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Exposure and Risk Assessment of Aflatoxin M1 in Children in Ethiopia: A Systematic Review, Meta-analysis, and Meta-regression

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Systematic Review

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Abstract

Aflatoxin M1 (AFM1), a byproduct of *Aspergillus flavus* and *A. parasiticus*, can get into milk, which is a healthy food. AFM1 induces a health risk, especially cancer risk. The study aimed to determine the pooled prevalence, mean concentration levels, and health risks associated with AFM1 in cow's milk in Ethiopia. The study followed PRISMA guidelines for executing a systematic review across databases, such as Google Scholar, ScienceDirect, and PubMed. Ultimately, fourteen research articles comprising 17 studies and a total sample size of 2,021 selected according to specific criteria and assessed for bias risk. A meta-analysis performed using STATA version 17. The results indicated that AFM1 was detected in 68.2% of the samples, with a 95% confidence interval between 55.3% and 81.1%. The data showed a significant difference (I^2 = 100%) and very little evidence of publication bias (p-value = 0.1082). The average amount of AFM1 was 0.316 µg/L, with amounts ranging from 0.163 µg/L to 0.469 µg/L. This level is higher than the European Union's limit of 0.05 µg/kg. Children in Ethiopia between the ages of six months and two years ingested 5.62 ng/kg/day of AFM1 through the consumption of cow's milk daily. The health risk assessment showed a significant cancer risk that threatens food safety and public health policies. Therefore, it suggests using mycotoxin binders in animal feed more and making monitoring procedures better.

Introduction

Milk is a full and healthy food that has vitamins, minerals, probiotics, lipids, proteins, and carbohydrates in it. Edwards and Younus (2024) say that raw milk may have healing properties that can help keep allergies and asthma at bay. Nevertheless, biological and chemical hazards can undermine the quality and safety of milk, resulting in foodborne illnesses that adversely impact public health and the economy (Lucey, 2015). Young children, the elderly, and people with weak immune systems are the most likely to get sick from AFM1 toxicity from drinking milk that has AFM1 in it. A study in Iran on AFM1 toxicity revealed that AFM1 is a primary chemical associated with the onset of cancer in children (Mortezazadeh and Gholami-Borujeni, 2023). The liver of cows changes aflatoxin B1 (AFB1) into AFM1 (Zebib et al., 2023), and AFM1 can be found in milk 12 to 24 hours after cows eat AFB1 (Kebede et al., 2024). When dairy cows eat animal feed that has more than 20 parts per billion of AFB1 in it, the levels of AFM1 in milk can go above safe levels (Mtimet et al., 2015). The International Agency for Research on Cancer (IARC) says that AFM1 is a Group I carcinogen, which means that having it in the liver increases the risk of hepatocellular carcinoma (HCC). As a result, HCC is prevalent in developing nations, such as Ethiopia (Liu and Wu, 2010). AFM1 is a big problem for food safety in Ethiopia because the weather can change a lot, which makes it easier for fungi that make mycotoxins to grow and spread. Also, the risk goes up even more because there aren't good rules in place to stop feed contamination. Kids who drink milk that has AFM1 in it could have health problems. It is essential to recognize that heat treatments, including autoclaving, pasteurization, and thermal inactivation, do not eradicate AFM1 (Sharma *et al*., 2019). Consequently, the existence of AFM1 in cow's milk presents both immediate and prolonged public health hazards (Sani and Nikpooyan, 2013).

Ethiopia produces about 960,000 tonnes of milk each year, which means that each person in the country drinks about 17.1 kg of milk (CSA, 1999; CSA, 2017). Nonetheless, numerous studies have documented the prevalence and concentration levels of AFM1 in milk. Studies have shown that the amount of AFM1 in milk can be anywhere from 0.038 µg/L to 0.690 µg/L (Gizachew et al., 2016; Tadesse et al., 2020; Admasu et al., 2021; Wubshet et al., 2022; Zebib et al., 2022). A significant study identified a 93% prevalence of AFM1 in the urine of schoolchildren (Gebreegziabher et al., 2022). There have been many studies on how common AFM1 is in milk and how high its levels are, but there hasn't been one that combines all of the studies to find the average level of AFM1 in cow milk. Zebib et al. (2023) also talked about exposure and health risk assessment of AFM1, but they only looked at a small number of raw milk samples (64) and only adults. This study makes it hard to conclude the health risks of AFM1 in children who drink cow's milk regularly. The Codex Alimentarius Commission and the US Food and Drug Administration set standards for AFM1 in milk at 0.5 µg/L, which is what Ethiopia did. However, this limit is higher than the European Union Commission's standard of 0.05 µg/L. The present study sought to determine the pooled prevalence and estimate the mean concentration levels of AFM1 in cow's milk, as well as to assess the exposure and health risks of AFM1 in children.

Methods

Search strategy

Following PRISMA guidelines, a literature search was performed to investigate the prevalence and concentration levels of AFM1 in milk in Ethiopia (Page *et al*., 2021). The search used the words "Aflatoxin M1 (AFM1)," "Prevalence and Concentration," "Milk," and

"Ethiopia." Research articles published from 2016 to 2024 were sourced from commonly assessed databases, including Science Direct, PubMed, and Google Scholar. The selected papers from each database were imported into Thomson Reuters' EndNote X7.8 software (version X7.8) to find and get rid of duplicates.

Inclusion and Exclusion Criteria

Studies chosen according to specific criteria to obtain the requisite data. The requirements included a full report in English that included a descriptive inquiry (prevalence) and the mean and standard deviation of AFM1 concentrations in cow's milk. The studies must also disclose the total number of samples and adhere consistently to established protocols for sampling, preparation, and analysis. Scientific articles that did not satisfy these criteria, including experimental studies, duplicates, or those lacking comprehensive information, were disqualified from consideration.

Data extraction

The amount of AFM1 in cow milk from a number of carefully chosen articles, including the mean and standard deviation, year of publication, regional state, detection method, total sample number, author name, and number of positive samples. The amount of AFM1 in cow's milk is given in µg/L. These data were put into a Microsoft Excel spreadsheet for more analysis.

Risk of bias assessment

The articles considered for the analysis were evaluated using a predetermined, standardized questionnaire to assess the risk of bias. A brief summary and visual representation of the risk of bias produced with the Cochrane Risk of Bias: Robvis Tool (McGuinness and Higgins, 2020).

Meta-analysis of data

STATA version 17 used to do a meta-analysis of the study's data by importing it from Microsoft Excel and using a random-effects model. Positive values, sample size, mean, and standard deviation used to analyze the prevalence and concentration of aflatoxin M1 in cow's milk. The study figured out the relative heterogeneity (H^2), the percentage of heterogeneity (I^2), and the between-study variance (Tau^2) to see how different things were. A Tau^2 value greater than 0 signifies genuine disparities in effect sizes among studies, while a Tau^2 value of 0 indicates the absence of true heterogeneity across studies.

The proportion of heterogeneity determined the total variation among the trials, with I^2 values greater than 75% showing a lot of heterogeneity. The variance is only due to sampling error within the study when H^2 equals 1. If H^2 is more than 1, though, it means that there is real heterogeneity. Relative heterogeneity is the ratio of the total variability in effect sizes to the variability anticipated from sampling error alone (Harrer *et al*., 2021). To examine the impact of moderators on the variability of effect sizes, meta-regression was utilized, employing the constrained maximum likelihood method to analyze heterogeneity (Harrer *et al*., 2021).

Subgroup analysis conducted to ascertain variability among studies. The current study was moderated by region, sample type, and analysis method. These moderators might make AFM1 more or less common. Aspergillus fungi that live in warm, humid places make AFB1, which gets into the feed that dairy cows eat. This makes AFM1 levels go up (Bellio *et al*., 2016). The other regional differences that are affecting the prevalence of AFM1 are the different ways that dairy farms operate, how they store feed, and the rules that limit how much AFM1 can be present (Miklos et al., 2020). The AFM1 analytical method introduces discrepancies among studies due to the superior sensitivity and trace detection capabilities of the HPLC method compared to the ELISA method (Bellio *et al*., 2016; Wood *et al*., 2021).

Exposure and Risk Assessment

Raw milk consumption data

Children in Ethiopia between the ages of six months and two years weigh an average of 8.43 kg and drink an average of 0.15 liters of milk per day (Ethiopian Public Health Institute (EPHI), 2013; Netherlands Development Organization (SNV) and Global Alliance for Improved Nutrition (GAIN), 2020).

Exposure Assessment of AFM1

Exposure is evaluated by computing the estimated daily intake (EDI) using body weight (bw), the individual consumer's intake rate of raw milk (IR), and the average AFM1 concentration (JECFA, 2017).

$$EDI = C_{ave} \text{ X } IR/bw$$

Risk Characterization

The health risk of AFM1 was evaluated using cancer risk (Belyhun *et al*., 2016), margin of exposure (MOE) (EFSA, 2005), and hazard index (HI) (Hooshfar *et al.,* 2020).

Margin of Exposure (MOE)

The Margin of Exposure (MOE) values are based on a reference value of 570 ng/kg body weight per day, which is based on how strong aflatoxin M1 (AFM1) is for causing hepatocellular carcinoma (HCC). This reference value is based on a two-year study of male Fischer rats and uses the estimated daily intake (EDI) to make its calculations. If the MOE value is higher than 10,000, it means that public health problems are not a big deal. The BMDL10 (benchmark dose level with a 10% confidence limit) is often used in MOE calculations. The benchmark dose is the amount of exposure that causes a small but measurable reaction.

$$MOE = \text{Reference value}/EDI$$

Hazard Index (HI)

The Hazard Index (HI) was calculated using the estimated daily intake of aflatoxin M1 (AFM1) and a reference value of 0.2 ng/kg body weight per day. This method helps figure out how much people are eating and how it affects their health. The acceptable reference value is found by dividing the TD50 of AFM1 (the dose that causes tumors in half of the tested animals, which would have stayed tumor-free at zero doses) by 5000, which is an uncertainty factor. This factor has a risk level of 1 in 100,000 (Gallardo *et al*., 2022). An HI of less than 1 usually means that people who drink raw milk are not at much risk.

$$HI = EDI/\text{Reference value}$$

Cancer Risk

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) looked at how likely it was for 100,000 people to get cancer if they were exposed to 1 ng of AFB1 (AFB1) per kg of body weight per day. The analysis showed that there could be as many as 0.049 more cancer cases per 100,000 people in populations that are negative for hepatitis B surface antigen (HBsAg) and 0.562 more cancer cases per 100,000 people in populations that are positive for HBsAg (JECFA, 2017).

Carcinogenicity studies, including tests on sensitive species like Fischer rats and rainbow trout, showed that Aflatoxin M1 (AFM1) is about one-tenth as potent as AFB1 (JECFA, 2017). So, the estimated risk of AFM1 causing cancer was 0.0562 more cases of cancer in people who were HBsAg-positive and 0.0049 more cases of cancer in people who were HBsAg-negative.

The study by Belyhun *et al.* (2016) found that 7.4% of people who were positive for the hepatitis B virus (HBV) were in the same group as the people in this study. For HBsAg-negative populations, a prevalence of 92.6% was determined by subtracting the HBV-positive rate from 100% (100 - 7.4%). The prevalence data were obtained via a systematic review and meta-analysis of five decades of published studies (1968–2015) from biomedical databases.

Average cancer potency = (0.0562 x HBsAg negative population) + (0.0049 x HBsAg positive

Populations/prevalence in Ethiopia)

Average cancer potency = (0.0562 x 0.072) + (0.0049 x 0.926)

Average cancer potency = 0.0000184 cases per person/yr. ng of aflatoxin/kg bw/day

= 1.84 cases per 10^5 persons/yr. of aflatoxin/kg bw/day

The population cancer risk of AFM1-induced hepatocellular carcinoma (HCC) assessed by multiplying the average AFM1 exposure estimates with the average cancer potency probability.

Cancer risk (cases/100,000 person/yr.) = EDI x Average cancer potency

Results

Systematic Review

The PRISMA guidelines were used to find and screen research articles on AFM1, as well as to set the rules for who could be included and who could not. Out of the forty-two research articles identified, fourteen included seventeen studies examining AFM1 in both raw and pasteurized cow milk, with a total sample size of 2,021 milk samples. Meta-analyses were utilized to ascertain the aggregated prevalence and estimated mean concentration of aflatoxin M1 (Figure 1).

Risk of bias assessment

A risk of bias assessment for AFM1 studies was conducted utilizing pre-established standard questionnaires, and ROBVIS offers a concise summary of the risk of bias (Figure 2). The evaluation encompassed fourteen research articles featuring seventeen studies. Two of these articles had a high risk of bias, and two others raised concerns about how the participants were chosen for the studies. The remaining ten research articles exhibited a minimal risk of bias and were chosen for the systematic review and meta-analysis. The overall risk in the study is mostly low, but about 25% of the data show some concern and high risk.

Meta-analysis

A meta-analysis showed that aflatoxin M1 was present in 68.2% of the samples, with 95% confidence that the true value is between 55.3% and 81.1% (Table 1). The estimated concentration of aflatoxin M1 is 0.316 µg/L, and 95% confident that the real value is between 0.163 µg/L and 0.469 µg/L (Table 3). The data extracted from seventeen selected studies regarding the prevalence and concentration level of AFM1 are heterogeneous; however, there is no substantial evidence for publication bias (Egger and Begg's test p-value exceeds 0.05) (Tables 1 and 3). The heterogeneity test evaluated the variability and reliability among selected studies. The heterogeneity test showed that Tau^2 = 739.879, I^2 = 100%, and H^2 = 1.6e+08 for the pooled prevalence (Table 1). Also, the percentage of heterogeneity (I^2 = 100%) and the relative heterogeneity (H) = 21,509.24 for estimates of AFM1 concentration level (Table 3) show that there is a lot of difference between studies.

Subgroup analysis aimed at investigating heterogeneity to identify variations in effect sizes among groups. The current meta-analysis groups, including study areas, sample types, and analysis methods, exhibited diverse effect sizes of studies, as indicated in Tables 2 and 4. Predictors lower the number of cases of AFM1 by 3.44 per year, 2.05 per region, and 4.49 per type of sample. They also lower the average AFM1 concentration level by 1.36% per year, 0.99% per region, and 0.02% per sample size. Moderators, on the other hand, raise the average AFM1 concentration level by 37.58% for each type of milk sample and 16.67% for each method of analysis. They also raise the prevalence of AFM1 by 0.9% per sample size and 33.66% per method of analysis. Methods of analysis elucidate 36.58% (p-value = 0.001) of the heterogeneity in aflatoxin M1 prevalence, while sample types account for 18.84% (p-value = 0.026) of the heterogeneity in mean aflatoxin M1 concentration. Tau^2, I^2, and H are still high (Tables 5 and 6).

The present exposure assessment and risk characterization study demonstrated substantial public health issues associated with AFM1 in cow's milk. Exposure assessment estimated the daily intake for children aged 6 months to 2 years at 5.62 ng/kg/day AFM1.

Risk characterization showed that children in Ethiopia who drank milk contaminated with aflatoxin M1 had a margin of exposure of 101.42, a hazard index of 28.1, and a cancer risk of 10.34 cases per 100,000 people per year (Table 7).

Discussion

A systematic review on the prevalence and concentration levels of AFM1 published from 2016 to 2024, followed PRISMA guidelines to make sure the data was reliable for a meta-analysis. The review included 14 research articles, which included 17 studies and about 2,012 samples. To evaluate the risk of bias, pre-established standard questionnaires and the Risk of Bias Visualizer (ROBVIS) were employed. The overall risk assessment showed that about 75% of the studies had a low risk of bias. In contrast, approximately 25% of the studies demonstrated a significant risk of bias or elicited concerns. The main problems with these studies had to do with biases in how participants were chosen. Weaknesses in determining sample sizes specifically impeded the adequate representation of the population in the studies conducted by Mesfin *et al*. (2018), Besufekad *et al*. (2018), Tadesse *et al*. (2020), and Kassa *et al*. (2020).

A meta-analysis of fourteen research articles, which included seventeen studies, was used to find the pooled prevalence and mean concentration level of aflatoxin M1. The analysis also sought to evaluate the existence of heterogeneity and publication bias within the studies. Subgroup analyses and meta-regression tests were also performed to investigate heterogeneity and pinpoint potential sources of variation. The meta-analysis employed a random-effects model in STATA version 17. Subgroup analyses sorted the studies into groups based on things like the number of samples, where the studies were done, the methods used to analyze them, and the types of milk samples.

A meta-analysis showed that the pooled prevalence of aflatoxin M1 (AFM1) is 68.2%, with a 95% confidence interval showing that the true value is between 55.3% and 81.1%. There have been many meta-analyses on AFM1 done all over the world. The pooled prevalence identified in this analysis aligns with the results of a prior study examining milk from the Middle East region (Rahmani *et al*., 2018) and Iran (Ahmadzadeh *et al*., 2015; Bahraman *et al*., 2019).

The mean concentration level of aflatoxin M1 is thought to be 0.316 µg/L, and 95% confident that the real value is between 0.163 µg/L and 0.469 µg/L. The maximum level of aflatoxin M1 allowed by the European Union is 0.05 µg/L (EU, 2023), but this level is still below Ethiopia's limit of 0.5 µg/L. The main reason aflatoxin M1 is in milk is that dairy cows that are nursing eat feed that has aflatoxin B1 (AFB1) in it. Moisture, humidity, storage conditions, drought, and insect infestations can all affect the growth of toxigenic fungi and the amount of toxins they make in animal feeds (Marin *et al*., 2013).

The examination of data from fourteen curated research articles, comprising seventeen studies on the prevalence and concentration levels of AFM1, indicates considerable variability. Even with this diversity, there is no significant indication of publication bias, as demonstrated by Egger's and Begg's tests, both producing a p-value exceeding 0.05. The heterogeneity tests for the chosen studies yield a statistic of 739.8792, signifying 100% heterogeneity, and a combined prevalence value of 1.6e+08. The estimates for AFM1 concentration levels also show a between-study variance (Tau^2) of 0.1008, a percentage of heterogeneity (I^2) of 100%, and a relative heterogeneity (H^2) of 21,509.24. These heterogeneity tests indicate that the discrepancies among the studies may arise from various factors, including variations in sample sizes, types of milk samples analyzed, analytical methods utilized, climatic conditions, regulatory disparities, and the particular interests of the researchers (Borenstein et al., 2017).

Subgroup analysis performed to investigate heterogeneity and ascertain which groups demonstrate variations in effect sizes. In the present meta-analysis, diverse factors, encompassing study domains, sample classifications, and analytical techniques, exhibited varying effect sizes. Meta-regression helps us understand how moderators explain the differences in effect sizes between the studies that were looked at. Predictors cut down on the amount of aflatoxin M1 (AFM1) by 3.44 per year, 2.05 per region, and 4.49 per sample type. They also lower the average amount of AFM1 by 1.36% per year, 0.99% per region, and 0.02% per sample size. On the other hand, moderators raise the number of AFM1 samples by 0.9% for each sample size and by 33.66 for each method of analysis. They also raise the average level of AFM1 by 37.58% for each type of milk sample and by 16.67% for each method of analysis. The methods of analysis explain a large part of the differences in the prevalence of aflatoxin M1 (36.58%, p-value = 0.001), while the types of samples explain a smaller part of the differences in the mean concentration of aflatoxin M1 (18.84%, p-value = 0.026). The values for Tau^2, I^2, and H^2 are still high (Tables 5 and 6). The ongoing heterogeneity at the meta-regression level suggests that there remains unexplained variability in the prevalence and mean concentration of AFM1, likely affected by unmeasured variables (Thompson and Higgins, 2002).

The current evaluation of exposure and risk associated with AFM1 in cow's milk indicates substantial public health issues. The exposure assessment figured out that children between the ages of 6 months and 2 years get 5.62 ng/kg/day of AFM1 every day. The risk characterization showed that drinking AFM1 contaminated milk in Ethiopia increases the risk of cancer by 10.34 cases per 100,000 people per year, the hazard index by 28.1 and the margin of exposure by 101.42. The European Food Safety Authority (EFSA) has set maximum limits for risk characterization, which means that a hazard index above one and a margin of exposure below 10,000 are bad for public health (EFSA, 2012). The current risk values estimated cancer risk about 10 cases per 100,000 people per year. This finding agrees with study from Kenya (Sirma *et al*., 2019) and Ghana (Kortei *et al*., 2022) indicated high-risk of liver cancer from exposure to AFM1. On the other hand, Ahlberg *et al*. (2018) from Kenya reported low risk of cancer from exposure to AFM1. This paper's strength is that it covers meta-analysis, meta-regression, exposure, and risk assessment in detail, giving a full picture of how AFM1 affects children aged 6 months to 2 years. However, there are some problems with the current assessment. Notably, it does not include unpublished studies such as theses, government and non-government reports, and preliminary studies. The study also did not include AFM1 reports from milk products such as cheese or yogurt, and there are differences in how the analyses are done. This study underscores the critical necessity for the implementation of mitigation measures to combat aflatoxin M1 in milk in Ethiopia. To take action, we should promote mycotoxin binders in animal feed, make storage better, and make national monitoring better.

Conclusion

In Ethiopia, many studies have looked at how common and high the levels of AFM1 are in cow's milk, but only a few have looked at exposure and health risks. The current study aimed to investigate the pooled prevalence, estimated mean concentration levels, and exposure risk to AFM1 in children using various methodologies, including systematic review, meta-analysis, meta-regression, and health risk assessment. It examined 14 research papers, which included 17 studies with 2,021 samples of milk. The bias risk assessment revealed that approximately 25% of the data exhibited significant concerns and a high likelihood of bias, whereas 75% demonstrated a low likelihood of bias. This meant that the meta-analysis could look at the data in more detail. The pooled prevalence of AFM1 was found to be 68.2%, and the mean concentration level was estimated to be 0.316 µg/L. The current mean concentration levels of AFM1 are lower than the Codex Alimentarius Commission's standard for AFM1, which is 0.5 µg/L. Nevertheless, higher than the European Union's standard for AFM1 is 0.05 µg/L. An exposure and health risk assessment calculated the daily intake of AFM1 from ingestion of AFM1 contaminated cow milk to be 5.62 ng/kg/day, indicating a margin of exposure of 101.42, a hazard index of 28.1, and a cancer risk of approximately 10.34 cases per 100,000 individuals in children aged 6 months to 2 years. These findings demonstrated a notable pooled prevalence and an estimated mean concentration level of AFM1 in cow's milk. Ethiopian children between the ages of 6 months and 2 years are also highly exposed to AFM1, which is dangerous to their health and can even cause cancer. These results raise concerns about food safety and public health because AFM1 is in cow's milk. This study warrants policymakers improving established regulatory policies and regulatory bodies in the execution of established regulatory policies and standards, regular monitoring, and control measures of AFB1 in animal feed.

Declarations

Conflict of interest

The authors declare that he has no conflicts of interest.

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Author Contribution

Kassaye BK - designed systematic review, collection of articles and data extraction, statistical analysis, and wrote the manuscript. Getahun MN - designed systematic review, collection of articles and data extraction, review, and editing the manuscript.All authors reviewed the manuscript.

Data Availability

In Research Square as Preprint with the DOI: 10.21203/rs.3.rs-7297234/v1 and LicenseCC BY 4.0.

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Tables

Table 1: Pooled prevalence and heterogeneity in studies on AFM1.

Study	Sample size	Prevalence	[95% Conf. interval]		Tau^2	I^2 (%)	H^2	Publication Bias	
								Egger test	Begg's test
			Lower	Upper				Prob > |z|	Prob > |z|
Tefera *et al*., 2022	240	77.920	77.919	77.921					
Zebib *et al*., 2022	64	62.500	62.378	62.622					
Zebib *et al*., 2022	64	67.200	67.087	67.313					
Admasu *et al*., 2021	100	99.000	98.857	99.143					
Tadesse *et al*., 2020	56	100.000	99.944	100.056					
Tadesse *et al*., 2020	52	100.000	99.863	100.137					
Kassa *et al*., 2020	20	15.000	14.956	15.044					
Mesfin *et al*., 2018	45	57.800	57.797	57.803					
Besufekad *et al*., 2018	10	60.000	59.975	60.025					
Gizachew *et al*., 2016	110	91.800	91.641	91.959					
Kebede *et al*., 2024	60	16.700	16.665	16.735					
Kebede *et al*., 2024	54	35.200	35.190	35.210					
Tadesse *et al*., 2024	60	56.600	56.267	56.933					
Zebib *et al*., 2023	96	100.00	99.944	100.056					
Geleta *et al*., 2024	90	84.400	84.398	84.402					
Tesfaye *et al*., 2024	810	63.900	63.867	63.933					
Fikadu et al., 2022	90	71.100	71.097	71.103					
theta	**2021**	**68.2**	**55.3**	**81.1**	**739.8792**	**100**	**1.6e+08**	**0.4861**	**0.3031**

Test of theta = 0; Z = 10.34; Test of homogeneity: Q = chi2 (16) = 3.5e+08; Prob > |z| = 0.0000; Prob > Q = 0.0000

Table 2: Subgroup analysis for pooled prevalence and heterogeneity of studies on AFM1.

Sub-Group	No. of studies	Prevalence	[95% conf. interval]		P-value	Tau^2	I^2%	H^2
			Lower	Upper				
Region								
Addis Ababa	1	56.600	56.267	56.933	0.000	0.000		
Amhara	2	57.000	-25.319	139.318	0.175	3527.997	100.000	1.2e+06
Oromia	12	70.883	55.937	85.830	0.000	697.864	100.000	7.9e+07
SNNPR	1	60.000	59.975	60.025	0.000	0.000		
Sidama	1	77.920	77.919	77.921	0.000	0.000		
Types of milk samples								
Pasteurized cow's milk	4	64.750	38.283	91.217	0.000	729.402	100.000	7.5e+05
Raw cow's milk	13	69.240	53.874	84.606	0.000	799.017	100.00	2.2e+08
Years of Publication								
2016	1	91.800	91.641	91.959	0.000	0.000		
2018	2	58.900	56.744	61.056	0.000	2.420	100.00	29835.62
2020	3	71.667	16.134	127.199	0.011	2408.333	100.00	2.2e+06
2021	1	99.000	98.857	99.143	0.000	0.000		
2022	4	69.680	63.289	76.072	0.000	42.537	100.00	9.2e+06
2023	1	100.000	99.944	100.056	0.000	0.000		
2024	5	51.360	28.424	74.296	0.000	684.708	100.00	1.5e+07
Method of analysis								
ELISA	9	84.024	72.425	95.624	0.000	315.214	100.00	3.2e+07
HPLC-FLD	8	50.362	32.759	67.966	0.000	654.320	100.00	6.0e+07
Overall								
Theta	**17**	**68.2**	**55.3**	**81.1**	**0.000**	**739.879**	**100**	**1.6e+08**

Table 3: Estimates of concentration level, heterogeneity, and publication bias of studies on AFM1.

Study	Mean of Conc. Level (µg/L)	[95% conf. interval]		Weight %	Tau^2	I^2 (%)	H^2	Publication Bias
		Lower	Upper					Begg's test
								Prob > |z|
Tefera *et al*., 2022	0.007	0.006	0.008	6.05				
Zebib *et al*., 2022	0.319	0.197	0.441	5.83				
Zebib *et al*., 2022	0.324	0.211	0.437	5.86				
Admasu *et al*., 2021	0.470	0.327	0.613	5.75				
Tadesse *et al*., 2020	0.970	0.914	1.026	6.00				
Tadesse *et al*., 2020	0.960	0.553	0.827	5.77				
Kassa *et al*., 2020	0.121	0.077	0.165	6.02				
Mesfin *et al*., 2018	0.054	0.051	0.057	6.05				
Besufekad *et al*., 2018	0.140	0.115	0.165	6.04				
Gizachew *et al*., 2016	0.410	0.251	0.569	5.68				
Kebede *et al*., 2024	0.047	0.012	0.081	6.03				
Kebede *et al*., 2024	0.046	0.036	0.056	6.05				
Tadesse *et al*., 2024	1.237	0.904	1.570	4.70				
Zebib *et al*., 2023	0.320	0.264	0.376	6.00				
Geleta *et al*., 2024	0.234	0.232	0.236	6.05				
Tesfaye *et al*., 2024	0.179	0.146	0.212	6.04				
Fikadu *et al*., 2022	0.044	0.041	0.047	6.05				
theta	**0.316**	**0.163**	**0.469**		**0.1008**	**100**	**21509.24**	**0.1082**

Test of theta = 0; z = 4.05; Test of homogeneity: Q = chi2 (16) = 56757.07; Prob > |z| = 0.0001; Prob > Q = 0.0000

Table 4: Subgroup analysis estimates the mean of the concentration level and heterogeneity in studies of AFM1.

Sub-Group	No. of studies	Mean of Conc. Level (µg/L)	[95% conf. interval]		P-value	Tau^2	I^2 (%)	H^2
			Lower	Upper				
Region								
Addis Ababa	1	1.237	0.904	1.570	0.000	0.000		
Amhara	2	0.289	-0.053	0.630	0.098	0.058	95.21	20.90
Oromia	12	0.300	0.138	0.461	0.000	0.080	99.99	9000.18
SNNPR	1	0.140	0.115	0.165	0.000	0.000		
Sidama	1	0.007	0.006	0.008	0.000	0.000		
Year of Publication								
2016	1	0.410	0.251	0.569	0.000	0.000		
2018	2	0.096	0.012	0.180	0.025	0.004	97.81	45.59
2020	3	0.593	0.1000	1.086	0.018	0.188	99.42	172.65
2021	1	0.470	0.327	0.613	0.000	0.000		
2022	4	0.164	-0.003	0.332	0.055	0.028	99.98	5989.97
2023	1	0.320	0.264	0.376	0.000	0.000		
2024	5	0.327	-0.082	0.736	0.117	0.213	99.98	4780.96
Types of milk samples								
Pasteurized milk	4	0.631	0.095	1.168	0.021	0.292	99.67	299.28
Raw milk	13	0.224	0.118	0.329	0.000	0.036	99.99	10073.66
Method of analysis								
ELISA	9	0.394	0.196	0.592	0.000	0.089	99.99	9132.79
HPLC-FLD	8	0.230	-0.005	0.466	0.055	0.113	99.99	10518.52
Overall								
Theta	**17**	**0.316**	**0.163**	**0.469**	**0.000**	**0.101**	**100**	**21509.24**

Table 5: Meta regression of prevalence by moderators.

Moderators	Coefficient	Std. error	Z	P> |z|	[95% conf. interval]		Tau^2	I^2 (%)	H^2	R^2 (%)
					Lower	Upper				
Publication year	-3.443984	3.344897	-1.03	0.303	-9.999862	3.111894	737.1	100	1.2e+08	0.37
Region	-2.054161	5.724013	-0.36	0.720	-13.27302	9.164698	782.5	100	6.6e+07	0.00
Types of sample	-4.489896	16.02084	-0.28	0.779	-35.89017	26.91038	785.1	100	1.8e+08	0.00
Sample size	0.0094124	0.0378844	0.25	0.804	-0.0648397	0.0836645	786	100	7.6e+07	0.00
Method of analysis	33.66196	10.52612	3.20	0.001	13.03114	54.29279	469.3	100	4.6e+07	36.58

Table 6: Meta regression of the mean of concentration level by moderators.

Moderators	Coefficient	Std. error	Z	P> |z|	[95% conf. interval]		Tau^2	I^2 (%)	H^2	R^2 (%)
					Lower	Upper				
Publication year	-0.013636	0.0409704	-0.33	0.739	-0.0939364	0.0666645	0.1069	99.99	17321.28	0.00
Region	-0.0099568	0.0679948	-0.15	0.884	-0.1432242	0.1233106	0.1082	99.99	9093.78	0.00
Types of sample	0.3758447	0.1690699	2.22	0.026	0.0444738	0.7072156	0.08178	99.99	18307.93	18.84
Sample size	-0.000261	0.0004415	-0.59	0.554	-0.0011264	0.0006044	0.1063	99.99	10232.60	0.00
Method of analysis	0.166703	0.1539033	1.08	0.279	-0.1349419	0.4683478	0.0974	99.99	9574.95	3.34

Table 7: Exposure and Health Risk Assessment of Consumption of AFM1 Contaminated Cow Milk in Children of Ethiopia.

Estimated Daily Intake (EDI) (ng/Kg/day)	Margin of exposure (MOE)	Hazard Index (HI)	Cancer risk (cases/10^5person/year
5.62	**101.42**	**28.1**	**10.34**

Figures

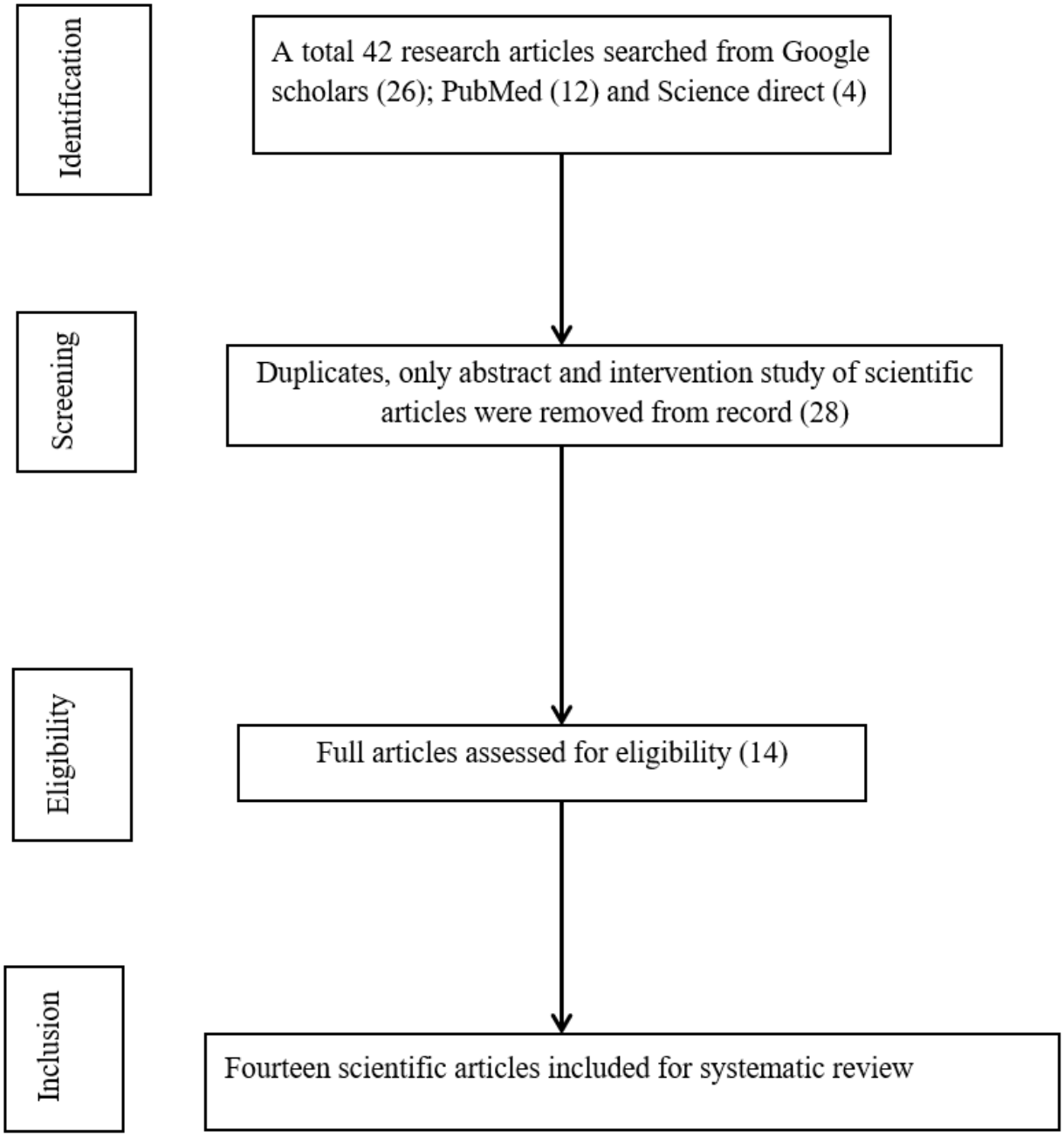
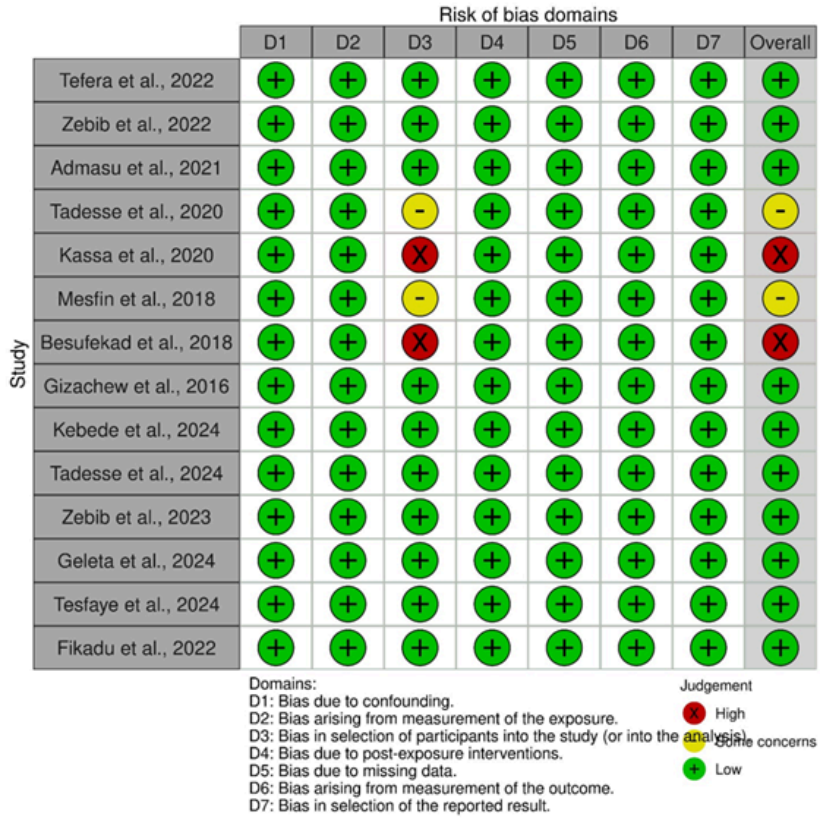


Figure 1

PRISMA flow diagram.

A: Risk of bias graph.



B: Risk of bias summary.

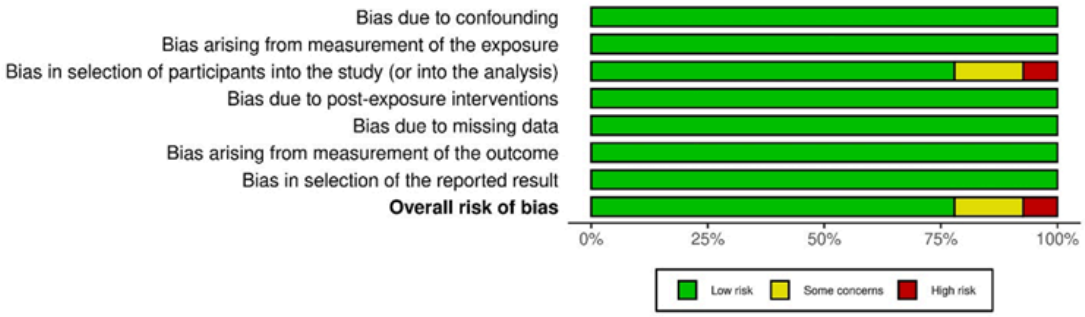


Figure 2

Risk of bias assessment for studies related to AFM1. (A) Risk of bias graph. (B) Risk of bias summary.