Aflatoxin M1 in Milk Worldwide from 1988 to 2020: A Systematic Review and Meta-Analysis

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Background. Aflatoxins are found in various types of food and animal feed. Food contamination with aflatoxin toxin is of particular importance today. Various studies have reported different prevalence of aflatoxin M1 in animal milk. Therefore, due to the importance of this toxin, its role in health, and lack of general statistics about it worldwide, the present study aimed to determine the prevalence of aflatoxin M1 in milk worldwide with a systematic review and meta-analysis study.

Methods. In this review study, national and international databases were extracted from SID, MagIran, IranMedex, IranDoc, Embase, ScienceDirect, Scopus, PubMed, and Web of Science (ISI) between January 1988 and February 2020. A random effects model was used for analysis, and heterogeneity of studies with an I² index was investigated. Data were analyzed using Comprehensive Meta-Analysis (version 2).

Results. The prevalence of aflatoxin M1 in milk worldwide from January 1988 to February 2020 in 122 articles with a sample size of 18921 was 79.1% (95% CI: 75.5–82.3%). Regarding the heterogeneity based on metaregression, there was a significant difference between the effect of the year of study (p ≤ 0.001) and sample size (p ≤ 0.001) with the prevalence of aflatoxin M1 in animal milk.

Conclusion. The results of this study show that the prevalence of aflatoxin M1 in milk is high worldwide. Therefore, considering the importance of the milk group and its products, special measures should be taken to protect the ration from aflatoxin molds and milk quality.

1. Introduction

Food contamination with aflatoxin toxin is of particular importance today, and global organizations such as the WHO, FAO, and Codex (the Codex Alimentarius Commission) have determined the maximum level of contamination of various foods [1].

Aflatoxin in humans causes acute and chronic poisoning. This toxin has teratogenic and carcinogenic effects [2]. Studies have shown the adverse effects of aflatoxin on the central nervous system, liver and kidney, brain injury, and death. If the human diet contains several mycotoxins, the symptoms of intoxication become more severe depending on the patient’s age, gender, and condition [3].

The long-term effects of taking small amounts of mycotoxins are different. The main effect of chronic mycotoxin poisoning, especially aflatoxins, is a variety of cancers, especially liver cancer [4].

Aflatoxins are found in a variety of foods and animal feed. Nowadays, methods of precise analysis have enabled the measurement of its concentration in micrograms of food [5].

Aflatoxin is divided into two major groups: B and G. Food contamination with aflatoxins is generally caused by inappropriate storage of foodstuff which leads to the contamination with Aspergillus fungi in various ways. Consumption of contaminated feed by poultry causes aflatoxin M1 to be present in milk, meat, and eggs [6, 7]. The permitted aflatoxin M1 level in dairy products is 0.05 mcg/liter [8].

Studies have shown that consumption of aflatoxin-containing foods is a cause of liver cancer in Qidong people in China [9]. In addition, four years of food inspection and
control in Cyprus showed contamination of many foods with this toxin [10].

Studies show that aflatoxin M1 in raw milk can be transferred to dairy products. In one study, adding 1.7 to 2 micrograms of aflatoxin to milk and production of cheese, it was observed that 40% of aflatoxin remained in cheese and 60% in whey [11].

Research on raw milk in Albania has also shown that the amount of aflatoxin M1 in winter milk is higher than in summer milk [12].

Almost all samples of dry matter, baby food, and yogurt in Italy and Kuwait are contaminated with aflatoxin M1, but its content value in dairy products was not considered a serious problem for the Italian people; however, for milk products, the principles of proper maintenance and proper livestock feeding have been suggested [13, 14].

Measurement of aflatoxin M1 by ELISA in Bursa, Turkey, showed that high-fat cheeses contained aflatoxin M1 more than the standard in Turkey [15, 16]. In some parts of the world, aflatoxin M1 contamination is not a serious health problem either, but contamination with this toxin has been reported. For example, studies of raw milk in Spain report contamination in only 33% of the samples [17].

Sampling of milk from supermarkets in Ribeirao, Brazil, showed that about 21% of the samples were contaminated with aflatoxin M1 and its content has been 24–50 ng/l. The results of this study showed that despite the high levels of contamination of pasteurized and sterile milk in Brazil, this is not a serious problem for people. However, more research and investigation is needed in this regard [18].

However, the examination of raw, pasteurized, sterile milk in Mexico shows aflatoxin M1 contamination and in another study, 40% of the samples collected contained more than 0.05 μg/L of aflatoxin M1 [19, 20].

Various studies have reported different prevalence of aflatoxin M1 in animal milk. However, no comprehensive study that shows the results of these studies as a whole globally has been found; therefore, due to the importance of this toxin, its role in health, and lack of general statistics about it worldwide, the present study was conducted to determine the prevalence of aflatoxin M1 in milk worldwide in a systematic review and meta-analysis study.

2. Methods

In this systematic review and meta-analysis study, the prevalence of aflatoxin M1 in animal milk worldwide was assessed based on studies conducted between January 1988 and February 2020. For this purpose, articles published in the databases of SID, MagIran, IranMedex, and IranDoc and international databases of Embase, ScienceDirect, Scopus, PubMed, and Web of Science (ISI) with keywords of Prevalence, Aflatoxin M1, and Milk were searched.

The selection criteria were the availability of full-text cross-sectional studies investigating the prevalence of aflatoxin M1 in animal milk. For more information, the sources of the articles were also reviewed for access to other articles.

2.1. Selection of Studies. Initially, all articles referring to the prevalence of aflatoxin M1 in milk worldwide were collected by researchers and acceptance was performed based on inclusion and exclusion criteria. Exclusion criteria included unrelated cases, case reports, interventional studies, duplication of studies, unclear methodology, and inaccessibility of the full text of the study. In order to reduce bias, articles were searched independently by two researchers and if there is a disagreement about a study, the article was reviewed by the refereeing supervisor. A total of 130 studies entered the third stage, i.e., qualitative evaluation.

2.2. Qualitative Evaluation of Studies. The quality of the articles was evaluated on the basis of the selected and related items of the STROBE 22-item checklist that could be evaluated in this study (study design, background, literature review, place and time of study, consequence, inclusion criteria, sample size, and statistical analysis). Previous studies have also referred to them. Articles referring to 6 to 7 criteria were considered as high-quality articles, and those that did not refer to 2 items and more than 2 items from the seven items were considered as medium and low methodological quality articles, respectively [21]. In the present study, 122 articles were entered into the systematic review and meta-analysis as high-quality and medium-quality studies, and seven articles had poor quality and were excluded.

2.3. Data Extraction. All final articles entered into the meta-analysis process were prepared by a prepared checklist. Checklists included article title, first author’s name, year of publication, study location, sample size, prevalence of aflatoxin M1 in milk, animal species, milk type, and implementation method.

2.4. Statistical Analysis. Since prevalence has binomial distribution, prevalence variance was calculated using a binomial distribution variance formula and weighted mean was used to combine prevalence rate of different studies. To evaluate the heterogeneity of the selected studies, the I² index test (heterogeneity was divided into three classes of less than 25% (low heterogeneity), 25–75% (moderate heterogeneity), and more than 75% (high heterogeneity)) was used.

Meta-regression analysis was used to investigate the relationship between the prevalence of aflatoxin M1 in animal milk worldwide with the year of study and sample size. The Begg and Mazumdar test at the significant level of 0.1 and its corresponding funnel plot were used to investigate the propagation error and also considering the high volume of samples. Data were analyzed using Comprehensive Meta-Analysis (version 2) software.

3. Results

In this study, all studies regarding the prevalence of aflatoxin M1 in milk worldwide without a time limit were systematically reviewed according to the PRISMA regulations. In the initial search, 1384 articles were identified, and 122
articles eventually published between January 1988 and February 2020 were entered into the final analysis (Figure 1).

The probability of bias in the results by the funnel plot and Begg and Mazumdar test at the significant level of 0.1 indicated no bias in the present study ($p = 0.102$) (Figure 2).

Based on the results of the test ($I^2: 95.1$) and due to the heterogeneity of the selected studies, a random effects model was used to combine the studies and the joint prevalence estimation. There were 122 articles with a sample size of 18921 individuals on the prevalence of aflatoxin M1 in animal milk worldwide with sample size, animal species, milk type, and execution method in each study; the specifications of the selected articles are presented in Table 1.

According to the results of the study, the prevalence of aflatoxin M1 in milk worldwide was 79.1% (95% CI: 75.5–82.3%) (Figure 3).

The middle point of each line represents the prevalence of aflatoxin M1 in milk worldwide in each study, and the rhombic figure shows the prevalence of aflatoxin M1 in milk worldwide for all studies.

Table 2 presents the results of the analysis of different subgroups by continents.
| Author, year, reference | Country | Sample size | Prevalence (%) | Species | Type | Detection method | Quality |
|-------------------------|---------|-------------|----------------|---------|------|------------------|---------|
| Kamkar, 2005, [22]      | Iran    | 111         | 75.7           | Cow     | Raw  | TLC             | Medium  |
| Karimi et al., 2007, [23]| Iran    | 110         | 99.5           | Cow     | Raw  | ELISA           | High    |
| Ghiasian et al., 2007, [24]| Iran    | 186         | 64.0           | Cow     | PTZ  | ELISA           | High    |
| Oveisi et al., 2007, [25]| Iran    | 128         | 99.6           | Cow     | PTZ  | ELISA           | High    |
| Tajkarimi et al., 2007, [26]| Iran    | 98          | 95.9           | Cow     | Raw  | HPLC            | Medium  |
| Tajik et al., 2007, [27]| Iran    | 144         | 99.7           | Cow     | Raw  | PTZ, ELISA      | High    |
| Tajkarimi et al., 2008, [28]| Iran    | 319         | 53.9           | Cow     | Raw  | HPLC            | High    |
| Seifidgar et al., 2008, [29]| Iran    | 120         | 98.3           | Cow     | Raw  | ELISA           | High    |
| Kamkar, 2008, [30]      | Iran    | 52          | 99.1           | Cow     | PTZ  | ELISA           | High    |
| Ghazani, 2009, [31]     | Iran    | 50          | 99.0           | Cow     | PTZ  | ELISA           | High    |
| Sadeghi et al., 2009, [32]| Iran    | 128         | 78.1           | Cow     | PTZ  | ELISA           | High    |
| Rahimi et al., 2009, [33]| Iran    | 236         | 90.3           | Cow     | Raw, PTZ, UHT | ELISA | High    |
| Riazipour et al., 2010, [34]| Iran    | 50          | 84.0           | Cow     | PTZ  | ELISA           | High    |
| Nemati et al., 2010, [35]| Iran    | 90          | 99.5           | Cow     | Raw, PTZ, UHT | ELISA | High    |
| Sani et al., 2010, [36] | Iran    | 196         | 99.7           | Cow     | PTZ  | ELISA           | High    |
| Fallah-1, 2010, [37]    | Iran    | 91          | 72.5           | Cow     | Raw  | PTZ, TLC        | Medium  |
| Fallah-2, 2010, [38]    | Iran    | 225         | 67.1           | Cow     | Raw  | PTZ, UHT        | ELISA   | High    |
| Mohammadian et al., 2010, [39]| Iran    | 272         | 94.5           | Cow     | Raw  | PTZ, UHT        | ELISA   | High    |
| Mohamadi and Alizadeh, 2010, [40]| Iran    | 80          | 99.4           | Cow     | PTZ  | UHT             | ELISA   | High    |
| Rahimi et al., 2010, [41]| Iran    | 311         | 42.1           | Cow, sheep, goat, camel, buffalo | Raw | ELISA           | High    |
| Heshmati and Milani, 2010, [42]| Iran    | 210         | 52.9           | Cow     | UHT  | ELISA           | High    |
| Seifidgar et al., 2011, [43]| Iran    | 72          | 99.3           | Cow     | PTZ  | ELISA           | High    |
| Kamkar et al., 2011, [44]| Iran    | 122         | 99.6           | Cow     | Raw  | ELISA           | High    |
| Movassagh, 2011, [45]   | Iran    | 49          | 99.0           | Cow     | UHT  | ELISA           | High    |
| Fallah et al., 2011, [46]| Iran    | 225         | 64.4           | Cow, sheep, goat | Raw | ELISA           | High    |
| Panahi et al., 2011, [47]| Iran    | 100         | 99.5           | Cow     | Raw  | ELISA           | High    |
| Rohani et al., 2011, [48]| Iran    | 72          | 50.0           | Cow     | Raw  | HPLC            | High    |
| Garmakhaney et al., 2011, [49]| Iran    | 74          | 85.1           | Cow     | Raw, PTZ, UHT | ELISA | High    |
| Rahimi-1 et al., 2011, [50]| Iran    | 149         | 95.3           | Cow     | PTZ  | UHT             | ELISA   | High    |
| Rahimi-2 and Ameri, 2011, [51]| Iran    | 150         | 46.7           | Cow, sheep, goat | Raw | ELISA           | High    |
| Rahimi-3 et al., 2011, [52]| Iran    | 60          | 40.0           | Cow     | PTZ  | ELISA           | High    |
| Behfar et al., 2012, [53]| Iran    | 100         | 99.5           | Cow     | PTZ  | ELISA           | High    |
| Mohamadi-Sani et al., 2012, [54]| Iran    | 42          | 97.6           | Cow     | PTZ  | ELISA           | High    |
| Behnamipour et al., 2012, [55]| Iran    | 75          | 99.3           | Cow     | PTZ  | ELISA           | High    |
| Movassagh and Adinehvand, 2013, [56]| Iran    | 90          | 99.5           | Cow     | Raw  | HPLC            | High    |
| Riahi-Zanjani and Balali-Mood, 2013, [57]| Iran    | 45          | 98.9           | Cow     | PTZ  | ELISA           | High    |
| Khosravi et al., 2013, [58]| Iran    | 2160        | 100.0          | Cow     | Raw  | ELISA           | High    |
| Sani and Nikpooyan, 2013, [59]| Iran    | 60          | 99.2           | Cow     | PTZ  | HPLC            | High    |
| Kamkar et al., 2014, [60]| Iran    | 120         | 75.0           | Cow, buffalo | Raw | ELISA           | High    |
| Moenian et al., 2014, [61]| Iran    | 311         | 92.0           | Cow     | Raw  | HPLC            | High    |
| Mahmoudiand Zare, 2014, [62]| Iran    | 30          | 98.4           | Buffalo | Raw | ELISA           | High    |
| Rezaei et al., 2014, [63]| Iran    | 40          | 98.8           | Cow     | Raw  | HPLC            | High    |
| Zanjani et al., 2015, [64]| Iran    | 45          | 98.9           | Cow     | Raw  | ELISA           | High    |
| Mahmoudi and Norian, 2015, [65]| Iran    | 288         | 56.6           | Cow     | Raw  | ELISA           | High    |
| Fallah et al., 2015, [66]| Iran    | 254         | 80.3           | Cow     | Raw  | ELISA           | High    |
| Author, year, reference | Country | Sample size | Prevalence (%) | Species | Type | Detection method | Quality |
|-------------------------|---------|-------------|----------------|---------|------|-----------------|--------|
| Barikbin et al., 2015, [67] | Iran | 59 | 94.9 | Cow | PTZ | ELISA | High |
| Rouhi et al., 2015, [68] | Iran | 120 | 8.3 | Cow | Raw, PTZ | ELISA | Medium |
| Najafian and Najafian, 2015, [69] | Iran | 100 | 99.5 | Cow | PTZ | ELISA | High |
| Mashak et al., 2016, [70] | Iran | 30 | 98.4 | Cow | UHT | HPLC | High |
| Hashemi, 2016, [71] | Iran | 180 | 55.6 | Cow | Raw, PTZ | ELISA | High |
| Bahrami et al., 2016, [72] | Iran | 172 | 65.7 | Cow, sheep, goat | Raw | ELISA | High |
| Mohammadi et al., 2016, [73] | Iran | 76 | 99.4 | Cow | PTZ | ELISA | High |
| Bolourchian et al., 2016, [74] | Iran | 221 | 26.7 | Cow | Raw | ELISA | High |
| Fallahi et al., 2016, [75] | Iran | 808 | 28.2 | Cow, sheep, goat, Camel | Raw | HPLC | High |
| Sohrabi and Gharahkoli, 2016, [76] | Iran | 49 | 81.6 | Cow | PTZ | ELISA | High |
| Palizban et al., 2016, [77] | Iran | 60 | 56.7 | Cow | Raw | ELISA | High |
| Taherabadi et al., 2016, [78] | Iran | 117 | 98.3 | Cow | Raw, PTZ | HPLC | High |
| Dakhili et al., 2016, [79] | Iran | 70 | 92.9 | Cow | Raw | ELISA | High |
| Shokri and Torabi, 2017, [80] | Iran | 70 | 85.7 | Camel | Raw | ELISA | High |
| Ghariby et al., 2017, [81] | Iran | 60 | 99.2 | Buffalo | Raw | ELISA | High |
| Xiong et al., 2018, [82] | China | 242 | 73.6 | Cow | PTZ, UHT | HPLC | High |
| Sumantri et al., 2019, [83] | Indonesia | 42 | 92.9 | Cow | Raw | ELISA | High |
| Nile et al., 2016, [84] | India | 200 | 45.5 | Cow | Raw | ELISA | High |
| Asghar et al., 2018, [85] | Pakistan | 156 | 91.7 | Cow | Raw | ELISA | High |
| Turkoglu and Keyvan, 2019, [86] | Turkey | 105 | 99.0 | Cow | Raw, PTZ, UHT | ELISA | High |
| Hassan et al., 2018, [87] | Qatar | 72 | 84.7 | Cow | PTZ, UHT | HPLC | High |
| Assem et al., 2011, [88] | Lebanon | 38 | 73.7 | Cow | Raw | ELISA | High |
| Cano-Sancho et al., 2010, [89] | Spain | 72 | 94.4 | Cow | UHT | ELISA | High |
| Arorini et al., 2016, [90] | Italy | 58 | 60.3 | Cow | Raw | UHT | ELISA | High |
| Duarte et al., 2013, [91] | Portugal | 40 | 27.5 | Cow | PTZ, UHT | HPLC | High |
| Rama et al., 2016, [92] | Kosovo | 178 | 80.9 | Cow | UHT, PTZ | ELISA | High |
| Santili et al., 2015, [93] | Brazil | 635 | 52.6 | Cow | Raw | HPLC | High |
| Molina et al., 2019, [94] | Costa Rica | 183 | 5.6 | Cow | Raw | HPLC | Medium |
| Elzupir and Elhussein, 2010, [95] | Sudan | 44 | 95.5 | Cow | Raw | — | HPLC | High |
| Kuboka et al., 2019, [96] | Kenya | 96 | 99.5 | Cow | Raw | ELISA | High |
| Mwanza et al., 2015, [97] | Egypt | 138 | 72.5 | Cow | Raw | ELISA | High |
| Dashdi et al., 2005, [98] | Kuwait | 177 | 79.7 | Cow, sheep, goat, camel | Raw, PTZ | ELISA | High |
| Polovinski-Horvatic et al., 2009, [99] | Serbia | 90 | 31.1 | Cow, sheep, goat | UHT, PTZ | TLC | High |
| Pathirana et al., 2010, [100] | Sri Lanka | 87 | 33.3 | Cow | Raw | HPLC | High |
| Muhammad et al., 2010, [101] | Pakistan | 84 | 81.0 | Cow | Raw | HPLC | Medium |
| Ruangwises and Ruangwises, 2010, [102] | Thailand | 240 | 99.8 | Cow | Raw | HPLC | High |
| Ertas et al., 2011, [103] | Turkey | 43 | 86.0 | Cow | Raw | ELISA | High |
| Ayoub et al., 2011, [104] | Egypt | 48 | 77.1 | Cow, sheep, goat | Raw, PTZ, UHT | ELISA | High |
| El Khoury et al., 2011, [105] | Lebanon | 138 | 40.6 | Cow | PTZ | ELISA | Medium |
| Kabak and Ozbek, 2012, [106] | Turkey | 40 | 20.0 | Cow | UHT | HPLC | High |
| Al Zuheir and Omar, 2012, [107] | Palestine | 40 | 85.0 | Cow, sheep, goat | Raw | ELISA | High |
| Sadia et al., 2012, [108] | Palestine | 232 | 76.3 | Cow | Raw, PTZ | ELISA | Medium |
| Abdallah et al., 2012, [109] | Saudi Arabia | 96 | 82.3 | Cow | UHT | ELISA | High |
| Siddappa et al., 2012, [110] | India | 45 | 66.7 | Cow | UHT | HPLC | High |
Table 1: Continued.

| Author, year, reference | Country       | Sample size | Prevalence (%) | Species | Type | Detection method | Quality |
|-------------------------|---------------|-------------|----------------|---------|------|-----------------|---------|
| Okeke et al., 2012, [111]| Nigeria       | 30          | 98.4           | —       | PTZ  | ELISA           | Medium  |
| Tsakiris et al., 2013, [112]| Greece     | 196         | 46.4           | —       | PTZ  | ELISA           | Medium  |
| Suliman and Abdalla, 2013, [113]| Sudan    | 143         | 98.6           | Cow     | Raw  | ELISA           | High    |
| Xiong et al., 2013, [114]| China        | 72          | 59.7           | —       | Raw  | ELISA           | High    |
| Ali-1 et al., 2014, [115]| Sudan        | 35          | 98.6           | Cow     | Raw  | ELISA           | High    |
| Ali-2 et al., 2014, [115]| Sudan        | 12          | 50.0           | Cow     | PTZ  | ELISA           | High    |
| Mulunda and Mike, 2014, [116]| South Africa| 125         | 85.6           | —       | Raw  | HPLC            | Medium  |
| Kos et al., 2014, [117]| Serbia       | 150         | 98.7           | Cow     | Raw  | ELISA           | High    |
| Han et al., 2013, [118]| China        | 200         | 36             | Cow     | Raw  | ELISA           | High    |
| Bilandžić-1 et al., 2014, [119]| Croatia| 337         | 99.9           | Cow     | Raw  | ELISA           | High    |
| Bilandžić-2 et al., 2014, [119]| Croatia| 32          | 98.5           | Cow     | Raw  | ELISA           | High    |
| Picinin et al., 2010, [120]| Brazil     | 129         | 14.0           | Cow     | Raw  | ELISA           | High    |
| Asi-1 et al., 2012, [121]| Pakistan    | 36          | 50.0           | Buffalo | Raw  | HPLC            | High    |
| Asi-2 et al., 2012, [121]| Pakistan    | 22          | 54.5           | Cow     | Raw  | HPLC            | High    |
| Asi-3 et al., 2012, [121]| Pakistan    | 24          | 54.2           | Goat    | Raw  | HPLC            | High    |
| Asi-4 et al., 2012, [121]| Pakistan    | 29          | 48.3           | Sheep   | Raw  | HPLC            | High    |
| Asi-5 et al., 2012, [121]| Pakistan    | 19          | 47.4           | Camel   | Raw  | HPLC            | High    |
| Lee et al., 2009, [122]| South Korea | 100         | 40.0           | Cow     | Raw  | ELISA           | High    |
| Elgerbi et al., 2004, [123]| Libya      | 49          | 71.4           | Cow     | Raw  | HPLC            | High    |
| Ghanem and Orfi, 2009, [124]| Syria     | 126         | 80.2           | Cow, sheep, goat | PTZ  | ELISA           | High    |
| Peng and Chen, 2009, [125]| Taiwan     | 144         | 69.4           | —       | PTZ  | HPLC            | High    |
| Kang’ethe et al., 2007, [126]| Kenya     | 391         | 45.5           | —       | —    | —               | Medium  |
| Sharma et al., 2019, [127]| India      | 150         | 42.7           | Cow     | Raw  | HPLC            | High    |
| Albar et al, 2019, [128]| Pakistan    | 960         | 72.3           | Cow, sheep, goat | Raw  | ELISA           | High    |
| Zakaria et al, 2019, [129]| Egypt       | 90          | 41.1           | —       | Raw, UHT | HPLC            | High    |
| Eker et al, 2019, [130]| Turkey      | 120         | 89.2           | —       | Raw  | ELISA           | High    |
| Tahira et al, 2019, [131]| Pakistan    | 570         | 99.9           | —       | Raw, UHT, PTZ | ELISA           | High    |
| Abbès et al, 2012, [132]| Tunisia     | 112         | 83.9           | Cow     | Raw  | ELISA           | High    |
| Peña-Rodas et al, 2018, [133]| USA        | 15          | 33.3           | Cow     | Raw  | ELISA           | High    |
| Venáncio et al, 2019, [134]| Brazil      | 35          | 85.7           | —       | Raw  | ELISA           | High    |
| Blanco et al, 1988, [17]| Spain       | 37          | 32.4           | Cow     | Raw  | —               | Medium  |
| Garrido et al, 2003, [18]| Brazil       | 60          | 21.7           | Cow     | Raw, UHT, PTZ | HPLC            | High    |
| Carvajal et al, 2003, [19]| Mexico      | 580         | 40.0           | Cow     | Raw  | HPLC            | High    |

*Thin-layer chromatography. ‡The enzyme-linked immunosorbent assay. †High-performance liquid chromatography. §Pasteurized. ¶Ultrahigh temperature.
### 3.1. Meta-Analysis of Results in Studies in Iran

Due to the high number of studies conducted in Iran, it was reported separately. Based on the results of the test ($I^2$: 95.7) and due to the heterogeneity of the selected studies, a random effects model was used to combine the studies. The probability of bias in the results by the Begg and Mazumdar test at the significant level of 0.1 indicated no bias in the present study ($p = 0.788$). There were 60 articles with a sample size of 10132 individuals on the results of the study in Iran, and the prevalence of aflatoxin M1 in milk in Iran was 88.5% (95% CI: 84.4–91.6%) (Figure 4).

Using metaregression, the relationship between the year of study and sample size with the prevalence of aflatoxin M1 in milk worldwide was investigated. The prevalence of aflatoxin M1 in milk increased worldwide as the year increased, and the prevalence decreased with increasing sample size; there was a significant difference between the prevalence of aflatoxin M1 and the sample size ($p ≤ 0.001$) and year of study ($p ≤ 0.001$) (Figures 5 and 6).

The results of metaregression, based on the year of study on each continent, showed that in the three continents of America, Africa, and Europe, with increasing year of study, the prevalence of aflatoxin M1 increases ($p < 0.001$) and decreases in Asia ($p ≤ 0.001$) (Figure 7).

### 4. Discussion

Mycotoxins are biological substances that affect the health, quality, and production of toxic fungi in foods due to the growth of toxigenic fungi. Therefore, the prevalence and amount of different mycotoxins in foods need to be constantly measured and planned in the food chain to minimize them in order to provide the health of consumers [34].

The potential risks of aflatoxin M1 in human health are particularly important in the development of liver cancer through milk and dairy products [74]. There have been various reports of the prevalence of aflatoxin M1 in milk samples [35]. In the present study, the prevalence of aflatoxin M1 in animal milk worldwide was 79.1%.

In a study by Ghaffarian Bahraman et al., the prevalence of aflatoxin M1 in buffaloes (86%), cows (86%), sheep (42%), goats (42%), and camels was reported (30%) [135]. The overall prevalence of raw milk aflatoxin in Iran, Turkey, Lebanon, Palestine, Egypt, and Syria was 76, 12, 67, 85, and 38%, respectively, in a systematic review and meta-analysis conducted by Rahmani et al. [136], and these two studies are in line with the present study.

The milk free of aflatoxin is considered desirable, but it is not easy to achieve this ideal. Therefore, all countries (depending on their particular circumstances) accept milk contamination with some amounts of this toxin [35].

The most effective way to prevent aflatoxin M1 contamination of milk is to reduce aflatoxin B1 in food and supplements used in dairy cattle. Therefore, it is recommended that the feed available be monitored permanently for the amount of aflatoxin contamination. Storage and harvesting of forage and other feedstuffs should be technically and hygienically performed, and feeds susceptible to molding, especially flour, bread, pulverized sugar beet pulp, and wet and moldy fodder should be removed from the diet of lactating animals [81].

| Study name     | Event rate | 95% CI Lower | 95% CI Upper | t-value | P-value |
|----------------|------------|--------------|--------------|---------|---------|
| Carvajal       | 0.400      | 0.361        | 0.440        | -4.384  | 0.000   |
| Venâncio      | 0.857      | 0.700        | 0.939        | 3.609   | 0.000   |
| Eker           | 0.892      | 0.822        | 0.936        | 7.177   | 0.000   |
| Zakaria        | 0.411      | 0.314        | 0.515        | -1.678  | 0.093   |
| Sharma         | 0.427      | 0.350        | 0.507        | -1.790  | 0.073   |
| Kang’ethe      | 0.455      | 0.406        | 0.505        | -1.768  | 0.077   |
| Peng           | 0.694      | 0.615        | 0.764        | 4.538   | 0.000   |
| Ghanem         | 0.802      | 0.723        | 0.862        | 6.250   | 0.000   |
| Asi -5         | 0.474      | 0.268        | 0.689        | -0.229  | 0.819   |
| Asi -3         | 0.542      | 0.346        | 0.725        | 0.408   | 0.683   |
| Biland         | -0.999     | 0.977        | 1.000        | 4.603   | 0.000   |
| Kos            | 0.987      | 0.948        | 0.997        | 6.046   | 0.000   |
| Suliman       | 0.986      | 0.946        | 0.996        | 5.976   | 0.000   |
| Siddappa       | 0.667      | 0.518        | 0.788        | 2.192   | 0.028   |
| Abdallah      | 0.823      | 0.733        | 0.887        | 5.746   | 0.000   |
| Sadia          | 0.763      | 0.704        | 0.813        | 7.571   | 0.000   |
| Ruangwises     | 0.998      | 0.968        | 1.000        | 4.362   | 0.000   |
| Polovinski-Horvatovi? | 0.311 | 0.224        | 0.414        | -3.491  | 0.000   |
| Mwanza         | 0.987      | 0.849        | 1.000        | 3.172   | 0.002   |

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**Figure 3**: Prevalence of aflatoxin M1 in milk worldwide and 95% confidence interval.
Table 2: Analysis of subgroups by continents.

| Continent | N   | Sample size | I²  | Begg and Mazumdar | Prevalence (95% CI) |
|-----------|-----|-------------|-----|-------------------|---------------------|
| Asia      | 92  | 14651       | 95.1| 0.110             | 82.6 (95% CI: 78.8–85.9) |
| Europe    | 14  | 1498        | 94.9| 0.381             | 79.1 (95% CI: 63.4–89.2) |
| Africa    | 9   | 1079        | 94  | 0.465             | 76.4 (95% CI: 61.7–86.7) |
| America   | 7   | 1637        | 93.7| 0.763             | 41.3 (95% CI: 30.1–53.5) |

Figure 4: Prevalence of aflatoxin M1 in milk in Iran and 95% confidence interval.

Given that the quality of milk received is the most important factor affecting the level of contamination of dairy products with aflatoxin M1 and the processes that take place at the plant (even if it is accompanied by the growth of fungi) have no role in producing this mycotoxin, factories must therefore avoid accepting contaminated milk to improve the quality of their products [28].

This is not possible in some cases due to the geographical location of the plant. Because the climatic conditions of dairy cattle affect their milk contamination, as the Tajkarimi study

Figure 5: Metaregression of the relationship between sample size and prevalence of aflatoxin M1 in milk.

in Iran also showed, milk contamination varies significantly across geographical regions [28].

The papers in this study used three methods of ELISA, HPLC, and TLC to measure aflatoxin M1, and the results of the three methods were not significantly different. All three methods have been introduced as valid methods in this regard [137].

Most of the papers used the ELISA method because it is an easy and inexpensive method for screening tests. It is said to be that one of the disadvantages of this method is that it shows the contamination higher than the actual amount [34].

The results of our systematic review and meta-analysis show that milk contamination rates are on the rise in most countries, and this could be a matter for policy makers to take seriously the quality of animal nutrition. However, this increase was not statistically significant, and in some countries, there was a declining trend, indicating that the health quality of animal nutrition in some countries increased.
According to thermal process sources, it does not have much effect on aflatoxin contamination [138]. Therefore, no specific reason was found to justify the difference between industrial and traditional farming as well as between different factories located in different regions. There is even some uncertainty about the effect of milk pasteurization process on aflatoxin M1 levels [139]. This lack of information and uncertainties makes it difficult to decide on the desired methods for decontamination and to determine acceptable levels of contamination in each area and factory, making it difficult to predict the extent of contamination. Although factors such as forage, climatic conditions, forage storage, and a number of other factors are considered important and effective in the rate of this contamination, but the extent and effect of any of the factors is not well clear. Therefore, a precise answer to this question needs special study in this field.

Considering the international public demand for food hygiene, contamination of mycotoxins by various foods has received much attention. This led to the development and evaluation of aflatoxin milk contamination by the GECFA Committee for Expert Evaluation of Toxicology in this field. The Codex, as an international organization, is responsible for providing food regulation facilities to facilitate trade and has established the aflatoxin M1 standard in milk at 0.05 ppb.

Which was higher than the standard value in most of the articles reviewed in different parts of the world. Therefore, by adopting appropriate measures to reach the standard level, it can reduce aflatoxin M1 levels in foods by training and changing nutritional culture.

4.1. Limitations. The fact that some samples were not randomly selected is one of the limitations of this study. Lack of identical reporting of articles, nonsimilarity of the implementation method, and lack of consistency and lack of full text of the papers presented at the conference can be mentioned.

5. Conclusion

The results of this study show that the prevalence of aflatoxin M1 in milk is high worldwide. The highest prevalence was also reported in Asia with 82.6, and the results of metaregression, based on the year of study on each continent, showed that in the three continents of America, Africa, and Europe, with increasing year of study, the prevalence of aflatoxin M1 increases and decreases in Asia. Therefore, due to the importance of the milk group and its products, special measures should be taken to protect the ration from aflatoxin molds and milk quality.

Data Availability

Datasets are available through the corresponding author upon reasonable request.
Ethical Approval

Ethical approval was received from the Ethics Committee of the Deputy of Research and Technology, Kermanshah University of Medical Sciences and Deputy of Research and Technology, Kermanshah University of Medical Sciences (3009778).

Disclosure

The funder has no role in the study process.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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