zones. Another study reported AFs contamination of 68% of poultry feeds from Morogoro region in Tanzania (Alfred and Mosha 2013). Contamination of animal feeds with AFs at levels higher than the acceptable limits of 10 µg /kg affects animal products such as milk and meat as suggested by (Obade et al. 2015; Kang'ethe and Lang'a 2009; Mohammed et al. 2016; EC 2006). As for Tanzania limits set by TZS 397: 2017, acceptable limits of animal feeds are 20 µg /kg.

Milk is one among nutritious food, thus, contamination of milk poses a threat to food safety and nutrition, especially in children who are the main consumers and one of the vulnerable subgroups in any population (Iqbal et al. 2015; Sumon et al. 2021). Even when milk is heated, AFM1 remains resistant to the heat, indicating the potential toxicity to human (Sumon et al. 2021). Between 2011 and 2018, milk production in Tanzania increased from 1.85 to 2.09 billion liters with its per capita consumption reaching 40 liters per annum (Lunogelo et al. 2020). Parallel to this and with an effort to combat undernutrition in children, there has been increasing advocacy on milk feeding to children, however there are no mechanisms to ensure safety of the milk (Galiè et al. 2021). Of a particular concern cow's milk is commonly used as complementary food among infant and young children, as a results, children are likely to be exposed to AFM1 as early as during the developmental window of the first 1000 days of life. With the increasing advocacy on milk feeding to children in conjunction with limited safety monitoring systems, studies to assess safety of milk are important to provide basis for further research, inform regulation systems and investors. Therefore, this study intended to determine AFM1 contamination of cow's milk and estimate the risk of exposure to these toxins among children through consumption of cow's milk.

Methods

Study Area and Design

The research was carried out in Morogoro Municipal in Morogoro region. The Municipal has high milk production and a good number of cattle keepers. In 2002, out of 762,952 labor force, 24,821 were classified as farmers while 889 were the cattle keepers (Mlozi et al. 2015); and in 2006, cattle population in Morogoro region was reported to be 4,170 (URT 2007). Cattle keepers in Morogoro Municipal practice zero grazing

mainly due to limited open pasture and fear of disease transmission in congested animal grazing. These cattle are mostly fed with hay and supplemented with cotton seed cake, maize bran and sunflower cake which are believed to increase milk production. Zero grazing and such feeding practices have been associated with AFs exposure in animals (Kitigwa et al. 2022), justifying the relevance of the area for this particular study.

In this study, we used a cross sectional study design to understand child feeding practices, consumption of cow's milk and collected milk samples for AFM1 analysis.

Recruitment of participants and Interviews

Study participants were randomly selected from a list of households with babies between the age of 6 to 36 months provided by the Ward Executive Office of Magadu Ward. The random selection was made using ENA for smart survey software to avoid bias. A sample size of 165 mother-child pairs was estimated using Cochran's formula (Cochran 1977) and recruited. Mothers were interviewed on feeding practices and feeding of cow's milk in the past 24-hours using a structured 24-hour recall adopted from the Tanzania National Nutrition Survey (TNNS 2018). Mothers were asked to recall all food items fed to the index child in the past 24 hours. Where plain cow's milk was fed, mothers were asked to estimate amount consumed by a child on their usual huosehold cup using milk or water. The amount were then transferred to a measuring cup and recorded. In addition, all ingredients used in preparation of mult-ingredient foods were itemized. Where cow's milk was listed, mothers were asked to estimate the amount used. Quantinties of cow's milk consumed plain or as an ingredient in other foods such as porridge or tea were summated to obtain cummulative milk intake within 24 hours. These amounts were further used to estimate exposure to AFM1 among children. The questionnaire was pre-tested to ascertain validity of the questions, using preliminary survey with 10 mother-child pairs who were residing at Kihonda ward in Morogoro region. These respondents were not part of the study but their characteristics matches the targeted sample population.

Collection of Cows' milk samples

In addition to the interviews, samples of cows' milk were collected from a subsample of 100 households. This subsample was obtained by a systematic sampling of every other household interviewed. An additional buffer of 20% to cover for households that might have no milk samples was considered. In total, 100 samples of raw cows' milk were aseptically collected and kept in a cool box at about 4 ^{o}C. At collection, milk samples were ascertained for water adulteration using AL-1272 Lactometer (India). Samples were transported within 6 hours to a recipient cold storage at -20^{o}C. After the entire sample collection, samples were transported on a portable car freezer at -20^{o}C to the laboratory at Nelson Mandela African Institution of Science and Technology (NM-AIST) and kept at -40°C prior to analysis of AFM1.

Analysis of AFM1 in cows' milk samples

Aflatoxins M1 in raw milk samples was analysed using competitive Enzyme Linked Immunosorbent Assay (ELISA) using the Helica Milk AFM1 ELISA kits Lot No. 070122 (Helica, USA) according to the manufacture's specification. The kits were stored at 2–8°C and were kept at room temperature for 30 minutes before use.

Samples were brought to room temperature prior to analysis. Then diluted with distilled water at a ratio of sample: water; 1:9 v/v. Diluted samples were vortexed on M1671033 ThermoScientific (China) vortex for 60 minutes and then centrifuged in a 5810 Hamburg (Germany) Centrifuge, at 2,000 g for 5 minutes. The supernatant fat free layer was pipetted into clean Eppendorf tubes. Then 200 µL of each sample or standards (0.0, 5.0, 10.0, 25.0, 50.0 and 100.0 pg/mL) were pipetted into the wells of AFM1-antibody-coated microtiter plate supplied with the kits. Each sample or standard were pipetted into duplicate wells on a specified order (Maleki et al. 2015). The plate was covered by the sealer and incubated at room temperature in dark for 2 hours. The plate was washed 3 times using washing buffer composed of Phosphate Buffered Saline (PBS) and 0.05% Tween^{20}* in 1 liter of distilled water. Then, the plate was tapped on absorbent paper towel to remove excess liquid. Then 100 µL of AFM1 Horse Radish Peroxidase (HRP) conjugate was added into both the standards and samples wells. The plate was covered by a sealer and incubated at room temperature for 15 minutes. Afterwards, the plate was washed 3 times using the wash buffer in order to remove the unbound conjugate. The plate was tapped on absorbent paper towel to remove excess liquid. Then 100 µL of Tetramethylbenzidine (TMB) substrate was added into both the standards and samples wells. The plate was covered by a sealer and incubated in dark at room temperature for 15 minutes (blue color developed). The reaction was stopped by adding 100 µL of stop solution into both the standard and sample wells. The color changed from blue to yellow. The absorbance of the yellow color was read at 450 nm using a BioTek ELx 808 Microplate reader, (USA) with the help of an installed software Gen 5 (v.2.09). The intensity of the color is inversely proportional to AFM1 concentration in the samples. A standard curve was used to calculate the concentration of AFM1 in the samples. Samples measuring above the standard curve were repeated following additional dilution of up to 25, 50 or 100 times. Samples were analyzed in duplicate and those with a coefficient of variation (CV) of greater than 7% were re-measured.

Method performance was tested using recovery from spiked milk samples at 0.005, 0.05 and 0.1 µg/L, precision as relative standard deviation (RSD%) and limit of detection (LoD) calculated as the sum of the mean concentration (n = 8) and a product of 2 times standard deviation of a zero calibrator (Armbruster and Pry, 2008).

Estimation of dietary exposure to AFM1

Dietary exposure of an indiviadual child to AFM1 through consumption of cow's milk was estimated using a deterministic approach described by JECFA (2010), and was calcuated by multiplying the consumption of cow's milk (L) by levels of AFM1 contamination (µg/L), divided by body weight (kg) (Eq. 1) and expressed in µg per kg of body weight per day (µg/kg/bw/day).

Estimated Daily Intake (EDI) of AFM1 due to consumption of cow's milk

= daily intake of cow's milk (L) *AFM1 contamination levels (µg/L) **/** individual bodyweight (kg) [1]

Characterization of a risk of exposure to AFM1

According to EFSA (2020) margin of exposure (MOE) is used to assess possible risks associated to a variety of genotoxic or carcinogenic substances. The MOE was calculated by dividing the established

Benchmark dose lower limit (BMDL) for aflatoxin B1 which is 0.4 µg/kg bw/day by toxin exposure levels (EDI) (Eq. 2), taking into account potency factor of 0.1.

$$MOE = BMDL_{10} / EDI \quad [2]$$

Where;

$BMDL_{10}$ is the Benchmark Dose Lower Limit for 10% increased cancer risk

EDI is the Estimated Daily Intakes (Dietary exposure) of AFM1 due to consumption of cow's milk, as calculated in Eq. 1.

Based on estimation of BMDL from animal data because it was inappropriate to use human data, EFSA (2020) concluded that AFM1 is capable of inducing liver cancer with a potency factor of one-tenth that of AFB1 (where a MOE of 100,000 which is equivalent to an exposure of 0.000004 µg/kg bw/day (0.4 µg/kg bw/day divided by 100,000) marked the cut-off point of low public health concern. This implies that MOE value less than 100,000 explains high risk to public health.

Ethical clearance

This study obtained ethical clearance (IHI/IRB/No: 09-2022) from Ifakara Health Institute Ethical Review Board (IHI-ERB). Research participants were informed of the aim of the study prior to interview, and were asked to consent. Those who consented to participate, provided verbal agreement and signed consent forms. Confidentiality of the information were maintained throughout the study.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 26 was used for data analysis. Descriptive statistics were used in reporting demographic information of the participants and complementary feeding practices including consumption of cows' milk.

Results

Social Demographic Characteristics of the Study Population

A total of 165 mother-child pairs were recruited. Nearly a half (49.7%) of the study participants were residing in urban side (Falkland and Kididimo), and 50.3% in the peri-urban (Konga and Magadu) side of Magadu ward. The mean age for mothers was 28.39 ± 6.0 years, and majority were within the age range of 20 to 34 years (78%). Majority of mothers were married (70.7%) and living in households with 2 to 4 members (69.1%). Almost half (49.9%) of mothers had primary school education. Business was the dominant occupation accounting for 67.3% of the surveyed mothers. The mean age of surveyed children was 21.3 ± 7.9 months. The mean weight of all children was 12.81 ± 2.9 kg (n = 165) which was further categorized into three, Table 1.

Table 1
Social Demographic Characteristics of Study Population

Variables	Urban (N = 82)		Peri-urban (N = 83)		Pooled (N = 165)	
	Frequency (n)	Percentages (%)	Frequency (n)	Percentages (%)	Frequency (n)	Percentages (%)
Age of the index child (months)						
6 to 11	10	12.2	15	18.1	25	15.2
12 to 23	38	46.3	29	34.9	67	40.6
24 to 36	34	41.5	39	47	73	44.2
Total	**82**	**100**	**83**	**100**	**165**	**100**
Sex of index child						
Boy	36	43.9	41	49.4	77	46.7
Girl	46	56.1	42	50.6	88	53.3
Total	**82**	**100**	**83**	**100**	**165**	**100**
Household size						
2 to 4 household members	53	64.6	61	73.5	114	69.1
≥ 4 household members	29	35.4	22	26.5	51	30.9
Total	**82**	**100**	**83**	**100**	**165**	**100**
Age of mothers (years)						
13 to 19	3	3.7	-	-	3	1.8
20 to 34	64	78	71	85.5	135	81.8
35 to 49	15	18.3	12	14.5	27	16.4
Total	**82**	**100**	**83**	**100**	**165**	**100**
Education level of mothers						
Above secondary school	20	24.4	14	16.9	34	20.6
No formal education	2	2.4	1	1.2	3	1.8

Variables	Urban (N = 82)		Peri-urban (N = 83)		Pooled (N = 165)	
	Frequency (n)	Percentages (%)	Frequency (n)	Percentages (%)	Frequency (n)	Percentages (%)
Age of the index child (months)						
Primary school education	18	22	34	41	52	31.5
Secondary school A-level	4	4.9	7	8.4	11	6.7
Secondary school O-level	38	46.3	27	32.5	65	39.4
Total	**82**	**100**	**83**	**100**	**165**	**100**
Marital status of mothers						
Divorced	2	2.4	1	1.2	3	1.8
Married	58	70.7	71	85.5	129	78.2
Single	20	24.5	11	13.3	31	18.8
Widow	2	2.4			2	1.2
Total	**82**	**100**	**83**	**100**	**165**	**100**
Occupation of mothers						
Business	49	59.8	62	74.7	111	67.3
Employed in formal sector	7	8.5	3	3.6	3	1.8
Employed in informal sector	26	31.7	1	1.2	8	4.8
Farmer			17	20.5	43	26.1
Total	**82**	**100**	**83**	**100**	**165**	**100**
Household head						
Husband/male partner	58	70.7	71	85.5	129	78.2
Mother (respondent)	24	29.3	11	13.3	35	21.2
Mother of the Respondent			1	1.2	1	0.6
Total	**82**	**100**	**83**	**100**	**165**	**100**

Variables	Urban (N = 82)		Peri-urban (N = 83)		Pooled (N = 165)	
	Frequency (n)	Percentages (%)	Frequency (n)	Percentages (%)	Frequency (n)	Percentages (%)
Age of the index child (months)						
Relationship with the household head						
Husband/ male partner	58	70.7	71	85.5	129	78.2
Mother of the Respondent			1	1.2	1	0.6
Self	24	29.3	11	13.3	35	21.2
Total	**82**	**100**	**83**	**100**	**165**	**100**
Participants location						
Falk land	38	46.3	-	-	38	23
Kididimo	44	53.7	-	-	44	26.7
Konga	-	-	43	51.8	43	26.1
Magadu	-	-	40	48.2	40	24.2
Total	**82**	**100**	**83**	**100**	**165**	**100**

Table 1

Complementary Feeding and Consumption of Cows' Milk

About thirty five percent (35.2%) of the surveyed children were fed cow's milk as part of complementary feeding 24 hours before the survey. Milk was consumed plain, incorporated in porridge and/or tea, at a frequency of 1 to 2 times a day. In total, milk consumption ranged from 62.5 to 500 ml per day with a median of 125 (125, 250) ml. Other foods consumed by children 24 hours before the survey were predominantly cereal-based foods (99%), followed by legumes (69%), leafy vegetables (53%) and fruits and vegetables (52%). Other food groups were consumed by less than half of the studied children, Fig. 1.

FIGURE 1

Occurance of Aflatoxin M1 in Cows' Milk

Aflatoxin M1 was detected in all samples (100%, n = 100) of raw cows' milk, at a range of 0.052 to 9.310 µg/L (LoD 0.002 µg/L). This contamination exceeded the limit of 0.05 µg/L set by European Union Commission Regulation (EU) No 165/2010 of 26 February 2010 (EC 2006), and Tanzania Bureau of Standards (TBS) (Mohammed et al. 2016) for AFM1 in raw cow milk. However, 97% of the samples had AFM1 that exceed the limit of 0.5 µg/L set by the United States Food and Drug Administration (FDA 2005)

for AFM1 in raw cow milk. When compared the peri-urban and urban samples, the median value of 2.218 (1.23, 2.89) µg/L for AFM1 in the peri-urban was slightly higher than that of the urban (2.046 (1.36, 2.37) µg/L) (Table 2).

Table 2
Occurrence of AFM1 in cow's milk

AFM1 (µg/L)		Location		Total
		Urban (n = 50)	Peri-Urban (n = 50)	(n = 100)
Median		2.046	2.218	2.076
IQR		1.026	1.705	1.230
Maximum		4.538	9.310	9.310
Minimum		0.052	0.710	0.052
Positive samples		50(100)	50(100)	100(100)
Negative samples		0(0.00)	0(0.00)	0(0.00)
EU/ Tanzania limits	AFM1 ≥ 0.05 µg/L	50(100)	50(100)	100(100)
	AFM1 ≤ 0.05 µg/L	0(0.00)	0(0.00)	0(0.00)
FDA limits	AFM1 ≥ 0.5µg/L	47(94)	50(100)	97(97)
	AFM1 ≤ 0.5µg/L	3(6)	0(0.00)	3(3)

Method performance test yield an average recovery of 91.3%, 100.9% and 96.7% for 0.005, 0.05 and 0.1 µg/L respectively, relative standard deviation (RSD%) of 1.3 and a limit of detection (LoD) of 0.0026 µg/L.

Table 2

Dietary Exposure and risk characterization to AFM1

The dietary exposure to AFM1 estimated in the current study ranged from 0.0024–0.077 µg/kg bw/day with a median of 0.019 (0.0016, 0.026) µg/kg bw/day. EFSA (2020) documented dietary exposure of > 0.000004 µg/kg bw/day entails a public health concern. In this study the highest EDIs of AFM1 0.0024–0.077 µg/kg bw/day were found for the consumption of cow's milk by the surveyed children where, any values of EDI found above 0.000004 µg/kg bw/day entails a public health concern. Furthermore, the estimated Margin of Exposure (MoE) ranged from 5.19- 166.76 with median of 20.68 (15.33, 25.40) which is < 100,000 and implying high risk of a negative impact on public health.

Discussion

This study surveyed 165 mother-baby dyads to understand feeding practices with particular interest in feeding of cows' milk in the past 24-hours, it further assessed AFM1 contamination of cows' milk and the risk of exposure to AFM1 due to consumption of cows milk among infants and young children (IYC) aged 6–36 months.

The surveyed children were reported to consume cow's milk in the previous 24 hours where, children between one and two years consumed more cows' milk (mean value of 171.88 ± 88.87 ml) than other age groups because at this age, breastfeeding is relatively reduced and gradually replaced by complementary feeding that include cows' milk. Children are also feeding on semi solid foods that in most cases are constituted by cows' milk. Reconstitution of solid and semi-solid foods with cows' milk was used to soothen the food for good taste, and also to help with milk intake in children who resist plain milk. Children between the age of 6–11 months were consuming less cows' milk because they are more breastfed, while those between 24–36 months consumed more of other foods including family meals than cows' milk. In Tanzania milk intake is reported at about 45 litres/ person per year (Katjiuongua and Nelgen 2014). Other study findings done in Tanzania and Kenya estimated that around 50–60% of people in a particular catchment area consume milk (Galiè et al. 2021) which include young children. The study further reported that, milk is consumed plain, mixed in porridge (a term describing a legume and grain-based dish made from a wide variety of ingredients), and tea.

All milk samples collected from household were found un-adultered. This was possible because the participants were informed of the collection of milk samples for educational purposes by the ward executive officer prior to the survey. Adultaration measure explains the quality of milk and that un-adultered milk had no interfere to the measurement of AFM1 concentration. The lactomiter reading was within the range of 1.026–1.032 g/ml (Small Holder Dairy Project (SDP 2004).

Aflatoxin M1 was detected in all raw cows' milk (n = 100) samples at levels exceeding the limit of 0.05 µg/L set by European Union Commission Regulation (EU) No 165/2010 of 26 February 2010 (EC 2006), and the Tanzania Bureau of Standards (TBS) (Mohammed et al. 2016) for AFM1 in raw cow milk. In 2017, Gonçalves et al. stated that, AFM1 excretion in raw milk is the major consequence of exposing cattle to aflatoxin B1. Approximately, 1–6% of AFB1 can be metabolized into AFM1 following ingestion of AFB1 contaminated feeds (Turna and Wu 2021; Tarannum et al. 2020). Higher AFM1 in cows' milk is prevalently reported in zero-grazing than in free-range. Zero-grazed dairy cattle are more likely to feed on contaminated feeds such as maize hay, bran, cotton and sunflower seed cakes that are frequently reported to contain high levels of AFs (Mohamed et al. 2016; Kitigwa et al. 2022; Kang'ethe and Lang'a 2009). This is because these feeds emanate mainly from reject and poor-quality grains, which are inadequately stored for a long time, thus provide conducive environment for mycotoxin production (Kang'ethe and Lang'a 2009).

While cereal bran used to feed dairy cattle is contaminated with aflatoxin B1, the milk obtained from the same cattle is found contaminated with aflatoxin M1 (Magoha et al. 2014). Two studies conducted in different locations in Tanzania by Mohamed et al. (2016) and Kitigwa et al. (2022) revealed AFM1 contamination in raw milk ranging from 0.026 to 2.01 µg/L (n = 37) and 0.03 to 43.98 µg/L (n = 141) respectively. A study conducted in Bangladesh which employed ELISA technique to assesses AFM1 in raw

(n = 50) and processed (n = 50) milk samples reported that, over 53% of the total sample (n = 100) tested positive for AFM1. Of the contaminated, 70% were raw milk samples at a range of 22.79-1489.28 ng/kg, mean 699.07 ng/kg (Tarannum et al. 2020). A study by Asghar et al. (2018) reported 91.7% contamination of AFM1 (ranging from 20 − 3090 ng /L with mean level of 317 ± 16.6 ng /L) in fresh milk (n = 143) out of 156 samples collected. Another study by Sumon et al. (2021) reported detection of AFM1 in 71.4% of raw milk samples (mean 41.1 ng/L, range 5.0–198.7 ng/L). In 2014, Magoha et al. reported that, all samples of human breast milk tested positive for AFM1 where more than 70% of the samples exceeded limit of 0.05 ng/L set by EU for milk and dairy products. This implies that, children are double exposed to AFM1 through consumption of both animal and human breast milk. In addition to consuming susceptible cows' milk, children were also fed with mainly cereal-based meals that have been frequently reported to contain aflatoxins (IRC 2015; Mollay et al. 2021). This implies double burden of exposure to both aflatoxins B and G, as well as AFM1.

Dietary exposure to AFM1 (0.0024–0.077 µg/kg bw/day) due to consumption of cows' milk among children exceeded the limit of not more than 0.000004 µg/kg bw/day reported in EFSA (2020), implying a significant concern to public health. Similarly, Ahlberg et al. (2018) reported mean AFM1 exposure of 0.8 ng/kg bw/day for all recruited children (< 3 years of age) in Kenya. Findings of Kortei et al. (2022) have reported the Estimated Daily Intakes (EDI) of AFM1 in raw cow milk samples from Greater Accra Region were 0.61, 0.36 and 0.14 ng/kg bw/day for infants, toddlers and children respectively.

Margin of Exposure (MOE) was way below 100,000 implying that, AFM1 intake through consumption of cows' milk can cause significant health problem (Kortei et al. 2022, EFSA 2020). A study by Kortei et al. (2022) reported Margin of Exposure (MoE) values ranging from 197.04 to 655.74 for infants (0–11 months), from 333.33 to 1111.11 for toddlers (12–35 months) and from 888.89 to 2857.14 for children (36 months to 10 years) in four regions of Ghana. Contrariwise a study by Milićević et al. (2021) reported higher values of MoE signifying no public health concern due to AFM1 among toddlers (1–3 years) and children (3–9 years) due to consumption of infant formula, fermented milk products, butter, milk beverages, sour cream, cheese, pasteurized and UHT milk and whey liquid in Serbia.

Conclusion

The present study reported consumption of cows' milk as part of complementary feeding among children, and the risk of exposure to AFM1. About 36% of children consumed plain and/or milk incorporated in porridge or tea. All cow milk samples collected were contaminated with AFM1 at levels exceeding the limit for AFM1 in raw milk set by Tanzania Bureau of Standard and the European Union. The study also reports high dietary exposure to AFM1 due to consumption of cow's milk and lower values of MOE implying high risk of public health concern. This study recommends that, advocacy for consumption of cows' milk to combat undernutrition in children should consider a holistic approach that takes into account the safety aspect of the milk.

Declarations

Acknowledgements

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Conflict of Interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Akeberegn, D., Alemneh, T. and Zewudie, D. 2018. Effects of Aflatoxin Contamination in Milk: A Review. *Merit Research Journal of Microbiology and Biological Sciences*, *6* (10): *118-128*.
2. Ahlberg, S., Grace, D., Kiarie, G., Kirino, Y., and Lindahl, J. 2018. A risk assessment of Aflatoxin M1 exposure in low and mid-income dairy consumers in Kenya. *Toxins*, *10*(9), 1–15. https://doi.org/10.3390/toxins10090348.
3. Alfred and Mosha. 2013. Surveillance of Aflatoxin B1 Contamination in Chicken Commercial Feeds in Morogoro. Tanzania. *Livestock Research for Rural Development*, *25* (3).
4. Armbruster DA, Pry T. 2008. Limit of blank, limit of detection and limit of quantitation. *Clinical Biochemistry Review*, Suppl 1(Suppl 1):S49-52. PMID: 18852857; PMCID: PMC2556583
5. Asghar, M. A., Ahmed, A., & Asghar, M. A. 2018. Aflatoxin M1 in fresh milk collected from local markets of Karachi, Pakistane. *Food Additives and Contaminants: Part B Surveillance*, *11*(3), 167-174.https://doi.org/10.1080/19393210.2018.1446459.
6. Bokhari, F., Aly, M., Kelany, A. Al and Rabah, S. 2017. Presence of Aflatoxin M1 in Milk Samples Collected from Jeddah, Saudi Arabia. *IOSR Journal of Pharmacy (IOSRPHR)*, *07*(05): 49–52. https://doi.org/10.9790/3013-0705014952.
7. Boni, S. B., Beed, F., Kimanya, M. E., Koyano, E., Mponda, O., Mamiro, D., Kaoneka, B., Bandyopadhyay, R., Korie, S. and Mahuku, G. 2021. Aflatoxin contamination in Tanzania: quantifying the problem in maize and groundnuts from rural households. *World Mycotoxin Journal*, *14* (4): 553-564.
8. Cochran, W. G. 1977. Sampling techniques (3rd ed.). John Wiley & Sons.
9. Lunogelo, H. Makene, F. and Gray, H. 2020. Dairy Processing in Tanzania. Project Brief. www.iiap.info.
10. EC Commission Regulation. 2006. Setting maximum levels for certain contaminants in foodstuffs as regards Aflatoxins.
11. EFSA. 2007. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. The EFSA Journal 446, 1-127. www.efsa.europa.eu.

12. EFSA. 2020. Risk assessment of aflatoxins in food. Scientific opinion. EFSA panel on contaminants in the food chain (CONTAM). *European Food Safety Authority Journal, 18*(3), 6040.
13. FDA. 2005. 527.400 Whole milk, Low fat milk, skim milk-aflatoxin M1 (CPG 7106.10).
14. Galiè, A., Farnworth, C.R., Njiru, N. and Alonso, S. 2021. Intra-Household Handling and Consumption Dynamics of Milk in Peri-Urban Informal Markets in Tanzania and Kenya: A Gender Lens. Sustainability,13 3449.
15. Gonçalves, L., Rosa, A. D., Gonzales, S. L., Manuela, M., Feltes, C., Badiale-furlong, E. and Dors, G. C. 2017. Incidence of Aflatoxin M 1 in fresh milk from small farms. *Food Science and Technology 37*, 11–15.
16. Gong, Y. Y., Watson, S., & Routledge, M. N. 2016. *Aflatoxin Exposure and Associated Human Health Effects a Review of Epidemiological Studies*. *March*. https://doi.org/10.14252/foodsafetyfscj.2015026.
17. Iqbal, S. Z., Jinap, S., Pirouz, A. A. and Faizal, A. R. A. 2015. Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review Trends in Food Science & Technology Aflatoxin M 1 in milk and dairy products, occurrence and recent challenges: A review. *Trends in Food Science & Technology*, *46*(1): 110–119. https://doi.org/10.1016/j.tifs.2015.08.005.
18. IARC. 1993. *Monograph on the evaluation of carcinogenic risks to humans. Some naturally occurring substances: Food items and constituents. Heterocyclic aromatic amines and mycotoxins* (Vol. 56). IARC Press.
19. IARC. 2012. A review of human carcinogens. Part F: Chemical agents and related occupations / IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2009: Lyon, France), ISBN 978 92 832 1323 9.
20. IARC. 2015. Chapter 7: Intervention strategies to reduce human exposure to aflatoxins and fumonisins. Mycotoxin control in low- and middle-income countries. IARC working group report no. 9. Accessed from https://www.ncbi.nlm.nih.gov/books/NBK350558/pdf/Bookshelf_NBK350558.pdf.
21. JECFA. 2010. Evaluation of Certain Contaminants in Food: Seventy-second Report of the Joint FAO/WHO Expert Committee on Food Additives (Technical Report Series No. 959 (pp. 1–105). Food and Agriculture Organization of the United Nations, World Health Organization. https://apps.who.int/iris/handle/10665/44514.
22. Kang'ethe E. K. and Lang'a K. A. 2009. Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya. *African Health Science 9* (4): *218-226*.
23. Katjiuongua, H. and Nelgen, S. Tanzania smallholder dairy value chain development: Situation analysis and trends. 2014. International Livestock Research Institute (ILRI).
24. Kitigwa, S. J., Kimaro, E. G., Nagagi, Y. P., Kussaga, J. B., Suleiman, R. A. and Matemu, A. 2022. Occurrence and associated risk factors of aflatoxin contamination in animal feeds and raw milk from three agroecological zones of Tanzania. *World Mycotoxins Journal 0* (0) 1-6.
25. Kortei, N. K., Annan, T., Kyei-Baffour, V., Essuman, E. K., Boakye, A. A., Tettey, C. O. and Boadi, N. O. 2022. Exposure assessment and cancer risk characterization of aflatoxin M1 (AFM1) through ingestion of

raw cow milk in southern Ghana. *Toxicology Reports 9* (2022) 1189–1197. https://doi.org/10.1016/j.toxrep.2022.05.015.

26. Kumar, A., Pathak, H., Bhadauria, S. and Sudan, J. 2021. Aflatoxin contamination in food crops: causes, detection, and management: a review. *Food Production, Processing and Nutrition*. *3* (17): 1-9. https://doi.org/10.1186/s43014-021-00064-y.
27. Magembe, K. S., Mwatawala, M. W., Mamiro, D. P. and Chingonikaya, E. E. 2017. Assessment of awareness of mycotoxins infections in stored maize (*Zea mays* L.) and groundnut (*Arachis hypogaea* L.) in Kilosa District, Tanzania. *International Journal of Food Contamination 4* (1): 1 – 8.
28. Magoha, H., Kimanya, M., De Meulenaer, B., Roberfroid, D., Lachat, C. and Kolsteren, P. 2014. Association between Aflatoxin M exposure through breast milk and growth impairment in infants from northern Tanzania. *World Mycotoxin Journal*, *7* (3): 277–284. https://doi.org/10.3920/WMJ2014.1705.
29. Magoha, H., De Meulenaer, B., Kimanya, M., Hipolite, D., Lachat, C. and Kolsteren, P. 2014. Fumonisin B1 contamination in breast milk and its exposure in infants under 6 months of age in Rombo, Northern Tanzania. *Food and Chemical Toxicology*, *74*, 112–116. https://doi.org/10.1016/j.fct.2014.09.008.
30. Milićević, D.R., Milešević, J., Gurinović, M., Janković, S., Đinović-Stojanović, J., Zeković, M. and Glibetić, M. 2021. Dietary Exposure and Risk Assessment of Aflatoxin M1 for Children Aged 1 to 9 Years Old in Serbia. Nutrients 2021, 13, 4450. https://doi.org/10.3390/ nu13124450.
31. Mlozi, M. R. S., Mtambo, M. M. A. and Olsen. J. E. 2015. Mindset of urban and peri-urban dairy cattle keepers in Morogoro, Tanga and Temeke Districts, Tanzania. *Livestock Research for Rural Development 27* (2) 2015.
32. Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC) [Tanzania Mainland], Ministry of Health (MoH) [Zanzibar], Tanzania Food and Nutrition Centre (TFNC), National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS) [Zanzibar] and UNICEF. 2018. Tanzania National Nutrition Survey using SMART Methodology (TNNS) 2018. Dar es Salaam, Tanzania: MoHCDGEC, MoH, TFNC, NBS, OCGS, and UNICEF.
33. Mohammed, S., Munissi, J. J. E., & Nyandoro, S. S. 2016. Aflatoxin M1 in raw milk and Aflatoxin B1 in feed from household cows in Singida, Tanzania. *Food Additives and Contaminants: Part B Surveillance*, *9* (2):85–90. https://doi.org/10.1080/19393210.2015.1137361.
34. Mollay, C., Kimanya, M., Kassim, N. and Stoltzfus, R. 2021. Main complementary food ingredients contributing to aflatoxin exposure to infants and young children in Kongwa, Tanzania. *Food Control, 135*; 108709.
35. Obade, M. 2015. Exposure of children 4 to 6 months of age to Aflatoxin in Kisumu County, Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *15*(2): 9949- 9963–9963.
36. Roshdy S. E., Omar, L. M., Sayed, R. H., Hassan, H., Hanafy, M. H. and Soliman, R. 2020. Reduction of milk contamination with Aflatoxin-M1 through vaccination of dairy cattle with Aflatoxin-B1 vaccine. *International Journal of Veterinary Science*, *9* (4):528-533.
37. Small Holder Dairy Project (SDP). 2004. Improve the quality of your milk and please your customers-training guide for small-scale informal milk traders in Kenya.

38. Sumon, A.H.; Islam, F., Mohanto, N.C., Kathak, R.R., Molla, N.H.; Rana, S., Degen, G.H., Ali, N. 2021. The presence of Aflatoxin M_1 in milk and milk products in Bangladesh. *Toxins*, *13* (440). https://doi.org/10.3390/toxins13070440.
39. Tarannum, N., Nipa, M. N., and Parveen, S. D. S. 2020. Aflatoxin M1 detection by ELISA in raw and processed milk in Bangladesh. *Toxicology Reports 7* (2020): 1339–1343. https://doi.org/10.1016/j.toxrep.2020.09.012.
40. Turna, N. S. and Wu, F. 2021. Aflatoxin M1 in milk: A Global Occurrence, Intake and Exposure Assessment. *Trends in Food Science & Technology 110*; 183-192.
41. Turner, P. C., Collinson, A. C., Cheung, Y. B., Gong, Y. Y., Hall, A. J., Prentice, A. M. and Wild, C. P. 2007. Aflatoxin exposure in utero causes growth faltering in Gambian infants. *International Journal of Epidemiology*, *36* (5), 1119–1125. https://doi.org/10.1093/ije/dym122.
42. UNICEF. 2017. First 1000 days the critical window to ensure that children survive and thrive.
43. WHO. 2018. Aflatoxins pose a serious health risk to humans and livestock. Food safety digests**.** Ref no.: WHO/NHM/FOS/RAM/18.1.

Figures

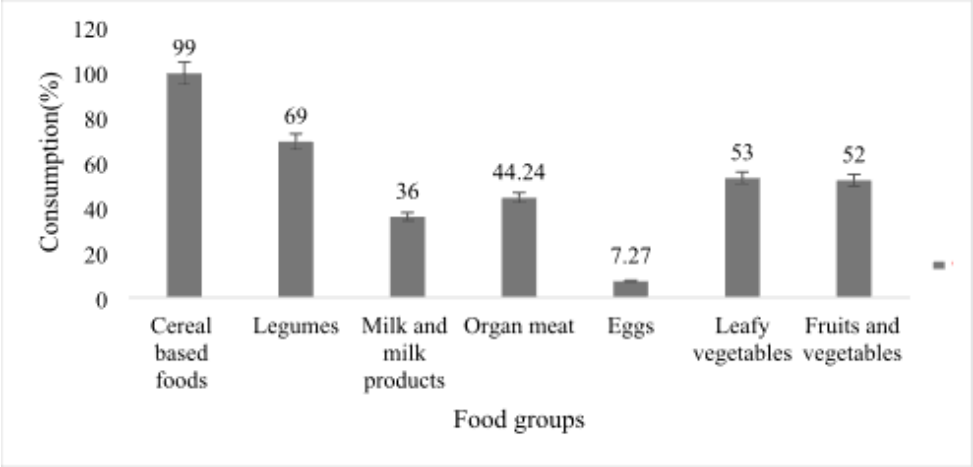

Figure 1

Consumption of milk and other food groups by the study children 24 hours before the survey