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SUPPLEMENTARY MATERIAL

Current research trends towards the control of protozoans in foods

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Keywords: parasites, fresh produce, shellfish, ddPCR, TAS, viability.

Supplementary Material S1. Search strategies, databases, last-search dates, deduplication and screening logic (PRISMA-S-style reporting).

The purpose of Supplement S1 is to provide proportionate transparency for evidence identification, consistent with SANRA principles for narrative reviews and PRISMA-S elements for search reporting where applicable.

The searches were designed to identify decision-relevant evidence on protozoa control across foods and connected reservoirs, focusing on: (i) matrix-adapted front-end processing (concentration, disruption/lysis, inhibitor management), (ii) inhibitor-resilient quantification (from qPCR to ddPCR escalation), (iii) sequencing-enabled attribution (TAS/mNGS), and (iv) intervention endpoints (detectability vs viability vs infectivity).

1. Information sources

Bibliographic databases: Scopus, Web of Science Core Collection, PubMed

Additional targeted sources: Agency and expert reports (e.g., FAO; FDA; FSIS/NACMCF), Reference-list screening from high-relevance primary studies and guidance; Preprint server (bioRxiv) for method-first evidence (interpreted cautiously as non-peer-reviewed)

Search dates and limits: Last updated: 2026-02-24

Time window: 2022-01-01 to 2026-02-24 (inclusive)

Language: English (primary); key agency documents in English

Document types prioritized: primary peer-reviewed research articles; authoritative guidance; preprints included only for clearly described methods with near-term operational relevance

2. Core concepts and term families

Protozoa / agents: protozoa, *Cryptosporidium*, *Giardia*, *Toxoplasma*, *Cyclospora*, oocyst*, cyst*

Matrices / One Health interfaces: food, fresh produce, leafy greens, lettuce, salad, berries, herbs, cilantro, shellfish, oyster*, mussel*, bivalve*, irrigation water, agricultural water, soil, wastewater, sewage

Methods / analytics: qPCR, quantitative PCR, ddPCR, digital PCR, metagenom*, shotgun sequencing, nanopore, amplicon sequencing, targeted amplicon sequencing, genotyp*, typing, source tracking, WBE, wastewater-based epidemiology

Viability / infectivity endpoints: viability, infectivity, RT-qPCR, reverse transcription, PMA, EMA, cell culture, animal model, inactivat*

3. Database-specific strategies

A complete, reproducible strategy is provided for Scopus, with representative strategies for Web of Science and PubMed. Minor refinements were used during screening (e.g., adding/removing specific matrix terms to increase precision for shellfish or *Cyclospora* genotyping).

Scopus (complete strategy)

TITLE-ABS-KEY(

protozoa OR cryptosporidium OR giardia OR toxoplasma OR cyclospora OR oocyst* OR cyst*

)

AND TITLE-ABS-KEY(

food OR "fresh produce" OR produce OR lettuce OR salad OR spinach OR berries OR cilantro OR herbs OR shellfish OR oyster* OR mussel* OR bivalve* OR irrigation OR "agricultural water" OR soil OR wastewater OR sewage OR "waste water"

)

AND TITLE-ABS-KEY(

qpcr OR "quantitative pcr" OR ddpcr OR "digital pcr" OR metagenom* OR "shotgun sequencing" OR nanopore OR "amplicon sequencing" OR "targeted amplicon sequencing" OR genotyp* OR typing OR "microbial source tracking" OR "source tracking" OR viability OR infectiv* OR "rt-qpcr" OR "reverse transcription" OR pma OR ema

)

AND (PUBYEAR > 2021 AND PUBYEAR < 2027)

Refinement notes: For *Cyclospora* traceback, a focused query added `Cyclospora` AND (`genotyping` OR `amplicon`) with produce-related terms.

For intervention endpoints, optional terms included: inactivat*, HPP, high pressure processing, cold plasma, and pulsed electric field*.

Web of Science Core Collection (representative)

TS=(protozoa OR Cryptosporidium OR Giardia OR Toxoplasma OR Cyclospora OR oocyst* OR cyst*)

AND TS=(food OR produce OR "leafy green*" OR lettuce OR salad OR berries OR herbs OR cilantro OR shellfish OR oyster* OR irrigation OR soil OR wastewater)

AND TS=(qPCR OR ddPCR OR "digital PCR" OR metagenom* OR nanopore OR "amplicon sequencing" OR genotyp* OR viability OR infectiv* OR PMA OR EMA OR RT-qPCR)

Refined by: Publication Years = 2022–2026

PubMed (representative)

((protozoa[Title/Abstract] OR cryptosporidium[Title/Abstract] OR giardia[Title/Abstract] OR toxoplasma[Title/Abstract] OR cyclospora[Title/Abstract])

AND (food[Title/Abstract] OR produce[Title/Abstract] OR lettuce[Title/Abstract] OR herbs[Title/Abstract] OR cilantro[Title/Abstract] OR salad[Title/Abstract] OR shellfish[Title/Abstract]

OR oyster*[Title/Abstract] OR irrigation[Title/Abstract] OR wastewater[Title/Abstract] OR soil[Title/Abstract])

AND (qpcr[Title/Abstract] OR ddpcr[Title/Abstract] OR "digital pcr"[Title/Abstract] OR metagenom*[Title/Abstract] OR nanopore[Title/Abstract] OR "amplicon sequencing"[Title/Abstract] OR genotyp*[Title/Abstract] OR viability[Title/Abstract] OR infectiv*[Title/Abstract] OR pma[Title/Abstract] OR ema[Title/Abstract] OR rt-qpcr[Title/Abstract]))

AND ("2022/01/01"[Date - Publication] : "2026/02/24"[Date - Publication])

Grey literature and agency sources: Targeted searches were performed on: (1) FAO open knowledge repository (protozoa and foods risk assessment); (2) FDA pages for *Cyclospora* prevention/response frameworks; and (3) FSIS/NACMCF reports on *Cyclospora* in produce. These sources were included when directly relevant to control program design, outbreak response, or risk assessment framing.

4. Deduplication and screening

Results were exported from each database and managed in a citation manager (e.g., Zotero). Then, duplicates removed using DOI matching and title/author checks. Screening was performed at title/abstract level for relevance to food-control decisions, followed by full-text checks for minimum methodological detail. Because the review is narrative (non-systematic), we do not provide a PRISMA flow diagram. Instead, all included sources are listed in the included-study register (Supplementary Material S2), alongside design class, matrix/system, and decision-grade elements emphasized.