The prevalence and mean concentration of *Listeria monocytogenes* in select ready-to-eat foods: soft cheeses, deli meats, and packaged salads: a protocol for a systematic review and meta-analysis

Katheryn J. Churchill1,2, Jan M. Sargeant1,2,3, Jeffrey M. Farber3,4, Annette M. O’Connor5, Ali M. Versluis6

**ADMINISTRATIVE INFORMATION**

**Title**

The prevalence and mean concentration of *Listeria monocytogenes* in select ready-to-eat foods: soft cheeses, deli meats, and packaged salads: a protocol for a systematic review and meta-analysis.

**Registration**

In accordance with the guidelines, our systematic review protocol will be registered with Systematic Review for Animal & Food (SYREAF). The systematic review will be reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines. This review protocol will follow the PRISMAP guidelines for review protocols.

**Authors**

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KC will serve as review leader, and therefore will be responsible for coordinating the review and preforming primary and secondary screening, data extraction, risk of bias assessment, and the meta-analysis. KC is responsible for preparing all drafts of the protocol and the final manuscript, with input from JF for content matters. KC developed the search strategy with input from JS. JS will act as the methodological expert, serving as a consultant for conducting the review. All authors contributed to the
development of the selection criteria, the risk of bias assessment strategy and data extraction criteria. All authors read, provided feedback and approved the final manuscript.

Amendments

In the event of deviation from the protocol during the review, the deviation and rationale for it will be reported.

Sponsor

No external funding was acquired for this project.

INTRODUCTION

Rationale

Listeria monocytogenes is a Gram-positive, facultative intracellular pathogen\(^3\) that is the causative agent of the foodborne disease listeriosis. The incidence of listeriosis is low, however it can have severe and sometimes fatal health consequences, especially among pregnant women, infants, the elderly, and immunocompromised individuals\(^3\). Listeria monocytogenes can survive common food processing conditions including extreme pH, low water activity, high salt concentration, and refrigeration temperatures\(^4\). This systematic review targets historically high-risk as well as emerging high-risk ready-to-eat foods, and will compare the prevalence and mean concentration of Listeria monocytogenes for each food type between geographical regions (i.e. Africa, Asia, Caribbean, Central America, Europe, North America, Oceania, South America, or unknown if not described in the abstract). This review will also investigate differences in mean concentration and prevalence in these foods over time, at different stages of processing, and between diagnostic methods if there is sufficient data to do so.

Objectives

The aim of this systematic review is to evaluate the prevalence and mean concentration of L. monocytogenes in select foods: soft cheeses, deli meats, packaged salads. If sufficient data is found, secondary questions will be addressed. To this end, the proposed systematic review will answer the following questions:

Primary Question

1. What is the prevalence and mean concentration of L. monocytogenes in soft cheeses, deli meats, and, packaged salad?

Secondary Questions

2. Is there a difference in the prevalence and mean concentration of L. monocytogenes in between geographical regions (See Appendix 3)?
3. Is there a difference in the prevalence and mean concentration of *L. monocytogenes* over time (i.e. by decade?)

4. Is there a difference in the prevalence and mean concentration of *L. monocytogenes* at different stages of processing e.g., processor, retail, point of consumption?

5. Is there a difference in the prevalence and mean concentration of *L. monocytogenes* between foods that are tested via the cultural method versus DNA diagnostic methods?

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**METHODS**

**Eligibility criteria**

Studies will be selected according to the criteria outlined below:

**Study designs**

We propose to include descriptive and analytical observational studies that measure prevalence or mean concentration of *L. monocytogenes* with two exceptions. Case-control studies and diagnostic test accuracy studies with artificial exposure that provide an estimate of the prevalence or mean concentration of *L. monocytogenes* will be excluded. Studies measuring incidence of *L. monocytogenes* will also be excluded. Experimental studies evaluating interventions to reduce *L. monocytogenes* in ready-to-eat (RTE) foods, review articles, editorials and testimonials, and short (<500 words) abstracts will not be relevant to the review.

**Participants**

Eligible RTE foods to be included in the review are soft cheeses, packaged salads, and deli meats, which are foods known to support the growth of *L. monocytogenes*.

- In the context of this review we define RTE foods as follows:
  - Ready-to-eat(RTE) food: “products in a form that is edible without additional preparation to achieve food safety and may receive additional preparation for palatability or aesthetic, gastronomic, or culinary purposes”
  - Soft cheese: “cheese [that] contains between 67 and 80% moisture”
  - Deli meat: “A ready-to-eat meat or poultry product that typically is sliced, either in an official establishment or after distribution from an official establishment, and typically is assembled in a sandwich for consumption”
  - Packaged salad: “fresh leafy greens whose leaves have been cut, shredded, sliced, chopped, or torn. The term “leafy greens” includes iceberg lettuce, romaine lettuce, leaf lettuce, butter lettuce, baby leaf lettuce (i.e., immature lettuce or leafy greens), escarole, endive, spring mix, spinach, cabbage, kale, arugula and chard. The term “leafy greens” does not include herbs such as cilantro or parsley. Lettuce and other leafy greens cut from their root in the field with no other processing are considered raw agricultural commodities (RACs) and are not included in the definition of “cut leafy greens””

**Outcome**

Prevalence or mean concentration of *L. monocytogenes* in included RTE foods.
Language

We will include articles reported in the English language.

Information sources

Multiple databases will be used to increase sensitivity of the search, and both published and unpublished literature will be eligible for inclusion. The following databases will be used to find published literature: MEDLINE® (Ovid interface), Web of Science™ (Thompson Reuters interface), and Agricola (ProQuest® interface). Grey literature will be identified via Conference Proceedings Citation Index-Science(CPCI-S) (Web of Knowledge interface), Science.gov (via http://www.science.gov/), ScienceResearch.com (via http://www.sciencere search.com/), and OpenGREY (via http://www.opengrey.eu/).

To ensure literature saturation, we will scan the reference list of included studies identified through the search.

Search strategy

No study design, date, or language limits will be imposed on the search. In the event of a non-English title/abstract, google translate will be used to convert to English, however this method will not be used at the full article screening; at this point non-English articles will be excluded and the reason will be documented. As relevant studies are identified at the full text screening level, reviewers will check for additional relevant cited and citing articles. The specific search strategies will be created with input from a Librarian with experience in systematic review searching. A draft MEDLINE search strategy is included in Appendix 1. The proposed MEDLINE strategy is included in Appendix 1. The librarian assisted in adapting the search strategy to the syntax and subject headings of the other databases. Search results will be uploaded into EndNote™ bibliographical management software (Philadelphia, PA, U.S.A.). Duplicates will be removed. Documentation of all search strategies and results will be provided in the final report.

Study records

Data management

Literature search results will be uploaded into online systematic review software (Distiller SR® (DSR), Ottawa, ON, Canada. Prior to the screening process, the questions will be piloted and tested by the reviewers on a subset of studies (200). Following pilot testing, the form will be adapted as recommended by the extractors to improve usability and completeness.

Selection process

Screening will be completed in two stages. The first stage of screening will be conducted via title and abstract screening. Two reviewers will screen articles independently, using evaluation questions. All questions will be answered using ‘yes’, ‘no’, or ‘unsure’. Questions will be answered stacked, such that, if a question is answered ‘no’ the reviewer will not answer the remaining questions. Questions for both level 1 and 2 screening are outlined in Appendix 2. Non-English abstracts found in level 1 will continue to level 2. If the full text article cannot be found in English, the article will be excluded based on the language requirement. Articles will be excluded if both reviewers answer ‘no’ to any of the questions. If
both reviewers answer ‘yes’ and/or ‘unsure’ to all questions for a citation, it will be retained for full article screening. Any disagreements will be resolved by consensus or arbitration. Full articles for abstracts deemed relevant at level one will be assessed in level two screening. If full text articles pass successfully through level two screening, they will enter the data collection process outlined below.

Data collection process
A data extraction form will be created in DistillerSR® (EvidencePartners, Ottawa, Canada). The extraction form will be piloted and tested by the data extractors on a subset of citations (5). Following pilot testing, the form will be adapted as recommended by the extractors to improve usability and completeness. The first author and one additional extractor will complete data extraction. Data extraction will be completed independently and the extractors will compare the data for consensus. If the extractors cannot answer a question, consensus will confirm that the data are unavailable to answer the question. In the event the data presented in a study are unclear, missing, or presented in a non-extractable or unusable form, authors will be contacted for clarification via email, and a follow-up email will be sent 2-weeks later. Authors will be provided 4-weeks from the initial contact to respond. Missing data will be noted in the report.

Data items
Using automatic features in Distiller SR® we will extract study identifiers as well as the year of publication. Location (geographic region and country) will be extracted from the article, and if it is not stated we will use the country of affiliation of the first author. Regions and their respective countries are outlined in Appendix 3. The specific study design will be extracted. We will also extract information on the specific food type, the stage of production where the sample was taken, and the detection method used (e.g., culture-based, DNA-based). Outcome related data will include the sample size, number (or proportion) of positive samples, and the prevalence and mean concentration of L. monocytogenes.

Outcomes and prioritization
The primary outcomes are the prevalence and mean concentration of L. monocytogenes in soft cheeses, deli meats, and packaged salads.

Risk of bias in individual studies
A modified version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool previously modified for prevalence studies, will be used to assess the risk of bias in each study, with additional modifications tailored to this review. The assessment criteria are outlined in Appendix 4. Results will be presented graphically or in a table and used in the interpretation of the review results.

Data synthesis
To summarize the findings, we propose to conduct a meta-analysis using the R Studio package (Boston, MA, U.S.A.) or Comprehensive Meta-Analysis (CMA) (Englewood, NJ, U.S.A.). A separate meta-analysis is planned for each study outcome (e.g. prevalence, mean concentration), stratified by food type (e.g. soft cheese, deli meats, packaged salad) using a random effects approach given the a priori assumption that
the study populations will compromise significant differences. Prevalence will be transformed using the Freeman-Tukey arcsine transformation in order to stabilize the variance. Summary effect measures will be reported as summary prevalence estimates.

Heterogeneity will be assessed using Cochran’s Q test and $I^2$. Potential sources of heterogeneity will be addressed with meta-regression and sub-group meta-analysis. Geographic region, time (decade) of study, diagnostic method, and stage of sampling (e.g. processor, trail, point of consumption) will be examined if there are at least two studies per category for the variable.
References


Appendix 1:

Preliminary search conducted in MEDLINE (Ovid platform) on 03/May/17

a) listeria [search line 1]

b) high risk foods [search lines 2-9]

<table>
<thead>
<tr>
<th>#</th>
<th>Searches</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(listeria or monocytogenes or listeriosis).ab,kf,ti. [ab= abstract, kf=keyword, ti=title]</td>
<td>19302</td>
</tr>
<tr>
<td>2</td>
<td>(deli or delis or delicatessen or charcuterie or cold meat* or cold cut* or lunch meat* or luncheon meat* or sandwich* or salami* or sliced salami* or pepperoni or chorizo or bologna or baloney or boloney or polony or sliced corned beef or sliced ham or sliced smoked ham or head cheese or sliced veal or sliced roast beef or mortadella or smoked meat or pastrami or beef pastrami or turkey pastrami or sliced tongue or sliced turkey breast or sliced chicken breast or chicken breast supreme or sliced mock chicken or pancetta or prosciutto or ham off the bone or chicken roll or roast beef).ab,kf,ti. [ab= abstract, kf=keyword, ti=title]</td>
<td>27147</td>
</tr>
<tr>
<td>3</td>
<td>(soft cheese or feta or brie or camembert or Neufchatel or queso blanco or chevre or blue vein* or mold ripe* or mould ripe* or soft ripe* or danish blue or gorgonzola or ricotta or munster or pont leveque or pont l’eveque or epoisses or coulommiers or unpasteurized or raw).ab,kf,ti. [ab= abstract, kf=keyword, ti=title]</td>
<td>55955</td>
</tr>
<tr>
<td>4</td>
<td>(salad or lettuce or leaf* vegetable* or escarole or endive or spring mix or cabbage or chard or spinach or romaine or kale or arugula or greens) ab,kf,ti. [ab= abstract, kf=keyword, ti=title]</td>
<td>19834</td>
</tr>
<tr>
<td>5</td>
<td>1 and 2 ab,kf,ti. [ab= abstract, kf=keyword, ti=title]</td>
<td>389</td>
</tr>
<tr>
<td>6</td>
<td>1 and 3 ab,kf,ti. [ab= abstract, kf=keyword, ti=title]</td>
<td>999</td>
</tr>
<tr>
<td>7</td>
<td>1 and 4 ab,kf,ti. [ab= abstract, kf=keyword, ti=title]</td>
<td>345</td>
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</tbody>
</table>

Appendix 2

Relevance screening level 1 questions:

<table>
<thead>
<tr>
<th>Question #</th>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Is this an original research study of eligible design?</td>
</tr>
<tr>
<td>2</td>
<td>Does the study involve one of the relevant ready-to-eat foods (soft cheese, deli meat, packaged salad)?</td>
</tr>
<tr>
<td>3</td>
<td>Does the study report on the prevalence or mean concentration of <em>Listeria monocytogenes</em> under natural exposure?</td>
</tr>
</tbody>
</table>
Appendix 3

Relevance screening level 1 questions:

<table>
<thead>
<tr>
<th>Question #</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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</tr>
<tr>
<td>3</td>
<td>Does the study report on the prevalence or mean concentration of <em>Listeria monocytogenes</em> under natural exposure?</td>
</tr>
<tr>
<td>4</td>
<td>Is the study written in English?</td>
</tr>
</tbody>
</table>

Appendix 4

Geographic Regions of the World

<table>
<thead>
<tr>
<th>Geographic Region</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>Afghanistan, Armenia, Azerbaijan, Bahrain, Bangladesh, Bhutan, Brunei, Burma, Cambodia, China, Cyprus, East Timor, Georgia, Hong Kong, India, Indonesia, Iran, Iraq, Israel, Japan, Jordan, Kazakhstan, Kuwait, Kyrgyzstan, Laos, Lebanon, Macau, Malaysia, Maldives, Mongolia, Nepal, North Korea, Oman, Pakistan, Philippines, Qatar, Saudi Arabia, Singapore, South Korea, Sri Lanka, Syria, Taiwan, Tajikistan, Thailand, Turkey, Turkmenistan, United Arab EmiratesCar, Uzbekistan, Vietnam, and Yemen.</td>
</tr>
<tr>
<td>Caribbean</td>
<td>Anguilla, Antigua and Barbuda, Aruba, The Bahamas, Barbados, Bermuda, British Virgin Islands, Cayman Islands, Cuba, Dominica, Dominican Republic, Grenada, Guadeloupe, Haiti, Jamaica, Martinique,Montserrat, Netherlands</td>
</tr>
</tbody>
</table>
Antilles, Puerto Rico, Saint Kitts and Nevis, Saint Lucia, Saint Vincent and the Grenadines, Trinidad and Tobago, Turks and Caicos Islands, and the U.S. Virgin Islands.

<table>
<thead>
<tr>
<th>Central America</th>
<th>Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>Albania, Andorra, Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Gibraltar, Greece, Holy See, Hungary, Iceland, Ireland, Italy, Kosovo, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Monaco, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Russia, San Marino, Slovak Republic, Slovenia, Spain, Serbia, Serbia and Montenegro, Sweden, Switzerland, Ukraine, and the United Kingdom.</td>
</tr>
<tr>
<td>North America</td>
<td>Canada, Greenland, Mexico, Saint Pierre and Miquelon, and the United States</td>
</tr>
<tr>
<td>Oceania</td>
<td>American Samoa, Australia, Christmas Island, Cocos (Keeling) Islands, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, Nauru, New Caledonia, New Zealand, Niue, Northern Mariana Islands, Palau, Papua New Guinea, Pitcairn Islands, Samoa, Solomon Islands, Tokelau, Tonga, Tuvalu, Vanuatu, and Wallis and the Futuna Islands.</td>
</tr>
<tr>
<td>South America</td>
<td>Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Falkland Islands, French Guiana, Guyana, Paraguay, Peru, Suriname, Uruguay, and Venezuela.</td>
</tr>
</tbody>
</table>
Modified version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool: criteria for prevalence studies

<table>
<thead>
<tr>
<th>Criteria #</th>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The final sample should be representative of the target population. At least one of the following should apply for the study (Yes[Y]/No[N]): 1. Sampled an entire target population 2. Randomly selected samples 3. Convenience sampling</td>
</tr>
<tr>
<td>2</td>
<td>Was the objective of the study to estimate prevalence or mean concentration or was this information a byproduct of another study design (Yes, study objective/No, secondary findings)?</td>
</tr>
<tr>
<td>3</td>
<td>Was the time from sampling to laboratory analysis consistent for all samples in the study (Y/N)?</td>
</tr>
<tr>
<td>4</td>
<td>Was the same laboratory methods used for all samples in the study (Y/N)?</td>
</tr>
</tbody>
</table>