Title: Comparative sensitivity and specificity of *Salmonella enterica* detection methods in equine feces: A systematic review

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ECH, AMO, and BAB conceptualized the study and drafted the review protocol. All authors provided input and final approval of the protocol. ECH will conduct the literature search. All authors will participate in screening, data collection, and risk of bias assessment. ECH will perform data analysis and draft the manuscript, with oversight by BAB and AMO. All authors will review and provide approval of the final manuscript.

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Abstract: Enriched fecal culture and PCR are commonly used for the detection of *Salmonella* in equine feces. However, there is a lack of reliable and generalizable information regarding the sensitivity and specificity of these tests, which hinders appropriate clinical decision-making in equine facilities. Therefore, in this systematic review, we will evaluate the available information on the diagnostic sensitivity and specificity of enriched *Salmonella* fecal culture and PCR in horses and assess the impact of study design, test protocol, and patient population characteristics on these measures of test accuracy.

Rationale: *Salmonella enterica* is among the most commonly reported causes of healthcare-associated infections in equine hospitals and a frequently cited reason for facility closure or restricted admissions.[1] The natural history of this disease, along with the limitations of commonly used *Salmonella* detection methods, hamper the identification of truly negative horses, and in turn, complicate the management of *Salmonella* in equine facilities. Both enriched aerobic culture and polymerase chain reaction (PCR) are frequently used for the diagnosis of equine salmonellosis, but our understanding of the accuracy of these tests remains incomplete. This issue partially stems from the variability in testing methods between studies and laboratories, which hinders the estimation of generalizable measures of test

accuracy.[2] Further, diagnostic test assessments for the detection of *Salmonella* tend to be performed on high-risk subgroups of horses (e.g., with colic or colitis), which can greatly impact estimates of test performance (e.g., sensitivity and specificity). Objective information about test reliability was recently identified by a panel of international experts as a critical need for improved infection control in equine populations.[3] Therefore, this review aims to identify, appraise, and synthesize available information on the accuracy (i.e., diagnostic sensitivity and specificity) of the tests most commonly used for the detection of *Salmonella* in equine fecal samples.

Clinical role of index test(s): In equine hospitals, culture and PCR are used as diagnostic tests among horses with clinical signs suggestive of *Salmonella* infection. Additionally, they are often used as screening tests for *Salmonella* surveillance as part of hospital infection control programs. PCR offers the advantage of a relatively fast turnaround time compared to aerobic culture;[4] however, it does not necessarily detect viable organisms. Therefore, *Salmonella* culture is used either alone or in tandem with PCR to confirm infection. Further, culture allows for *Salmonella* characterization through serogrouping, serotyping, and antimicrobial susceptibility testing. Because *Salmonella*-infected horses tend to shed low numbers of the bacteria, and equine feces are a rich microbial environment, fecal enrichment in non-selective and/or selective media is typically performed as an initial step in *Salmonella* culture or PCR. Therefore, in this review, any variations of enriched culture or enriched PCR (e.g., non-selective fecal enrichment in buffered peptone water and/or selective fecal enrichment in tetrathionate or selenite broth) will be considered as the index tests, with subgroup analyses performed to assess the impact of enrichment broth type on test sensitivity and specificity.

Objectives: The primary objective of this systematic review is to examine and appraise the existing literature in order to compare the diagnostic sensitivity and specificity of enriched fecal culture and PCR for the detection of fecal *Salmonella* shedding in horses. Secondarily, we aim to identify factors related to study design, patient population, and test protocol that drive heterogeneity in the diagnostic sensitivity and specificity of these tests.

Table 1: Definitions for study objectives

Population	Horses tested for *Salmonella* by enriched fecal culture and/or enriched fecal PCR
Index Tests	Enriched fecal culture and enriched fecal PCR
Target Condition	Fecal *Salmonella* shedding (including both clinical and subclinical shedding)
Outcome	Diagnostic sensitivity and specificity of the index tests

Eligibility criteria: Eligibility criteria will include publication in English with no restriction on date or publication type. Both published and non-published (grey literature) studies are eligible, provided they report the results of a primary research study of diagnostic test assessment on equine fecal samples using an eligible study design, including:

- Cross-sectional diagnostic studies: studies with a primary objective of assessing diagnostic test accuracy in which the presence of the target condition is unknown among study subjects at the time of enrollment, and both the index and reference tests are performed on the same individuals

- Experimental studies: studies of diagnostic test accuracy in which the index and reference tests are performed on experimentally inoculated samples or samples from experimentally infected individuals
- Field studies/outbreak investigations: studies with a primary objective of assessing disease presence/absence among the study population
- Diagnostic case-control studies: diagnostic accuracy studies in which the presence of the target condition is known (and accepted as the true state of health/disease) before the index test is performed

Information sources: A literature search will be conducted in a range of relevant bibliographic databases and other information sources containing both published and unpublished (grey) literature. Table 2 presents the resources to be searched.

Table 2: Databases and information sources to be searched via UGA Libraries

Database/Information Source	Interface/URL
PubMed	PubMed (UGA Libraries)
CAB Abstracts/CAB Archive	EBSCO*host* (UGA Libraries)
Web of Science	Web of Science (UGA Libraries)
Agricola	EBSCO*host* (UGA Libraries)
PubAg	USDA (UGA Libraries)

In addition, a hand-search of the table of contents of the following relevant conference proceedings from the previous 5 years if conference reports are >500 words: Proceedings of the International Symposium on *Salmonella*, Proceedings of the American Association of Veterinary Laboratory Diagnosticians/United States Animal Health Association (AAVLD/USAHA) Annual Meeting*;* and we will check the reference lists of all included studies for any eligible studies that may have been missed by the database searches.

Search strategy: A search strategy designed to identify studies on comparative use of *Salmonella* testing methods in horses is presented in Table 3.

The search was based on 3 concepts:

1) Population – horses;
2) Target condition — *Salmonella* shedding; and
3) Index tests — comprising 3 concepts
    a) terms related to the testing methods,
    b) terms related to diagnostic test performance, and
    c) terms related to analytic methods.

As part of developing this search we reviewed the reference lists of an older 1985 paper and a newer 2016 paper to determine that this search strategy was performing as intended.[5,6]

Table 3: Search strategy to identify studies on the comparative use of *Salmonella* testing methods in horses in CAB Abstracts/CAB Archive, PubMed, Agricola, Web of Science, and PubAg; July 13, 2023)

Search number	Search Parameter	Search Strings	Number of Returns				
			CAB	PubMed	Agricola	Web of Science	PubAg
1	Population	horse* OR equid* OR	244,687	205,348	58,242	453,145	24,072

		equine* OR equus OR mare* OR gelding* OR stallion* OR pony OR ponies OR foal*					
2	Target condition	salmonell* OR enterica	77,975	103,352	23,371	122,121	3,902
3	Diagnostic test performance	roc OR "roc curve*" OR "receiver operating characteristic*" OR auc OR "area under the curve"	24,353	311,858	24,642	293,152	202
4	Diagnostic test performance	sensitivity OR specificity OR "predictive value" OR "likelihood ratio" OR accuracy OR correlation OR "false negative*" OR "false positive*" OR “latent class” OR bayes*	991,976	7,708,585	553,180	6,044,657	141,005
5	Index test	culture OR enrich* OR pre-enrich* OR preenrich* OR selenite OR tetrathionate OR “buffered peptone water” OR BPW OR “rappaport-vassiliadis” OR “RV” OR R10 OR “polymerase chain reaction” OR PCR OR rPCR	1,188,782	3,277,550	473,372	4,263,079	521,034

		OR rtPCR OR r-PCR OR rt-PCR OR qPCR OR q-PCR					
6	Diagnostic test	3 OR 4 OR 5	2,090,545	9,933,280	985,048	10,003,482	641,996
7	Exclude *Salmonella* serotype Abortusequi	abortusequi OR abortus-equi OR "abortus equi"	370	256	22	200	2109
8	Final search	1 AND 2 AND 6 NOT 7	464	480	106	446	6

Study records:

Data management: Search results will be downloaded in a tagged format into bibliographic software (EndNote, Clarivate Analytics, Philadelphia, PA, USA). Results from resources that do not allow export in a format compatible with EndNote will be saved in Word or Excel documents, as appropriate, and manually de-duplicated. Search results from EndNote will be uploaded into online systematic review software (Covidence®, Melbourne, VIC, Australia) and de-duplicated. Reviewers will have training in epidemiology and systematic review methods. Before both abstract and full-text screenings, data extraction, and risk-of-bias assessment for diagnostic tests, the reviewers assigned to each step will undergo training to ensure consistent data collection using forms created in Covidence®.

Selection process: In the first round of screening, abstracts and titles will be screened for inclusion using the eligibility criteria from ITEM 6 and the screening questions. Two reviewers will independently evaluate each citation for relevance using the following screening questions:

1. Does the study involve assessment of a diagnostic test for the detection of *Salmonella* spp. (other than *Salmonella enterica* serovar Abortusequi) in equine fecal samples?

    - ☐ Yes - next question
    - ☐ Unclear - next question
    - ☐ No – exclude

2. Does the study involve assessment of at least one of the diagnostic tests of interest (enriched fecal culture, enriched fecal PCR)?

    - ☐ Yes - include for full-text assessment
    - ☐ Unclear - include for full-text assessment
    - ☐ No – exclude

Citations will be excluded if both reviewers respond "no" to any of the questions. If one reviewer says "yes", the citation will move to full-text assessment. A pre-test will be conducted by all reviewers on the first 5 abstracts to ensure clarity of questions and consistency of understanding of the questions.

Following title/abstract screening, eligibility will be assessed through full-text screening using the following questions. Two reviewers will independently evaluate the full-text articles, with any disagreements resolved by consensus. If consensus cannot be reached, a third reviewer will be consulted.

1. Correct population: Is the study population horses?
    - ☐ Yes – next question

- ☐ No – exclude

2. Correct target condition: Does the study target *Salmonella* spp. (other than *Salmonella enterica* serovar Abortusequi)?
   - ☐ Yes – next question
   - ☐ No – exclude
3. Correct index tests: Does the study assess enriched fecal culture or enriched fecal PCR?
   - ☐ Yes – next question
   - ☐ No – exclude
4. Correct outcome: Does the study report on test sensitivity, specificity, or diagnostic test accuracy/performance (i.e., data to calculate diagnostic sensitivity and/or specificity)?
   - ☐ Yes – next question
   - ☐ No – exclude

***Data collection process*:** Data will be extracted by two reviewers working independently. Consensus will resolve any disagreements or, if consensus cannot be reached, a third reviewer will be consulted. Authors will not be contacted to request missing data or to clarify published results. A form for data extraction will be created for this review in Covidence® and pre-tested on 2 full-text articles to ensure question clarity.

Definitions for data extraction: Data will be extracted from each study in the form of a 2x2 contingency table indicating the number of true positive, false positive, true negative, and false negative test results as classified by the index test and reference standard used in the study. If these data are unavailable, the reported sensitivity and specificity of the index test, and their respective confidence intervals, will be collected. Additionally, data on the following covariates will be extracted:

Table 4: Covariate definitions for data extraction

Category	Variable	Definition/Levels
Study features	Year	Year of study publication
	Country	Country where study was conducted
	Study type	- Cross-sectional diagnostic study - Experimental study (experimental infection) - Experimental study (experimental inoculation of samples) - Diagnostic case-control study - Field study/outbreak investigation
	Clinical setting	- Referral hospital (i.e., equine healthcare setting providing specialty/advanced care) - Primary care (i.e., non-referral equine healthcare setting) - Research/teaching (i.e., setting in which horses are primarily used for research and/or teaching purposes, such as a university herd) - Field (i.e., equine facility not involved in healthcare or research such as a farm, boarding facility, or competition venue) - Not reported
	Analysis method	- Frequentist - Bayesian latent class analysis (*if BLCA used, indicate whether or not tests were considered conditionally independent*)
	Sample size*	*If not reported, write NR.* - Total number of horses included in the study

Category	Variable	Definition/Levels
Characteristics of study population	Age*	*Report measures of age in years. Report TWO decimal places for all values. If any value is not reported, write NR. If the study is NOT a diagnostic case control study, leave the rows for case and control horses BLANK. For example, if a cross-sectional study reports a mean age of 12.5 years with a standard deviation of 3.15 (but does not report median, standard error, minimum, or maximum age), in the first row, report 12.50 for mean, 3.15 for standard deviation, and NR for median, standard error, minimum, and maximum; leave the second and third rows blank.* - Measure of central tendency (*mean or median; indicate which reported*) - Measure of dispersion (*standard deviation or standard error; indicate which reported*) - Minimum - Maximum - Not reported
	Sex*	*Report proportions as decimals with TWO decimal places. Calculate the proportion if necessary. If any value is not reported (or cannot be calculated from the provided data), write NR. If the study is NOT a diagnostic case control study, leave the rows for case and control horses BLANK. For example, if the study population is reported as 50% female, report 0.50. If there are 25 castrated males in a total study population of 100, report 0.25.* - Proportion female - Proportion male intact - Proportion male castrated - Not reported
	Disease type*	*Report proportions as decimals with TWO decimal places. Calculate the proportion if necessary. If any value is not reported (or cannot be calculated from the provided data), write NR. If the study is NOT a diagnostic case control study, leave the rows for case and control horses BLANK. For example, if 50% of the population is reported to be healthy, report 0.50. If there are 25 horses with gastrointestinal disease in a total study population of 100, report 0.25.* - Proportion with no disease (healthy) - Proportion with gastrointestinal disease (e.g., colic, colitis) - Proportion with non-gastrointestinal disease (e.g., respiratory, musculoskeletal, reproductive) - Not reported
	Purpose of sample collection*	- Research (i.e., collected exclusively to evaluate diagnostic test performance) - Surveillance (i.e., collected as part of existing, routine procedures for *Salmonella* surveillance in the facility) - Clinical (i.e., collected at the discretion of clinician due to suspicion of *Salmonella* infection) - Outbreak (i.e., collected for the purpose of *Salmonella* detection in an existing outbreak scenario, either from clinically healthy or diseased horses) - Not reported
	Hospitalization*	*Report proportions as decimals with TWO decimal places. Calculate the proportion if necessary. If any value is not reported (or cannot be calculated from the provided data), write NR. If the study is NOT a diagnostic case control study, leave the rows for case and control horses BLANK. For example, if 50% of the population was hospitalized, report 0.50. If there are*

Category	Variable	Definition/Levels
		25 horses that were hospitalized in a total study population of 100, report 0.25. - Proportion hospitalized (including horses that were ever hospitalized during the study period) - Proportion not hospitalized (including horses that were never hospitalized during the study period) - Not reported
	Survival*	*Report proportions as decimals with TWO decimal places. Calculate the proportion if necessary. If any value is not reported (or cannot be calculated from the provided data), write NR. If the study is NOT a diagnostic case control study, leave the rows for case and control horses BLANK. For example, if 50% of the population survived throughout the study period, report 0.50 in the "survived" column. If 25 horses died or were euthanized during the study period in a total study population of 100, report 0.25 in the "died/euthanized" column.* - Proportion of study population that survived throughout study period - Proportion of study population that died or was euthanized throughout study period - Not reported
	Definition of cases	*If a diagnostic case-control design was used, indicate how a "case" horse was defined. If not reported, write NR. If a diagnostic case-control design was NOT used, write NA.*
	Definition of controls	*If a diagnostic case-control design was used, indicate how a "control" horse was defined. If not reported, write NR. If a diagnostic case-control design was NOT used, write NA.*
	Index test(s)	*Definition: the test that is either (1) defined as the index test by the investigators or (2) described as the primary test under evaluation in the study title or objectives. If neither of these criteria are specified, the LESS sensitive test should be selected as the index test.* - Enriched fecal culture - Enriched fecal PCR
Sampling/test protocol	Reference/ comparison test	*Definition: the test that is either (1) defined as the reference test by the investigators or (2) compared against the index test. If neither of these criteria are specified, the MORE sensitive test should be selected as the reference test.* - Enriched fecal culture - Enriched fecal PCR - Experimental inoculation of samples (i.e., samples considered Salmonella-positive or -negative based on experimental inoculation) - Experimental infection (i.e., samples considered Salmonella-positive or -negative based on experimental infection of study subjects)
	Individual or pooled†	- Individual – fecal sample(s) collected from a single horse tested separately from those collected from other horses - Pooled – fecal samples from multiple horses combined for testing - Not reported
	Amount†	- Amount of feces in each sample subjected to *Salmonella* testing *If provided, indicate mass in grams. Otherwise, indicate amount in the level of detail provided (e.g., 1 swab, 1 fecal ball). If samples were pooled for testing, indicate how many samples were included in the pool. For example, if 5 1-g fecal samples were pooled, write "5 x 1 g." If not reported, write NR.*

Category	Variable	Definition/Levels
	Non-selective pre-enrichment media†	- Buffered peptone water - Other (*specify*) - None - Not reported
	Selective enrichment media†	- Tetrathionate broth - Selenite broth - Rappaport-Vassiliadis (R10) broth - Other (*specify*) - None - Not reported
	Plating media†	- XLT4 - XLD - Hektoen Enteric - Brilliant Green - MacConkey - None - Other (*specify*) - Not reported
	Incubation temperature†‡	*Indicate the temperature of pre-enrichment, enrichment, and/or plating media incubation for the index and reference tests. Report temperature in degrees Celsius but include only a whole number with no units (e.g., if media was incubated at 35°C, report 35). If incubation temperature is given as a range, report the lower and upper limits with a hyphen between (e.g., if media was incubated at 35-40°C, report 35-40). If any values are not reported, write NR. If non-selective pre-enrichment, selective enrichment, or plating media were not used for either the index or reference test, write NA in that cell. For example, if the index test is a PCR with only a selective enrichment step, write NA for non-selective pre-enrichment and plating media.* - Temperature, in degrees Celsius, of pre-enrichment, enrichment, and/or plating media incubation
	Incubation time†‡	*Indicate the time of pre-enrichment, enrichment, and/or plating media incubation for the index and reference tests. Report time in hours but include only a whole number with no units (e.g., if media was incubated for 24 hours, report 24). If incubation time is given as a range, report the lower and upper limits with a hyphen between (e.g., if media was incubated for 24 to 48 hours, report 24-48). If any values are not reported, write NR. If non-selective pre-enrichment, selective enrichment, or plating media were not used for either the index or reference test, write NA in that cell. For example, if the index test is a PCR with only a selective enrichment step, write NA for non-selective pre-enrichment and plating media.* - Time, in hours, of pre-enrichment, enrichment, and/or plating media incubation
	PCR type†	- Conventional (end-point) PCR - Reverse transcriptase PCR (RT-PCR) - Quantitative/real-time PCR (qPCR) - Quantitative/real-time reverse transcriptase PCR (real time RT-PCR or RT-qPCR) - Not reported - Not PCR

Category	Variable	Definition/Levels
	PCR manufacturer†	*If not reported, write NR. If not applicable (test is not a PCR assay), write NA.* - Commercial (*specify manufacturer*) - In-house
	PCR target†	*If not reported, write NR. If not applicable (test is not a PCR assay), write NA.* - Region of the *Salmonella* genome targeted for PCR amplification
	Ct value†	*If not reported, write NR. If not applicable (test is not a PCR assay), write NA.* - Cycle threshold (Ct) value indicative of a negative test
	Time lag between sample collection for the index and reference tests	*Report a whole number to the nearest hour. For example, if a sample was collected for the index test 12 hours after sample collection for the reference test, write 12. If time lag is given as a range, report the lower and upper limits with a hyphen between (e.g., if sample collection occurred 12 to 24 hours apart, write 12-24). If the index and reference test were performed on the same sample, write "same sample." If not reported, write NR. If not applicable (index and reference tests performed on different horses, as in a diagnostic case-control study), write NA.* - Time (in hours) between collection of the fecal samples used for the index and reference test, if performed on the same horse
	Time lag between sample collection and test performance†	*Report a whole number to the nearest hour. For example, if there was a 24-hour delay between fecal sample collection and performance of the index test, write 24 in the "index test" column. If time lag is given as a range, report the lower and upper limits with a hyphen between (e.g., if sample collection and test performance occurred 12 to 24 hours apart, write 12-24). If the reference test was performed immediately upon sample collection, write 0 in the "reference test" column. If not reported, write NR.* - Time (in hours) between sample collection and test performance
	Salmonella serogroup(s)*	*If not reported, write NR. If only serotypes were reported, write NR and see next question.* - *Salmonella* serogroup(s) identified within the study population
Salmonella characteristics	*Salmonella* serotype(s)*	*If not reported, write NR.* - *Salmonella* serotype(s) identified within the study population
	Inoculating dose	*If not reported, write NR. If not applicable (not an experimental study), write NA. Report using the same units reported in the study.* - Inoculating dose of *Salmonella* used to infect horses or to spike into fecal samples, if applicable (experimental study)
Test results	Per-sample or per-horse reporting of test results	- Results were reported on a per-sample basis (i.e., test results from individual samples were reported) - Results were reported on a per-horse basis (i.e., multiple samples were collected from the same horse and interpreted in parallel or series to classify the horse as Salmonella-negative or -positive) - Not reported
	Definition of positive horse	*If results were reported on a per-horse basis, indicate how a Salmonella-positive horse was defined. If not reported, write NR. If results were NOT reported on a per-horse basis, write NA.*
	Definition of negative horse	*If results were reported on a per-horse basis, indicate how a Salmonella-negative horse was defined. If not reported, write NR. If results were NOT reported on a per-horse basis, write NA.*

* Collected separately for case and control horses (if diagnostic case-control study)

† Collected separately for index and reference tests

‡ Collected separately for pre-enrichment, enrichment, and plating media

If data from more than one diagnostic test comparison or more than one study population are reported, complete additional data extraction forms and bias assessment forms as necessary.

Risk of bias and applicability: Risk of bias for diagnostic test assessments will be performed using a modified QUADAS-2 — A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies (www.quadas.org).[7] This tool will be pre-tested on 3 full-text articles to ensure question clarity.

Reviewers will assign 'risk of bias' using the following guidelines:

Domain: Patient selection	**Risk of Bias**			
	Signaling question	**Yes**	**No**	**Unclear**
	Was a consecutive or random sample of patients enrolled?	Sampling method is explicitly described as either consecutive or random	Sampling method is explicitly described as a method **other than** consecutive or random	Sampling method is not described in adequate detail to determine if it was consecutive, random, or other
	Was a case-control design avoided?	The *Salmonella* shedding status of horses in the study was **not** known prior to performance of the index test	The *Salmonella* shedding status of horses in the study was known prior to performance of the index test	Insufficient detail is provided to determine whether or not the *Salmonella* shedding status of horses in the study was known prior to performance of the index test
	Did the study avoid inappropriate exclusions?	Horses were **not** excluded based on factors likely associated with *Salmonella* shedding status	Horses were excluded based on factors likely associated with *Salmonella* shedding status	Insufficient detail is provided to determine whether or not horses were excluded based on factors likely associated with *Salmonella* shedding status
	Could the selection of patients have introduced bias?	**Risk level**		
		Low	**High**	**Unclear**
		Answers to two or more "Patient selection" signaling questions are "Yes"	Answers to two or more "Patient selection" signaling questions are "No"	Answers to two or more "Patient selection" signaling questions are "Unclear" **OR** fewer than 2 answers were classified as either "Yes," "No," or "Unclear"
Domain: Index test	**Risk of Bias**			
	Signaling question	**Yes**	**No**	**Unclear**
	Were the index tests results interpreted without knowledge	Index test was performed prior to the reference/comparison	Investigators knew the results of the reference/	Insufficient detail is provided to determine whether

	of the results of the reference/ comparison test?	test OR investigators were blinded to results of the reference/comparison test when index test was performed	comparison test when the index test was performed	investigators knew the results of the reference/ comparison test when the index test was performed
	If a threshold was used, was it pre-specified?	Threshold value for the index test (e.g., Ct value for PCR or number of consecutive negative cultures to consider a horse *Salmonella*-negative) was specified prior to performance of the index test	Threshold value for the index test (e.g., Ct value for PCR or number of consecutive negative cultures to consider a horse *Salmonella*-negative) was **not** specified prior to performance of the index test	Insufficient detail is provided to determine whether a threshold value for the index test was specified prior to performance of the index test
	Could the conduct or interpretation of the index test have introduced bias?	**Risk level**		
		Low	**High**	**Unclear**
		Answers to two "Index test" signaling questions are "Yes"	Answers to two "Index test" signaling questions are "No"	Answers to two "Index test" signaling questions are "Unclear" **OR** fewer than two answers were classified as either "Yes," "No," or "Unclear"
Domain: Reference/ comparison test	**Risk of Bias**			
	Signaling question	**Yes**	**No**	**Unclear**
	Is the reference/ comparison test likely to correctly classify the target condition?	The results of the reference/comparison test are likely to demonstrate the true *Salmonella* shedding status of the horses tested in this study population	The results of the reference/ comparison test are unlikely to demonstrate the true *Salmonella* shedding status of the horses tested in this study population	Insufficient detail is provided to determine whether the results of the reference/ comparison test will demonstrate the true *Salmonella* shedding status of the horses tested in this study population
	Were the reference/ comparison test results interpreted without knowledge of the results of the index test?	Reference/ comparison test was performed prior to the index test OR investigators were blinded to results of the index test when reference/comparison test was performed	Investigators knew the results of the index test when the reference/ comparison test was performed	Insufficient detail is provided to determine whether investigators knew the results of the index test when the reference/ comparison test was performed
	Could the reference/	**Risk level**		
		Low	**High**	**Unclear**

	comparison test, its conduct, or its interpretation have introduced bias?	Answers to two "Reference/ comparison test" signaling questions are "Yes"	Answers to two "Reference/ comparison test" signaling questions are "No"	Answers to two "Reference/ comparison test" signaling questions are "Unclear" **OR** fewer than 2 answers were classified as either "Yes," "No," or "Unclear"
Domain: Flow and timing	**Risk of Bias**			
	Signaling question	**Yes**	**No**	**Unclear**
	Was there an appropriate interval between index test(s) and reference test?	The index and reference/comparison tests were performed on the same specimen (or on specimens collected from the same animal at the same timepoint)	The index and reference/ comparison tests were performed on different specimens	Insufficient detail is provided to determine the interval between performance of the index and reference/ comparison tests
	Did all patients receive a reference/ comparison test?	All horses included in the study were tested for *Salmonella* using the reference/ comparison test	There are horses in the study population that were **not** tested for *Salmonella* using the reference/ comparison test	Insufficient detail is provided to determine whether all horses in the study population were tested for *Salmonella* using the reference/ comparison test
	Did patients receive the same reference/ comparison test?	All horses included in the study were tested for *Salmonella* using the same reference/ comparison test	Different reference/ comparison tests for *Salmonella* were performed on different horses included in the study	Insufficient detail is provided to determine whether all horses included in the study received the same *Salmonella* reference/ comparison test
	Were all patients included in the analysis?	All members of the study population were included in the analysis of diagnostic test performance	Some members of the study population were excluded from the analysis of diagnostic test performance	Insufficient detail is provided to determine whether all members of the study population were included in the analysis of diagnostic test performance
	Could the patient flow have introduced bias?	**Risk level**		
		Low	**High**	**Unclear**
		Answers to three or more "Flow and timing" signaling questions are "Yes"	Answers to three or more "Flow and timing" signaling questions are "No"	Answers to two or more "Flow and timing" signaling questions are "Unclear" **OR** fewer than three answers

				were classified as either "Yes" or "No"

Diagnostic accuracy measures: The diagnostic sensitivity and diagnostic specificity of the index tests will be evaluated.

Synthesis of results: Results will be summarized using forest plots for the reported sensitivity and specificity of enriched culture and enriched PCR. If feasible, the impact of enrichment method on diagnostic accuracy outcomes will also be visualized within the forest plots.

Meta-analysis: Depending upon the data network formed by the resulting data, we will perform either a pairwise comparison of the tests of interest or, if feasible, a network meta-analysis of diagnostic tests.[8-11]

Additional analyses: We also propose to conduct subgroup analyses on the covariates to evaluate the impact of enrichment method, study design, disease status, clinical setting, and bias on diagnostic accuracy outcomes. If feasible, we will conduct a meta-regression of the variable's study year and disease prevalence as a source of between-study variation. Publication bias will be assessed through construction of a funnel plot, and the overall quality of evidence provided by this review will be classified as high, moderate, low, or very low using the GRADE approach.[12,13]

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