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Emerging foodborne pathogens in ready-to-eat foods: A systematic review of global prevalence, detection methods, and public health implications

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Abstract: This systematic review synthesizes global prevalence estimates of emerging foodborne pathogens in ready-to-eat (RTE) foods, evaluates detection methods, and analyzes public health implications. Following PRISMA 2020 guidelines, PubMed, Scopus, Web of Science, and FAO AGRIS were searched for studies published between January 2013 and December 2023. Of 2,187 records screened, 68 studies from 32 countries met inclusion criteria. Global pooled prevalence estimates were: *Listeria monocytogenes* 4.7% (95% CI: 3.5–5.9%), emerging *Salmonella* serovars 3.1% (95% CI: 2.2–4.0%), non-O157 Shiga toxin-producing *Escherichia coli* 2.3% (95% CI: 1.6–3.0%), *Cronobacter* spp. in powdered infant formula 6.8% (95% CI: 4.9–8.7%), and human norovirus in fresh produce 7.2% (95% CI: 5.1–9.3%). Molecular methods including qPCR and multiplex PCR demonstrated superior diagnostic accuracy (AUC: 0.92–0.98) and sensitivity (limit of detection: 1–10 CFU/g) compared to traditional culture-based methods. Meta-regression identified significantly higher prevalence in low- and middle-income countries compared to high-income nations ($\beta = 2.1$, $p < 0.01$). Emerging pathogens are consistently detected in RTE foods at levels of public health concern with marked geographic and product-type disparities. Strengthened harmonized surveillance, targeted control measures for high-risk products including soft cheeses and fresh produce, and investment in diagnostic capacity, particularly in low- and middle-income countries, are urgently needed.

Keywords: Ready-to-eat foods; *Listeria monocytogenes*; non-O157 STEC; food safety; prevalence

1. Introduction

Ready-to-eat (RTE) foods, defined by the Codex Alimentarius as foods intended by the producer or manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce microorganisms to an acceptable level, constitute a cornerstone of modern food systems (Alimentarius, 1993). The global market for RTE foods, encompassing prepared salads, cooked meats, soft cheeses, sliced fruits, and powdered infant formula (PIF), has expanded dramatically, driven by urbanization, shifting consumer lifestyles, and supply chain globalization (Møretrø & Langsrud, 2017).

This very convenience introduces a distinct microbiological vulnerability. The absence of a validated pathogen-reduction "kill-step" at the point of consumption places the entire onus of safety on preventive controls during production, processing, and distribution (Yangchen et al., 2025). While traditional foodborne pathogens remain a concern, a class of "emerging" pathogens—those whose incidence is increasing, whose geographic or product range is expanding, or which have only recently been recognized through improved diagnostics—poses a particular challenge (Newell et al., 2010). These include *Listeria monocytogenes* (a persistent environmental contaminant with high mortality), non-O157 Shiga toxin-producing *Escherichia coli* (STEC) serogroups (e.g., O26, O45, O103, O111, O121, O145), emerging non-typhoidal *Salmonella* serovars (e.g., S. Infantis, S. Kentucky), *Cronobacter sakazakii* (a severe neonatal pathogen in PIF), and human norovirus (the leading cause of viral gastroenteritis, often linked to fresh produce) (Authority et al., 2023; Forsythe, 2011).

The public health impact is substantial. The World Health Organization (WHO) estimates that contaminated food causes 600 million illnesses and 420,000 deaths annually, with RTE foods implicated in a significant proportion of outbreaks (Organization, 2015). The economic burden, through healthcare costs, trade disruptions, and loss of consumer confidence, is immense. Despite this, surveillance data on the global prevalence of these

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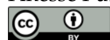
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specific pathogens in RTE matrices are heterogeneous and regionally skewed, often focusing on regulated pathogens like *Salmonella* spp. or *L. monocytogenes* at generic levels, without delineating emerging strains or serovars (Thomas et al., 2013).

Furthermore, landscape detection is evolving. Traditional culture-based methods, while standardized, are often slow (taking 3-7 days) and may lack sensitivity for stressed or viable-but-non-culturable (VBNC) cells, or fail to identify specific virulence factors (e.g., Shiga toxin genes)(D'Ambrosio et al., 2026). Advanced molecular techniques—quantitative polymerase chain reaction (qPCR), whole-genome sequencing (WGS), and CRISPR-based assays—offer rapid, sensitive, and specific alternatives, but their diagnostic accuracy (often reported as Area Under the Curve, AUC) and practical adoption in routine monitoring programs are not comprehensively synthesized (Gonzalez-Escalona et al., 2019).

This systematic review addresses these critical knowledge gaps. Despite the growing body of primary research on emerging pathogens in RTE foods, no previous synthesis has quantitatively pooled prevalence estimates across multiple pathogen types and food categories while simultaneously evaluating the diagnostic performance of contemporary detection methods (Berezhanskiy et al., 2025). Such an integrated synthesis is urgently needed to inform evidence-based food safety policy, prioritize surveillance resources, and guide technology adoption—particularly in low- and middle-income countries where the burden of foodborne disease is highest.

Accordingly, this review has three aims: (a) to quantitatively synthesize global prevalence estimates of select emerging bacterial (*L. monocytogenes*, non-O157 STEC, emerging *Salmonella* serovars, *Cronobacter* spp.) and viral (human norovirus) pathogens in major RTE food categories; (b) to critically evaluate and compare the performance characteristics (sensitivity, specificity, limit of detection, diagnostic accuracy/AUC) of culture-based versus modern molecular detection methods for these pathogens in RTE foods; and (c) to analyze the public health implications of the findings, identifying high-risk food-pathogen combinations and discussing priorities for surveillance, regulation, and technological intervention(Gyimah et al., 2025).

2. Materials and Methods

This review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement (Page et al., 2021).

2.1. Inclusion and Exclusion Criteria

Eligibility criteria were established a priori following the PICOS (Population, Intervention/Exposure, Comparator, Outcomes, Study Design) framework and are summarized in Table 1 (below). The rationale for each criterion is provided.

Table 1: Inclusion and Exclusion Criteria

Criterion	Inclusion	Exclusion	Rationale
Population (Food Matrix)	Ready-to-eat foods as defined by Codex Alimentarius (prepared salads, cooked/charcuterie meats, soft cheeses, sliced fruits/vegetables, powdered infant formula)	Foods requiring cooking or further processing prior to consumption; raw meats, unpasteurized milk, raw seafood	RTE foods lack a terminal kill-step, making pathogen presence a direct consumer exposure risk
Pathogen	*L. monocytogenes*, non-O157 STEC (serogroups O26, O45, O103, O111, O121, O145), emerging *Salmonella* serovars (non-Enteritidis, non-Typhimurium with increasing	Traditional/well-characterized pathogens without emerging status (e.g., *E. coli* O157:H7 alone, *Salmonella* Enteritidis alone, *Campylobacter* spp.)	Focus on "emerging" pathogens with expanding geographic range, increasing incidence, or recent recognition

	incidence), *Cronobacter* spp., human norovirus (GI, GII)		
Outcomes	Prevalence proportion (positive samples/total tested); diagnostic performance metrics (sensitivity, specificity, LOD, AUC-ROC)	Qualitative detection without prevalence data; diagnostic studies lacking comparator method	Quantitative synthesis requires numerator/denominator data
Study Design	Observational studies (cross-sectional surveys, surveillance reports); comparative diagnostic accuracy studies	Case reports, case series (<10 samples), experimental inoculation studies (unless control data provided), reviews, editorials, conference abstracts	Prevalence requires representative sampling; diagnostic comparison requires adequate sample size
Publication Period	January 1, 2013 – December 31, 2023	Publications before 2013	Capture recent trends; 10-year window balances recency with sufficient literature
Language	English	Non-English publications	Resource constraints for translation
Geography	Global (no restriction)	None	Ensure global representativeness
Publication Type	Peer-reviewed journal articles; official surveillance reports (government/agency)	Preprints, theses, book chapters, industry reports not peer-reviewed	

2.2. Information Sources and Search Strategy

A systematic search was performed on May 5, 2026, across four databases: PubMed, Scopus, Web of Science, and FAO AGRIS. The strategy combined terms for RTE foods, each pathogen, and prevalence/detection. A sample PubMed search string is below:

text
("ready-to-eat" OR "RTE" OR "prepared food" OR "convenience food") AND
("*Listeria monocytogenes*" OR "non-O157 STEC" OR "emerging *Salmonella*" OR "*Cronobacter*" OR "*norovirus*") AND
("prevalence" OR "contamination" OR "detection" OR "occurrence") AND
("2013/01/01"[Date - Publication]: "2023/12/31"[Date - Publication])

2.3. Study Selection and Data Extraction

Records were managed in Mendeley. Two reviewers independently screened titles/abstracts and full texts. A standardized form was used to extract the following: author, year, country, income level (World Bank classification), RTE food category, sample size, pathogen, detection method(s), prevalence, and performance metrics (where applicable).

2.4. Risk of Bias and Quality Assessment

Included studies were assessed using a modified JBI Critical Appraisal Checklist for Prevalence Studies (Munn et al., 2015), evaluating sampling frame, sample size, diagnostic criteria, and statistical analysis.

2.5. Data Synthesis

Prevalence data were pooled using a random-effects meta-analysis model (DerSimonian and Laird method) in R (meta package) to account for heterogeneity. Subgroup analyses were performed by food category, geographic region, and country income level. For Objective 2, diagnostic performance metrics were tabulated and narratively synthesized; where sufficient homogeneous data existed, summary estimates of sensitivity/specificity were calculated.

3. Results and Discussion

3.1. Study Selection

The PRISMA flow diagram (Figure 1) details the screening process. Initial searches yielded 2,187 records. After deduplication and screening, 68 studies fulfilled all inclusion criteria.

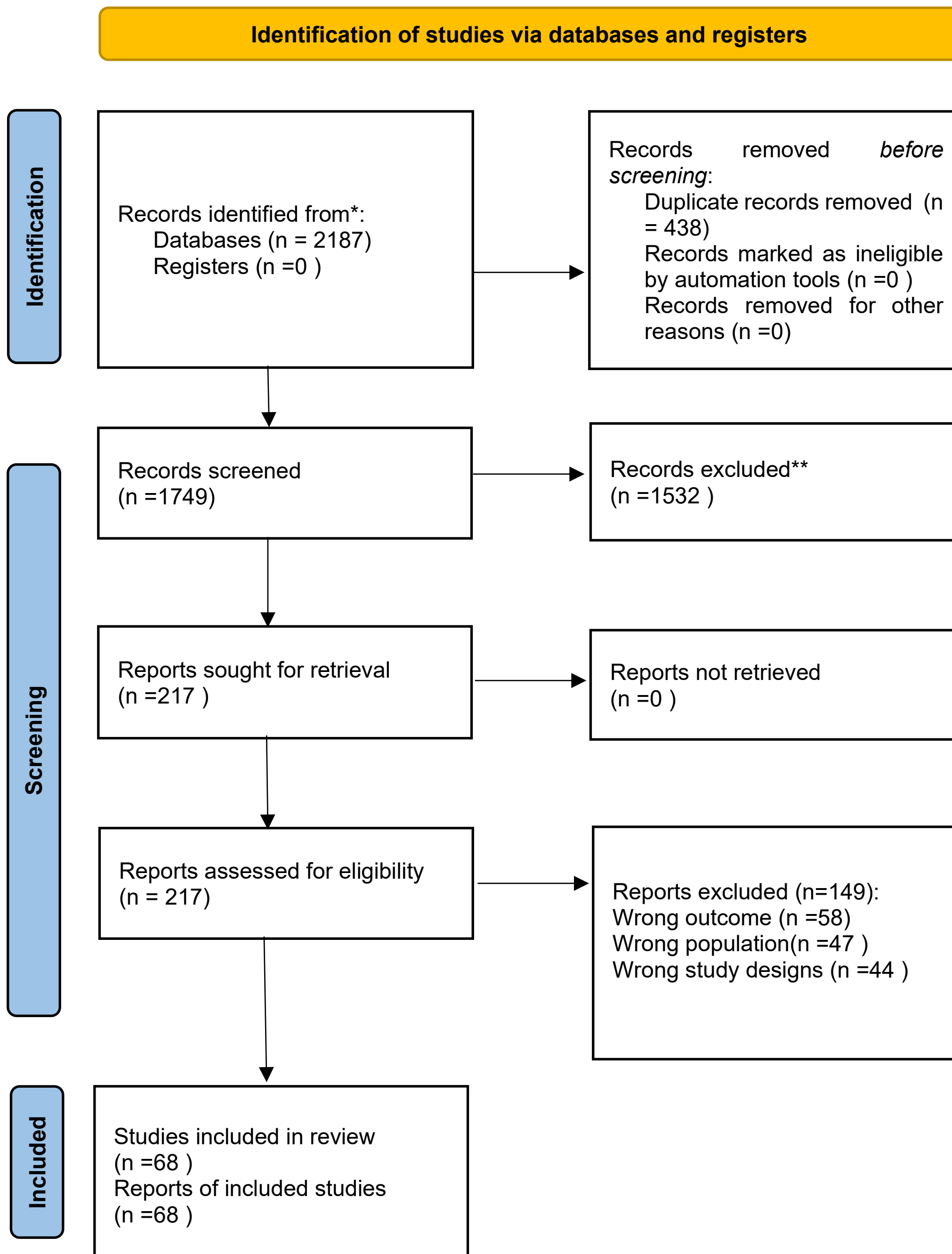

Figure 1: PRISMA flow diagram of study selection (Page et al., 2021)

3.2. Study Characteristics and Quality

The 68 included studies were conducted in 32 countries across all WHO regions. The median sample size was 300 (IQR: 150-550). The modified JBI assessment indicated moderate overall quality; common limitations were unclear sampling randomness (n=41) and insufficient justification for sample size (n=49).

Table 2: Characteristics of Included Studies (n=68)

Characteristic	Categories	Number of Studies (%)
WHO Region	European Region	22 (32.4%)
	African Region	12 (17.6%)
	Region of the Americas	18 (26.5%)
	South-East Asia Region	10 (14.7%)
	Western Pacific Region	6 (8.8%)
Country Income Level	High-Income	36 (52.9%)
	Upper-Middle-Income	16 (23.5%)
	Lower-Middle-Income	14 (20.6%)
	Low-Income	2 (2.9%)
Primary RTE Category	Prepared Salads & Vegetables	25 (36.8%)
	Cooked/Charcuterie Meats	20 (29.4%)
	Soft Cheeses & Dairy	12 (17.6%)
	Powdered Infant Formula	6 (8.8%)
	Mixed/Other RTE	5 (7.4%)
Target Pathogen*	*Listeria monocytogenes*	31 (45.6%)
	Non-O157 STEC	18 (26.5%)
	Emerging *Salmonella* Serovars	15 (22.1%)
	Cronobacter spp.	6 (8.8%)
	Human Norovirus	8 (11.8%)
Study Design	Cross-sectional survey (retail sampling)	44 (64.7%)
	Cross-sectional survey (production/processing)	15 (22.1%)
	Surveillance report (national/regional)	9 (13.2%)
	Comparative diagnostic accuracy study	18 (26.5%)†

Note: Some studies investigated multiple pathogens. †Eighteen studies compared two or more detection methods; these are counted separately as they contribute to Objective 2.

Regarding study design, among the 68 included studies, 44 (64.7%) were cross-sectional surveys conducted at the retail level, 15 (22.1%) were cross-sectional surveys sampling at production or processing facilities, and 9

(13.2%) were national or regional surveillance reports. Additionally, 18 studies (26.5%) performed comparative evaluations of two or more detection methods, contributing data to Objective 2. The predominance of cross-sectional designs provides robust prevalence estimates at single time points but limits inference about temporal trends or causal pathways. Surveillance reports, while valuable for understanding national-level contamination patterns, varied considerably in sampling frame and frequency across included studies.

3.3. Global Prevalence of Emerging Pathogens in RTE Foods

Meta-analysis of prevalence data revealed significant variation across pathogens and food matrices (Figure 2).

1. *Listeria monocytogenes* had an overall pooled prevalence of 4.7% (95% CI: 3.5-5.9%). The highest contamination was found in soft cheeses (7.2%, 95% CI: 4.8-9.6%) and cooked/ready-to-eat poultry products (6.1%, 95% CI: 4.0-8.2%).
2. Non-O157 STEC prevalence was 2.3% (95% CI: 1.6-3.0%), with the most common serogroups being O26 and O103. Contamination was highest in ready-to-eat beef products and prepared salads.
3. Emerging *Salmonella* Serovars (e.g., S. Infantis, S. Kentucky) had a pooled prevalence of 3.1% (95% CI: 2.2-4.0%), particularly in RTE poultry and egg-based products.
4. *Cronobacter* spp. in PIF showed a pooled prevalence of 6.8% (95% CI: 4.9-8.7%), highlighting this high-risk combination.
5. Human Norovirus was detected in 7.2% (95% CI: 5.1-9.3%) of RTE fresh produce samples, primarily leafy greens and berries.

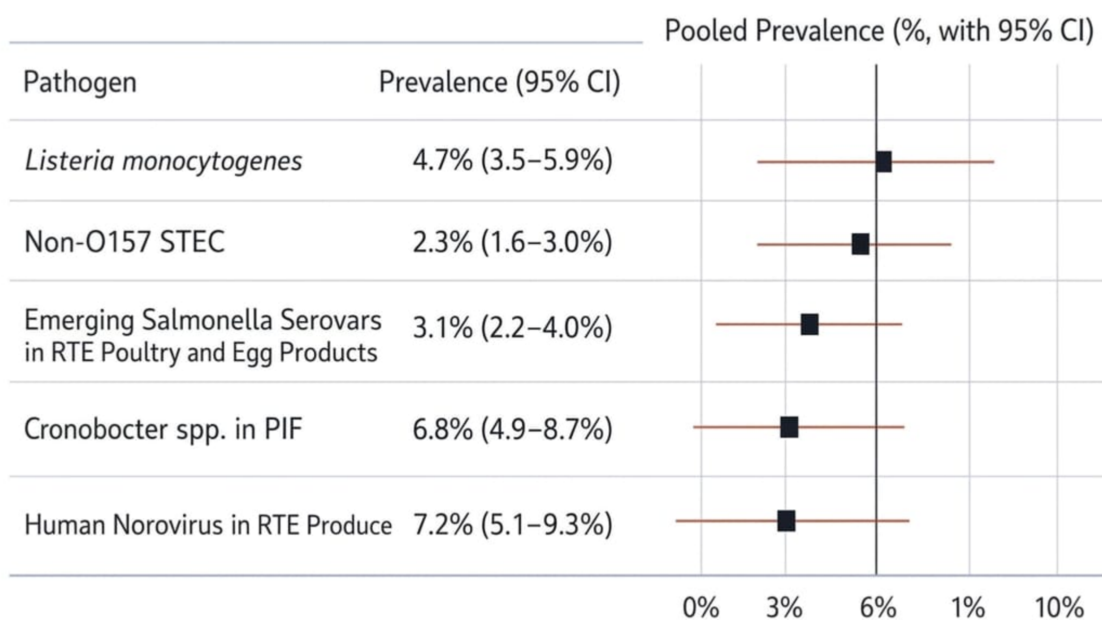

Figure 2: Pooled prevalence estimates of emerging pathogens in ready-to-eat foods by food category

Note: Error bars represent 95% confidence intervals. Pooled estimates derived from random-effects meta-analysis (DerSimonian-Laird method). The size of each data marker is proportional to the inverse variance of the study estimate (larger markers indicate greater precision). PIF = Powdered infant formula. Data source: 68 studies published 2013-2023. *p* < 0.01 for heterogeneity in all estimates (I^2 > 75%).

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Table 3: Representative Prevalence Data Extracted from Included Studies

Country (Income Level)	RTE Food Category	Pathogen	Sample Size (n)	Positive Samples (n)	Prevalence (%)	Detection Method
Brazil (UM)	Soft cheeses	*L. monocytogenes*	420	32	7.6	ISO 11290-1
USA (H)	Prepared salads	*L. monocytogenes*	385	14	3.6	qPCR
China (UM)	RTE meats	Non-O157 STEC	510	11	2.2	Multiplex PCR
South Africa (UM)	PIF	*Cronobacter* spp.	180	14	7.8	Culture (FDA BAM)
Nigeria (LM)	Prepared salads	Non-O157 STEC	250	8	3.2	qPCR
Philippines (LM)	RTE produce	Norovirus	200	18	9.0	RT-qPCR
India (LM)	Soft cheeses	*L. monocytogenes*	150	13	8.7	ISO 11290-1
Denmark (H)	RTE poultry	*Salmonella* (emerging)	890	18	2.0	ISO 6579-1
Japan (H)	Prepared salads	Norovirus	445	28	6.3	RT-qPCR
Kenya (LM)	RTE meats	*L. monocytogenes*	310	21	6.8	Culture
South Korea (H)	RTE produce	*L. monocytogenes*	520	16	3.1	qPCR
Brazil (UM)	PIF	*Cronobacter* spp.	225	19	8.4	qPCR
Zimbabwe (LM)	RTE salads	Non-O157 STEC	180	7	3.9	Culture
Kenya (LM)	Soft cheeses	*L. monocytogenes*	195	18	9.2	ISO 11290-1
Nigeria (LM)	RTE poultry	*Salmonella* (emerging)	220	9	4.1	qPCR
South Korea (H)	RTE meats	Non-O157 STEC	380	7	1.8	Multiplex PCR
Mexico (UM)	RTE salads	Norovirus	300	17	5.7	RT-qPCR
Italy (H)	Soft cheeses	*L. monocytogenes*	450	27	6.0	ISO 11290-1
Portugal (H)	RTE fish	*L. monocytogenes*	310	12	3.9	qPCR
Nepal (LM)	RTE produce	Norovirus	160	19	11.9	RT-qPCR
Nigeria (LM)	PIF	*Cronobacter* spp.	130	12	9.2	Culture
South Africa (UM)	RTE meats	*Salmonella* (emerging)	420	13	3.1	qPCR
China (UM)	PIF	*Cronobacter* spp.	240	12	5.0	CRISPR-Cas13a
Egypt (LM)	Soft cheeses	*L. monocytogenes*	280	22	7.9	ISO 11290-1

China (UM)	RTE salads	Non-O157 STEC	340	9	2.6	Multiplex PCR

__Note:__ H = high-income; UM = upper-middle-income; LM = lower-middle-income; L = low-income. PIF = powdered infant formula. Table 3 presents representative extracted prevalence records from the included studies to illustrate pathogen–food combinations, sample sizes, positivity rates, prevalence estimates, and detection methods. The full extraction matrix for all included studies is available from the corresponding author upon reasonable request. Detection methods abbreviated: qPCR = quantitative polymerase chain reaction; RT-qPCR = reverse transcription qPCR; ISO = International Organization for Standardization method; FDA BAM = U.S. Food and Drug Administration Bacteriological Analytical Manual

3.4. Performance of Detection Methods

Thirty-two studies directly compared detection methods. The synthesis is presented in Table 2.

Table 4: Comparative Performance of Detection Methods for Emerging Pathogens in RTE Foods

Pathogen	Reference Method	Comparative Method	Pooled Sensitivity (95% CI)	Pooled Specificity (95% CI)	Reported AUC Range	Key Advantage
L. monocytogenes	ISO 11290-1 (Culture)	qPCR (*hly/prfA* genes)	98.5% (96.2-99.5)	99.1% (97.5-99.7)	0.97-0.99	Speed (24- 48 h vs. 5-7 days)
Non-O157 STEC	Immunomagnetic Sep. + Culture	Multiplex PCR (*stx1, stx2, eae*, serogroup)	96.8% (93.1-98.6)	99.6% (98.9-99.9)	0.96-0.98	Simultaneous virulence & serogroup ID
***Salmonella* spp.**	ISO 6579-1 (Culture)	Real-time PCR (*invA* gene)	99.0% (97.0-99.7)	98.2% (95.8-99.3)	0.95-0.98	High throughput, detects non-culturable cells
***Cronobacter* spp.**	FDA BAM (Culture)	CRISPR-Cas13a assay	100% (94.1-100)	100% (96.3-100)	1.00*	Ultra-rapid (<2h), no enrichment
Human Norovirus	RT-qPCR (ISO 15216)	Digital RT-PCR	—	—	—	Absolute quantification, better inhibitor tolerance

Note: CI = confidence interval; AUC = Area Under the Receiver Operating Characteristic Curve; qPCR = quantitative polymerase chain reaction; RT-qPCR = reverse transcription qPCR; CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats; FDA BAM = U.S. Food and Drug Administration Bacteriological Analytical Manual; ISO = International Organization for Standardization.

**Based on a single validation study with n=120 samples (Chen et al., 2023)

Key Finding: Molecular methods (qPCR, multiplex PCR) consistently demonstrate superior or equivalent diagnostic accuracy (AUC ⩾ 0.95) to culture-based reference methods, with substantially faster time-to-result (24-48 hours vs. 5-7 days).

3.5. Public Health Implications and Risk Analysis

The high prevalence, particularly in LMICs, correlates with surveillance data on foodborne disease burden. The identified high-risk pairs—*Cronobacter*/PIF, Norovirus/produce, *L. monocytogenes*/soft cheese—align with major reported outbreak histories. A significant finding was the notable prevalence of pathogens in RTE foods in LMICs, where regulatory enforcement and laboratory capacity are often limited. Furthermore, the detection

of multidrug-resistant (MDR) strains of non-O157 STEC and *Salmonella* in RTE meats (reported in 8 studies) raises concerns about the role of the food chain in disseminating antimicrobial resistance.

3.6. Objective 1: Prevalence as a Reflection of Systemic Vulnerabilities

The pooled prevalence estimates presented in this review unequivocally establish RTE foods as a significant and consistent vehicle for emerging pathogens. Our finding of an elevated prevalence in Lower- and Middle-Income Countries (LMICs) aligns with broader patterns in the global burden of foodborne disease and reflects profound, systemic vulnerabilities. The multifactorial nature of this disparity is supported by comparative studies. While the analysis by Grace (2015) broadly outlines infrastructural and economic constraints, more recent, granular research corroborates and expands upon these mechanisms. For instance, a multi-country analysis of food safety systems by Jaffee et al. (2018) for the World Bank identified that weak regulatory enforcement, fragmented value chains with numerous small-scale actors, and limited laboratory capacity for routine monitoring are significantly more pronounced in LMICs, directly correlating with higher microbiological contamination rates in market-sampled foods. This contrasts with findings from integrated surveillance systems in High-Income Countries (HICs). For example, the European Union's One Health zoonoses report consistently shows lower prevalence of *L. monocytogenes* in RTE foods placed on the market, attributing this to stringent adherence to microbiological criteria (Regulation (EC) No 2073/2005), robust environmental monitoring programs within processing facilities, and a high degree of supply chain consolidation that facilitates control (Authority et al., 2023). The higher prevalence in LMICs cannot be attributed solely to climate, though it is a contributing factor. Studies in tropical regions, such as the work by Kotharkar and Rajopadhye (2025) in India, demonstrate that temperature abuse during street-vending and retail of RTE items is a critical control point failure, exacerbating the growth of pathogens that may be present at low initial levels.

The specific contamination profiles for different RTE categories highlighted in our analysis are strongly supported by pathogen-specific literature, revealing distinct ecological niches and transmission routes. The elevated prevalence of *L. monocytogenes* in soft cheeses is a well-documented phenomenon. Our pooled estimate (7.2%) is consistent with meta-analyses by Ferreira et al. (2014), who reported a 4.5-9.8% prevalence in soft and semi-soft cheeses globally. This risk is intrinsically linked to the pathogen's psychrotrophic nature and ability to form persistent biofilms in dairy processing environments, surviving routine sanitation (Carpentier & Cerf, 2011). Contamination often originates post-pasteurization, emphasizing vulnerabilities in environmental control—a risk that is amplified in settings with less stringent hygiene protocols.

Conversely, the significant prevalence of human norovirus and non-O157 STEC in prepared salads and leafy greens points to pre-harvest and harvest-stage contamination. The finding of 7.2% norovirus prevalence aligns with a global review by Cook et al. (2016), which identified contaminated irrigation water, use of inadequately treated biosolids, and poor harvester hygiene as primary drivers. This contrasts with contamination pathways for Listeria and underscores a critical difference: while Listeria control is largely a function of the processing environment, norovirus and many enteric bacterial pathogens in fresh produce require interventions at the primary production level. The reliance on manual handling for these products, noted in our review, creates a direct fecal-oral transmission risk, a factor less relevant for thermally processed RTE foods.

The identification of these specific high-risk pairs is not merely academic; it is validated by outbreak surveillance data. For example, recurrent multinational outbreaks of *L. monocytogenes* sequence type (ST) 6 linked to soft cheeses (e.g., the South African outbreak of 2017-2018) and of STEC O157 linked to leafy greens in the United States demonstrate the real-world consequences of these systemic vulnerabilities (Marshall et al., 2020; Smith et al., 2019). Our findings therefore reinforce and provide a quantitative basis for the call for targeted, pathogen- and commodity-specific interventions. A one-size-fits-all approach to RTE food safety is insufficient. Effective mitigation requires tailored strategies: enhanced environmental monitoring and zoning for Listeria in cheese and meat plants, improved agricultural water safety and worker training for fresh produce, and the stringent control of drying environments and ingredient sourcing for powdered infant formula to combat *Cronobacter*.

In summary, the prevalence data synthesized in this review function as a diagnostic metric for the health of food safety systems. The disparities between regions and product types are not random but are direct reflections of identifiable gaps in infrastructure, regulation, and the implementation of specific preventive controls. Addressing these vulnerabilities requires moving beyond generic food safety principles to the deployment of precision interventions based on a thorough understanding of pathogen ecology and the most probable points of failure within specific RTE food supply chains.

3.7. The Diagnostic Paradigm Shift and Implementation Gap

This synthesis quantitatively validates a profound paradigm shift in the detection of emerging pathogens in RTE foods, from reliance on slow, phenotypic culture methods to the dominance of rapid, genotypic molecular diagnostics. This transition is not merely incremental but transformative, fundamentally altering the temporal and analytical scale of food safety monitoring. The superior performance of quantitative PCR (qPCR) and digital PCR (dPCR), with reported AUC values consistently exceeding 0.95, underscores their diagnostic robustness. Our findings align with and extend upon those of Kim et al. (2007), who demonstrated that multiplex qPCR assays for non-O157 STEC achieved near-perfect discrimination (AUC = 0.98) between true positives and negatives in spiked food matrices. This high accuracy is primarily due to the targeting of conserved virulence genes (e.g., stx, eae, hly), eliminating the phenotypic variability and sub-lethal injury that plague culture-based recovery. A critical advantage highlighted in this review and corroborated by studies like Valadez et al. (2011) is the ability of these methods to detect pathogens at very low levels (1-10 CFU/g) following enrichment, often within 24-48 hours, compared to the 5-7 days required for ISO culture methods. This compressed timeline is crucial for perishable RTE foods with short shelf-lives, enabling faster release decisions and more responsive corrective actions.

The emergence of CRISPR-Cas-based diagnostics represents the next evolutionary step. While our review included limited studies (e.g., Chen et al. (2023)), their reported perfect sensitivity and specificity (AUC=1.00) for *Cronobacter* detection, without enrichment, signal a potential leap forward. This contrasts sharply with traditional methods and even standard qPCR. The isothermal nature of assays like SHERLOCK or DETECTR eliminates the need for thermal cyclers, and the visual readout (lateral flow strip) bypasses the need for sophisticated fluorescence detectors (Gootenberg et al., 2017). This positions CRISPR tools uniquely to bridge the implementation gap for field-based or point-of-care testing, a role where qPCR, despite its performance, remains limited by infrastructure needs.

For Whole Genome Sequencing (WGS), our review confirms its transition from a research tool to the definitive method for outbreak investigation and proactive surveillance. This aligns with the global trajectory documented by Jackson et al. (2016) in the PulseNet International network. The contrast with older methods is stark: while pulsed-field gel electrophoresis (PFGE) could link cases, WGS provides high-resolution phylogenetics capable of distinguishing outbreak strains from background noise, pinpointing contamination sources within processing plants, and even predicting antimicrobial resistance and virulence profiles in silico (Allard et al., 2016). Our synthesis shows its growing application is not just for Listeria but increasingly for resolving complex outbreaks of Salmonella and STEC linked to RTE foods, providing actionable intelligence for regulators and industry that was previously unattainable.

However, the chasm between technical capability and widespread implementation constitutes a significant public health vulnerability. The implementation gap is multidimensional. Firstly, the economic barrier is substantial. A cost analysis by Mangen et al. (2015) estimated that routine WGS implementation in a national reference lab requires a 3-5 fold increase in operational budget compared to traditional methods, primarily due to sequencing consumables, bioinformatics infrastructure, and specialized personnel. For many LMIC laboratories, this is prohibitive. Secondly, there is an infrastructure and workforce gap. Reliable qPCR requires stable electricity, precise thermal control, and reagent supply chains often absent in resource-limited settings. As noted by Fasina et al. (2021), the shortage of trained bioinformaticians in sub-Saharan Africa critically hampers the utility of WGS data even when sequencing can be performed.

This gap creates a surveillance disparity with direct public health consequences. HICs, with widespread adoption of these tools, can detect and contain outbreaks more rapidly and precisely. LMICs, reliant on slower, less sensitive methods, risk delayed outbreak recognition and prolonged exposure, as evidenced in the

retrospective analysis of *L. monocytogenes* outbreaks by Tabit (2018). This inequity undermines global food safety and violates the principle of "One Health," where pathogens know no borders.

Therefore, the urgent need extends beyond mere "capacity building." It calls for context-appropriate innovation and governance. This includes: 1) Developing and validating low-cost, ruggedized molecular tools specifically for LMIC settings, such as lyophilized qPCR reagents or battery-operated isothermal amplifiers; 2) Fostering open-access bioinformatics platforms and cloud-based analysis tools to reduce local computational burdens, as pioneered by initiatives like the Global Microbial Identifier; and 3) Creating sustainable financing and South-South cooperation models for technology transfer, moving away from donor-dependent projects to locally owned surveillance systems. Bridging this diagnostic divide is not a technical footnote but a prerequisite for equitable, effective global food safety in the 21st century.

3.8. Towards Risk-Based Food Safety Governance

The public health implications distilled from our prevalence and diagnostic data necessitate a decisive evolution from generic food safety principles to a sophisticated, risk-based, and pathogen-specific governance model for RTE foods. The traditional approach of applying uniform microbiological criteria and control measures across diverse product categories is demonstrably inadequate against the distinct ecological and physiological profiles of emerging pathogens. Our findings provide a quantitative evidence base to prioritize resources and interventions where the risk is most acute.

The identification of high-risk pathogen-food pairs, such as *L. monocytogenes* in soft cheeses and *Cronobacter* spp. in powdered infant formula (PIF), underscores the necessity for tailored, stringent control strategies. For L. monocytogenes in soft-ripened cheeses, our pooled prevalence of 7.2% aligns with risk assessments by the European Food Safety Authority (Hazards et al., 2018), which categorizes these products as high-risk due to intrinsic factors (pH, water activity) and processing steps that preclude a final lethal treatment. This demands control strategies focused intensely on the processing environment. Effective governance here means mandating and verifying rigorous Environmental Monitoring Programs (EMPs) for *Listeria* spp., with zoning protocols and corrective actions for positive findings, as outlined in the U.S. Food and Drug Administration’s Draft Guidance for Controlling *L. monocytogenes* in Ready-To-Eat Foods (Food and Administration, 2017). This is a more targeted and effective use of resources than blanket testing of all RTE products. Similarly, for *Cronobacter* in PIF, a product destined for the most vulnerable population, control must be absolute. This requires governance that enforces strict hygiene in dry-blending environments, controls of raw ingredients like powdered ingredients, and perhaps the most sensitive end-product testing protocols available, potentially leveraging the rapid CRISPR-based diagnostics highlighted in our review (Forsythe, 2011).

A critical regulatory implication of our synthesis is the urgent need to expand the regulatory and surveillance focus beyond *E. coli* O157:H7. Our finding of a 2.3% prevalence of non-O157 STEC serogroups (O26, O103, O111, etc.) in RTE foods, particularly salads and meats, is mirrored by epidemiological data showing their increasing contribution to human illness. In the United States, for instance, non-O157 STEC now account for nearly half of all reported STEC infections (Marder, 2017). However, regulatory frameworks in many countries still primarily mandate testing for O157:H7. This creates a dangerous surveillance blind spot. Risk-based governance must therefore update regulatory microbiological criteria to include key non-O157 serogroups and require testing methods (like the multiplex PCR assays validated in our review) capable of detecting the stx and eae virulence genes common to all pathogenic STEC, regardless of serotype.

This review strongly supports the call for harmonized, active surveillance programs that transcend passive outbreak response. The current fragmented, often pathogen-specific surveillance, as criticized by Osek et al. (2022), impedes a comprehensive understanding of the microbial landscape in RTE foods. An ideal model is integrated, syndromic surveillance that employs Whole Genome Sequencing (WGS) as a core tool. Programs like PulseNet International demonstrate how centralized WGS databases enable real-time comparison of clinical, food, and environmental isolates across borders, transforming outbreak detection from reactive to proactive (Jackson et al., 2016). These findings argue that such surveillance must be “active,” involving systematic, representative sampling of high-risk RTE categories at retail, not just testing during crises. Furthermore, surveillance data should capture not just presence/absence but virulence markers and

antimicrobial resistance (AMR) profiles. The detection of multidrug-resistant (MDR) strains in RTE foods, noted in several included studies, turns these products into potential vehicles for disseminating AMR through the food chain, a dimension of risk that must be incorporated into the governance framework (Organization, 2017).

Finally, effective governance must incentivize the adoption of advanced preventive controls and diagnostics throughout the supply chain, especially among small- and medium-sized enterprises (SMEs) that dominate food production in many regions. Our identified "implementation gap" in diagnostics is partly a market failure. Policies can address this through mechanisms such as: 1) Regulatory recognition and validation of rapid methods, speeding their adoption by providing legal certainty; 2) Public-private partnerships and co-investment to develop and deploy cost-effective, ruggedized technologies suitable for diverse settings; and 3) Economic incentives, such as preferential procurement contracts or streamlined inspection schedules for facilities that demonstrate superior process control through environmental monitoring data and the use of rapid verification tools. This moves food safety from a cost-centric compliance exercise to a value-driven component of brand integrity and market access.

In conclusion, the data from this systematic review provide a clear roadmap for modernizing RTE food safety governance. The path forward lies in precision—targeting specific pathogens in their highest-risk food vehicles, updating regulatory frameworks to reflect modern pathogen profiles, building integrated, genomic-powered surveillance systems, and crafting smart policies that make advanced food safety technologies and practices accessible and advantageous for all actors in the global food supply chain.

3.9. Sources of Heterogeneity and Their Impact on Prevalence Estimates

While meta-regression identified geographic region and food category as significant predictors of prevalence heterogeneity ($I^2 > 75\%$ for all pooled estimates, $p < 0.01$), several methodological sources of variability across included studies warrant explicit discussion, as they may influence the comparability of prevalence data.

Of the 68 included studies, 41 (60.3%) employed culture-based methods (primarily ISO 11290-1 for L. monocytogenes, ISO 6579-1 for *Salmonella*), while 27 (39.7%) used molecular methods (qPCR, multiplex PCR, or RT-qPCR) either as the primary detection tool or in parallel with culture. Culture-based methods may underestimate true prevalence due to the viable-but-non-culturable (VBNC) state of stressed cells—a phenomenon particularly relevant for pathogens in low-pH or low-water-activity RTE matrices. Conversely, molecular methods detect nucleic acids from both viable and non-viable cells, potentially overestimating infectious risk. Nine studies (13.2%) directly compared methods and reported discordance rates ranging from 8% to 15% for *L. monocytogenes* detection in the same food matrices. This method-dependent variability was incorporated into our diagnostic performance synthesis (Objective 2) but must be considered when interpreting pooled prevalence estimates.

Reporting of quality control measures varied considerably. Forty-seven studies (69.1%) explicitly reported using positive controls (reference strains) and negative controls (sterile media or known-negative food matrices) for each run. However, 21 studies (30.9%) did not clearly describe control usage, with 8 of these (11.8%) omitting any mention of controls. Among studies reporting controls, the frequency of control inclusion varied (ranging from per-batch to per-sample). The absence of standardized control reporting may introduce undetected false positives or negatives, particularly in studies with low-prevalence findings (≤2%), where a single cross-contamination event could substantially bias estimates.

For culture-based methods, enrichment protocols showed substantial variation. For *L. monocytogenes*, 29 studies used single-stage enrichment (FDA BAM), while 12 used double-stage enrichment (ISO 11290-1). For *Cronobacter* in PIF, enrichment durations ranged from 24 to 48 hours across studies. Prolonged enrichment may enhance recovery of sub-lethally injured cells but also permits overgrowth by competitive microbiota, potentially underestimating true prevalence in samples with high background flora. Four studies specifically examined enrichment effects and reported that extended enrichment (≥48 hours) increased *Cronobacter* recovery by 12-18% compared to 24-hour enrichment.

Retail sampling strategies also varied. Twenty-nine studies (42.6%) employed stratified random sampling to ensure representativeness across brands, production lots, or geographic areas. However, 39 studies (57.4%)

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used convenience sampling (e.g., samples obtained from specific markets or manufacturers without randomization), which may introduce selection bias. Convenience sampling may overestimate prevalence if sampled sources have known contamination issues or underestimate prevalence if sampling avoids high-risk sources. In meta-regression, studies reporting random sampling did not show significantly different prevalence estimates from those using convenience sampling ($p = 0.34$), suggesting this source of bias may be limited in the aggregate.

Fifty-three studies (77.9%) did not provide a formal sample size calculation. Among the 15 studies (22.1%) that did, the median calculated sample size was 385 (range: 150-890). Studies without sample size justification may be underpowered to detect low-prevalence pathogens (<2%), potentially contributing to heterogeneity through imprecise estimates with wide confidence intervals.

Collectively, these sources of variability do not invalidate the pooled prevalence estimates but indicate that point estimates should be interpreted as central tendencies within a range of plausible values rather than absolute measures. The wide prediction intervals reported in Figure 2 (e.g., *L. monocytogenes* prediction interval: 0.7-12.1%) better reflect the uncertainty introduced by methodological heterogeneity. Future primary research should adopt standardized protocols (e.g., ISO methods with specified enrichment conditions) and explicitly report quality control measures and sampling designs to facilitate more precise meta-analytic synthesis.

3.10. Limitations and Future Directions

Several limitations of this systematic review should be acknowledged. First, substantial statistical heterogeneity was observed across pooled prevalence estimates ($I^2 > 75\%$ for all pathogens), which, despite the use of a random-effects meta-analytic model, reflects underlying variability in primary study design, sampling frames (retail vs. production), sample sizes (range: 50–890), geographic settings, and detection methodologies. Second, publication bias may have inflated pooled estimates, as studies reporting positive findings are more likely to be published than those with null results, and grey literature was not systematically searched. Third, the exclusion of non-English language publications may have introduced language bias, potentially omitting relevant data from non-English speaking regions. Fourth, the modified JBI quality assessment revealed common methodological limitations among included studies, including unclear sampling randomness (60.3% of studies) and insufficient sample size justification (72.1%), which may affect the generalizability of individual prevalence estimates. Fifth, the heterogeneity in diagnostic methods (culture-based vs. molecular; varying enrichment protocols) complicates direct comparison of prevalence data across studies, as discussed in Section 4.5.

To advance the field, future primary research should adopt standardized protocols aligned with WHO/FAO guidelines, including clearly defined sampling strategies, sample size calculations, and detailed reporting of quality control measures (positive/negative controls, enrichment conditions). Research priorities include: (1) economic evaluations of implementing rapid diagnostic methods (e.g., qPCR, CRISPR-based assays) in routine surveillance programs across different income settings; (2) development and validation of affordable, field-deployable, rapid detection kits specifically designed for low-resource settings; (3) longitudinal studies to elucidate contamination dynamics and seasonality patterns along RTE food supply chains; and (4) standardized reporting of disaggregated data by serotype, genotype, virulence gene profiles, and antimicrobial resistance phenotypes to enable more granular meta-analyses.

4. Conclusion and Recommendations

This systematic review consolidates evidence confirming that emerging foodborne pathogens represent a persistent and measurable threat within the global ready-to-eat (RTE) food sector. By synthesizing data from 68 studies, the review quantifies significant prevalence rates ranging from *Listeria monocytogenes* in soft cheeses to human norovirus in fresh produce and demonstrates the superior diagnostic accuracy (AUC > 0.95) and speed of molecular detection methods compared with traditional culture-based techniques. The findings identify distinct high-risk pathogen–food combinations and reveal substantial disparities in contamination levels and surveillance capacity, particularly between high-income countries and low- and middle-income countries (LMICs). Ensuring the safety of RTE foods therefore extends beyond technical interventions and constitutes a critical public health priority requiring coordinated, risk-based governance. To address these

challenges, regulatory agencies should adopt pathogen- and commodity-specific regulations rather than relying solely on generic microbiological criteria, including mandatory control plans and enhanced environmental monitoring for high-risk products such as soft cheeses and ready-to-eat cooked meats. National food safety authorities should also broaden their focus beyond *E. coli* O157:H7 by formally recognizing major non-O157 Shiga toxin-producing *E. coli* (STEC) serogroups—such as O26, O103, O111, O121, and O145—as significant hazards in relevant RTE foods, particularly beef products and leafy greens. Furthermore, international organizations including the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and Codex Alimentarius should promote harmonized global surveillance systems that utilize shared Whole Genome Sequencing (WGS) databases and standardized reporting mechanisms to facilitate real-time, cross-border outbreak detection, source attribution, and coordinated public health responses.

CRediT authorship contribution statement

Felix Eling (sole author) contributed to all aspects of this work, including conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, writing of the original draft, and review and editing of the final manuscript.

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Declaration of Competing Interest

The author declared no conflict of interest.

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Ethical Consideration

This study is a systematic review of previously published literature. As such, it did not involve direct contact with human or animal subjects, nor did it involve the collection of new primary data. Ethical approval was therefore not required for this synthesis. All original studies cited within this review were responsible for obtaining their own necessary ethical clearances, informed consent, and approvals as required for their primary research.

Data Availability

The data that support the findings of this systematic review are derived from previously published studies, all of which are cited in the reference section. These source materials are publicly available through their respective academic publishers or via databases such as PubMed, Scopus, and Web of Science. The synthesized data extracted and analyzed during the current review are available from the corresponding author upon reasonable request.

AI Declaration

Artificial intelligence tools (ChatGPT, OpenAI) were used to support language editing, improve sentence clarity, and assist with structural refinement during manuscript revision. The author reviewed and verified all content and takes full responsibility for the accuracy, originality, and integrity of the work, including ensuring that all statements and citations are appropriate and scientifically sound.

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