Rapid Systematic Review Protocol

Project title: A rapid systematic review of the literature on the association between *Salmonella enterica* harborage in cattle at various life stages and *Salmonella enterica* burden at harvest

Registration: This protocol will be registered in the knowledge synthesis section of the K-Rex repository (https://krex.k-state.edu/handle/2097/42518).

Authors, contacts, and contributions:

Dr. John E. Ekakoro (JEE), Center for Outcomes Research and Epidemiology (CORE), College of Veterinary Medicine, Kansas State University, jekakoro@vet.k-state.edu, Co-Principal Investigator.

- Coordination of team members
- Study design development
- Development of rapid review tools
- Database search
- Relevance screening
- Data extraction
- Risk of bias assessment
- Synthesis
- Manuscript development and approval for publication

Dr. David Edache (DE), Center for Outcomes Research and Epidemiology (CORE), College of Veterinary Medicine, Kansas State University, edachedave@vet.k-state.edu

- Calibration and testing of the rapid review tools
- Relevance screening
- Data extraction
- Synthesis
- Manuscript development and approval for publication

Dr. Natalia Cernicchiaro (NC), Center for Outcomes Research and Epidemiology (CORE), College of Veterinary Medicine, Kansas State University, ncernic@vet.k-state.edu, Principal Investigator.

- Overall guidance on the rapid systematic review process
- Study design development
- Data extraction
- Synthesis
- Manuscript development and approval for publication

Dr. David Renter (DR), Center for Outcomes Research and Epidemiology (CORE), College of Veterinary Medicine, Kansas State University, drenter@vet.k-state.edu, Co-Principal Investigator.

- Study design development
- Manuscript development and approval for publication

Ms. Margaret Costello (MC), Department of Animal & Dairy Sciences, College of Agricultural and Life Sciences, University of Wisconsin-Madison, mkcostello@wisc.edu

- Calibration and testing of the rapid review tools
- Relevance screening
- Data extraction
- Risk of bias assessment
- Synthesis
- Manuscript development and approval for publication

Dr. Sara E. Gragg (SEG), Department of Animal & Dairy Sciences, College of Agricultural and Life Sciences, University of Wisconsin-Madison, sgragg@wisc.edu

- Study design development
- Data extraction
- Synthesis
- Manuscript development and approval for publication

Dr. Steven C. Ricke (SCR), Director Meat Science and Animal Biologics Discovery Program, Department of Animal & Dairy Sciences, College of Agricultural and Life Sciences, University of Wisconsin-Madison, sricke@wisc.edu

- Study design development
- Data extraction
- Synthesis
- Manuscript development and approval for publication

Tentative Project Start date: January 01, 2024. Project end date: December 31, 2024

A breakdown of the time frame is given below.

Activity	Done by	Month
Calibration and testing of the relevance screening, and risk of bias assessment tools	JEE	January-April 2024
Calibration and testing of the data extraction tool	JEE, MC, DE,	January-March 2024
Database search	JEE	February 2024
Relevance screening	JEE	March-April 2024
Data extraction	JEE, MC, DE, NC, SEG and SCR	May to August 2024
Risk of bias assessment	JEE	May to August 2024
Synthesis	JEE, MC, DE, NC, SEG and SCR	September-October 2024

Manuscript writing	JEE, MC, DE, SEG, SCR, DR, and NC	November-December 2024
Manuscript submission to journal	NC	December 2024

Sponsor: The National Cattlemen's Beef Association (NCBA)

Role of sponsor: Other than providing the funds for this study, the sponsor has not played any role in developing this protocol.

Introduction

Non-typhoidal *Salmonella*, is a major cause of foodborne illness in humans in the United States leading to about 1.35 million human infections, 26,500 hospitalizations, and 420 deaths (CDC, 2023). Food-producing animals such as cattle are reservoirs of *Salmonella* (Heredia and García, 2018) posing a serious food safety concern (Xie et al., 2016). Animals that have recovered from initial *Salmonella* infection may continue to harbor the bacteria in their lymph nodes and internal organs, a phenomenon referred to as carrier status, and may intermittently or continuously shed high numbers of the bacteria in their feces or milk (Nielsen et al., 2004). *Salmonella* harborage in cattle has been studied by several research groups who have demonstrated the presence of this pathogen in cattle lymph nodes (Brichta-Harhay et al., 2012; Gragg Sara E. et al., 2013; Webb et al., 2017; Wottlin et al., 2022), hence posing a transmission risk to consumers via ground beef (Xie et al., 2016). Cattle type, season, geographic region where cattle farm is located are risk factors for *Salmonella* harborage in peripheral lymph nodes and feces of cattle at harvest (Webb et al., 2017; Wottlin et al., 2022). In addition to the mentioned risk factors, the life stage of cattle may be an important risk factor for *Salmonella* harborage. A study conducted in the northeastern United States (Cummings et al., 2009), found wide disparity in the incidence of *Salmonella* among cattle at different life stages with pre-weaned female calves having the highest incidence and the lowest incidence reported in adult cows. Additionally, calves harvested for veal have been found to harbor *Salmonella* (Nielsen et al., 2011). Despite the existing literature on *Salmonella* harborage, we do not have a comprehensive understanding of the link between *Salmonella* harborage in cattle at various life stages and the burden of *Salmonella* at harvest (from stunning, skinning, evisceration, chilling to fabrication into beef cuts, ground beef, and other beef products). Given its importance as a foodborne pathogen and the risk of its transmission to consumers through beef products, there is a need for an in-depth understanding of the association between pre-harvest harborage and the impact on post-harvest burden of this pathogen in beef carcasses, ground beef and other beef products. This knowledge will inform efforts aimed at mitigating *Salmonella* in beef products. This study will review the existing scientific literature to fill this knowledge gap and identify key areas for future research and will be conducted as a collaboration between Kansas State University and the University of Wisconsin-Madison.

Objectives

The objectives of this rapid review are to:

1. Assess scientific literature on the link between *Salmonella* harborage in all cattle breeds or cattle type including beef-on-dairy cross animals at various life stages from birth up to transportation to the slaughterhouse and lairage, and the burden of *Salmonella* at harvest.
2. Identify knowledge gaps and provide recommendations for future research.
3. Collaborate with UW investigators to prepare a comprehensive review and assessment of the literature.
4. Submit final review manuscript(s) to peer-reviewed scientific journals for publication.

Rapid review question

What is the association between *Salmonella* prevalence and concentration in all cattle breeds at various life stages from calf hood to lairage with *Salmonella enterica* prevalence and concentration at harvest (from stunning, skinning, evisceration, chilling to fabrication into beef cuts, ground beef, and other beef products)?

Methods

This protocol is based on the guidance provided by Cochrane Rapid Reviews Methods Group (Garritty et al., 2021).

Inclusion criteria:

- Studies must report on non-typhoidal *Salmonella*.
- Study must have been conducted in the United States
- Studies must be on *Salmonella* harborage in cattle of any breed or cattle type and including beef-on-dairy cross animals and cull dairy cows.
- Experimental and observational studies.
- Studies must be on *Salmonella enterica* burden in cattle at various life stages up to transportation to the slaughterhouse and lairage.
- Studies must report on *Salmonella* burden at harvest at stunning, skinning, evisceration, chilling to fabrication into beef cuts, ground beef, and other beef products.
- Studies must report on prevalence and/or concentration of *Salmonella* in the United States. Studies should report on any or all *Salmonella* serotypes. Studies reporting on *Salmonella* at the species level (*Salmonella* spp.) will also be included.
- Studies reporting on *Salmonella* in feces collected directly from the rectum or other intestinal contents, external farm environment (e.g., pastures, non-mammalian vectors e.g., insects, rodents, wild birds), internal animal housing environment (e.g., pen-floor samples), animal feed, water samples from water troughs, environmental related matrices during transportation and in the lairage e.g., transportation trucks), lymph nodes, hides, pre-and post-evisceration carcass swabs, internal organs (such as rumen, livers, hearts, kidneys), meat cuts, and ground beef.
- Studies must be published in English, in peer-reviewed journals.
- Studies must reflect original research including meta-analyses.

- Data from existing relevant systematic reviews will be used to reduce the time spent on data extraction, and to minimize the risk of duplication of already synthesized evidence.
- No publication date limits will be set.

Exclusion criteria:

In general, any study that does not meet all inclusion criteria will be excluded from the review. Studies that will be excluded from the review include:

- Studies reporting on *Salmonella* in animals other than cattle.
- Studies conducted outside the United States
- When only citations and/or abstracts are available and full text articles are missing (attempts will not be made to contact authors).
- Studies published in languages other than English.
- Narrative reviews will be excluded.
- Grey literature including dissertations and theses.
- Case reports, case series and surveys will be excluded.
- Studies reporting on validation of diagnostic test performance will be excluded. If such studies report on prevalence and concentration of *Salmonella*, only the section reporting prevalence or concentration will be considered.
- Studies reporting on anti-*Salmonella* activity of medicinal products will be excluded. If such studies include negative controls where applicable, and report on prevalence and/or concentration of *Salmonella*, only the section reporting on these aspects will be considered.

Information sources:

Scholarly databases to be searched will include PubMed (Medline) and Scopus.

Search Strategy:

A comprehensive list of search terms for identifying potentially relevant literature will be compiled and these search terms will be related to the population of interest (cattle of all breed types at different life stages and the outcomes of interest (*Salmonella* prevalence and concentrations in different sample types pre and post slaughter). The search strategy will be tailored to the specifications of each database. Search terms within each component of the rapid review question will be combined using "OR" and the population and outcome components will be linked using "AND" as summarized the table below. A manual search for additional publications will be performed by scanning through the reference lists of the retrieved publications.

Database	Search terms	Number of publications retrieved as of 2/23/2024

PubMed (Medline)	Salmonella OR Salmonella enterica AND (ruminant OR bovine OR cattle OR cow OR steer OR beef OR calf OR calves OR heifer OR yearling OR young cattle OR young beef OR young dairy OR adult cattle OR adult beef OR adult dairy OR bull OR bullock OR cull OR dairy OR veal OR weaned OR stockers OR backgrounders OR finished OR feedlot OR feedyard) AND (prevalence OR percent positive OR Positives OR isolation OR identification OR concentration OR concentrations OR Bacterial levels OR Bacterial counts OR Bacteria counts) AND (faeces OR feces or fecal OR faecal or manure OR transportation OR lairage OR skinning OR evisceration OR environment OR animal feed OR pen-floor samples OR pen floor OR pen OR pen-surface OR pen surface OR water OR water troughs OR lymph nodes OR hides OR carcass OR internal organs OR rumen OR heart OR kidney OR liver OR meat cuts OR ground meat OR ground beef).	3,634
Scopus	(TITLE-ABS-KEY(Salmonella) OR TITLE-ABS-KEY(Salmonella AND enterica) AND TITLE-ABS-KEY(ruminant OR bovine OR cattle OR cow OR steer OR beef OR calf OR calves OR heifer OR yearling OR bull OR bullock OR cull OR	787

	dairy OR veal OR weaned OR stockers OR backgrounder OR finished OR feedlot OR feedyard) AND TITLE-ABS-KEY(prevalence OR percent positive OR Positives OR isolation OR identification OR concentration OR concentrations OR levels OR counts) AND TITLE-ABS-KEY(faeces OR feces or fecal OR faecal or manure OR transportation OR lairage OR skinning OR evisceration OR environment OR feed OR floor OR water OR troughs OR lymph OR nodes OR hides OR carcass OR organs OR rumen OR heart OR kidney OR liver OR meat OR ground OR beef))	

Data management:

Abstracts from all databases will be downloaded into the Covidence systematic review software (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia) by a single reviewer (JEE), and duplicates removed (JEE). Reference lists of any systematic review articles will be evaluated for relevant articles. Retrieved articles will be screened for relevance at two levels. In the first level, only titles and abstracts of the retrieved articles will be reviewed, and irrelevant articles discarded. Then, full-text papers of only articles that passed the relevance screening or were deemed unclear during the title and abstract screening will be screened at the second level. After relevance screening, data will be extracted from the relevant papers, followed by evidence synthesis.

Relevance screening /study selection

The following steps will be used for the relevance screening:

- Citations retrieved from the search engines will be downloaded as Research Information Systems (RIS) files and then uploaded to Covidence. This will be followed by deduplication using the deduplication function in Covidence.
- A standardized relevance screening tool will be developed by JEE and piloted using 50 abstracts by JEE. Amendments to the tool will be made as deemed necessary.
- All the title and abstract screening will be performed by JEE using the finalized relevance screening tool.

Supplementary screening will be performed on only articles deemed unclear during the title and abstract screening.

Data extraction:

- Data extraction will be conducted using a pre-tested data extraction tool. The data extraction tool will be calibrated using the approach described for the relevance screening tool.
- Using the finalized data extraction tool, full text articles will be assessed for data extraction in duplicate by teams of two reviewers working independently. JEE will pair with SEG, MC with SCR, and DE with NC. Any discrepancies will be resolved by consensus or by a third reviewer (JEE, MC, DE, NC, SEG and SCR). When necessary, JEE or any other team member will join any of the other extraction teams to supplement extraction efforts. Full-text articles will be excluded during the extraction stage if both reviewers in an extraction team agree that it is irrelevant and should be excluded.
- Data from existing systematic reviews (if any) will be extracted to reduce the time spent on data extraction and to minimize the likelihood of possible duplication of already synthesized knowledge. However, the methodological and reporting quality of these systematic reviews will be assessed prior to extraction.

Data items

The following information will be extracted from each eligible publication: the article title, all authors, first affiliation, journal, volume, pages, year of publication, year of study, study location in the United States, cattle type (beef, dairy, beef-on-dairy crosses), age (calves, yearlings, adults), life stages (calf hood, weaned, feedlot, cull, transportation, lairage), sampling location (farm, ranch, slaughterhouse, feedlot), production system, study type (research article, systematic review or meta-analysis), study design (experimental, observational), sample size, sample type tested, number of samples tested, type of diagnostic test used, number of *Salmonella* positive samples, serotype(s) identified.

Outcomes and prioritization

The outcomes of interest that will be extracted include:

- *Salmonella* prevalence in lymph nodes, feces, hides, farm environmental samples, carcass swabs, meat cuts, ground beef; reported as proportions or percentages, risk ratios, incidence rate ratios with the associated confidence intervals and p-values. Crude or adjusted measures of association will be considered. This data will be extracted for each serotype if serotype(s) is reported in the publication.
- *Salmonella* concentration (reported as colony-forming units per gram (CFU/g) or Cycle Threshold (CT) values for molecular quantification) in feces, lymph nodes, environmental samples, hides, beef carcasses, ground beef, and other beef products. This data will be extracted for each serotype if serotype(s) is reported in the publication.

These outcomes of interest will be extracted alongside information on the study period (year and month) the study was conducted, the season the study was conducted, and the geographic characteristics of the study (state, city, region where the study was conducted).

Risk of bias assessment:

- Risk of bias assessment of key outcomes will be conducted using a standardized risk of bias assessment tool (where applicable).
- The risk of bias will be rated by a single reviewer (JEE).

Data synthesis:

- The characteristics of each included study will be summarized in the "characteristics of included studies table."
- A narrative synthesis, using a tabular approach, will be conducted to interpret the collective evidence fully. The narrative synthesis will be organized around the population of interest and outcomes question framework elements.
- If appropriate, a meta-analysis will be performed for only important outcomes.

Other considerations

- Post hoc changes to the protocol (e.g., eligibility criteria, search strategy) will be allowed for efficiency and as part of an iterative process.
- Any amendments to the protocol will be tracked, documented, and dated. A pre-tested template will be used to summarize any protocol amendments including a description of the change and the rationale. An updated protocol with the amendments will be posted in the K-Rex repository. Any amendments made will be reported in the manuscript write up.

References

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NIELSEN, L.R., D.L. BAGGESEN, S. AABO, M.K. MOOS, and E. RATTENBORG, 2011: Prevalence and risk factors for Salmonella in veal calves at Danish cattle abattoirs. *Epidemiology & Infection* **139**, 1075–1080, DOI: 10.1017/S0950268810002591.

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