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REVIEW ARTICLE

Aflatoxin M1 Detection Frequency in Cheese: A Meta-analysis of Recovered Evidence with a Systematic Literature Update

Mustafa ATASEVER [1(*)], Rabia Nurselin KALAYCI [1], Halil ÜZÜM [1], Meryem AYDEMİR ATASEVER [1]

[1] Atatürk University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, TR-25240 Erzurum - TÜRKİYE

(*) **Corresponding author:**
Mustafa Atasever
Phone: +90 532 557 29 31
Fax: +90 442 231 72 44
E-mail: atasever@atauni.edu.tr

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Abstract

Reported aflatoxin M1 (AFM_1) detection in cheese depends on contamination, sampling and analytical thresholds. We reanalyzed recovered historical evidence and integrated a systematic database update covering 1 August 2023 through 19 August 2026. The revised primary analysis combined one non-overlapping aggregate per study using logit random-effects meta-analysis with restricted maximum likelihood and study-level robust inference. Sensitivity analyses examined the influence of study inclusion and available quality appraisals, while subgroup and moderator analyses explored differences by analytical method and publication year. The primary set comprised 111 study clusters, 11.369 samples and 5.938 positives. Pooled detection frequency was 56.2% (95% CI 47.4-64.6%); the crude sample-weighted fraction was 52.2%. Heterogeneity was substantial (I^2=95.4%; 95% prediction interval 3.6-97.8%). The secondary 271-stratum model gave 56.3% (95% CI 47.3-65.0%). Among 141 strata from 53 clusters with usable LOD information, the exploratory year-adjusted association per tenfold higher LOD was OR 0.393 (95% CI 0.154-1.004; P=0.051). A separate concentration synthesis included 12 studies. Historical selection decisions and some appraisals could not be recovered, and regional database coverage remained incomplete. These estimates describe the recovered evidence and should not be interpreted as universal prevalence or proof of complete global retrieval.

Keywords: Aflatoxin M1, Cheese, Detection frequency, Evidence reconstruction, Limit of detection, Meta-analysis

Introduction

Aflatoxin M1 (AFM_1), the hydroxylated metabolite of aflatoxin B_1 (AFB_1), can enter milk after lactating animals consume AFB_1-contaminated feed and can persist during dairy processing. In cheese, association with casein and partitioning into curd make measured AFM_1 occurrence dependent on both contamination and the product-specific analytical process [1-3].

The global evidence base is large but methodologically heterogeneous. Studies differ in cheese classification, milk source, geographic setting, sampling season, sample size, analytical platform, immunoaffinity cleanup, limit of detection (LOD), limit of quantification (LOQ), recovery, confirmatory testing, and treatment of non-detects. These features can influence whether a sample is classified as positive and whether concentration summaries from different studies are commensurable. Apparent occurrence heterogeneity may therefore reflect both true contamination and measurement-system heterogeneity. Related analytical surveys in cheese and other dairy products illustrate this measurement context [4,5].

Earlier global and regional syntheses already established that AFM_1 occurs in cheese [6-8]. An updated synthesis is useful only when its contribution and source limitations are explicit. The present work examines analytical measurement, reconstructs an auditable quantitative dataset and separates study-level inference from within-study subgroup reporting. It does not establish an entirely independent body of evidence or methodological superiority over earlier reviews.

The objectives were to summarize detection frequency and a restricted concentration outcome in recovered cheese studies, incorporate a formal literature update, assess sensitivity to study dependence and source discrepancies, and examine exploratory associations with analytical method, publication year and LOD.

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Material and Methods

Design and Registration

This archive-based meta-analysis combined recovered historical evidence with a systematic literature update. PROSPERO CRD420251248533, Version 1.0, was registered on 16 September 2026, after the update cutoff. The record states that no separate full protocol was written. The registry lists an earlier planned completion date of 3 February 2026, whereas the literature update reported here covered 1 August 2023–19 August 2026. Registration was therefore retrospective. Departures from the registered plan included use of logit REML with study-level primary inference rather than Freeman-Tukey pooling alone, separate descriptive benchmark reporting instead of pooled legal-exceedance synthesis, and omission of planned publication-bias analyses for the reasons described below. PRISMA 2020 items were followed where retained records permitted [9]; a complete historical flow could not be reconstructed.

Information Sources and Selection

The historical archive names PubMed/MEDLINE, Scopus and Web of Science through July 2023. It records 2.358 identified records, 1.745 at a combined deduplication/initial-screening checkpoint, 273 full texts and 147 eligible studies. Original exports and individual reasons for 126 full-text exclusions were not fully retained. Quantitative data were recovered for 114 studies; 94 originally contributed frequency effects and 93 remained after excluding one ambiguous numerator. The 33 unrecovered and 20 originally non-contributing recovered studies lack retrospective study-specific exclusion reasons. These are archived checkpoints, not a newly reproduced search.

The update covered 01 August 2023-19 August 2026. Searches combined AFM_1/aflatoxin M1 with cheese, dairy or milk concepts; exact interface-specific syntax was not fully retained. PubMed/MEDLINE, Scopus and Web of Science yielded 178, 328 and 289 date-eligible records (795 total); DOI, PMID and normalized-title matching left 385 unique records. The retained Scopus export contained 390 year-filtered records, including 62 subsequently removed as pre-window. A supplementary ScienceDirect archive contained 1.017 raw and 796 DOI-prioritized unique records; 102 were dated before the window and 34 after its endpoint. Issue and first-online dates for the latter were not fully reconciled. ScienceDirect records were not added to the formal 795.

Revision reopened the study by Kos et al.[10] (U0119) after its archived non-cheese exclusion proved incorrect. The updated register contains 358 other archived exclusions and 27 source-relevant reports: 18 primary frequency contributors and nine quantitative holds. Other archived decisions were preserved, without claiming a fresh independent rescreen of all 385 records. Turkish National Academic Index (TR-Dizin), the National Thesis Center of the Council of Higher Education (YÖK) theses, SID, CNKI and SciELO/LILACS were not comprehensively searched. Retrieving a Türkiye-focused review through its Turkish National Academic Index (TR Dizin)/publisher listing was targeted retrieval; proposed cheese-variety searches were not executed.

Eligibility and Data Reconciliation

Eligible studies reported naturally occurring AFM_1 in cheese and an extractable denominator with at least one quantitative frequency, concentration or threshold outcome. Eligible methods included ELISA/EIA, HPLC/UHPLC, LC-MS/MS, TLC and other validated immunochemical or chromatographic methods. Reviews, editorials, non-data conference abstracts, artificial-spiking or detoxification experiments without separable natural-occurrence data, non-cheese studies and reports without extractable cheese-specific outcomes were excluded from frequency synthesis.

Only non-overlapping observed sample strata were retained. Laboratory replicates, validation spikes and duplicated overall/component summaries were excluded. Hisada et al.[11] reported 152/259, whereas the former extraction used 152/246 after omitting 13 negative imported cheeses. Four complete annual cells (28/61, 65/80, 13/23 and 46/95) replaced 22 incomplete country/year cells; four additional confirmation purchases were excluded. The study by Aydemir Atasever et al.[4] was removed from primary frequency pooling because 65 kashar positives conflict with 80%, 15 negatives and concentration-bin counts totaling 60. Both 65/75 and 60/75 were retained as conditional sensitivities. Separately coherent threshold counts remained usable.

The study by Kos et al.[10] reports cheese results but provides a cheese LOQ of 37 ng/kg without a recovered LOD and incompletely separates other cheese categories; it remains a qualitative/threshold record. Unresolved numerators, below-LOQ negative definitions and threshold exceedances were not converted into detection counts. The primary model contains 111 study aggregates from 271 strata. Source checks and available JBI appraisals do not constitute complete independent verification of every report.

Outcome Definitions and Appraisal

Detection frequency was the source-defined AFM_1-positive count divided by tested samples. ELISA cutoffs, matrix recoveries, LOD/LOQ and confirmation practices varied; detection, quantification and benchmark exceedance were kept distinct. Pavicich et al. [12] report

an AFM_1 LOD of 20 ng/kg in the text and 10 ng/kg in a table; LOD was omitted from the main moderator dataset and both alternatives tested. The study by Kiermeier et al.[13] reports presumptive positives with negative mass-spectrometric proof and was examined in an exclusion sensitivity. The pooled outcome is source-defined detection, not uniformly confirmed chemical identification.

The JBI prevalence checklist [14,15] summarized available study-level judgments. Seventy-two archived appraisals matched the 93 historical primary clusters; 21 were unavailable and marked not assessed. Twenty-six update appraisals were recovered; Kos et al.[10] and the excluded study by Aydemir Atasever et al. [4] have separate provisional appraisals. No fresh independent duplicate appraisal, unsupported total score or risk category was claimed. A sensitivity analysis retained only clusters with archived appraisals.

Statistical Analysis

Positives and denominators were summed across non-overlapping strata within each study, producing one primary logit effect per study. For zero/all-positive cells, 0.5 was added to positives and one to the denominator. Between-study variance used REML. Study-level sandwich inference used CR1 scaling, t-based intervals and G−1 degrees of freedom. The secondary 271-stratum model used diagonal weights $1/(v_i+\tau^2)$, study-summed score contributions and CR1 factor $G/(G-1) \times (k-1)/(k-p)$ [16]. Neither study aggregation nor clustered inference resolves unidentified overlap between separate reports.

Sensitivities restored alternative counts from Aydemir Atasever et al.[4], excluded the study by Kiermeier et al. [13], restricted to available archived JBI records and compared study-level with stratum-level estimates. The primary year regression used study aggregates; exploratory period, method and LOD models used strata with clustered inference. LOD was $\log_{10}$-transformed with centered year. Method, country, recovery and cheese characteristics were not simultaneously adjusted. No causal or multiplicity-adjusted confirmatory claim was made. Q, I^2 and prediction intervals described heterogeneity; stratum-level Q and I^2 were descriptive because strata were correlated.

Concentration pooling required one all-sample-compatible mean, SD and denominator per study; positive-only means and unresolved non-detect handling were excluded. Log-scale inverse-variance random-effects pooling estimated the geometric mean of study-level means, with conventional model-based normal intervals. Planned funnel/Egger and trim-and-fill analyses were omitted because dependence, extreme heterogeneity and boundary proportions impeded interpretation; absence of reporting bias was not inferred. No formal certainty grade was assigned.

A separate descriptive outcome divided above-benchmark counts by all tested samples. Selected 250 and 500 ng/kg benchmarks were not pooled or treated as representative. Source claims about applicable law were not adopted automatically: the EU milk maximum of 0.050 µg/kg is not a universal cheese maximum, and processing/concentration requires product-specific interpretation [3,17]. Python, NumPy and SciPy code and versioned CSV inputs reproduce the numerical results.

Ethics committee approval was not required because only published aggregate data were analyzed.

Results

Evidence and Source Reconciliation

The primary set contained 111 study clusters (93 historical, 18 update), 11.369 tested samples and 5.938 positives *(Fig. 1; Table 1)*. Replacing 22 cells from Hisada et al.[11] with four complete annual cells (+13 samples) and removing four cells from Aydemir Atasever et al.[4] (−304 samples) explains the change from 293 strata/112 clusters/11.660 samples to 271 strata/111 clusters/11.369 samples. A retained reconciliation log accounts for all original 112 clusters. Study-level estimates are shown in *Fig. 2 (panels a-c)*. Selected primary reports span different products, countries and periods [18-47]; a retained source index links all 111 plotted studies to their publications.

The review by Ötün [48] lists 60 publications and excludes theses. Its crosswalk identifies 28 publications represented in the primary set, Aydemir Atasever et al.[4] represented only in sensitivities and 31 not represented; two other Türkiye clusters lie outside its list. Eligibility, sample overlap and data recovery for unrepresented reports remain unresolved. Publication/cluster unit differences do not establish completeness, and secondary percentages were not imported without checking primary counts.

Detection Frequency and Sensitivity

Table 1. *Revised evidence characteristics*

Characteristic	Value
Primary independent study clusters	111
Extracted non-overlapping strata	271
Historical/update clusters	93 / 18
Tested/AFM_1 positive	11,369 / 5,938
Crude sample-weighted detection	52.2%
Historical archived appraisals available	72 of 93; 21 unavailable
Update source records	27; 26 archived appraisals plus provisional appraisal for Kos, 2024 [10]
LOD moderator data	141 strata from 53 clusters

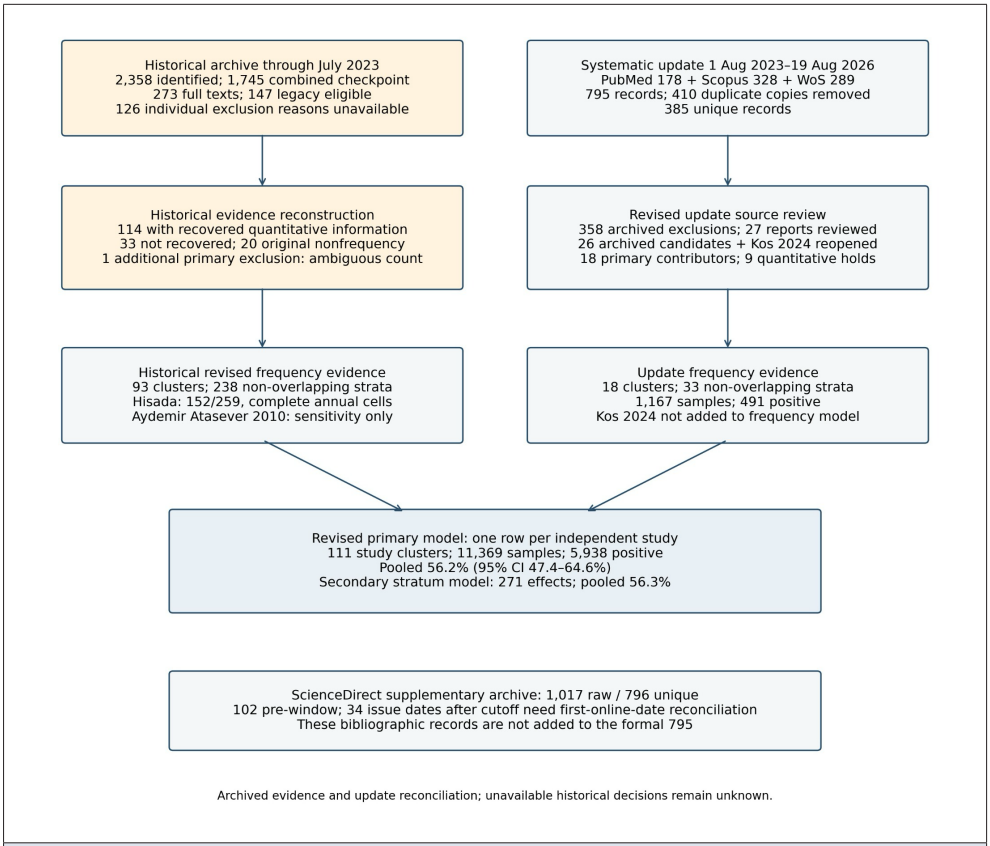


Fig 1. Historical archive checkpoints, revised update source review and analytical reconciliation. Unknown historical reasons are not supplied as known exclusions

The primary study-level pooled detection frequency was 56.2% (95% CI 47.4-64.6%); I^2 was 95.4% and the prediction interval was 3.6-97.8% *(Table 2)*. The crude sample-weighted proportion was 52.2% (5.938/11.369). The crude proportion gives each sample equal weight, whereas the random-effects estimate combines study-level logits with precision and heterogeneity weights before back-transformation; the estimands differ.

The secondary stratum model gave 56.3% (95% CI 47.3-65.0%). Restoring either count from Aydemir Atasever et al.[4] gave 56.3% (47.7-64.6%); excluding the study by Kiermeier et al.[13] gave 56.0% (47.2-64.5%); restricting to available appraisals gave 56.9% (47.8-65.6%). Historical and update study-level estimates were 56.8% (47.5-65.7%) and 54.3% (28.4-78.0%), respectively. Stable averages do not resolve incomplete retrieval or uncertain reporting.

Exploratory Subgroup and Moderator Results

Analytical-method and country subgroup estimates are secondary stratum-level summaries with study-clustered uncertainty *(Table 3; Fig. 3, Fig. 4)*. They must not be interpreted as causal platform effects or national rankings. Sparse-country intervals were wide.

Table 2. *Primary and sensitivity detection frequency analyses*

Analysis	Effects	Studies	Samples	Pooled	95% CI
Primary one row/study	111	111	11369	56.2%	47.4-64.6%
Secondary stratified model	271	111	11369	56.3%	47.3-65.0%
Historical one row/study	93	93	10202	56.8%	47.5-65.7%
Update one row/study	18	18	1167	54.3%	28.4-78.0%
Restore Aydemir Atasever, 2010 [4] (65/75)	112	112	11673	56.3%	47.7-64.6%
Restore Aydemir Atasever, 2010 [4] (60/75)	112	112	11673	56.3%	47.7-64.6%
Remove Kiermeier, 1977 [13]	110	110	11172	56.0%	47.2-64.5%
Available archived JBI	90	90	9005	56.9%	47.8-65.6%

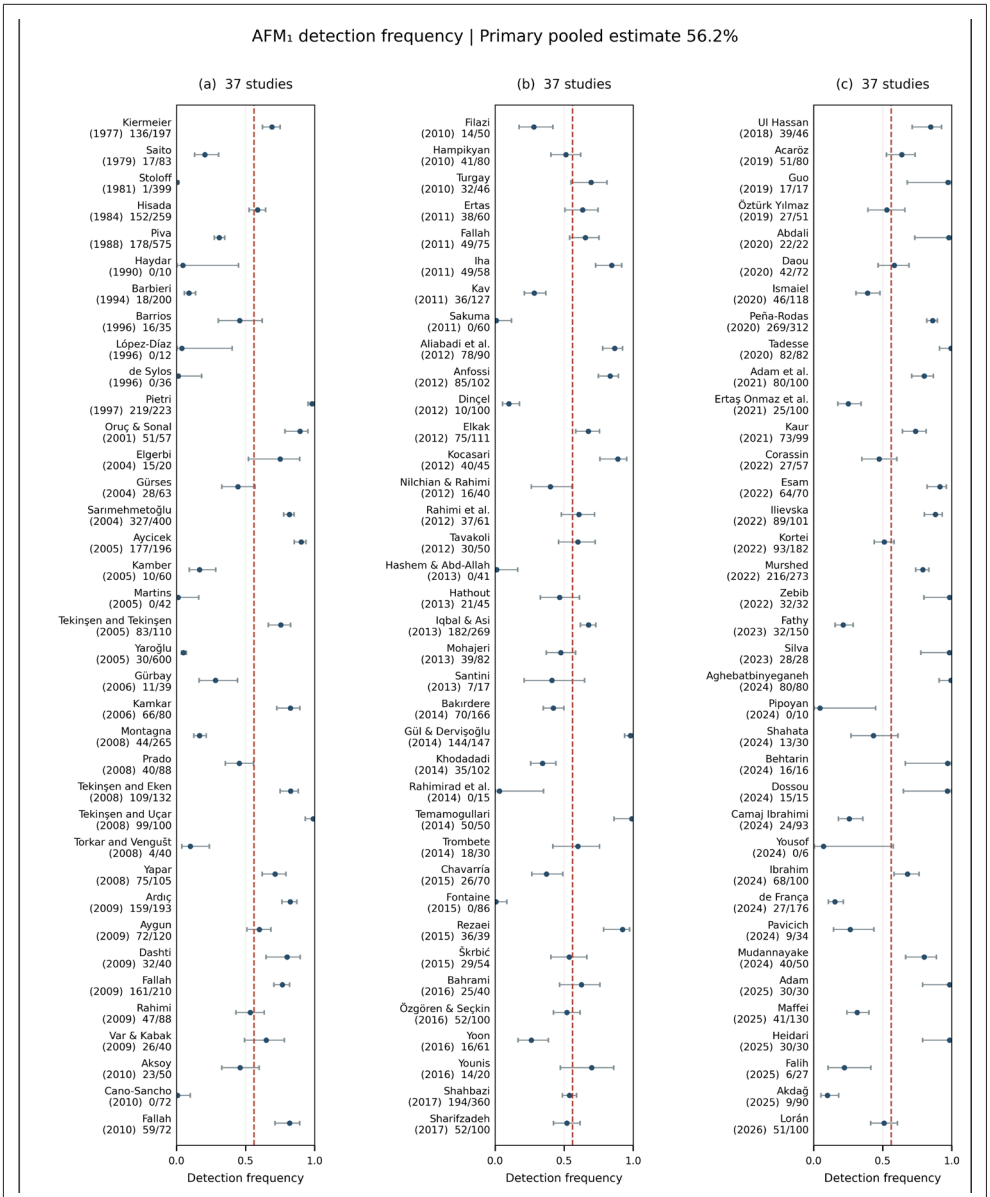


Fig 2. Study-level AFM_1 detection-frequency estimates for all 111 primary study clusters, arranged in panels a-c within one frame (37 studies per panel). Points and horizontal bars indicate study estimates and 95% confidence intervals. Boundary cells use the stated continuity correction. The dashed line indicates the primary pooled estimate of 56.2%. Labels show author, publication year and positive/tested counts. The complete source index is available from the corresponding author upon reasonable request

The primary study-level year coefficient was positive (OR 1.045 per year, 95% CI 1.004-1.088; P=0.032). The secondary stratum-level year model shown in *Fig. 5* gave OR 1.044 (95% CI 1.008-1.082; P=0.018). The historical-versus-update comparison was imprecise (OR 0.805, 95% CI 0.283-2.287; P=0.682). These differently parameterized exploratory comparisons do not demonstrate a causal temporal trend *(Table 4)*.

Table 3. *Secondary analytical method subgroup estimates*

Method	Strata	Clusters	Samples	Pooled	95% CI
Chromatography	100	45	3777	50.3%	37.1-63.5%
Immunoassay	155	59	6071	62.3%	49.2-73.8%
Other/NR	16	8	1521	32.3%	8.8-70.1%

A study can contribute strata to more than one analytical-method group; cluster counts therefore do not sum to the overall number of studies

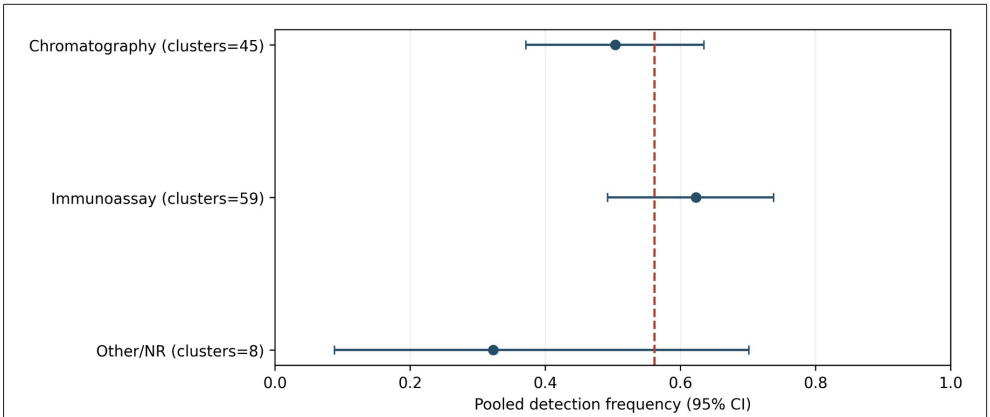


Fig 3. Secondary stratum-level pooled detection frequency by analytical method; study-clustered 95% confidence intervals. Dashed line: primary study-level estimate. The same study may appear in more than one method subgroup

The revised LOD model included 141 strata from 53 clusters. A tenfold higher LOD corresponded to OR 0.393 (95% CI 0.154-1.004; P=0.051). The confidence interval includes 1. Using 10 ng/kg for the ambiguous LOD reported by Pavicich et al.[12] gave OR 0.408 (95% CI 0.163-1.019; P=0.055); using 20 ng/kg gave OR 0.394 (95% CI 0.156-0.997; P=0.049). Both alternatives included 143 strata from 54 clusters. LOD availability is selective and this model does not adjust simultaneously for analytical method or other study-level confounders.

Concentration and Threshold Outcomes

Twelve studies met the restricted concentration criteria. The back-transformed pooled log-mean was 145.1 ng/kg (95% CI 75.3-279.8); I^2=99.8% *(Table 5)*. This secondary result is highly heterogeneous and should not be used as a population exposure estimate.

Threshold outcomes were separate *(Table 6)*. Elkak et al. [20] reported 13/111 above 250 ng/kg (11.7%); 13/75 (17.3%) instead conditions on detected-positive samples. Other source benchmarks were 4/40 for Bahrami et al.[49] and 16/120 for Aygun et al.[29] above 250 ng/kg, and 8/80 for Hampikyan et al.[50] above 500 ng/kg. No pooled legal-exceedance estimate was calculated because harmonized product-, date- and jurisdiction-specific limits were unavailable.

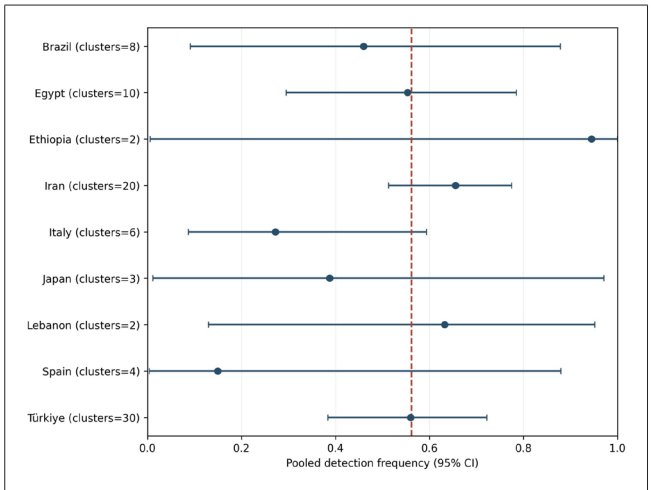


Fig 4. Secondary country summaries for countries with at least two study clusters; intervals are imprecise and do not rank national safety

Discussion

The revised synthesis indicates common but highly variable source-defined AFM_1 detection in the recovered cheese evidence. A mean near 56% does not imply that any particular cheese market has that detection frequency. The wide prediction interval, selective geographic coverage and measurement-dependent outcome definition are more informative than a single global percentage.

The revision changes both the data and the inferential emphasis. Restoring omitted negatives in Hisada et al. [11] and treating an internally inconsistent numerator separately

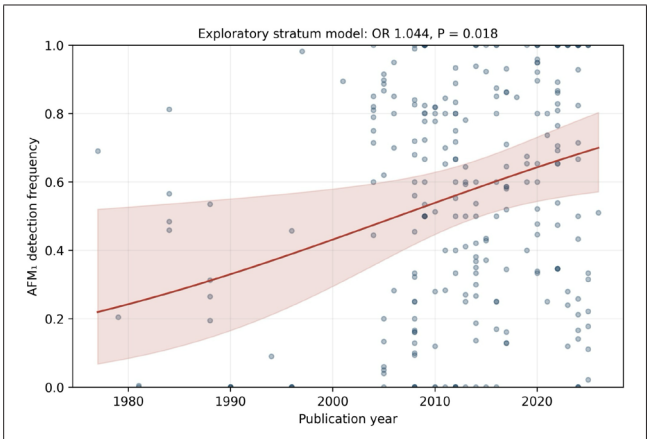


Fig 5. Exploratory stratum-level publication-year meta-regression with robust 95% confidence band. The separate primary study-level coefficient is reported in *Table 4*. The plotted OR 1.044 and P=0.018 refer to the secondary stratum-level model, not the primary study-level regression (OR 1.045; P=0.032)

Table 4. Exploratory meta-regression			
Term	**OR**	**95% CI**	**P-value**
Primary year	1.045	1.004-1.088	0.032
Secondary year	1.044	1.008-1.082	0.018
Year adjusted for method	1.040	1.002-1.079	0.042
Chromatography vs immunoassay	0.615	0.295-1.282	0.193
Other vs immunoassay	0.485	0.119-1.967	0.308
Update vs historical	0.805	0.283-2.287	0.682
Year in LOD model	1.009	0.967-1.053	0.682
Tenfold higher LOD	0.393	0.154-1.004	0.051

Table 5. Restricted concentration synthesis			
Subset	**Studies**	**Geometric Mean ng/kg**	**95% CI ng/kg**
Integrated	12	145.1	75.3-279.8
Historical	6	210.3	60.7-728.5
Update	6	100.6	68.6-147.5

Table 6. Selected source-reported threshold exceedance			
Study	**Threshold ng/kg**	**Above/ Tested**	**Percent**
Elkak, 2012 [20]	250	13/111	11.7%
Bahrami, 2016 [49]	250	4/40	10.0%
Aygun, 2009 [29]	250	16/120	13.3%
Hampikyan, 2010 [50]	500	8/80	10.0%
Aydemir Atasever, 2010 [4]	250	63/304	20.7%
Aydemir Atasever, 2010 [4]	500	30/304	9.9%

Source-reported benchmarks are descriptive and do not establish current legal non-compliance. The two rows for Aydemir Atasever et al.[4] represent different thresholds applied to the same sample set and are reported descriptively

are necessary even when the pooled percentage changes little. Making study-level aggregation primary also prevents a publication with many strata from receiving multiple primary contributions. The secondary clustered model preserves analytical detail but its point estimate can depend on how strata are partitioned; robust standard errors alone do not remove that dependence.

The LOD association remains negative but does not meet the conventional 5% threshold after correction. It is exploratory and may reflect analytical era, sampling, matrix, platform, recovery or selective reporting. Similarly, a positive publication-year coefficient in one model and a null period contrast do not establish increasing contamination. These sensitivities support cautious interpretation rather than preservation of the former significant result.

Earlier cheese meta-analyses [6-8] reuse part of the same primary literature, with different geographic scope, time windows and definitions. Complete included-study lists were not recovered for an exact overlap fraction. This work is an updated reanalysis of recoverable data, not a fully independent evidence base. The crosswalk of the review by Ötün [48] exposes Türkiye coverage gaps without establishing historical exclusion reasons. The study/source links and supporting bibliographic records are retained by the authors.

Limitations include unavailable individual historical exclusions and an incomplete 147→114→93 membership trail, 21 missing historical JBI appraisals, incomplete regional/grey-literature searches, unexecuted cheese-variety queries and update decisions not independently re-adjudicated. Reopening the study by Kos et al. [10] shows that retrieval and selection are not fully closed. Unknown sample overlap across reports also remains unresolved. Missing records cannot be replaced with assumed exclusion reasons.

Source-reported benchmark counts should not be equated with current legal non-compliance. Definitive legal-exceedance synthesis requires primary legislation, product classification, sampling date and compatible all-sample counts for every dataset [3,17]. The selected examples cannot substitute for that review.

Future surveillance should report sampling frames and dates, cheese type and milk source, analytical confirmation/recovery, numerical LOD/LOQ, below-threshold counts, exact numerators/denominators and all-sample concentration statistics. Preserved screening and appraisal logs would improve separation of contamination variation from measurement and reporting differences.

Conclusion

In the recovered evidence, study-level AFM_1 detection frequency pooled to 56.2%, with substantial heterogeneity and uncertainty. Corrected source data and a study-level primary model produced similar average estimates across several sensitivities, but the LOD association was not statistically conclusive. Incomplete historical selection and appraisal records, regional retrieval gaps and threshold differences prevent a claim of complete global coverage or universal prevalence.

Declarations

Availability of Data and Materials: The extracted study-level data, source index, screening and reconciliation records, available quality appraisals, search documentation, PRISMA reporting checklist, and analysis and plotting code are retained by the authors and are available from the corresponding author upon reasonable request. Unavailable historical selection decisions and appraisals remain identified as unavailable.

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Competing Interests: The authors declare that they have no competing financial or non-financial interests.

Declaration of Generative Artificial Intelligence (AI): ChatGPT (OpenAI) was used for language editing and assistance with analysis and plotting code. The authors reviewed and verified the outputs and take full responsibility for the manuscript, tables, and figures.

Ethical Compliance Declaration: This study used published aggregate data and involved no new human or animal experiments; study-specific ethics committee approval was therefore not applicable. The authors declare that the study was conducted in accordance with all applicable animal welfare and research ethics principles, institutional and national/international regulations, and the requirements of the relevant ethics committees and funding/supporting institutions, where applicable. The authors accept full responsibility for the ethical integrity of the study.

Protocol Registration: PROSPERO CRD420251248533, registered 16 September 2026 after the search cutoff; no prospective-registration claim is made.

Authors' Contributions: MA: study conception, data verification, formal analysis, manuscript preparation, critical revision, supervision, and correspondence. RNK, HÜ, and MAA: study conception, data verification, formal analysis, manuscript preparation, and critical revision. All authors are responsible for reviewing and approving the final manuscript and for the integrity of their contributions.

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