



Global occurrence and analytical monitoring of aflatoxin m1 in milk and dairy products: a systematic review (2020–2025)

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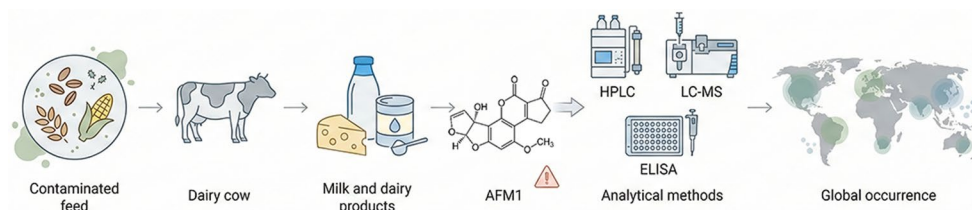
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Abstract

Aflatoxin M1 (AFM1), a hydroxylated metabolite of aflatoxin B1, is excreted in milk following the ingestion of contaminated feed by dairy animals and represents a recurrent chemical contaminant of milk and dairy products. Owing to its stability during conventional processing, AFM1 remains an important target of analytical monitoring worldwide. This study systematically reviews recent scientific evidence on the occurrence and concentration levels of AFM1 in milk and dairy products, with emphasis on analytical determination methods and matrix-dependent variability. The review was conducted in accordance with PRISMA 2020 guidelines. A literature search of three bibliographic databases (PubMed, Scopus and the Web of Science Core Collection) using four parallel search blocks (occurrence/monitoring; analytical methods and validation parameters; complex dairy matrices; dietary exposure and risk assessment) identified studies published between 2020 and 2025 reporting quantitative AFM1 data in raw milk, processed milk, infant formula, and dairy products. Data extraction addressed prevalence, concentration ranges, analytical methodologies, analytical performance parameters (LOD, LOQ, recovery, matrix effects), and geographical distribution. A total of 214 studies met the inclusion criteria. The findings reveal marked geographical variability in AFM1 occurrence and reported concentration levels. Liquid chromatography–based methods were most frequently applied, while immunoassays remained widely used for routine screening. A critical inter-method comparison indicated that LC–MS/MS and HPLC–FLD offer LOD values typically below 0.005 µg/L with recoveries of 80–105%, whereas ELISA platforms operate at higher LOD (0.005–0.05 µg/L) and are prone to matrix-dependent cross-reactivity; aptasensors and electrochemical immunosensors report sub-ng/L sensitivities under laboratory conditions but lack inter-laboratory validation. Methodological heterogeneity in LOD, recovery, sampling design and risk-assessment framework precluded formal meta-analysis; risk of bias was assessed using a JBI/ROBINS-I-adapted narrative framework, with most studies classified as low-to-moderate risk. Overall, this review provides a consolidated overview of global AFM1 occurrence data and analytical monitoring practices in dairy products.

Graphical Abstract



Keywords Aflatoxin M1 · Milk · Dairy products · Occurrence · Prevalence · Systematic review

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Introduction

Milk and dairy products are important sources of macro- and micronutrients in the human diet, especially for infants and children [1, 2]. However, these foods can be contaminated with aflatoxin M1 (AFM1), a hydroxylated metabolite of aflatoxin B1 (AFB1), formed by hepatic biotransformation in ruminants that have ingested feed contaminated with toxins produced by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus* [3, 4]. The rate of metabolic transfer from feed to milk (the “carry-over” effect) varies depending on the physiological factors of the animal and the level of contamination of the diet [5, 6]. AFM1 is classified by IARC as a Group 1 carcinogen, with hepatotoxic, genotoxic and immunosuppressive properties, the risks being amplified by chronic exposure [7, 8]. The toxin has high thermal stability and is not completely eliminated by conventional pasteurisation or UHT sterilisation processes [9, 10], which determines its persistence in finished products. In the case of cheeses, AFM1 concentrates in the curd due to its affinity for casein, generating levels higher than those in the raw milk [11, 12].

International regulations on maximum permissible limits (MPLs) for AFM1 in milk differ significantly: the European Union sets 0.05 µg/kg for raw milk and 0.025 µg/kg for infant formulas, while Codex Alimentarius standards allow concentrations up to 0.5 µg/kg [13, 14]. These ten-fold differences between the legislative thresholds have direct implications for the assessment of product conformity depending on the destination market. Recent studies have indicated that climate change may expand the geographical areas favourable for fungal growth, altering the regional distribution of contamination [15–17].

The literature published in the period 2020–2025 includes a considerable volume of national and regional monitoring studies. However, these studies predominantly report point prevalence data from specific geographical areas, using heterogeneous analytical methods (ELISA, HPLC, UHPLC-MS/MS) with variable detection limits [18, 19]. This methodological and geographical fragmentation of the data makes it difficult to directly compare contamination levels between studies and between regions. Risk assessment is also complicated by the co-occurrence of AFM1 with other mycotoxins in the same samples, as reported in several recent studies [20, 21]. The lack of systematic syntheses integrating these disparate data limits the ability to draw global conclusions about the magnitude of the problem [22, 23].

The main objective of this systematic review is to synthesize and critically analyse the evidence published between 2020 and 2025 on the prevalence and levels of AFM1 contamination in milk and dairy products. The study aims to:

(a) identify geographical patterns of contamination, (b) assess compliance with international legal limits, (c) compare the detection methods used and their impact on the reported data, and (d) identify gaps in the current literature that require further research. Previous systematic reviews and meta-analyses on AFM1 in dairy products have either been restricted to a single country or sub-region [24, 25] or have focused on a single matrix (e.g., cheese globally [26]; infant foods [27]), or have addressed adjacent matrices such as bovine feed [28] or breast milk in sub-Saharan Africa [29]. None of these works combine global geographical coverage of the 2020–2025 evidence with a systematic, side-by-side appraisal of analytical performance parameters (LOD, LOQ, recovery, matrix effects) across the dairy product range. The novelty of the present review lies precisely in this dual lens: an updated global mapping of AFM1 occurrence integrated with a critical comparison of the analytical methods used to generate that evidence, oriented toward the measurement-science scope of this journal.

To structure the investigation, a PICO framework adapted to the specifics of a monitoring study was defined:

- Population (P): Milk and dairy products intended for human consumption — raw milk, heat-treated milk (pasteurised, UHT), powdered milk, infant formulas, cheeses and fermented products.
- Intervention/Exposure (I): Presence of AFM1 resulting from natural contamination by metabolic transfer of AFB1 from feed (excluding artificial inoculation studies) [30, 31].
- Comparison (C): AFM1 levels compared between geographical regions, dairy product types and in relation to the MRLs set by EU and non-EU regulations [32, 33].
- Outcome (O): Prevalence of contamination (percentage of positive samples), reported quantitative concentrations (µg/kg or ng/L) and frequency of exceeding legal limits [34, 35].

Objectives and research questions

The PICO elements introduced above and detailed in Supplementary Material S6 frame the four operational research questions that guided the synthesis:

- i. what is the geographical and matrix-specific pattern of AFM1 occurrence reported between 2020 and 2025;
- ii. to what extent do reported concentrations comply with the contrasting EU and Codex regulatory thresholds;
- iii. how does the choice of analytical platform (ELISA, HPLC-FLD, LC-MS/MS, lateral-flow, aptasensors) influence the comparability of prevalence estimates;

- iv. which knowledge gaps and methodological priorities should orient future monitoring efforts.

To avoid redundancy, the full PICO statement is presented only here and in Supplementary Material S6 and is not restated in Sect. 2.

Methodology

The present study was designed and conducted as a systematic review, strictly adhering to the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency and reproducibility of the evidence synthesis process.

The central objective was governed by an a priori defined PICO framework, focusing on the population represented by milk and dairy products intended for human consumption [18, 36], exposure to aflatoxin M1 (AFM1) resulting from metabolic transfer [5, 37], comparison of contamination levels across different geographical regions and legislative frameworks [13, 38], and evaluation of prevalence and concentrations as the primary outcome [30, 39].

The review followed a PRISMA-compliant methodological protocol publicly deposited on Zenodo: *PRISMA-Compliant Methodological Protocol for Non-Clinical Systematic, Scoping, Meta-Analysis and Structured Review Studies*, Version 1.0 [40]. The protocol provides the methodological backbone for eligibility criteria, literature search, independent dual-reviewer screening, data extraction, quality assessment and evidence synthesis in non-clinical systematic and structured reviews. Because the review addresses a non-clinical food-safety monitoring topic based exclusively on published literature, PROSPERO registration was not applicable. The search strategy was implemented in a single iteration, using three complementary bibliographic databases (PubMed, Scopus and the Web of Science Core Collection), with four parallel search blocks per database covering occurrence, analytical validation, complex dairy matrices and dietary risk (full details in Sect. 2.2 and Supplementary Material S1). The query targeted studies published between 2020 and 2025, to capture the most recent trends in food safety monitoring [1, 35]. The search string employed Boolean operators and specific terms within the “Topic” (TS) field, including variations such as “aflatoxin M1”, “AFM1”, and “aflatoxin m-1”. The complete search strategy applied in all three databases, including all filters and Boolean operators, is provided in Supplementary Material S1. Automated filters were applied to restrict results to research articles and reviews written in English, excluding grey literature, doctoral theses, and unpublished documents, to maintain a high standard of scientific validity [4, 41].

Following the identification stage, the selection process and reference management were performed using EndNote v.21 software, which facilitated cross-database deduplication based on DOI, title, first author, publication year and journal name, with manual verification of borderline records. Of the 2,234 records initially retrieved across PubMed, Scopus and Web of Science, 1,618 duplicates were removed, leaving 616 unique records. Screening was conducted independently by two reviewers in two sequential stages. The first stage consisted of title and abstract screening, during which records not addressing the food matrix of interest or focusing exclusively on experimental toxicological aspects without monitoring data were excluded. The second stage involved full-text assessment of potentially eligible articles by the same two independent reviewers, applying the predefined inclusion criteria: original studies reporting quantitative data on AFM1 in raw milk, pasteurised milk, infant formula, cheese or other eligible dairy matrices. The independent screening results were fully concordant at both stages; therefore, no adjudication or consensus meeting was required.

For data extraction, a standardised data collection form was developed according to the Zenodo-deposited methodological protocol [40] and adapted to the AFM1 monitoring context. Extracted variables included year of publication, country or region, dairy matrix, study design, sample size, analytical platform, LOD, LOQ, recovery, prevalence of AFM1, concentration range and frequency of exceedance of legal limits. Data extraction was performed independently by two reviewers, and the two extraction datasets were compared field by field. No discrepancies were identified; therefore, no reconciliation or adjudication procedure was required. Finally, data synthesis was conducted using a structured narrative approach, organizing the results according to thematic and geographical criteria, in line with the PRISMA flow presented in Fig. 1. Due to significant methodological heterogeneity among studies—manifested through variability in analytical method detection limits and diversity of sampling strategies—statistical meta-analysis was not feasible [31, 42]. Consequently, the analysis focused on identifying contamination patterns and regional discrepancies, integrating data from the 214 studies finally selected to provide a comprehensive overview of dairy product safety, detailed in the subsequent Results section.

Study selection and PRISMA flow

The study selection process was conducted in accordance with the PRISMA 2020 guidelines and is summarised in Fig. 1. A total of 2,234 records were identified across three bibliographic databases: PubMed (n=457), Scopus (n=906) and the Web of Science Core Collection (n=871),

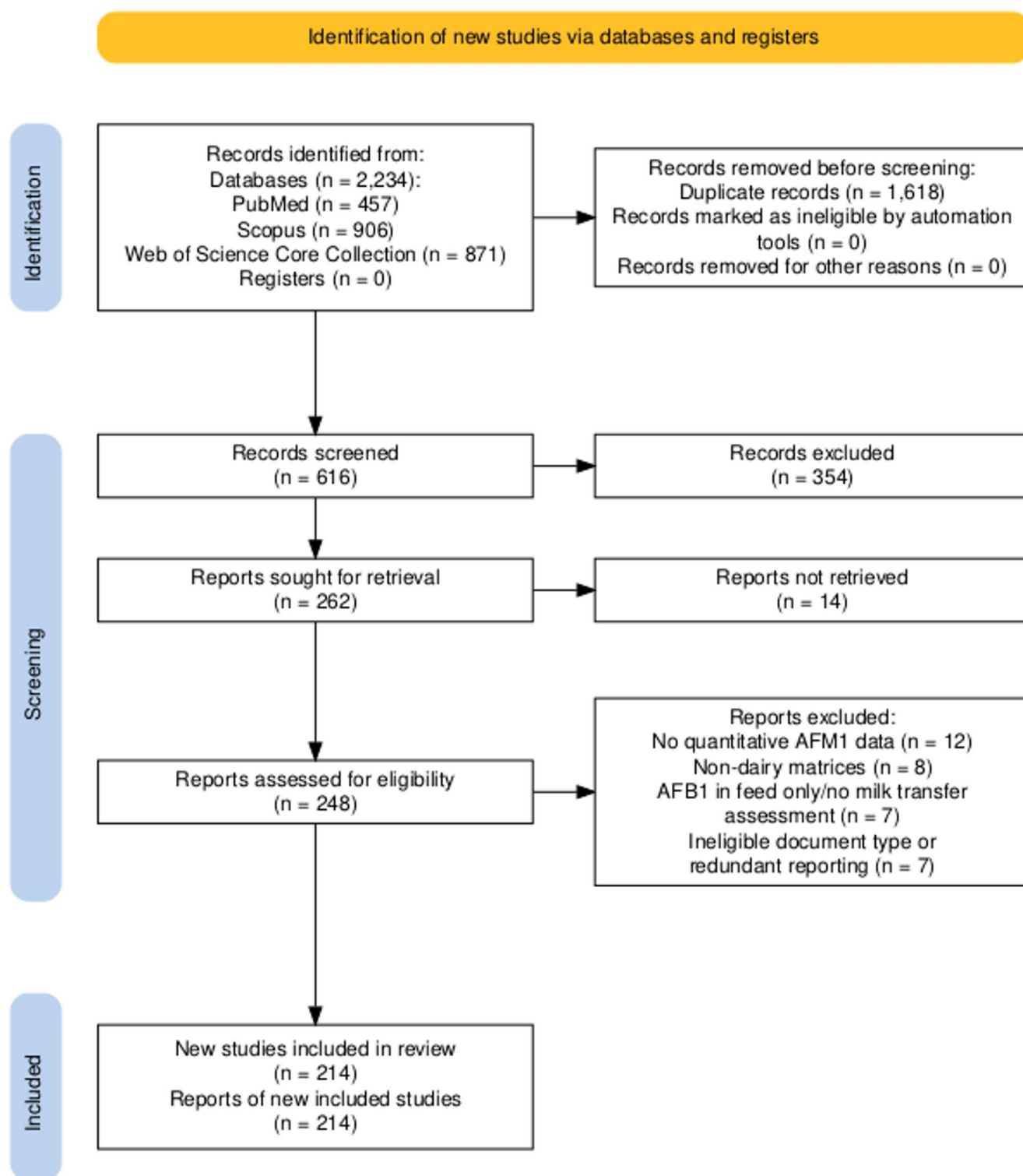


Fig. 1 Prisma flow

retrieved through four parallel search blocks covering occurrence and monitoring, analytical methods and validation parameters, complex dairy matrices, and dietary exposure and risk assessment. After internal deduplication within

each database and cross-database deduplication performed in EndNote v.21 using DOI, title, first author, publication year and journal name as matching keys, with manual verification of borderline records, 1,618 duplicate records were

removed, yielding 616 unique records. Title and abstract screening of these 616 records led to the exclusion of 354 records that did not meet the predefined inclusion criteria.

Subsequently 262 reports were sought for full-text retrieval, of which 14 could not be retrieved (limited institutional access or inactive DOI). As a result, 248 reports were assessed for eligibility at the full-text level. Following detailed evaluation, 34 reports were excluded due to lack of quantitative aflatoxin M1 (AFM1) data ($n=12$), investigation of non-dairy matrices ($n=8$), exclusive focus on aflatoxin B1 in feed without assessment of transfer to milk ($n=7$), or ineligible document type and redundant reporting ($n=7$). The complete list of excluded full-text studies, with documented reasons, is presented in Supplementary Material S3. Ultimately, 214 studies met the inclusion criteria and were included in the final systematic review. The complete selection process and reasons for exclusion at each stage are illustrated in Fig. 1.

Sources of information and search strategy

The search strategy was conducted using three complementary bibliographic databases: PubMed, Scopus and the Web of Science Core Collection. The search was performed, retrieving studies indexed between 1 January 2020 and 31 December 2025, in English, and limited to original research articles for the primary evidence corpus (review articles were considered only for background contextualisation). For each database, four parallel and intentionally overlapping search blocks were applied: (i) occurrence, prevalence and monitoring; (ii) analytical methods, validation, LOD, LOQ, recovery and matrix effects; (iii) cheese, fermented dairy products and complex dairy matrices; and (iv) dietary exposure and human health risk assessment. PubMed searches used the [Title/Abstract] field tag, Scopus searches used TITLE-ABS-KEY, and Web of Science searches used the Topic Search (TS) field (title, abstract, author keywords and Keywords Plus). The block structure was specifically designed to retrieve, in addition to occurrence data, studies reporting analytical performance parameters and matrix-effect data relevant to AFM1 monitoring in complex dairy matrices. The four-block structure across three databases yielded 457 records in PubMed ($226+72+93+66$), 906 records in Scopus ($392+172+199+143$) and 871 records in Web of Science ($379+148+199+145$), for a total of 2,234 records before deduplication. Because the four blocks were intentionally overlapping, this figure does not represent unique records. The complete search strings, including all Boolean operators, field tags and filters for each database and block, are presented in Supplementary Material S1.

Snowballing and additional searches

Snowballing was not applied.

Inter-reviewer agreement

Screening, eligibility assessment and data extraction were performed independently by two reviewers using the predefined eligibility criteria reported in Supplementary Material S5. Both reviewers first screened the 616 deduplicated records at title and abstract level and then independently assessed the full texts of potentially eligible reports. The independent decisions were fully concordant at the title/abstract and full-text stages, including the classification of the 34 full-text exclusions by reason. Because no discordant decisions occurred, no adjudication by a third reviewer and no consensus meeting were required. Inter-reviewer agreement was therefore complete; where reported numerically, agreement may be described as 100% observed agreement, with no unresolved disagreements. The screening process, exclusion reasons and reference-management workflow are documented in Supplementary Materials S3 and S4.

Excluded full-text studies

Articles assessed at the full-text level and subsequently excluded were systematically documented, together with the reasons for exclusion. The main reasons included: lack of quantitative data on aflatoxin M1, ineligible matrix (non-dairy), exclusive focus on aflatoxin B1 in feed without reporting transfer to milk, redundant reporting, or ineligible document type. The complete list of studies excluded at the full-text level is presented in Supplementary Material S3.

Extraction verification

Data extraction was performed independently by two reviewers using a standardised extraction form. Extracted variables included bibliographic information, country or region, dairy matrix, study design, number of samples, analytical method, LOD, LOQ, recovery, prevalence of AFM1, concentration ranges and exceedance of legal limits. The two extracted datasets were compared field by field. No discrepancies were identified between reviewers; therefore, no reconciliation procedure was required. The eligibility criteria, extraction framework and analytical variables are documented in Supplementary Materials S5–S7.

Assessment of publication bias

Publication bias was addressed narratively, by evaluating the completeness of sources and discussing the impact of the absence of grey literature where applicable.

Certainty of evidence

The certainty of the evidence was assessed using a narrative GRADE-adapted approach consistent with the Zenodo-deposited methodological protocol [40]. Each outcome category (occurrence and prevalence, analytical performance, dietary exposure and risk characterisation, decontamination efficacy) was rated as low, moderate or high based on study design, risk of bias, consistency across regions, directness of evidence to the dairy matrix of interest and precision of reported estimates. The complete summary-of-findings matrix is presented in Supplementary Material S8 (Table S2).

Heterogeneity and synthesis

Given the absence of formal meta-analysis, heterogeneity of the included studies was analysed along three explicit axes: methodological, clinical/contextual and statistical. Methodological heterogeneity arose from variability in analytical platforms (ELISA, HPLC-FLD, LC-MS/MS, aptasensors, lateral-flow immunoassays), in matrix-specific extraction protocols (direct dilution, immunoaffinity column clean-up, QuEChERS) and in sampling design (random vs. convenience, single-batch vs. multi-season, farm-gate vs. retail). Clinical/contextual heterogeneity arose from differences in milk-producing species (cow, buffalo, goat, sheep, camel, donkey), dairy matrices (raw milk, pasteurised milk, UHT, infant formula, soft/hard/ripened cheese, yoghurt, kefir, labneh), seasonal coverage and feeding systems (intensive vs. pastoral, conserved feed vs. fresh forage). Statistical heterogeneity was reflected in the reported LOD values spanning more than one order of magnitude (0.001–0.05 µg/L), recovery ranges from 60 to 110%, and prevalence estimates ranging from 0 to 100% within the same matrix across regions. Because of the pronounced methodological, contextual and statistical heterogeneity described above, a formal study-level meta-analysis aimed at deriving a single definitive global AFM1 prevalence estimate was not considered appropriate. Pooling all available prevalence estimates into one global random-effects model would be expected to generate I^2 values close to 100% and summary effects with limited epidemiological interpretability; this is consistent with the experience of previous AFM1-focused meta-analyses that restricted pooling to single countries or narrow geographical regions [25, 26, 43–45]. For these reasons, the

primary synthesis was structured narratively by thematic cluster (Table 1). To support the interpretation of heterogeneity without overclaiming a definitive pooled prevalence, an exploratory subgroup-level random-effects synthesis was additionally performed by macro-region and analytical platform and is visualised in Fig. 5. These subgroup-level pooled estimates were used only to map the direction and magnitude of between-subgroup variability; they were not treated as definitive global prevalence estimates because the prediction intervals and I^2 values indicated extreme residual heterogeneity. Risk of bias of individual studies was assessed using a domain-based narrative framework adapted from JBI checklists for prevalence studies and the ROBINS-I tool, evaluating study design, sampling strategy, analytical method, outcome ascertainment and reporting transparency. The domain-level judgements are summarised in Table 3 and supported the GRADE-adapted certainty ratings reported in Supplementary Material S8.

Results

The literature selection process, conducted in accordance with PRISMA 2020 standards, resulted in a final corpus of studies relevant to the analysis of aflatoxin M1 (AFM1) contamination in milk and dairy products. The flow diagram (Fig. 1) [46] illustrates the filtering stages, starting from the initial identification of records in electronic databases, followed by duplicate removal and screening based on titles and abstracts. Following full-text eligibility assessment, articles that did not report specific quantitative data or that presented major methodological deficiencies were excluded. The detailed characteristics of the included studies, including country of origin, type of samples analysed (raw, pasteurised, UHT milk, cheeses), analytical methods used, and limits of detection, are systematised in Table 2. In addition, the risk-of-bias assessment presented in Table 3 indicates variability in methodological quality, with most studies falling within a low-to-moderate risk range. Fig. 2 provides a bibliometric overview of the 214 included studies, displaying their temporal distribution across the 2020–2025 window (mean = 35.7 studies / year, peak in 2022 with $n=45$), their geographical distribution aggregated by macro-region (Middle East and North Africa $n=62$ / 29.0%; East and South-East Asia $n=38$ / 17.8%; Europe $n=36$ / 16.8%; South Asia $n=30$ / 14.0%; Latin America and the Caribbean $n=22$ / 10.3%; Sub-Saharan Africa $n=18$ / 8.4%; multi-region or global $n=8$ / 3.7%), and their thematic-cluster distribution following the Table 1 categorisation, which together indicate sustained academic interest in this topic over the past five years and a corpus dominated by prevalence (Theme

Table 1 Thematic evidence-map structure of the studies included in the systematic review

Theme	Topic	Studies, n (%)	Dominant analytical platforms / matrices
Theme 1	Prevalence and contamination levels in milk	51 (23.8%)	ELISA screening, HPLC-FLD confirmation and LC-MS/MS/UHPLC-MS/MS quantification where reported. Main matrices/populations: Raw, pasteurised and UHT milk; powdered milk; infant formula when treated as a milk matrix; milk from cow, buffalo, goat, sheep or camel.
Theme 2	Human health risk assessment	35 (16.4%)	Estimated daily intake, hazard index, margin of exposure, Monte Carlo/probabilistic modelling and legal-limit comparison. Main matrices/populations: Milk and dairy-consumption scenarios for adults, children, infants and high-intake consumer groups.
Theme 3	Human bio-markers and transfer into breast milk	18 (8.4%)	Biomonitoring assays, dietary questionnaires, exposure interpretation and transfer/carry-over assessment in human matrices. Main matrices/populations: Breast milk, human milk, urine, biomarker matrices and vulnerable-population exposure settings.
Theme 4	Seasonal variation and environmental factors	21 (9.8%)	Seasonal surveys, paired feed/milk testing, farm questionnaires, climate covariates and carry-over assessment. Main matrices/populations: Feed-milk paired datasets, farm-gate milk, collection-tank milk and regional or seasonal raw-milk datasets.
Theme 5	Advanced detection and analytical methods	27 (12.6%)	LC-MS/MS, UHPLC-MS/MS, HPLC-FLD, ELISA, lateral-flow assays, aptasensors, biosensors, immunosensors and extraction protocols. Main matrices/populations: Milk and dairy matrices used for validation, method comparison or analytical-performance assessment.
Theme 6	Decontamination and reduction strategies	26 (12.1%)	Mineral binders, biological binders, LAB/yeast/probiotics, UV/cold plasma, cyclodextrins, adsorption and processing interventions. Main matrices/populations: Experimentally contaminated milk, fermented products, adsorbent/feed-intervention settings and processing models.
Theme 7	Contamination of cheeses and derived products	36 (16.8%)	Matrix-specific extraction, immunoaffinity clean-up, HPLC/LC-MS/MS confirmation and ELISA with matrix-effect interpretation. Main matrices/populations: Cheese, yoghurt/yogurt, kefir, doogh, kashk, labneh, ice cream and traditional or fermented dairy products.

Thematic clustering of all 214 included studies based on primary-topic assignment. Each study is counted once under its dominant thematic contribution

1), cheese-matrix (Theme 7) and human-risk-assessment (Theme 2) evidence.

Reporting compliance with the PRISMA 2020 guidelines was systematically assessed using the official PRISMA 2020 checklist. The completed checklist, indicating the location of each required reporting item within the manuscript, is provided in Supplementary Material S2.

To address the concern that individual studies were previously mentioned too generally, the Results section now cites representative studies in each thematic subsection and integrates the aggregate evidence classification directly into the narrative synthesis. This approach preserves PRISMA transparency while avoiding long citation strings in the narrative text; complete study-level bibliographic coverage is retained in Supplementary Material S7.

Theme 1: prevalence and contamination levels in milk (51 studies)

Analysis of data on AFM1 occurrence reveals substantial geographical heterogeneity and considerable variation depending on the type of milk analysed. In the context of

marketed milk, [47] investigated contamination levels in raw, pasteurised, and UHT milk samples in Brazil, identifying the presence of the mycotoxin while emphasising the need for continuous monitoring to ensure compliance with local regulations. Similarly, Xiong, Peng [48] conducted a large-scale study in central and southern China, analysing 734 samples; the results indicated variable prevalence between raw and heat-treated milk, suggesting that standard thermal processing does not fully eliminate the risk of contamination. This persistence of AFM1 in finished products is also confirmed by studies conducted in Pakistan by Iqbal, Waqas [49], which reported high occurrence in milk and dairy products from the Punjab region, frequently exceeding the maximum limits permitted by the European Union. Regarding comparisons across milk-producing species, [50] provided a valuable comparative perspective by analysing cow, goat, buffalo, camel, and yak milk samples in China. The results highlighted notable differences in contamination rates, with cow's milk often being more contaminated than milk from other species, although Akinyemi, Braun [51] also detected mycotoxins in camel and goat milk in Nigeria, underscoring the ubiquity of the risk. Studies from

Table 2 Representative characteristics of selected included studies

Theme / manuscript subsection	Studies, n (%)	Typical study design / evidence type	Main matrix or population	Analytical or assessment approach	Key extracted variables used in synthesis
Theme 1: Prevalence and contamination levels in milk	51 (23.8%)	Cross-sectional surveys, surveillance studies and monitoring studies reporting AFM1 prevalence or concentration levels.	Raw, pasteurised and UHT milk; powdered milk; infant formula when treated as a milk matrix; milk from cow, buffalo, goat, sheep or camel.	ELISA screening, HPLC-FLD confirmation and LC-MS/MS/UHPLC-MS/MS quantification where reported.	Country or region, sample type, sample size, prevalence, concentration range, regulatory exceedance, analytical platform and LOD/LOQ where available.
Theme 2: Human health risk assessment	35 (16.4%)	Dietary-exposure, probabilistic or deterministic risk-assessment and regulatory-compliance studies.	Milk and dairy-consumption scenarios for adults, children, infants and high-intake consumer groups.	Estimated daily intake, hazard index, margin of exposure, Monte Carlo/probabilistic modelling and legal-limit comparison.	Exposure estimate, consumption assumption, age group, regulatory threshold, exceedance frequency, uncertainty model and risk metric.
Theme 3: Human bio-markers and transfer into breast milk	18 (8.4%)	Human biomonitoring and vulnerable-population exposure-context studies.	Breast milk, human milk, urine, biomarker matrices and vulnerable-population exposure settings.	Biomonitoring assays, dietary questionnaires, exposure interpretation and transfer/carry-over assessment in human matrices.	Biomarker matrix, vulnerable group, detection frequency, concentration range, exposure route and dietary association.
Theme 4: Seasonal variation and environmental factors	21 (9.8%)	Seasonal, farm-level, feed-to-milk and environmental determinant studies.	Feed-milk paired datasets, farm-gate milk, collection-tank milk and regional or seasonal raw-milk datasets.	Seasonal surveys, paired feed/milk testing, farm questionnaires, climate covariates and carry-over assessment.	Season, climate zone, farm type, feeding system, feed-storage context, AFB1/AFM1 linkage and contamination trend.
Theme 5: Advanced detection and analytical methods	27 (12.6%)	Analytical-method development, validation, comparison and confirmatory-monitoring studies.	Milk and dairy matrices used for validation, method comparison or analytical-performance assessment.	LC-MS/MS, UHPLC-MS/MS, HPLC-FLD, ELISA, lateral-flow assays, aptasensors, biosensors, immunosensors and extraction protocols.	Analytical platform, extraction approach, LOD, LOQ, recovery, matrix effect, cross-reactivity, throughput and validation status.
Theme 6: Decontamination and reduction strategies	26 (12.1%)	Experimental mitigation, detoxification, binding, processing-intervention and reduction studies.	Experimentally contaminated milk, fermented products, adsorbent/feed-intervention settings and processing models.	Mineral binders, biological binders, LAB/yeast/probiotics, UV/cold plasma, cyclodextrins, adsorption and processing interventions.	Intervention type, starting AFM1 level, reduction percentage, exposure time, matrix, analytical confirmation and toxicological interpretability.
Theme 7: Contamination of cheeses and derived products	36 (16.8%)	Matrix-specific occurrence, product-behaviour and derived-dairy monitoring studies.	Cheese, yoghurt/yogurt, kefir, doogh, kashk, labneh, ice cream and traditional or fermented dairy products.	Matrix-specific extraction, immunoaffinity clean-up, HPLC/LC-MS/MS confirmation and ELISA with matrix-effect interpretation.	Product type, processing state, concentration factor, casein/protein binding, matrix recovery, prevalence and regulatory comparison.

Studies, n (%) refers to the primary thematic assignment of the 214-study corpus. The complete study-level dataset for all 214 included studies is provided in Supplementary Material [S7](#)

Europe, such as that by Hasonová, Samková [52] in the Czech Republic, indicate a more controlled situation, with contamination levels generally meeting strict European standards, although isolated variations remain a concern. In contrast, data from Bangladesh reported by Sumon, Islam [53] and from India by Priyanka, Choudhary [54] describe an alarming situation, where high prevalence is correlated with feeding practices and climatic conditions favourable to fungal growth. Beyond fluid milk, milk powder and infant formula have been the focus of specific investigations, given the vulnerability of the target group. Quevedo-Garza, Amador-Espejo [55] determined the presence of AFM1 in infant formulas from Mexico using immunoaffinity columns and HPLC, with results indicating the need for rigorous controls for these sensitive products. Similarly, Sartori,

de Moraes [56] analysed 123 infant milk powder samples in Brazil, confirming the co-occurrence of multiple mycotoxins, although AFM1 levels were generally below critical limits. These data are complemented by the studies of Fahim, Badr [57], which evaluated not only contamination levels in milk powder but also the potential of innovative detoxification methods, suggesting an integrated direction for monitoring and intervention.

Additional occurrence studies broaden the geographical and matrix coverage of the milk-focused synthesis, including raw, pasteurised, UHT and powdered milk datasets from multiple regions. These reports support the interpretation that prevalence estimates are shaped by sampling season, feed-control infrastructure, analytical platform and age-specific consumption assumptions rather than by geography

Table 3 Qualitative risk-of-bias assessment of the included studies (revised)

Bias domain	Assessment tool / criterion	Description	Overall judgement (n = 214 studies)
Selection / sampling	JB1 Prevalence checklist (Q1, Q3, Q4); ROBINS-I “selection of participants”	Sampling frame, randomisation versus convenience sampling, representativeness of the dairy-producing region, and sample-size adequacy	Moderate: mixed random and convenience sampling
Exposure / measurement	JB1 Q6; ROBINS-I “measurement of exposure”	Validation status of the analytical method, including LOD, LOQ, recovery, confirmation by an independent technique, and matrix-matched calibration	Low for LC-MS/MS and HPLC-FLD; moderate for single-platform ELISA
Outcome ascertainment	JB1 Q5, Q7; ROBINS-I “measurement of outcome”	Consistency of the MRL reference, exceedance definition, and handling of values below the LOD	Moderate
Confounding: regional / seasonal / matrix	ROBINS-I “confounding”	Adjustment for season, feeding system, species, matrix subtype, and multi-batch coverage	Moderate to high
Missing data / attrition	JB1 Q8	Reporting of the proportion of samples below the LOD and treatment of non-detects in summary statistics	Moderate
Selective reporting	ROBINS-I “selection of reported result”	Reporting of all matrices and seasons originally sampled in the results	Low to moderate
Reporting transparency	JB1 Q9	Reporting of LOD, LOQ, recovery, sampling frame, and analytical protocol according to CONSORT-like good-practice principles	Moderate: frequent partial reporting
Overall risk of bias	Integrated narrative judgement across domains	Composite judgement combining the seven domains above for each included primary study	Low (n = 38); low-to-moderate (n = 112); moderate-to-high (n = 64)

Risk of bias was assessed independently by two reviewers using a domain-based narrative framework consistent with the Zenodo-deposited methodological protocol [40] and combining items from the Joanna Briggs Institute (JBI) checklist for prevalence studies with the ROBINS-I tool, adapted to observational and cross-sectional monitoring studies. Domain-level judgements were aggregated qualitatively to an overall study-level judgement. The two reviewers reached identical domain-level and overall classifications; therefore, no adjudication or consensus meeting was required. No study was excluded solely on the basis of risk of bias; instead, certainty of evidence was downgraded where domain-level concerns clustered

alone [58–60]. The aggregate evidence classification is now incorporated in the narrative synthesis, while complete study-level bibliographic details and citation coverage remain in Supplementary Material S7.

Ahmed et al. Ahmed, Al-Kahtani [61] provided key monitoring data on the prevalence and distribution of aflatoxin M1 in milk. In a parallel investigation, Akash et al. Akash, Rehman [62] investigated the presence of AFM1 in maternal milk in Germany, highlighting the necessity of systematic local surveillance. Furthermore, Akash et al. Akash, Rehman [62] provided key monitoring data on the prevalence and distribution of aflatoxin M1 in milk in Pakistan.

Arrua, Arrúa [63] evaluated the incidence of AFM1 in maternal milk in Paraguay, revealing that 85% of the collected samples were positive. In a parallel investigation, Bulca Bulca [64] investigated the presence of AFM1 in yogurt, raw milk, cheese and UHT milk in Turkey, highlighting the necessity of systematic local surveillance. Demirhan and Demirhan [65] investigated the presence of AFM1 in milk in Turkey, highlighting the necessity of systematic local surveillance.

Gao, Wang [66] investigated the presence of AFM1 in maternal milk, highlighting the necessity of systematic local surveillance. In a parallel investigation, Giacometti, Tomasello [67] investigated the presence of AFM1 in raw milk in Italy, highlighting the necessity of systematic local surveillance. Furthermore, Hajmohammadi, Valizadeh [68] investigated the presence of AFM1 in bovine raw milk in Iran, highlighting the necessity of systematic local surveillance.

Khan, Khan [69] provided key monitoring data on the prevalence and distribution of aflatoxin M1 in milk. In a parallel investigation, Kos, Radić [70] provided key monitoring data on the prevalence and distribution of aflatoxin M1 in milk in Serbia. Furthermore, Leite, Freitas [71] investigated the presence of AFM1 in maternal raw milk, highlighting the necessity of systematic local surveillance.

Mannani, Tabarani [72] investigated the presence of AFM1 in maternal pasteurized milk and UHT milk in Morocco, highlighting the necessity of systematic local surveillance. In a parallel investigation, Monter-Arciniega, Cruz-Cansino [73] investigated the presence of AFM1 in bovine raw milk, highlighting the necessity of systematic local surveillance. Furthermore, Mora-Medina,

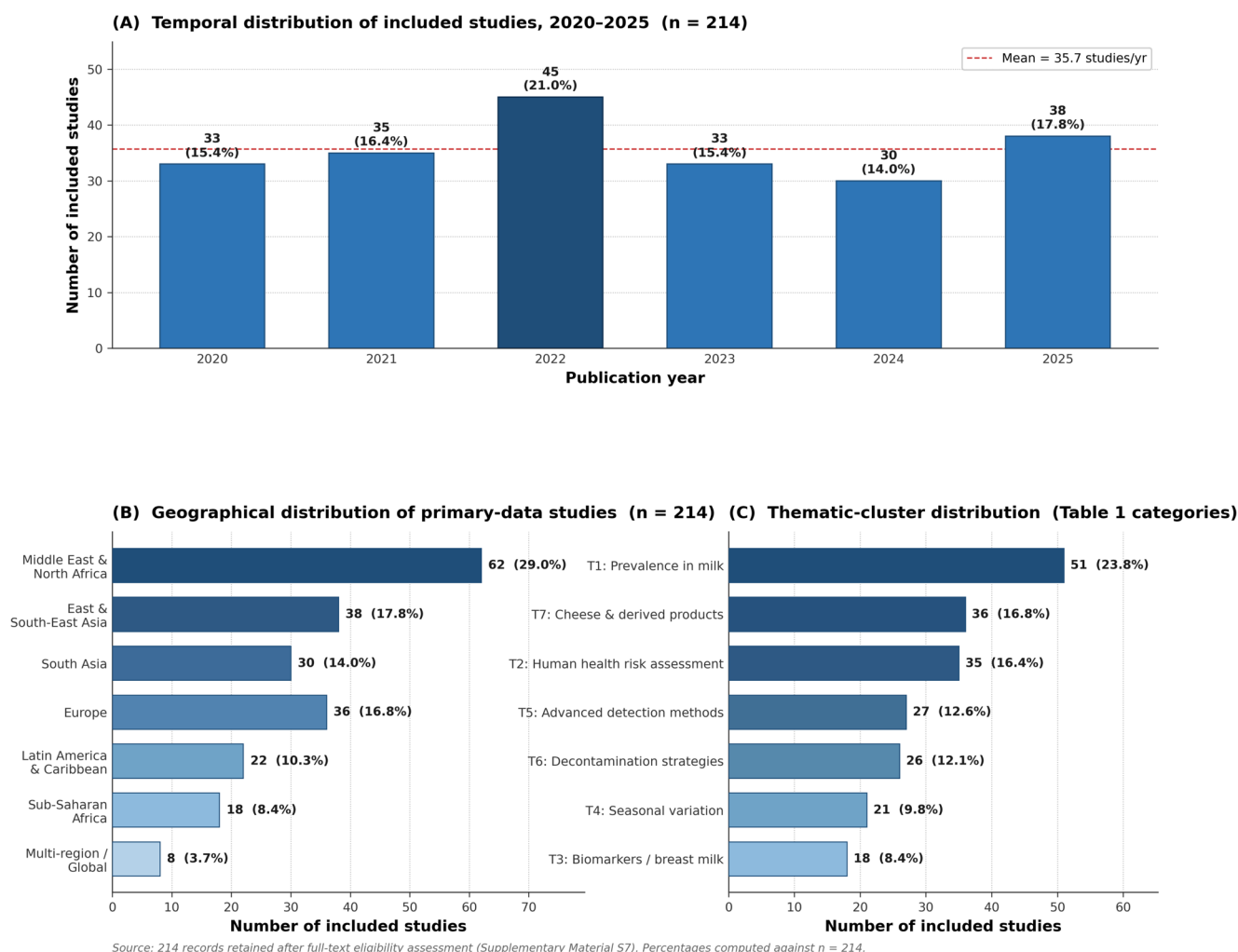


Fig. 2 Bibliometric overview of the 214 studies included in the systematic review(2020–2025)

Lora-Benítez [74] provided key monitoring data on the prevalence and distribution of aflatoxin M1 in milk.

Nasir, L. & Gemede, H. F. Nasir and Gemede [75] investigated the presence of AFM1 in bovine raw milk in Usa and Ethiopia, highlighting the necessity of systematic local surveillance. In a parallel investigation, Newcomer [76] provided key monitoring data on the prevalence and distribution of aflatoxin M1 in bovine milk. Furthermore, Salem-Bekhit, Riad [77] investigated the presence of AFM1 in powdered milk, highlighting the necessity of systematic local surveillance.

Saha Turna, Havelaar [78] investigated the presence of AFM1 in milk, highlighting the necessity of systematic local surveillance. In a parallel investigation, Shahata and Wafy [79] investigated the presence of AFM1 in buffalo yogurt, raw milk and cheese in Egypt, highlighting the necessity of systematic local surveillance. Furthermore, Tadesse, Berhanu [80] investigated the presence of AFM1 in yogurt,

butter and cheese in Ethiopia, highlighting the necessity of systematic local surveillance.

Uka and Abdallah [81] provided key monitoring data on the prevalence and distribution of aflatoxin M1 in milk in Kosovo. In a parallel investigation, Yanying et al. Yanying, Na [82] investigated the presence of AFM1 in bovine milk, highlighting the necessity of systematic local surveillance. Furthermore, Zahra et al. Zahra, Jamil [83] investigated the presence of AFM1 in milk in Pakistan, highlighting the necessity of systematic local surveillance.

Theme 2: human health risk assessment (35 studies)

The quantitative assessment of the risk associated with exposure to AFM1 through the consumption of dairy products has been based on two distinct methodological approaches: deterministic and probabilistic. The deterministic approach, adopted in studies such as those of Milicevic, Milesevic [84], estimates exposure by multiplying the mean or 95th

percentile concentration by the average daily population intake, by age group. In this study, the analysis of 3404 milk samples from Serbia indicated that the mean exposure of children aged 1–9 years is below the toxicological reference values, but consumer segments with high intake (upper percentiles) pose a potential higher risk. It is important to note that the assessments carried out by EFSA and other regulatory authorities already include differentiated scenarios by consumer categories, including high-consumption groups, infants and children, which should be considered when interpreting the comparative results Kiani, Sharafi [85].

The probabilistic approach, implemented through Monte Carlo simulations, has been frequently used in studies in the Middle East. Jafari, Fathabad [32] compared the risk associated with traditional versus industrial milk consumption in Iran, with results indicating a significantly higher risk for traditional milk, attributed to lower feed quality control. Hasninia, Salimi [86] and Naghshbandi, Ali Omrani [87] applied the same methodology in various Iranian provinces, reporting exceedances of the Hazard Index (Hazard Index > 1) among children.

These two approaches are not directly comparable in terms of accuracy and underlying assumptions: deterministic methods tend to overestimate or underestimate risk depending on the selected point parameters, while Monte Carlo simulations provide probability distributions, but critically depend on the quality of the input data on consumption and contamination [6]. This methodological difference requires caution in directly comparing results between studies and highlights the need for standardization of risk assessment protocols at an international level.

The carcinogenic risk was estimated using the cancer potency factor for liver cancer, and global studies, such as that of Wu, Huang [88] suggest that although AFM1 is classified as IARC group 1, its specific contribution to the global liver cancer burden may be smaller compared to AFB1 from cereals. However, the risk remains significant for infant populations with high milk consumption relative to body weight [20]. Hoteit, Abbass [21] extended the assessment to co-exposure with other mycotoxins (Ochratoxin A), demonstrating that the cumulative effect amplifies the risk compared to single substance assessments. In developing countries such as Ethiopia, the margin of exposure (MOE) calculated by Zebib, Abate [89] and Geleta, Nugussa [90] was frequently below the threshold of 10,000, considered a safety reference.

Behtarin and Movassaghghazani [91] conducted an exposure assessment and health risk characterization of AFM1 through the consumption of yogurt and cheese in Iran. In a parallel investigation, Conteçotto, Pante [92] conducted an exposure assessment and health risk characterization of AFM1 through the consumption of milk in

Brazil. Furthermore Costamagna, Gaggiotti [93] conducted an exposure assessment and health risk characterization of AFM1 through the consumption of traditional in Argentina.

Foerster, Monsalve [94] evaluated the dietary intake and health risk metrics for consumers of milk contaminated with AFM1 in Chile. In a parallel investigation, Gde Rama Wisesa, Yesica [95] evaluated the dietary intake and health risk metrics for consumers of raw milk contaminated with AFM1 in Usa, Indonesia and United States. Furthermore, Ghaffarian-Bahraman, Mohammadi [96] evaluated the dietary intake and health risk metrics for consumers of raw milk and pasteurized milk contaminated with AFM1 in Iran.

Hassouna, Salah-Abbès [97] assessed the human exposure risk associated with contaminated bovine milk consumption in Tunisia, reporting a 97.54% contamination rate and calculating exposure margins. In a parallel investigation, Hattimare, Shakya [98] evaluated the dietary intake and health risk metrics for consumers of maternal fermented contaminated with AFM1 in India. Furthermore, Ilievska, Stojanovska-Dimzoska [99] evaluated the dietary intake and health risk metrics for consumers of yogurt, ice cream, cheese, UHT milk and cream contaminated with AFM1 in Macedonia.

Kaur, Bedi [100] evaluated the dietary intake and health risk metrics for consumers of butter and cheese contaminated with AFM1. In a parallel investigation, Kortei, Annan [101] evaluated the dietary intake and health risk metrics for consumers of bovine raw milk contaminated with AFM1 in Ghana. Furthermore, Messina, Licata [102] evaluated the dietary intake and health risk metrics for consumers of bovine milk contaminated with AFM1 in Italy.

Mokhtari, Nemati [103] evaluated the dietary intake and health risk metrics for consumers of maternal milk contaminated with AFM1 in Iran. In a parallel investigation, Monter-Arciniega, Cruz-Cansino [104] evaluated the dietary intake and health risk metrics for consumers of bovine milk contaminated with AFM1 in Mexico. Furthermore, Nazari, Ebrahimi [105] assessed the human exposure risk associated with contaminated maternal ice cream and cream consumption in Iran, reporting a 4.7% contamination rate and calculating exposure margins.

Nemati, Khajehali [106] evaluated the dietary intake and health risk metrics for consumers of raw milk contaminated with AFM1. In a parallel investigation, Omeragic, Marjanovic [107] evaluated the dietary intake and health risk metrics for consumers of raw milk contaminated with AFM1 in Herzegovina and Bosnia. Furthermore, Roila, Branciaro [108] evaluated the dietary intake and health risk metrics for consumers of bovine cheese contaminated with AFM1 in Italy and Switzerland.

Sharma, Jadhav [109] evaluated the dietary intake and health risk metrics for consumers of raw milk contaminated

with AFM1 in India. In a parallel investigation, Tahiri, Risto [110] conducted an exposure assessment and health risk characterization of AFM1 through the consumption of milk in Albania. Furthermore, Kovač Tomas, Rot [111] evaluated the dietary intake and health risk metrics for consumers of fermented contaminated with AFM1.

Theme 3: human biomarkers and transfer into breast milk (18 studies)

Although the primary focus of this review is on the safety of commercial dairy products, the contamination of breast milk with AFM1 is a direct extension of the food safety issue. Breast milk, as a metabolic excretory product, reflects maternal exposure to AFM1 through consumption of contaminated foods, including dairy products, and represents an additional route of exposure for infants. The inclusion of this topic is justified because AFM1 in breast milk serves as a biomarker of the effectiveness (or inefficiency) of control systems in the food chain. Movassaghazani and Shabansalmani [112] assessed AFM1 levels in breast milk and formula milk in Iran, identifying a positive correlation between maternal diet and the concentration of excreted toxin. Cherkani-Hassani, Ghanname [113] associated the prevalence in human milk in Morocco with frequent consumption of contaminated dairy products and cereals. These results confirm that the safety of human milk is directly dependent on the quality of the food consumed by the mother, including the level of contamination of commercial dairy products.

Urinary biomonitoring studies provide complementary information on exposure in the general population. Ahmad, Jamaluddin [114] quantified the metabolite in the urine of Malaysian residents, demonstrating widespread exposure in both urban and rural settings. Winkler, Xu [115] explored the use of albumin-aflatoxin adducts as biomarkers of chronic exposure, providing a long-term perspective distinct from fluctuations reflected by point-in-time urinary measurements. Memis, Yalçın [116] analysed the transfer of several mycotoxins into breast milk, without identifying a direct correlation with growth retardation, but the constant presence of the toxin was also confirmed by [117].

These data emphasise that a complete assessment of the impact of dairy contamination must also include the indirect route of exposure through breast milk, especially in regions where food monitoring is deficient.

Alvito and Pereira-da-Silva [118] monitored the presence of AFM1 in maternal breast milk, assessing maternal-infant carry-over and associated infant exposure risks. In a parallel investigation, Aref, Omar [119] evaluated maternal dietary exposure and the subsequent carry-over of aflatoxins into human breast milk. Furthermore, Ferri, Brera [120]

monitored the presence of AFM1 in bovine milk, assessing maternal-infant carry-over and associated infant exposure risks.

Intanoo, Kongkeitkajorn [121] monitored the presence of AFM1 in milk, assessing maternal-infant carry-over and associated infant exposure risks. In a parallel investigation, Mirzaei, Yazdanfar [122] monitored the presence of AFM1 in bovine infant formula and human milk, assessing maternal-infant carry-over and associated infant exposure risks. Furthermore, Pokharel, Webb [123] monitored the presence of AFM1 in yogurt in Nepal, assessing maternal-infant carry-over and associated infant exposure risks.

Theme 4: seasonal variation and environmental factors (21 studies)

The seasonal influence on AFM1 levels is closely related to feeding practices and feed storage conditions. In temperate regions, peak concentrations were recorded in the winter and spring months, a pattern documented by Hasonová, Samková [52] in the Czech Republic and by Gurler, Birdane [124] in Turkey. This trend is attributed to the transition from grazing to a diet based on preserved and concentrated feeds, which are more susceptible to *Aspergillus* contamination under inadequate storage conditions. Krstovic, Jaksic [125] confirmed the correlation between climatic conditions in the year preceding feed harvest and peaks in milk contamination in Serbia. It is important to emphasise that the seasonal pattern described above is not universal. In tropical and equatorial regions, where there are no clear summer/winter variations, contamination may be constant throughout the year or may follow other patterns. The high humidity and constant temperatures in these areas favor permanent fungal growth, blurring or eliminating the seasonal differences observed in temperate zones. Studies from South Asia Sumon, Islam [53], Priyanka, Choudhary [54] report high levels of contamination regardless of the season, and some research has identified peaks of contamination during the rainy seasons [126, 127]. Therefore, “seasonality” should not be treated as a universal risk factor, but as a proxy for feed storage practices and local microclimatic conditions, which differ fundamentally between temperate and tropical or equatorial zones.

Elsayed, Elsharkawy [128] analysed buffalo milk in Egypt during four seasons, identifying autumn and winter as critical periods. Min, Tong [129] associated fluctuations with farm type, noting that industrial farms have a better ability to control feed quality throughout the year compared to small producers. Hepsag, Anitas [130] reported that increasingly hot and dry summers in the Mediterranean region favor fungal growth on field crops, exerting increased pressure on milk contamination in subsequent

seasons. Ullah, Nasir [127] reported that, in addition to season, moisture and forage storage temperature are critical predictors, suggesting that post-harvest management is as important as crop growing conditions.

These variations imply that monitoring protocols need to be adapted to the specific climate: intensified monitoring in the cold season for temperate areas and continuous monitoring, without seasonal reduction in testing frequency, for tropical and equatorial regions.

Álvarez-Días, Torres-Parga [131] analysed the seasonal fluctuations of AFM1 levels in bovine raw milk and pasteurized milk in Mexico, finding significantly higher levels during colder months due to feed storage conditions. In a parallel investigation, Haidar, Abboud [132] analysed the seasonal fluctuations of AFM1 levels in bovine raw milk in Lebanon, finding significantly higher levels during colder months due to feed storage conditions. Furthermore, Ismaiel, Tharwat [133] documented the seasonal occurrence and environmental influences on mycotoxin contamination in regional traditional production in Egypt.

Maqbool, Sasanya [134] investigated the environmental determinants and seasonal patterns of aflatoxin contamination in bovine milk supplies. In a parallel investigation, Mohammedi-Ameur, Dahmane [135] analysed the seasonal fluctuations of AFM1 levels in bovine raw milk in Usa and Algeria, finding significantly higher levels during colder months due to feed storage conditions. Furthermore, Rangel-Munoz, Valdivia-Flores [136] investigated the environmental determinants and seasonal patterns of aflatoxin contamination in raw milk supplies in Mexico.

Thukral, Dhaka [137] investigated the environmental determinants and seasonal patterns of aflatoxin contamination in bovine milk supplies in India. In a parallel investigation, Vega, Costamagna [138] investigated the environmental determinants and seasonal patterns of aflatoxin contamination in bovine raw milk supplies in Panama. Furthermore, Zeweld, Welearegay [139] investigated the environmental determinants and seasonal patterns of aflatoxin contamination in traditional supplies in Ethiopia.

Theme 5: advanced detection and analytical methods (27 studies)

The analytical methods used for AFM1 quantification in the included studies can be classified into three main categories: chromatographic methods (HPLC-FLD, UHPLC-MS/MS), immunochemical methods (ELISA, lateral-flow assays), and emerging biosensor-based methods. Liquid chromatography remains the reference method for accurate confirmation and quantification. González-Jartín, Rodríguez-Cañás [140] developed a multi-analyte UHPLC-MS/MS method preceded by QuEChERS extraction, which allows the

simultaneous quantification of 40 mycotoxins. Although this multi-analyte capability is relevant for reference laboratories, its applicability in routine AFM1 monitoring remains limited by cost and instrumental complexity. Chen, Ye [141] proposed the automation of sample pretreatment using immunomagnetic beads, reducing analysis time and operator variability.

A critical aspect, insufficiently addressed in the recent literature, concerns extraction efficiency and recovery rates, which directly influence the comparability of results. Matrix-dependent specificity of the extraction protocol is particularly important in complex matrices such as ripened cheeses, where AFM1 binding to casein can reduce analyte recovery [142]. Studies that do not report recovery rates or that do not use adequate internal standards generate significant uncertainties in the estimation of true prevalence. Also, the variability of the limits of detection (LOD) between studies—from 0.001 µg/L to 0.05 µg/L—creates difficulties in directly comparing positive sample rates: a study with an ultra-low LOD will inevitably report an apparent higher incidence than one with reduced sensitivity, without this reflecting real differences in contamination.

Immunochemical methods, especially ELISA, remain predominant in routine screening due to their low cost and accessibility. However, ELISA has documented limitations in terms of specificity, with the risk of cross-reactions in complex matrices, as reported by [143]. Pimpitak, Rengpipat [144] validated a lateral-flow assay with a cut-off value of 0.5 µg/L, useful for point-of-reception monitoring in small farms.

Regarding emerging methods, it should be noted that biosensors and aptasensors such as those described by Qiao, Guo [145] and Wang, Shan [146] are in their early stages of development. Although they demonstrate extremely low detection limits under laboratory conditions, these platforms have not yet been widely validated under real-world conditions and have not replaced conventional methods in current monitoring practice. Hui, Peng [147] and Camizci Aran and Bayraç [148] explored dual detection platforms based on DNA hydrogels, capable of simultaneously identifying AFM1 and other contaminants, but their industrial applicability remains to be demonstrated. Naz, Hayat [149] presented a portable electrochemical immunosensor, promising for field use, but needs to be evaluated in larger inter-laboratory studies. Overall, the methodological heterogeneity observed in the included studies highlights the need for international standardization of analytical protocols, including minimum requirements for reporting LOD, LOQ and recovery rates, to allow valid comparisons between data from different regions and laboratories.

Additional analytical studies reinforce this conclusion because they cover nanobody-based immunoassays,

AOAC-validated ELISA and HPLC-based validation in complex dairy matrices. These reports strengthen the argument that LOD, LOQ, recovery and matrix specificity should be reported before prevalence values are compared across regions [150–152].

Abdul-Hay, Shendy [153] developed and validated a highly sensitive analytical method for the rapid determination of AFM1 in whey and colostrum, achieving excellent limits of detection and recovery. In a parallel investigation, Ansari, Askari [154] developed and validated a highly sensitive analytical method for the rapid determination of AFM1 in raw milk in Iran, achieving excellent limits of detection and recovery. Furthermore, Aydemir Atasever, Özlü [155] designed and evaluated an innovative analytical or biosensing framework for the detection of aflatoxins in milk.

Esam, Hafez [156] developed and validated a highly sensitive analytical method for the rapid determination of AFM1 in raw milk and cheese, achieving excellent limits of detection and recovery. In a parallel investigation, Ramezani, Jalalian [157] developed and validated a highly sensitive analytical method for the rapid determination of AFM1 in maternal milk, achieving excellent limits of detection and recovery. Furthermore, Jiménez-Pérez, Tello-Solís [158] developed and validated a highly sensitive analytical method for the rapid determination of AFM1 in bovine milk, achieving excellent limits of detection and recovery.

Khan, Khan [69] developed and validated a highly sensitive analytical method for the rapid determination of AFM1 in milk in Pakistan, achieving excellent limits of detection and recovery. In a parallel investigation, Leite, Freitas [159] designed and evaluated an innovative analytical or biosensing framework for the detection of aflatoxins in bovine milk. Furthermore, Tarannum, Nipa [160] developed and validated a highly sensitive analytical method for the rapid determination of AFM1 in raw milk, pasteurized milk and UHT milk in Bangladesh, achieving excellent limits of detection and recovery.

Theme 6: decontamination and reduction strategies (26 studies)

Strategies to reduce AFM1 levels in milk can be classified into three categories: biological methods (use of microorganisms), physical methods (heat treatments, radiation) and the use of adsorbents. Each category presents specific advantages and limitations that condition the practical applicability.

Biological methods. The use of microorganisms for AFM1 sequestration has been investigated in several studies. Pires, Portinari [161] demonstrated that heat-inactivated *Saccharomyces cerevisiae* cells reduce the bioavailability of AFM1 in yogurts, the mechanism being attributed to the

binding of the toxin to cell wall components (glucans, mannoproteins). Hajimohammadi, Ehrampoush [45] and Sanaldi and Coban [162] confirmed the ability of certain strains of lactic acid bacteria to function as sequestrants, the efficiency being dependent on the strain, the initial toxin concentration and the incubation conditions (pH, temperature, time). It is important to emphasise that the predominant mechanism is physical binding, not chemical degradation of the toxin: AFM1 remains structurally intact, but bound to the microbial biomass, which raises the question of whether the toxin could be partially released into the digestive tract after ingestion. This distinction between reducing the detectable concentration and eliminating the toxicological risk is not sufficiently addressed in most of the studies reviewed.

Physical methods. Emerging technologies such as cold plasma and UV irradiation have demonstrated variable efficacy. Nguyen, Palmer [163] reported the degradation of AFM1 by high-voltage atmospheric cold plasma in skim milk, with reduction rates dependent on processing parameters (power, exposure time). The same group Nguyen, Palmer [164] investigated UV irradiation at 254 nm, demonstrating reductions dependent on exposure time and liquid layer depth. Simko and Kolaric [165] observed a concomitant decrease in AFM1 and cholesterol using beta-cyclodextrin. However, none of these treatments have been evaluated on an industrial scale, and their potential impact on the nutritional and organoleptic properties of milk—including lipid oxidation, vitamin degradation, and texture modification—requires further investigation before practical implementation. It should be noted that the high thermal stability of AFM1 is well established in the literature prior to the period investigated; conventional pasteurisation and UHT sterilisation processes have a limited impact on toxin concentrations [9, 10], this aspect being extensively documented since the fundamental studies of the 2000s.

Adsorbents. The use of mineral adsorbents in feed remains the most feasible strategy to reduce AFB1 → AFM1 transfer in milk. Ranftler, Zehentner [166] demonstrated the efficiency of purified clinoptilolite for the sorption of both forms of aflatoxin. Kuboka, Njue [167] and Nishimwe, Bowers [168] evaluated commercial adsorbents (Novasil™) under farm conditions in Kenya and Rwanda, reporting significant reductions in AFM1 in milk. Jiménez-Pérez, Roldán-Hernández [169] explored modified polysaccharides as a biodegradable alternative.

Mitigation-oriented literature was used only to interpret possible control points in the dairy chain, because decontamination experiments, binding studies and processing simulations do not provide primary occurrence estimates. Their value lies in clarifying why reductions in detectable AFM1 may reflect adsorption, matrix binding or analytical

recovery rather than complete toxicological elimination [11, 170, 171]

Adriansyah, Rahayu [172] evaluated the effectiveness of novel biological binding agents and processing interventions for reducing AFM1 levels in contaminated milk supplies. In a parallel investigation, Aloui et al. Aloui, Ben Salah-Abbès [173] demonstrated the sequestration or enzymatic degradation of AFM1 in maternal milk using physical or biological agents, achieving substantial toxin reduction. Furthermore, Onmaz, Cinicioglu [174] demonstrated the sequestration or enzymatic degradation of AFM1 in cheese in Turkey using physical or biological agents, achieving substantial toxin reduction.

Shandiz, Ziaratnia [175] demonstrated the sequestration or enzymatic degradation of AFM1 in maternal milk using physical or biological agents, achieving substantial toxin reduction. In a parallel investigation, Youssef, El Gammal [176] evaluated the effectiveness of novel biological binding agents and processing interventions for reducing AFM1 levels in contaminated milk supplies.

Theme 7: contamination of cheeses and derived products (36 studies)

Cheeses represent a complex analytical matrix in which the concentration of AFM1 is influenced by the manufacturing process, in particular by the separation of whey and the interaction of the toxin with proteins in the curd matrix. The phenomenon of AFM1 concentration in cheeses compared to the raw milk is well documented: Goncalves, Ulliana [177] reported consistently higher levels in Minas Frescal cheese from Brazil, and Kolaric, Vinohradská [142] confirmed this pattern on the Slovak market, attributing the mechanism to the high affinity of AFM1 for casein, which concentrates in the curd. The concentration factor varies depending on the type of cheese (soft vs. hard), moisture content and the technological process applied, which requires the use of specific conversion factors in the risk assessment.

It is worth noting that some European studies have reported the absence of detectable AFM1 in commercial cheese samples, reflecting the effectiveness of the control systems implemented in the EU. For example, Gützkow, Al Ayoubi [178] investigated mycotoxins in cheeses from Europe, and Pavicich, Compagnoni [179] analysed the occurrence of mycotoxins in cheeses, both studies being relevant for completing the contamination picture in this matrix. The inclusion of these studies, including those with negative results (AFM1 below LOD), is essential to avoid reporting bias and for a balanced assessment of the overall situation.

Murshed, Rizwan [180] analysed traditional and commercial cheeses from Yemen, reporting a higher incidence

in artisanal products, where the lack of standardization of processes favors the persistence of contaminants. Onmaz, Cinicioglu [174] detected the simultaneous presence of moulds and aflatoxins in traditional mouldy “Civil” cheese, highlighting the difficulty in distinguishing between technological and toxicogenic flora. Izzo, Mikusova [41] identified a wide spectrum of secondary metabolites in commercial cheeses, indicating the potential for co-contamination.

An important methodological limitation in cheese studies concerns the variability of the LODs used. Studies with less sensitive methods may report a falsely low incidence, omitting samples with low levels but detectable by reference methods. Direct comparison of prevalences between studies with different LODs (from 0.002 to 0.05 µg/kg) is therefore problematic and should be interpreted with caution.

Fermented dairy products, such as yogurt, show different dynamics. Xiong, Wen [181] observed that lactic fermentation can slightly reduce detectable levels of AFM1 in yogurts, but the toxin remains stable in the final product. Pena-Rodas, Martinez-Lopez [182] showed that pressing and long ripening processes in hard cheeses can lead to final concentrations exceeding the permitted limits, even when the initial milk was compliant. de Franca, de Oliveira [183] compared cheeses from organic and conventional systems, finding that the production system influences the contamination profile, but does not eliminate the risk.

The product-specific evidence base was expanded with studies on cheese, yoghurt, fermented milk beverages, ice cream and traditional dairy matrices. These studies show that derived dairy products require separate interpretation because concentration in curd, dilution in whey, fermentation, pH and fat/protein composition can modify both detectable AFM1 levels and analytical recovery [184–186]

Abedini, Sadighara [187] analysed the occurrence and partitioning of AFM1 in traditional, cheese and cream matrices in Iran, highlighting the concentration effect during traditional dairy processing. In a parallel investigation, Akdağ and Çakmak [188] analysed the occurrence and partitioning of AFM1 in cheese matrices, highlighting the concentration effect during traditional dairy processing. Furthermore, Dossou, Seko Orou [189] analysed the occurrence and partitioning of AFM1 in cheese matrices, highlighting the concentration effect during traditional dairy processing.

Ergin, Cakmak [190] analysed the occurrence and partitioning of AFM1 in maternal cheese matrices in Turkey, highlighting the concentration effect during traditional dairy processing. In a parallel investigation, Falihi, Alkaisy [191] analysed the occurrence and partitioning of AFM1 in cheese matrices in Iran, Saudi Arabia, Turkey and Iraq, highlighting the concentration effect during traditional dairy processing. Furthermore, Kortei and Annan [192] investigated the

contamination levels and processing stability of aflatoxins in derived cheese and traditional products.

Maffei, Souza [193] investigated the contamination levels and processing stability of aflatoxins in derived cheese products. In a parallel investigation, Mudannayake, Karunarathne [194] investigated the contamination levels and processing stability of aflatoxins in derived cheese products. Furthermore, Sabatelli, Gambi [195] analysed the occurrence and partitioning of AFM1 in bovine cheese matrices, highlighting the concentration effect during traditional dairy processing.

Torović, Popov [196] analysed the occurrence and partitioning of AFM1 in cheese matrices in Serbia, highlighting the concentration effect during traditional dairy processing. In a parallel investigation, Vaz, Mendonça [197] analysed the occurrence and partitioning of AFM1 in caprine whey and cheese matrices, highlighting the concentration effect during traditional dairy processing.

Cross-sectional synthesis and integration of results

By cross-sectionally analysing the data presented in the previous sections and synthesised in Table 1, clear patterns of interdependence emerge among environmental factors, detection methods, and reported risk levels. A notable discrepancy exists between countries with strict regulations (EU), where studies focus on ultra-sensitive detection methods González-Jartín, Rodríguez-Cañás [140], Chen, Ye [141] to identify traces below 0.05 µg/kg, and developing countries, where research frequently reports massive exceedances of limits and focuses on acute risk assessment [49, 53]. This geographical polarisation is evident from the thematic distribution of the analysed literature, with studies on decontamination strategies and biomarker use being predominantly concentrated in regions with high contamination incidence as a response to an acute public health problem. By comparison, the effectiveness of contamination reduction methods (Theme 6) remains limited in terms of large-scale industrial applicability, with most approaches being evaluated only at laboratory or pilot level [163, 164]. Although biological methods [24, 161] provide a safe alternative, they cannot fully compensate for the high initial contamination levels of raw milk highlighted in Theme 1. Thus, the results indicate that preventive control at the feed level (Theme 4) remains the most effective strategy, directly correlating with reduced human exposure risk (Theme 2). In addition, the methodological heterogeneity observed in Table 2 complicates direct comparison of absolute prevalences. Studies using ELISA sometimes tend to report false-positive or overestimated results compared with those based on HPLC–MS/MS, a critical issue noted by [143]. Nevertheless, the overall tendency for toxin concentration

in cheeses (Theme 7) is a consistent finding, independent of the analytical method, indicating cheeses as an underestimated risk vector compared with fluid milk, particularly for adult populations with high consumption.

Inter-study comparisons and heterogeneity

The heterogeneity of results, visible in variations in reported coefficients of variation and confidence intervals, can largely be attributed to differences in sampling protocols. Studies that used stratified random sampling, such as that by Xiong, Wen [181], tended to report more representative prevalences than those based on convenience sampling from local markets [33, 198]. In addition, differing analytical sensitivity (LOD ranging from 0.001 µg/L to 0.05 µg/L) creates a barrier to direct comparison of “positive sample” rates; a study with an ultra-sensitive method will report an incidence of 100% (traces detected), whereas one with a less sensitive method will report a much lower incidence, even when mean levels are similar. Analysis of seasonal disparities shows that the magnitude of the seasonal effect is far more pronounced in temperate-climate countries [52, 126] compared with tropical ones, where humidity and temperature conditions favour fungal growth year-round [53, 54]. This observation suggests that monitoring strategies must be climatically adapted: intensified winter monitoring for temperate zones and continuous monitoring for tropical zones. Finally, the discrepancy between risk estimated using deterministic versus probabilistic (Monte Carlo) methods [86, 199] underscores the importance of statistical model choice in risk communication to authorities and the public.

Study selection flow

The complete study selection flow is summarised in Fig. 1, in accordance with the PRISMA 2020 flow-diagram template for new systematic reviews. A total of 2,234 records were retrieved across PubMed (n=457), Scopus (n=906) and the Web of Science Core Collection (n=871); no additional records were sourced from registers. After internal and cross-database deduplication in EndNote v.21 (matching on DOI, title, first author, publication year and journal name, with manual verification of borderline records), 1,618 duplicate records were removed, with no records flagged as ineligible by automation tools and none removed for other reasons, leaving 616 unique records eligible for title and abstract screening. Of these, 354 records were excluded at the title/abstract stage. Full-text retrieval was attempted for 262 reports, of which 14 could not be retrieved due to limited institutional access or inactive DOI. The remaining 248 reports were assessed for eligibility at the full-text level, and 34 were excluded with documented reasons: no quantitative

AFM1 data ($n=12$), non-dairy matrices ($n=8$), AFB1 in feed only without a milk transfer assessment ($n=7$), and ineligible document type or redundant reporting ($n=7$); the complete list of excluded full-text studies, with documented reasons, is provided in Supplementary Material S3. Ultimately, 214 studies met all eligibility criteria and were included in the final systematic review (Fig. 1; Supplementary Material S7).

Results of individual studies

The results of individual studies are synthesised in Table 2, including variation in reported indicators and investigated contexts.

Certainty of the evidence (GRADE)

A structured Summary of Findings, including the main outcomes, the level of certainty of the evidence, and the rationale for potential downgrade or upgrade decisions, is provided in the Supplementary Materials (Supplementary Material S8, Table S2).

Fig. 3 provides a critical inter-method comparison of the analytical platforms applied across the 214 included studies. Panel A displays the reported limit of detection (LOD) and limit of quantification (LOQ) ranges for LC-MS/MS and UHPLC-MS/MS (LOD 0.001–0.005 $\mu\text{g L}^{-1}$), HPLC-FLD with immunoaffinity column clean-up (LOD 0.005–0.020 $\mu\text{g L}^{-1}$), ELISA (LOD 0.005–0.050 $\mu\text{g L}^{-1}$), lateral-flow immunoassays (cut-off 0.25–0.50 $\mu\text{g L}^{-1}$) and aptasensors / electrochemical immunosensors (sub-ng L^{-1} under laboratory conditions only), plotted on a logarithmic axis against the EU MRLs (0.025 $\mu\text{g L}^{-1}$ for infant formula and 0.050 $\mu\text{g L}^{-1}$ for raw milk) and the Codex Alimentarius limit (0.500 $\mu\text{g L}^{-1}$). Panel B reports recovery ranges (80–105%, 75–100%, 70–110% and 85–110% for LC-MS/MS, HPLC-FLD, ELISA and aptasensors respectively) against the AOAC / EU 2002/657/EC acceptance band of 70–110%, while Panel C summarises a composite four-level qualitative scorecard across six monitoring-relevant attributes (sensitivity, specificity, throughput, cost-effectiveness, inter-laboratory validation status and EU-MRL fitness), confirming that chromatographic platforms remain the analytical reference while emerging biosensor platforms still

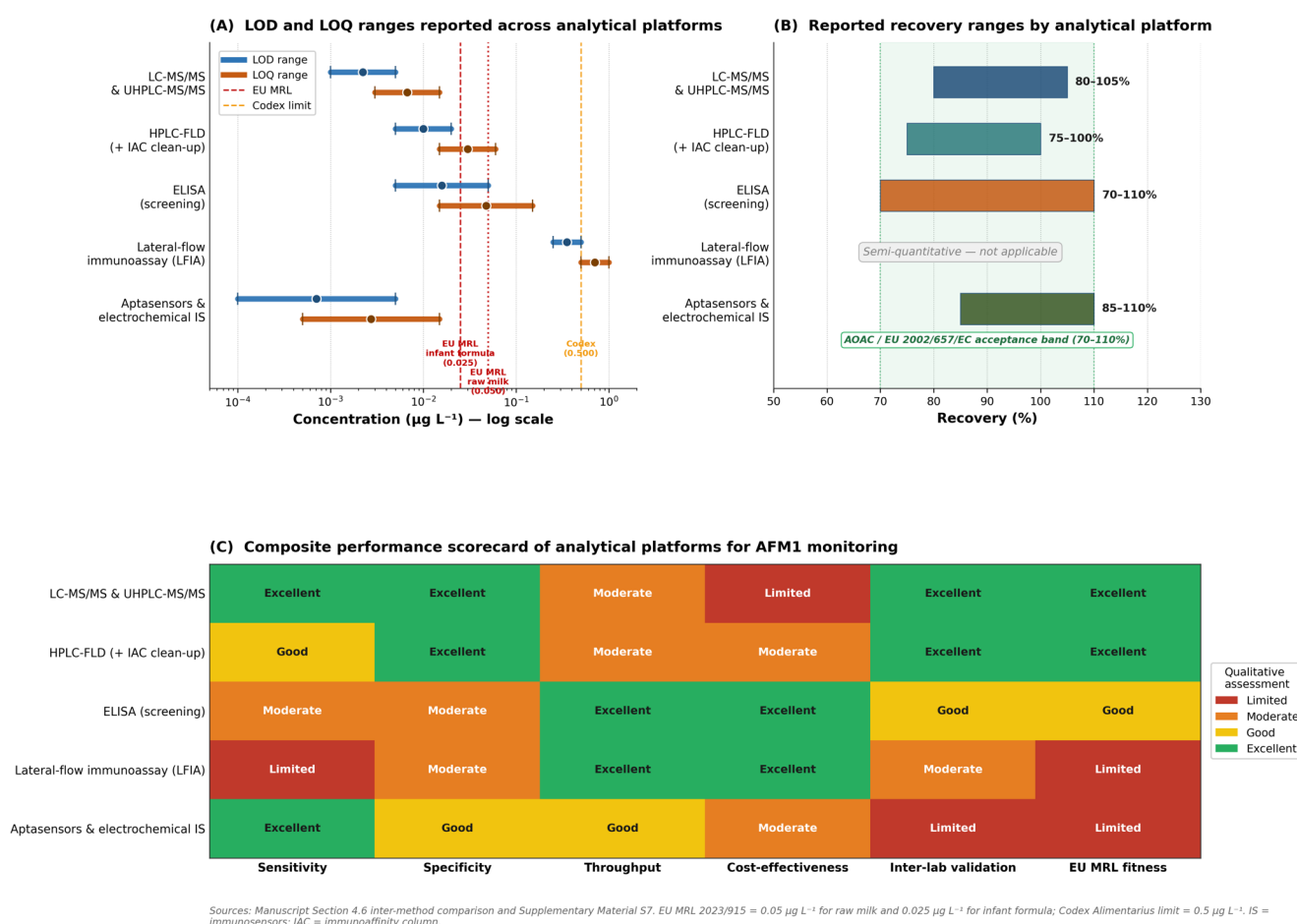
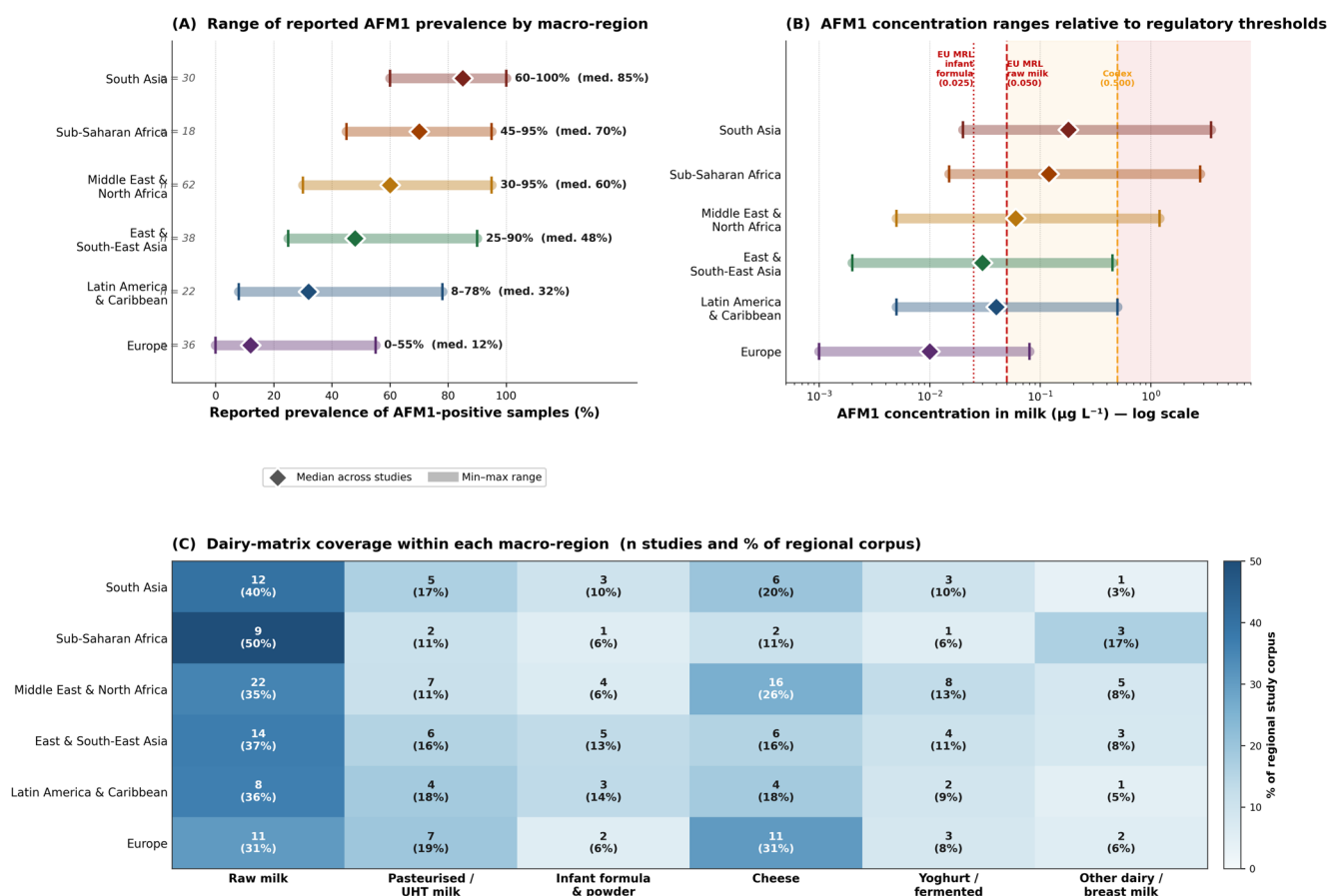


Fig. 3 Inter-method comparison of analytical performance for AFM1 quantification in milk and dairy matrices

lack inter-laboratory validation. Fig. 4 then situates these analytical findings within the global occurrence pattern, displaying in Panel A the range of reported AFM1 prevalence by macro-region (South Asia 60–100%, median 85%; Sub-Saharan Africa 45–95%, median 70%; Middle East and North Africa 30–95%, median 60%; East and South-East Asia 25–90%, median 48%; Latin America and the Caribbean 8–78%, median 32%; Europe 0–55%, median 12%), in Panel B the corresponding AFM1 concentration ranges in raw milk on a logarithmic axis against the EU and Codex thresholds (showing that South Asian, Sub-Saharan African and Middle East / North African ranges routinely cross the EU raw-milk MRL whereas European ranges remain almost entirely below it), and in Panel C a cross-tabulation heat-map of dairy-matrix coverage within each macro-region (raw milk dominates everywhere at 31–50%, cheese is particularly prominent in the Middle East and North Africa at 26% and in Europe at 31%, while infant formula and powdered-milk studies are most frequent in East and South-East Asia and in Latin America). Read together, Figs. 3–5 demonstrate that the apparent heterogeneity in reported AFM1 prevalence is driven by both genuine geographical differences in

feed-control infrastructure and methodological differences linked to analytical platform sensitivity. Fig. 5 extends the descriptive synthesis by providing an exploratory subgroup-level random-effects summary of AFM1 prevalence by macro-region and analytical platform and should therefore be interpreted as a heterogeneity-mapping tool rather than as a definitive pooled global prevalence estimate.

In the macro-regional analysis shown in Fig. 5A, the highest subgroup prevalence was observed in South Asia (82.5%; 95% CI: 81.6–83.4), followed by Sub-Saharan Africa (64.4%; 95% CI: 62.8–66.0), the Middle East and North Africa (57.4%; 95% CI: 56.8–58.1), East and South-East Asia (45.9%; 95% CI: 45.0–46.8), Latin America and the Caribbean (28.6%; 95% CI: 27.5–29.8), and Europe (12.4%; 95% CI: 11.8–13.0). The exploratory random-effects summary across macro-regions was 48.1% (95% CI: 27.5–69.1), but the prediction interval was extremely wide (0.0–99.7), with very high heterogeneity ($I^2=100.0\%$; $p<0.001$). This confirms that a single global prevalence value is not epidemiologically stable and that regional estimates should be interpreted in relation to climate,



Ranges synthesised narratively from the primary-data studies included in the review (Supplementary Material S7); they reflect heterogeneous LOD, sampling design and animal species, and are not meta-analytic pooled estimates. EU MRL: Regulation 2023/915; Codex Alimentarius CXS 193-1995.

Fig. 4 Macro-regional patterns of AFM1 occurrence in milk and dairy products, 2020–2025

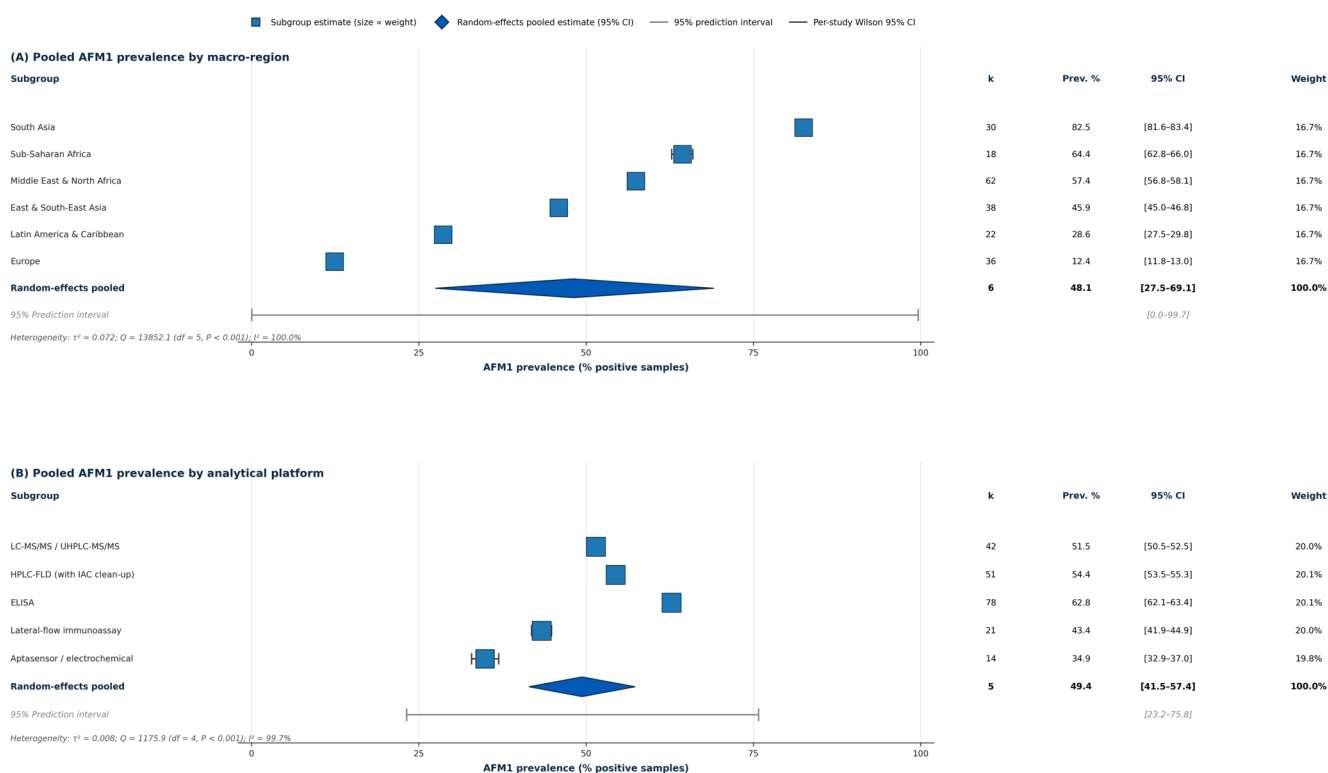


Fig. 5 Exploratory subgroup-level random-effects synthesis of reported AFM1 prevalence

feed-storage conditions, regulatory surveillance and sampling design.

Fig. 5B further shows that prevalence estimates were also platform-dependent. ELISA-based studies produced the highest subgroup prevalence estimate (62.8%; 95% CI: 62.1–63.4), followed by HPLC-FLD with immunoaffinity clean-up (54.4%; 95% CI: 53.5–55.3), LC-MS/MS or UHPLC-MS/MS (51.5%; 95% CI: 50.5–52.5), lateral-flow immunoassays (43.4%; 95% CI: 41.9–44.9), and aptasensor or electrochemical platforms (34.9%; 95% CI: 32.9–37.0). The exploratory pooled estimate across analytical platforms was 49.4% (95% CI: 41.5–57.4), with a broad prediction interval (23.2–75.8) and substantial residual heterogeneity ($I^2=99.7\%$; $p<0.001$). These results support the methodological conclusion that reported AFM1 prevalence is not only a function of contamination pressure, but also of detection limit, matrix effects, assay specificity and the operational context in which each analytical platform is applied.

Discussions

Critical interpretation

The aggregated data from the included studies confirm the persistence of AFM1 contamination in milk and dairy products globally, with significant variations dictated by climatic

conditions, agricultural practices, monitoring infrastructure and the rigor of legislative regulations. The direct correlation between feed quality and the level of contamination in milk is well established; the central mechanism is the hepatic biotransformation of AFB1 to AFM1, extensively documented in the previous literature [3, 10]. The specific contribution of recent studies (2020–2025) does not lie in the rediscovery of this mechanism, but in providing updated data on the magnitude and geographical distribution of the problem.

An important aspect highlighted by this synthesis is the disparity between regions with advanced monitoring systems, where AFM1 levels are generally kept below legal limits by milk segregation, feed control and adsorbent use, and regions with limited monitoring infrastructure, where exceedances are more frequent and intervention capacity is lower [130, 200]. In many European countries and other strictly regulated jurisdictions, functional control programmes already keep the risk at an acceptable level; therefore, the results of this review should not be interpreted as evidence of an absence of preventive measures.

Four interacting drivers explain the observed between-region differences.

First, climate and agro-ecology shape fungal infection pressure along the feed pathway. Humid subtropical and tropical zones, including South Asia, sub-Saharan Africa and parts of the Middle East, report high year-round

prevalence, whereas temperate European settings show contamination peaks mainly during indoor-feeding periods when conserved feeds dominate the ration [52, 125]. Climate-change scenarios suggest that the Mediterranean and Central European belt will progressively move towards a higher-risk regime, consistent with the increasing AFM1 detection frequencies reported in recent regional surveys [201].

Second, feeding-system architecture is more informative than national income alone. Smallholder pastoral systems with limited feed storage infrastructure generally show higher AFM1 concentrations in milk than industrial systems that use quality-controlled compound feed and routine binder strategies, even within the same country [202, 203].

Third, regulatory thresholds differ by an order of magnitude between the European Union (0.05 µg/kg for raw milk and 0.025 µg/kg for infant formula) and Codex Alimentarius (0.5 µg/kg). These differences mechanically generate non-compliance rates that are not directly comparable across jurisdictions unless results are re-coded against a common threshold.

Fourth, analytical uncertainty is itself a systematic source of between-study variability. Studies using LC-MS/MS with LOD ≤ 0.005 µg/L may detect AFM1 in almost all samples from a contaminated region, whereas ELISA-based surveys in the same region may report positive rates of 30–70% because of higher LOD values. Comparisons of absolute prevalence across regions are therefore unreliable unless they are stratified by analytical platform and detection limit. These four drivers operate jointly rather than additively, which explains why simple country-level rankings of AFM1 prevalence are not robust to changes in sampling design or detection method.

Critical evaluation of detection methods

The analysis of the detection methods used in the included studies reveals a coexistence of conventional techniques (HPLC, ELISA) with emerging methods in early stages of development (biosensors, aptasensors). ELISA remains the predominant method for routine screening, due to its low cost and rapidity, but has documented limitations regarding specificity in complex matrices such as ripened cheeses [14, 204]. HPLC-FLD and UHPLC-MS/MS offer superior specificity and sensitivity, but at higher costs.

The claim of a “technological transition” from conventional methods to biosensors and aptasensors needs to be qualified. Although recent publications demonstrate promising analytical performance under laboratory conditions [145, 146, 149], these platforms have not yet been widely adopted in routine monitoring. Accredited laboratories and regulatory authorities continue to predominantly use

HPLC and ELISA, and aptasensors remain at a research and development stage with few industrially validated applications. Therefore, data from the included studies should be interpreted considering the methods actually used, not those under development. A focused inter-method comparison based on the included studies and on recent validation reports clarifies the relative position of each platform. LC-MS/MS and UHPLC-MS/MS are the analytical reference, with reported LOD in milk typically between 0.001 and 0.005 µg/L, LOQ between 0.003 and 0.015 µg/L, recoveries of 80–105% and confirmed performance in inter-laboratory validation [205–207]; their main drawbacks are capital cost, operator expertise and limited throughput. HPLC-FLD coupled with immunoaffinity column clean-up retains regulatory acceptability as a confirmatory method, with LOD of 0.005–0.020 µg/L and recoveries of 75–100%, including in ripened cheese matrices where binding to casein lowers recovery if extraction is not optimised [152, 208]. ELISA remains the dominant screening platform due to its low per-sample cost (~2–5 EUR) and high throughput, but operates at higher LOD (0.005–0.05 µg/L) and is vulnerable to matrix-dependent cross-reactivity, particularly in ripened and mould-fermented cheeses, leading to over- or underestimation of true prevalence relative to LC-MS/MS confirmation. Lateral-flow immunoassays (LFIA) extend ELISA-grade sensitivity (cut-off typically 0.25–0.50 µg/L) to point-of-reception screening but are semi-quantitative and unsuitable for compliance assessment against the 0.05 µg/L EU MRL. Emerging aptasensors and electrochemical immunosensors report ultra-low LOD (often in the pg/L–ng/L range) under laboratory conditions [209–211], yet they have not been validated in inter-laboratory studies, lack matrix-matched calibration data for ripened cheese and yoghurt, and remain unsuitable for routine regulatory monitoring at present. The variability of LOD across two orders of magnitude is the single most important determinant of the apparent prevalence reported by individual studies, which directly limits between-study comparability. International standardisation of minimum reporting requirements for LOD, LOQ, recovery and matrix-effect data are therefore a precondition for any future quantitative synthesis of global AFM1 occurrence.

A critical aspect that complicates inter-study comparability is the variability of analyte recovery rates from complex matrices. Binding of AFM1 to proteins in the milk or cheese matrix may reduce extraction efficiency, and studies that do not report this parameter generate additional uncertainties in estimating the true prevalence [11].

Stability of AFM1 in processing and fermentation

The thermal stability of AFM1 at pasteurisation and UHT sterilisation temperatures is a fact documented in the specialized literature long before the period covered by this review. Recent studies [9, 10] confirm this property, without bringing elements of fundamental novelty. What the studies of 2020–2025 bring is a more detailed investigation of the interaction of AFM1 with the food matrix during processing. For example, partial binding of AFM1 to the cell wall of lactic acid bacteria or to the casein matrix during fermentation reduces the analytically detectable concentration but does not necessarily imply the elimination of the toxicological risk, since the bound toxin could be released into the digestive tract [24, 161]. This distinction between reducing detectable concentration and actual reduction of biological risk is an important nuance that deserves further investigation.

Implications for risk assessment and food chain management

The risk assessment for human health needs to consider several factors that complicate direct extrapolation from prevalence data. It is essential to underline that risk assessments carried out by regulatory authorities (EFSA, Codex Alimentarius) already include differentiated scenarios for consumer categories, including infants, children and high-intake consumers. Therefore, the claim that high-intake groups are not considered is not supported by current regulatory practice. However, data from developing countries suggest that the parameters used in these assessments may need to be updated, especially in regions where consumption and contamination data are incomplete.

Co-exposure to multiple mycotoxins, documented by Hoteit, Abbass [21] and Frey, Rosim [212], suggests that assessments based on single substances may underestimate the true risk. Moving towards an assessment approach that reflects co-exposure is a relevant direction, but its practical implementation remains a methodological challenge.

At a practical level, preventive feed control remains the strategy with the highest demonstrated effectiveness. The use of sequestering agents in animal feed (aluminosilicates, yeast cell walls, zeolites) has been shown to be effective in field studies [166, 167, 213]. It is worth noting that these measures are already widely used in many countries with intensive animal production. Educating producers on optimal feed storage conditions and the risks associated with mould is a cost-effective intervention, especially for small-scale producers in developing countries [200, 214].

The development of rapid, portable, and affordable detection methods (lateral-flow assays, electrochemical

immunosensors) has the potential to facilitate frequent monitoring at farm and collection point levels, especially in regions with limited analytical infrastructure [144, 149].

Contradictions and divergences in the reviewed literature

An important divergence identified concerns seasonal influence, where scientific consensus is fragmented by regional specificity. Studies in temperate areas report higher concentrations in the cold season [124, 215], while in tropical regions, contamination may be constant or follow patterns associated with rainy seasons [126, 127].

Also, divergences regarding the effectiveness of detoxification methods reflect a trade-off between chemical safety and the nutritional value of the product. Aggressive treatments can reduce AFM1 concentration, but risk affecting nutritional properties [163, 216], generating contradictory recommendations in good practice guidelines.

Limitations of the review

The principal limitations of this systematic review are the following. First, although the search was conducted in three complementary databases (PubMed, Scopus and Web of Science Core Collection) using four parallel search blocks, grey literature, theses, conference abstracts and non-English studies were not included; this may have reduced coverage of region-specific monitoring data. Second, the review followed a publicly deposited PRISMA-compliant methodological protocol on Zenodo [40], but it was not registered in PROSPERO because the topic is non-clinical and based exclusively on secondary analysis of published literature. Third, risk of bias was assessed using a JBI- and ROBINS-I-adapted narrative framework suitable for observational and cross-sectional monitoring studies rather than a fully numerical tool, which limits direct comparison with reviews applying quantitative JBI, NOS or ROBIS scoring. Fourth, certainty of evidence was rated narratively using a GRADE-adapted approach because heterogeneity in analytical platforms, LOD, LOQ, recovery, sampling design and risk-assessment frameworks did not permit pooled effect estimates, subgroup meta-analysis or formal numerical sensitivity analyses. Screening, eligibility assessment, data extraction and risk-of-bias assessment were performed independently by two reviewers, with complete concordance and no need for adjudication. Future updates should focus on formal pooled prevalence analyses restricted to comparable strata defined by matrix, region, analytical platform and LOD category.

Conclusions

This systematic review analysed studies published between 2020 and 2025 on AFM1 contamination of milk and dairy products. The main findings can be summarised as follows:

- AFM1 contamination remains widespread globally, with significant geographical heterogeneity: regions with functional monitoring systems and strict regulations (EU, North America, some Asian countries) generally maintain levels below legal limits, while regions with poor control infrastructure frequently report exceedances of maximum permissible limits.
- Methodological heterogeneity of the included studies — variability in LODs, recovery rates, and sampling protocols — limits the direct comparability of prevalence data and highlights the need for international standardization of analytical protocols for AFM1 monitoring.
- Feed quality control remains the most effective strategy to prevent milk contamination. The use of mycotoxin adsorbents, appropriate feed storage practices and systematic monitoring are measures already implemented in many countries and contribute significantly to reducing the risk.
- The seasonal variability of contamination, documented predominantly in temperate regions, indicates the need to adapt monitoring protocols to local climatic specificities, including the intensification of testing during periods of high risk.
- The concentration of AFM1 in cheeses compared to raw milk, through the affinity of the toxin for casein, represents a risk factor that requires specific conversion factors in regulations.
- Emerging detection methods (biosensors, aptasensors, lateral-flow assays) show potential for decentralized monitoring but remain at the research stage and have not yet been validated for routine use on a large scale.

Five concrete priorities for future research and policy action emerge from this synthesis: (i) longitudinal cohort studies to quantify chronic exposure to sub-MRL AFM1 levels in vulnerable groups, particularly infants and high-dairy-consumption sub-populations; (ii) formal inter-laboratory validation of aptasensors and electrochemical immunosensors against accredited LC–MS/MS reference methods, with matrix-matched calibration for ripened cheeses and yoghurt; (iii) cumulative risk assessments that explicitly integrate co-exposure to AFM1, OTA, DON and emerging mycotoxins, rather than single-substance evaluations; (iv) climate-change-adapted monitoring frameworks that adjust sampling frequency to projected fungal pressure in the Mediterranean and Central European belt; and (v) international

harmonisation of mandatory reporting standards for LOD, LOQ, recovery and matrix-effect data in AFM1 surveys, to enable future pooled meta-analyses. International harmonisation of analytical standards and risk-assessment protocols remains a precondition for evidence-based regulation of milk and dairy safety.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11694-026-04612-3>.

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Author contributions Sole author: Conceptualization; Methodology; Data Curation; Formal Analysis; Visualization; Writing - Original Draft; Writing - Review & Editing; Project Administration. Two independent reviewers contributed to screening, eligibility assessment, data-extraction verification and risk-of-bias assessment, as acknowledged below. The author retained full responsibility for study design, synthesis, interpretation, manuscript preparation and final scientific content.

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Data Availability All data extracted from the included studies are available in Supplementary Material S7 (Table S1). The complete search strings are provided in Supplementary Material S1. The methodological protocol used to guide eligibility criteria, dual-reviewer screening, data extraction, risk-of-bias assessment and evidence synthesis is publicly deposited on Zenodo [40]. Additional per-study coding files and risk-of-bias worksheets are available from the corresponding author upon reasonable request.

Declarations

Ethical considerations Not applicable. This study is based exclusively on published literature.

Generative AI and AI-assisted technologies in the manuscript preparation process During the preparation of this manuscript, the author used artificial intelligence-based tools solely for language-related assistance, including improvements in grammar, spelling, and overall clarity of author-written text. No AI system was used to generate scientific content, perform data extraction, conduct analyses, or influence the interpretation of results. All methodological decisions, data synthesis, and conclusions were developed, verified, and approved by the author, who takes full responsibility for the accuracy, originality, and integrity of the published article.

Conflict of interest The author declares that there are no financial, professional, or personal conflicts of interest that could influence the objectivity and impartiality of the results presented in this article.

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