DETECTION, DRIVERS, AND DURATION OF SHEDDING OF *SALMONELLA ENTERICA* IN HORSES

by

EMILY COOK HERRING

(Under the Direction of Brandy A. Burgess)

ABSTRACT

Salmonella enterica is among the most common causes of healthcare-associated infections in horses. Outbreaks in veterinary hospitals and other equine facilities are often costly in terms of morbidity, mortality, and financial impact. While the detection and prevention of *Salmonella* infections in horses have long been recognized as a priority of equine infection control programs, these efforts continue to be a challenge, in part due to remaining gaps in our understanding of the epidemiology of this pathogen in horses, as well as limitations of available diagnostic tests. To address the lack of objective information about the sensitivity and specificity of the most commonly used tests to detect *Salmonella* in equine fecal samples – culture and polymerase chain reaction (PCR) – we first performed a systematic review of the literature to evaluate the performance of these tests, which revealed wide variability in reported test methods. In the second study, we compared the performance of a novel rapid test for *Salmonella* in horses against culture and PCR, demonstrating the utility of the rapid test as a point-of-care screening test for use in equine facilities, and providing objective information about the sensitivity and specificity of existing tests. Next, we addressed two key pieces of the epidemiology of *Salmonella* in horses that have been poorly described to date – the duration of fecal shedding

among infected horses, and the relationship of *Salmonella* shedding and the equine gastrointestinal microbiome. By following *Salmonella*-positive horses over time, we demonstrated that they shed the bacteria in their feces for a median of thirteen days but are likely to shed longer if they have experienced clinical illness. Additionally, we provided preliminary evidence that among horses with subclinical *Salmonella* infections, gastrointestinal microbiome composition may be associated with *Salmonella* shedding patterns over time. Altogether, this work fills key knowledge gaps that have historically hindered the effective management of *Salmonella*-positive horses in hospital settings and the provision of evidence-based clinical guidance to the owners of these horses. The tools and evidence provided herein bring us closer to reducing the negative impact of *Salmonella* infections on equine populations.

INDEX WORDS: Horse, Equine, Healthcare-associated infections, Veterinary hospital, Infection control, Diagnostic testing, *Salmonella enterica*

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EMILY COOK HERRING

B.S., Clemson University, 2016

D.V.M., The University of Georgia, 2020

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2025

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EMILY COOK HERRING

Major Professor:	Brandy Burgess
Committee:	Travis Glenn Paul Morley Nikki Shariat

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2025

DEDICATION

To Blake, who did not know he was signing up for nine more years of school when we moved to Georgia in 2016. Thank you for your unwavering patience and support.

ACKNOWLEDGEMENTS

I would like to thank the organizations whose support made this work possible, including Morris Animal Foundation, the National Science Foundation, and the Foundation for the Horse.

To the many equine clinicians and horse owners who collected samples and completed surveys as part of this research, thank you for your interest, time, and dedication to improving equine health.

To Drs. Glenn, Morley, and Shariat, thank you for your guidance and support throughout this process, and to all of the co-authors who contributed to this work, thank you for all of your hard work and feedback.

To Dr. Burgess, a few sentences are hardly sufficient to express how grateful I am for your mentorship over the past eight years. Thank you for encouraging and challenging me. You really are a unicorn.

To Nichelle, thank you for being a constant source of moral support and humor over the past five years. This would've been a lot less fun without you.

To Molly and Jenny, thank you for being there through all the high and lows since day one of vet school. I'm so grateful for your friendship.

And finally, to my family, thank you for a lifetime of encouragement, support, and laughs. I love you guys.

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CHAPTER 1

INTRODUCTION

This body of research aims to build upon the existing knowledge of a leading cause of healthcare-associated infections (HAIs) in horses – *Salmonella enterica*. The clinical, financial, and public health impact of infections caused by this agent have been increasingly acknowledged in veterinary medicine. However, key gaps remain in our understanding of its epidemiology, hindering the development and implementation of targeted interventions. The studies described herein address these gaps, first tackling the diagnosis and management of salmonellosis in horses, then zooming out to explore the drivers of this disease. Altogether, this research provides novel insights into the epidemiology of this disease, paving the way for more effective approaches to management and prevention.

HAIs and their impact on public health are well-recognized and have been extensively studied in human medicine; however, our understanding of the epidemiology of HAIs in veterinary medicine remains limited. In both settings, a HAI is defined as an infection that a patient contracts as a result of receiving medical care in a facility providing healthcare services, including both inpatient and outpatient settings. These infections may be identified by laboratory confirmation of the presence of an infectious agent, or they can be defined by clinical syndromes (i.e., a combination of symptoms indicative of a HAI), with or without the determination of a causative infectious agent.[1] According to recent estimates, approximately 1 in every 31 patients in a U.S. human hospital has a HAI.[2] These infections increase a patient's length of stay in the hospital by an average of eight days, and often longer depending on the type and severity of

infection.[3,4] Further, HAIs increase the risk of death among hospitalized patients.[5-7] The extended duration of hospitalization, as well as additional medications, diagnostics, procedures, and other HAI-related expenses amount to an estimated increase of at least $10,000 per patient in healthcare costs, and collectively, preventable HAIs pose a burden of approximately $6 billion annually in the U.S.[4] Quantification of the economic and societal impact of HAIs has prompted the implementation of infection prevention programs globally, and in the U.S., such programs have contributed to decreases in overall rates of HAIs in recent decades.[2,8,9] While certain organisms and infection types continue to pose challenges to infection control programs, state and national HAI reporting systems allow for continued monitoring of progress such that targeted prevention efforts can be developed, assessed, and improved over time.[1]

In contrast, we know relatively little about the impact of HAIs in animals. Some infections that are often acquired in veterinary healthcare settings, such as salmonellosis or certain antimicrobial-resistant infections, may be reportable to public health or agricultural agencies at the state level. However, these reporting requirements are typically intended to investigate and prevent outbreaks of zoonotic or economically significant diseases among humans or livestock. There are no comprehensive, centralized reporting systems dedicated to HAIs in veterinary medicine; therefore, our understanding of the epidemiology of these infections is limited to facility-level reports, which often describe only a single outbreak, and few multi-center studies.[10-17] This sporadic reporting of HAIs makes it challenging to draw generalizable conclusions about the burden of these diseases and assess trends over time across facilities, regions, and species.

However, available data indicate that HAIs have a substantial impact on the health of veterinary patients, and that these infections can place a considerable economic strain on

veterinary healthcare facilities. In a survey of 38 American Veterinary Medical Association (AVMA)-accredited veterinary teaching hospitals, 82% of institutions reported experiencing at least one outbreak of a HAI within the preceding 5 years, with 45% of institutions reporting more than one outbreak.[12] In both small and large animal species, HAI outbreaks have resulted in severe clinical disease, with case fatality rates as high as 30-60%.[13,15,18,19] Oftentimes, such instances of high morbidity and mortality, coupled with extensive contamination detected within a veterinary hospital environment, prompt partial or complete facility closure to halt transmission and facilitate outbreak mitigation efforts. In fact, in the same survey of AVMA-accredited hospitals, 58% of surveyed institutions reported restricting patient admissions within the prior five years as the result of a HAI.[12] Collectively, these statistics underscore not only the ubiquity of HAIs in veterinary medicine, but also their impact on patient welfare, in terms of both the direct health consequences in infected animals, as well as lost opportunities to provide care for additional patients due to facility closures.

While both small and large animal species are affected by HAIs in veterinary healthcare settings, available evidence suggests that horses may be among the most commonly impacted species. In a multicenter study of hospitalized horses with gastrointestinal disorders, 19.7% of horses experienced a syndrome indicative of a HAI during hospitalization.[11] Comparatively, in a similar study of companion animals hospitalized in critical care units, only 16.3% of dogs and 12.0% of cats experienced a HAI-related syndrome.[10] Further, among AVMA-accredited veterinary teaching hospitals that reported restricting patient admissions due to a HAI, 68% indicated that they had restricted admissions of horses, and equine pathogens including *Salmonella* and equine herpesvirus 1 (EHV-1) were indicated as the most common reasons for facility closure.[12] In addition to this disproportionate impact of these infections on hospitalized

equine populations, HAIs in horses are of particular interest given the unique role of horses within both human and animal communities. Horses are used for a variety of purposes, including, but not limited to, athletic competition, companionship, and agricultural labor, and as such, they often share space, resources, and close contact with humans and other animal species. In the U.S., 86% of horses share contact with other domestic animals, including food animals, and 68% share contact with wildlife.[20] Therefore, equine HAIs that are zoonotic or have the potential for interspecies transmission can be detrimental not only to equine health, but also to public and environmental health. This underscores the impact of one of the most common HAI-related concerns in horses – salmonellosis – which poses a risk to the health of human, equine, and other animal populations alike.

Salmonella is the most commonly reported cause of HAI outbreaks in AVMA-accredited veterinary teaching hospitals,[12] and while this statistic includes outbreaks in both large and small animal hospitals, the multitude of reports describing *Salmonella* outbreaks in hospitalized equine populations suggests that horses are disproportionately impacted.[13,15,16,21-26] In horses, clinical salmonellosis is typically characterized by acute colitis, and in foals, septicemia is a common manifestation.[27,28] However, *Salmonella*-infected horses may present with more nonspecific clinical signs, such as fever, inappetence, lethargy, colic, and leukopenia, either alone or in conjunction with diarrhea.[22,28] Further, horses often shed *Salmonella* in the absence of or prior to the development of clinical signs.[16,22,27] Outbreaks of salmonellosis in hospitalized horses are often characterized by a high incidence of severe clinical disease, resulting in case fatality rates exceeding 35%.[13,15,24] These outcomes, in addition to extensive environmental contamination, have necessitated facility closures to facilitate outbreak mitigation efforts.[13,15] Ultimately, the costs of these efforts, including decontamination, facility remediation, surveillance, decreased

caseload, and coverage of patient bills, have been reported to exceed $4 million for a single hospital.[13] Interestingly, outbreaks of *Salmonella* in equine hospitals often occur in spite of existing infection control efforts specifically targeted towards *Salmonella* detection and prevention.[13,16,22,24] Failures of such efforts highlight the unique challenges associated with the prevention and control of equine salmonellosis, which are largely attributable to the epidemiology of this disease and the associated gaps in our understanding of it. Currently, much of our knowledge of the epidemiology of *Salmonella* in horses is derived from case reports and case series describing outbreaks,[13,15,16,21,27,29] as well as cross-sectional or retrospective case-control studies investigating risk factors associated with salmonellosis.[30-37] These studies are typically limited to a single population of hospitalized horses or a single herd affected by an outbreak, making it challenging to generalize results across equine populations from different geographic regions, with various clinical presentations, and affected by different *Salmonella* serotypes. As such, basic aspects of the epidemiology of equine salmonellosis remain undescribed.

For example, the duration of *Salmonella* shedding has been investigated among individual affected horses and herds,[29,38] and in few small, prospective longitudinal studies of equine salmonellosis.[37,39] However, these reports are insufficient to provide a generalizable estimate of *Salmonella* shedding duration among affected horses, or to describe factors associated with duration of shedding. This, in turn, hinders the ability of equine clinicians, facility managers, and owners to make sound, evidence-based decisions regarding the management of *Salmonella*-positive horses. These decisions are further complicated by limitations in our ability to detect *Salmonella* in equine feces. Horses, especially those with subclinical salmonellosis, tend to shed low numbers of *Salmonella* and do so intermittently;[39,40]

this limits the sensitivity of *Salmonella* culture, and as such, 3 to 5 consecutive cultures are typically recommended to determine that a horse is truly *Salmonella*-negative.[40-42] Further, culture results take several days to finalize, allowing for shedding, environmental contamination, and transmission to occur in the meantime. PCR offers a faster turnaround time and a higher sensitivity for *Salmonella* detection in equine feces, but it does not necessarily detect viable organisms; therefore, compared to culture, positive results are less likely to represent true positives.[43] Additionally, the specific procedures used for *Salmonella* detection in equine fecal samples, including both culture and PCR, are poorly standardized and vary widely by laboratory.[43-46] This variability precludes the determination of standard estimates of test sensitivity and specificity, and in turn, muddies the interpretation of test results for the end user. Together, these limitations highlight the need for affordable, reliable diagnostic tests that allow for rapid detection of *Salmonella* in equine feces, as well as an unbiased assessment of the diagnostic value of existing tests. To address these barriers to the management and diagnosis of equine salmonellosis, this research first explores the sensitivity and specificity of existing diagnostic tests for equine salmonellosis (i.e., culture and PCR) via a systematic review of the literature, then assesses the diagnostic value of these tests in comparison to a novel rapid test. Next, we describe the duration of *Salmonella* shedding among infected horses in a multicenter prospective study. While these studies will provide equine clinicians and owners with an improved understanding of how to manage this disease in-hospital and on-farm, they do not address the remaining gaps in knowledge of the drivers of salmonellosis in horses. It has long been hypothesized that *Salmonella* shedding is associated with alterations in the equine gut microbiome, and manipulation of the gastrointestinal flora has been proposed as a treatment for equine salmonellosis.[39,40] However, the impact of *Salmonella* infection on the equine

gastrointestinal microbiome, and vice versa, remains poorly described. Therefore, the next study in this research takes a step back from disease diagnosis and management to consider equine salmonellosis in the broader context of the microbial ecology of the gut.

Together, these studies offer an exploration of the epidemiology of salmonellosis in horses, with each study aimed at filling a key gap in our existing knowledge of these infections to inform a multifaceted approach to disease prevention and management. We start by performing an in-depth assessment of existing and novel diagnostics, then by providing an estimate of shedding duration in infected horses, both of which will be instrumental in assisting equine clinicians, facility managers, and owners in making informed biosecurity and infection control decisions. Next, we explore salmonellosis in the context of the equine gastrointestinal microbiome, aiming to identify drivers of *Salmonella* shedding to open the door for the development of interventions targeting the microbial ecology of the gut. Ultimately, this body of research aims to provide tools and evidence that will aid in improving the health of horses receiving veterinary care, and in turn, the health of the animals, people, and environment with which they share their lives.

CHAPTER 2

CLINICAL PERFORMANCE OF *SALMONELLA* DETECTION METHODS IN EQUINE FECES: A SYSTEMATIC REVIEW [a]

[a] Herring, E.C., Aceto, H.W, Morley, P.S., O'Connor, A.M., Pusterla, N., Rankin, S.C., and B.A. Burgess. To be submitted to *Equine Veterinary Journal*.

Abstract

Salmonella enterica is an important cause of gastrointestinal disease in horses and a frequent challenge to infection control programs in equine facilities. Enriched fecal culture and PCR are commonly used as diagnostic and screening tests for *Salmonella*, but available information about the sensitivity and specificity of these tests is limited. Therefore, we performed a systematic review of the literature to compare the diagnostic sensitivity and specificity of enriched fecal culture and PCR for the detection of fecal *Salmonella* shedding in horses, and to identify characteristics of studies, patients, and tests that drive test performance estimates. A literature search of five electronic databases was conducted. Abstracts and full texts were screened, and studies were selected for the review based on inclusion of the appropriate study population, target condition, index tests, and outcome measures. Data including study population characteristics, sampling and test protocols, and test performance outcomes were extracted using an electronic form in an online systematic review software. Risk of bias was evaluated using a quality assessment tool. Test sensitivity and specificity were summarized using forest plots, but a formal meta-analysis was not conducted. Thirty diagnostic test comparisons from 19 studies met the inclusion criteria. Study design and test methods were highly variable among the included studies, and reporting of demographic and clinical characteristics of the study population were often incomplete. Specificity estimates for both enriched fecal culture and PCR were both consistently high, while sensitivity estimates for PCR were higher and less variable than those for culture. Heterogeneity and incomplete reporting across studies precluded the generation of summary estimates of test performance. Improved consistency and reporting of diagnostic test methods for *Salmonella* detection in horses is warranted to facilitate evidence-

based evaluations of test performance and appropriate clinical recommendations for the application of these tests.

Introduction

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.[12] In horses, clinical salmonellosis is typically characterized by acute colitis, and in foals, septicemia is a common manifestation.[27,28] However, *Salmonella*-infected horses may present with nonspecific clinical signs or shed *Salmonella* in the absence of or prior to the development of clinical signs; this shedding often occurs intermittently.[16,22,27,28,39,40]

Both aerobic culture and polymerase chain reaction (PCR) are frequently used for the diagnosis of equine salmonellosis; however, many variations of these tests exist. Because horses tend to shed low numbers of *Salmonella*, and feces are a rich microbial environment, *Salmonella* culture and PCR often involve a selective enrichment step.[43,47,48] However, *Salmonella* detection methods vary widely between different studies and laboratories, and there is not an established gold standard.[43,44,46-52]In equine hospitals, culture and PCR are used not only as diagnostic tests, but also as screening tests for *Salmonella* surveillance as part of hospital infection control programs. While PCR offers the advantage of a relatively fast turnaround time compared to aerobic culture,[43] *Salmonella* culture is used either alone or in tandem with PCR to confirm infection and allow for serogroup and serotype determination, and antimicrobial susceptibility testing.

In spite of the key role that fecal culture and PCR play in the management and prevention of *Salmonella* infections, our understanding of the accuracy of these tests remains incomplete

due to variability in test methods and the performance of diagnostic test assessments on high-risk subgroups of horses (e.g., with colic or colitis), which can greatly impact estimates of test sensitivity and specificity. In equine facilities, these limitations muddy the interpretation of test results for the end user, potentially resulting in the implementation of increased biosecurity and infection control measures when such efforts are unnecessary, or failure to implement such measures in the face of false negative results. 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.[53] Therefore, this review aimed to identify, appraise, and synthesize available information on the diagnostic sensitivity and specificity of enriched fecal culture and PCR for the detection of *Salmonella* in equine fecal samples, and to identify factors related to study design, patient population, and test protocol that drive heterogeneity in the diagnostic sensitivity and specificity of these tests.

Methods

Protocol, registration, and reporting guidelines

A systematic review protocol was developed and published prior to conducting the study (**Appendix 2A**).[54] This study is reported according to the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies (PRISMA-DTA; **Appendix 2B**).[55]

Eligibility criteria

Studies were eligible for inclusion if they evaluated the diagnostic sensitivity and/or specificity of one or both of the index tests of interest – enriched fecal culture and enriched fecal PCR – for the detection of fecal *Salmonella* spp. shedding in a population of horses. Only studies

published in English were considered for inclusion, with no restriction on date or publication type. Studies evaluating the detection of *Salmonella enterica* serovar Abortusequi were excluded. Both published and non-published (grey literature) studies were eligible, provided they reported 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, experimental studies, field studies or outbreak investigations, or diagnostic case-control studies.

Information sources

Electronic searches of PubMed®, Centre for Agricultural Biosciences (CAB) Abstracts/CAB Archive, Web of Science, Agricola, and PubAg were performed via the University of Georgia Libraries interface. Searches were completed on January 26, 2024. In addition, a hand search of the tables of contents of the Proceedings of the American Association of Veterinary Laboratory Diagnosticians/United States Animal Health Association (AAVLD/USAHA) Annual Meeting from the previous five years were performed. Reference lists of all included studies were also searched for eligible studies that were missed by database searches.

Search strategy

The search strategy (**Table 2.1**) was comprised of three concepts: population (horses); target condition (*Salmonella* shedding), and index tests (including test methods, diagnostic test performance parameters, and analytic methods). Search results were downloaded into bibliographic software (EndNote™, Clarivate Analytics, Philadelphia, PA, USA) and automatically deduplicated.

Study selection

Search results from EndNote™ were uploaded into an online systematic review software (Covidence®, Melbourne, VIC, Australia), which performed additional deduplication. Two rounds of screening were then performed by a group of reviewers with expertise in epidemiology and systematic review methods; reviewers also underwent training to ensure understanding of data collection using forms created in Covidence®. In the first round of screening, abstracts and titles were evaluated for inclusion based on the defined eligibility criteria: the study population includes horses, the index tests under evaluation include enriched fecal culture and/or enriched fecal PCR, the target condition is *Salmonella* spp. (excluding *S. enterica* serovar Abortusequi), the reported outcomes include diagnostic sensitivity and/or diagnostic specificity (or test result data that allow for the calculation of these measures), and the study is either a cross-sectional diagnostic study, an experimental study (including experimental infection or experimental inoculation of fecal samples), a field study/outbreak investigation, or a diagnostic case-control study. Each title and abstract were screened by two independent reviewers; if both determined that the eligibility criteria were not met, the citation was excluded. Otherwise, the citation proceeded to full text screening. Using the same criteria, two independent reviewers assessed the full text of each article to determine its eligibility for inclusion. If both reviewers agreed that the eligibility criteria were met, the study progressed to data extraction. Any disagreements were resolved by consensus. If consensus could not be reached, a third reviewer was consulted, and the majority opinion was accepted. Reviewers did not evaluate any studies that they had co-authored.

Data collection process

A form for data extraction was created in Covidence® and pre-tested on two full-text articles to ensure question clarity. Using this form, data from each study were extracted by two reviewers working independently. Authors were not contacted to request missing data or to clarify published results. A consensus data extraction form was finalized by comparing the responses of both reviewers. Disagreements between reviewers were resolved by consensus, or by consulting a third reviewer if consensus could not be reached. Reviewers did not evaluate any studies that they had co-authored.

Items for data extraction

Study features, including year of publication, country where the study was conducted, study design (cross-sectional diagnostic study, experimental study [experimental infection of horses], experimental study [experimental inoculation of fecal samples], diagnostic case-control study, field study/outbreak investigation), clinical setting (referral hospital, primary care, research/teaching, field), analysis method (frequentist or Bayesian latent class analysis), and sample size (including both number of horses and number of samples) were extracted for each study. Additionally, for observational studies and studies involving experimental infection of horses, study population characteristics were collected, including age (in years; measures of central tendency and dispersion collected), sex (proportion female, proportion male intact, proportion male castrated, proportion male unspecified), disease type (proportion with no disease, proportion with gastrointestinal disease, proportion with non-gastrointestinal disease), purpose of sample collection (research, surveillance, clinical, outbreak), hospitalization (proportion ever hospitalized, proportion never hospitalized), and survival (proportion survived,

proportion died/euthanized). For diagnostic case-control studies, definitions of case and control horses were also collected. The type of index test (enriched fecal culture or enriched fecal PCR) and type of comparison test (enriched fecal culture, enriched fecal PCR, or – for experimental studies – experimental inoculation of samples or experimental infection of horses) was extracted for each study. Additionally, details about each reference and comparison test were collected, including whether individual or pooled samples were used, amount of feces used for testing, type of non-selective pre-enrichment media, type of selective enrichment media, type of plating media, incubation temperature, incubation time, PCR type (conventional/end-point or quantitative/real-time), PCR kit manufacturer, PCR target, cycle threshold value (for qPCR), time lag between sample collection for index and comparison tests, and time lag between sample collection and performance of each test. Characteristics of *Salmonella* isolates, including serogroup(s), serotype(s), and – for experimental studies – inoculating dose, were also collected from each study. If a data item was not applicable for a given study, that item was recorded as "not applicable." Otherwise, if a data item was missing from a study, it was recorded as "not reported." If data from more than one diagnostic test comparison or more than one study population were reported in a single study, multiple data extraction forms were completed as necessary.

Risk of bias assessment

The risk of bias of each study included in the review was evaluated using a modified Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2; **Appendix 2A**).[56] This tool included signaling questions related to four domains of bias: patient selection, index test, reference/comparison test, and flow and timing. If multiple data extraction

forms were completed to accommodate reporting of multiple diagnostic tests comparisons or study populations, an additional risk of bias assessment was completed for each data extraction form. Answer options for each signaling question were "Yes or N/A," "No," and "Unclear." Risk of bias for each domain was assigned as "Low" or "High" if the majority of answers to the signaling questions in each domain were "Yes or N/A" or "No," respectively. Risk of bias for a given domain was assigned as "Unclear" if the majority of answers to the signaling questions in a given domain were "Unclear," or if no single answer option made up the majority of responses. The risk of bias assessment tool was pre-tested by reviewers on three full-text articles to ensure question clarity.

Diagnostic accuracy measures

To assess the primary outcomes of interest – the diagnostic sensitivity and specificity of each index test – test result data were extracted in the form of a two-by-two table comparing the results of the index test to the results of the comparison test. These data were collected on a per-sample basis and/or a per-horse basis (i.e., if multiple samples were collected from the same horse and interpreted in parallel or in series). If results were reported on a per-horse basis, the definitions of a *Salmonella*-positive and -negative horse were also extracted. If test result data were not reported, but test sensitivity and/or specificity were calculated, these values were collected, along with their confidence intervals. Sensitivity and specificity and their 95% Wilson's confidence limits were calculated from per-sample test result data using the *epiR* package in R (version 4.1.3).[57]

Synthesis of results

Results were summarized using forest plots for the reported sensitivity and specificity of enriched culture and enriched PCR. Separate forest plots were created for each type of test comparison; that is, within each forest plot, all comparisons evaluated the same type of index test against the same type of reference test.

Meta-analysis

The protocol specified that either a pairwise comparison of the tests of interest or a network meta-analysis of diagnostic tests would be performed, depending upon the nature of the resulting data. However, given the variability in study design and test methods used across studies included in the review, a formal meta-analysis was determined to be infeasible.

Additional analyses

Funnel plots for both test sensitivity and test specificity were constructed to evaluate publication bias. The GRADE system was used to guide the discussion of quality of evidence, including the GRADE categories risk of bias, consistency, indirectness, precision, and publication bias.

Results

Study selection

The number of studies identified in the literature search, screened for inclusion, and included in the final review is illustrated in **Figure 2.1**. Of the 1622 studies identified through a search of electronic databases and 5 references retrieved from the grey literature

(USAHA/AAVLD Annual Meeting Proceedings), 19 studies were ultimately included in the final review. These 19 studies included 30 separate evaluations of diagnostic test performance that were included in the review.

Study characteristics

Characteristics of the 30 diagnostic test evaluations from 19 articles included in the review are summarized in **Table 2.2**. Year of publication ranged from 1979 to 2023, with a median publication year of 2010. Of the 19 studies, 12 (63.2%) were performed in the United States, 2 (10.5%) in India, 2 (10.5%) in Iran, 1 (5.3%) in Brazil, and 1 (5.3%) in Canada; country of study conduct was not reported for 1 publication. The most common study design was a cross-sectional diagnostic study, which comprised 12 of the 19 studies (63.2%). Other study designs included benchtop experimental studies (i.e., involving experimental inoculation of equine fecal samples with *Salmonella*; 5/19 [26.3%]), experimental studies involving experimental infection of horses with *Salmonella* (1/19 [5.3%]), and field studies/outbreak investigations (2/19 [10.5%]). One study (Bohaychuk 2007) included both a cross-sectional diagnostic study component and a benchtop experimental component. Clinical setting in which the studies were conducted included field settings (5/19 [26.3%]), referral hospitals (7/19 [36.8%]), and research/teaching settings (4/19 [21.1%]). Clinical setting was not reported in 4/19 studies (21.1%). One study (Slovis 2014) included horses from both a referral hospital and field setting. Purpose of sample collection included research (15/19 studies [78.9%]), surveillance sampling (4/19 [21.1%]), and clinical suspicion of *Salmonella* infection (3/19 [15.8%]). Purpose of sample collection was not reported in 1 study (5.3%). Three (15.8%) studies (Cohen 1996, Ekiri 2016, and Pusterla 2010) reported multiple purposes of sample collection. Of the 30 diagnostic test

evaluations reported across all studies, 14 (46.7%) included enriched fecal PCR as the index test, while in 16 comparisons (53.3%), enriched fecal culture was the index test. The reported comparison or reference tests included enriched fecal culture (13/30 [43.3%]) and enriched fecal PCR (6/30 [20.0%]). In the remaining diagnostic test evaluations, either experimental inoculation of fecal samples with *Salmonella* (10/30 [33.3%]) or experimental infection of horses with *Salmonella* (1/30 [3.3%]) was considered the reference.

Enriched fecal culture (**Table 2.3**) methods varied across studies. Of the 29 diagnostic test evaluations that included enriched fecal culture, the amount of feces used for culture was reported for 17 methods (58.6%); among these methods, the median amount of feces used for culture was 5 g (range: 1 – 10 g). Among the 13 reported culture methods that included a non-selective pre-enrichment step, median pre-enrichment incubation temperature and time were 37°C (range: 35 – 43°C) and 18 hours (range: 6 – 24 hours), respectively. Buffered peptone water was the only non-selective pre-enrichment media used among these culture methods (**Table 2.3**). In contrast, four different selective enrichment media were used for culture, among which, tetrathionate broth was the most common (**Table 2.3**). In three diagnostic test comparisons, two different selective enrichment media (tetrathionate broth and Rappaport-Vassiliadis broth) were used in parallel; however, their results were not reported separately, so they were considered a single culture method in the current study. Of the 29 diagnostic test evaluations that included enriched fecal culture, selective enrichment incubation temperature and time were reported for 27 and 28 culture methods, respectively. The median enrichment incubation temperature and time were 37°C (range: 35 – 43°C) and 24 hours (range: 12 – 36 hours), respectively. Six different plating media were utilized across the 29 reported culture methods (**Table 2.3**). Hektoen enteric agar was used most frequently. In 13 diagnostic test

evaluations, two different plating media were used in parallel, but results were not reported separately; in these instances, the paired plating media were considered part of a single culture method. Combinations of plating media used included Hektoen enteric/brilliant green (8/13, 61.5%), brilliant green/MacConkey (2/13, 15.4%), XLT4/Rambach (2/13, 15.4%), and XLT4/brilliant green (1/13, 7.7%).

Reported PCR methods also varied across studies (**Table 2.4**). Among the 20 diagnostic test evaluations including enriched fecal PCR, the amount of feces used for PCR was reported for 14 methods (70.0%); among these methods, the median amount of feces used for testing was 4.5 g (range: 1 – 10 g). Six PCR methods (30.0%) included a non-selective pre-enrichment step, while in 2 reported PCR methods (10.0%), it was unclear if a pre-enrichment step was included (**Table 2.4**). The most commonly used non-selective pre-enrichment media was buffered peptone water. Pre-enrichment incubation temperature and time were reported for four PCR methods. Among these methods, the median pre-enrichment incubation temperature and time were 36°C (range: 35 – 37°C) and 21 hours (range: 18 – 24 hours), respectively. Three different selective enrichment media – tetrathionate broth, selenite broth, and Rappaport-Vassiliadis broth – were used for PCR, among which, tetrathionate broth was most common (13/20 comparisons [65.0%]; **Table 2.4**). In two diagnostic test comparisons, two different selective enrichment media (tetrathionate broth and Rappaport-Vassiliadis broth) were used in parallel, but results were not reported separately; in these instances, the paired enrichment media were considered to be part of a single PCR method. Overall, qPCR (55.0%) was more commonly used than conventional (end-point) PCR (40.0%), and the majority of reported PCR methods utilized an in-house assay (85.0%) rather than a commercial assay (10.0%). The most commonly reported PCR target was the *invA* gene (70.0%). In two diagnostic test comparisons, multiple PCR targets were reported

(*invA*/*ttrC* and *invA*/*invE*). Cycle threshold values were not reported for 81.8% of qPCR methods.

Among the 30 diagnostic test evaluations included, 25 (83.3%) and 23 (76.7%) reported using individual fecal samples for the index and comparison tests, respectively, while 4 (13.3%) reported using pooled fecal samples for both the index and comparison tests. The use of individual or pooled fecal samples was not reported for one index test (3.3%) and two comparison tests (6.7%), and for one study (Owen 1979), this question was not applicable for the comparison test because the experimental infection status of horses was considered the reference standard in this study. The index and comparison tests were performed on the same sample in 28 of 30 (93.3%) diagnostic test comparisons. For one comparison (3.3%), time lag between sample collection for the index and reference tests was not reported, and for the remaining comparison (from Owen 1979), this question was not applicable because the experimental infection status of horses was considered the reference standard. Time lag between sample collection and index test performance was not reported in 27 of 30 diagnostic test comparisons (90.0%), and time lag between sample collection and comparison test performance was not reported in 17 of 19 applicable (non-experimental) diagnostic test comparisons (89.5%). *Salmonella* serotypes identified by the tests under evaluation were reported in 23 of 30 (76.7%) diagnostic test comparisons (**Supplementary Table 2.1**). All studies used a frequentist method of data analysis (i.e., no Bayesian latent class analyses were included). Test results were reported on a per-sample basis for all 30 of the included diagnostic test comparisons and on a per-horse basis for only three comparisons (10.0%). Only results reported on a per-sample basis were considered for further analysis.

Reporting of demographic variables was inconsistent among included studies. Of the 15 studies for which study population demographics were collected (observational studies and studies involving experimental infection of horses with *Salmonella*), 33.3% reported information on the age distribution of the population (including a measure of central tendency and/or a measure of dispersion); 46.7% reported information on sex (proportion female, proportion castrated male, proportion intact male, and/or proportion male [unspecified]); 53.3% reported information on the disease status of the study population (proportion healthy, proportion with gastrointestinal disease, and/or proportion with non-gastrointestinal disease); 53.3% reported information on hospitalization status of the study population (proportion hospitalized and/or proportion not hospitalized); and 13.3% reported information on survival among the study population (proportion survived and/or proportion died/euthanized).

Risk of bias and applicability

Risk of bias for each diagnostic test evaluation, as quantified by the modified QUADAS-2 tool, is detailed in **Table 2.5**. Among the 30 diagnostic test comparisons from the 19 included studies, risk of bias in the Patient Selection domain was classified as "low" for 14 comparisons (46.7%) and "unclear" for 16 comparisons (53.3%). For the Index Test domain, risk of bias was "low" for five comparisons (16.7%) and "unclear" for 25 comparisons (83.3%). For the Comparison Test domain, risk of bias was "low" for 12 comparisons (40.0%) and "unclear" for 18 comparisons (60.0%). Risk of bias in the Flow/Timing domain was categorized as "low" for 29 diagnostic test comparisons (96.7%) and "unclear" for one comparison (3.3%).

Results of individual studies

Forest plots summarizing the diagnostic sensitivity and specificity of each test under evaluation in the included studies are presented in **Figure 2.2** (for experimental studies) and **Figure 2.3** (for observational studies). Among experimental studies in which enriched fecal culture was compared against experimental inoculation of fecal samples (**Figure 2.2A**), specificity estimates were quite uniform across the five diagnostic test comparisons from three publications, ranging from 96% (95% confidence interval [CI]: 80.5%, 99.3%) to 100% (95% CI: 72.2%, 100%). In contrast, sensitivity estimates were more variable, ranging from 57.5% (95% CI: 42.2%, 71.5%) to 100% (95% CI: 96.7%, 100%). Among the culture methods under evaluation in these studies, sensitivity tended to be higher for those involving selective enrichment in tetrathionate broth compared to those only involving selective enrichment in Rappaport-Vassiliadis or selenite broth. Among the three experimental studies in which enriched fecal PCR was compared against experimental inoculation of fecal samples (**Figure 2.2B**), sensitivity ranged from 86.7% (95% CI: 70.3%, 94.7%) to 99.1% (95% CI: 95.1%, 99.8%), and specificity ranged from 96.0% (95% CI: 80.5%, 99.3%) to 100% (95% CI: 72.2%, 100%). In the one study evaluating enriched fecal culture in horses experimentally infected with *Salmonella* (Owen 1979; **Figure 2.2C**), the sensitivity estimate for tetrathionate-enriched culture was the lowest of all estimates generated from experimental studies at 41.2% (95% CI: 21.6%, 64.0%), while specificity was estimated at 100% (95% CI: 34.2%, 100%).

Among the observational studies included in the review, there were three diagnostic test comparisons from one study (Babu 2008) in which two enriched fecal culture methods were compared against one another. In this study, all three culture methods under evaluation (*Salmonella* selective enrichment broth-enriched, selenite-enriched, and Rappaport-Vassiliadis-

enriched) demonstrated 100% specificity (95% CI: 98.2%, 100%) compared to tetrathionate-enriched culture. However, the sensitivity of these methods ranged from 0% (95% CI: 0%, 9.9%) for SSEB- and SEL-enriched culture to 14.3% (95% CI: 6.3%, 29.4%) for RV-enriched culture compared to TET-enriched culture (**Figure 2.3A**). Five diagnostic test comparisons from four observational studies evaluated the sensitivity and specificity of enriched fecal culture compared to enriched fecal PCR. Among these comparisons, specificity estimates for enriched fecal culture were consistently high, ranging from 92.3% (95% CI: 87.0%, 95.5%) to 100% (95% CI: 99.5%, 100%). Sensitivity estimates were lower and more variable, ranging from 20.0% (95% CI: 5.7%, 51.0%) to 57.1% (95% CI: 25.0%, 84.2%) (**Figure 2.3B**). Ten diagnostic test comparisons from nine studies evaluated the sensitivity and specificity of enriched fecal PCR compared to enriched fecal culture. Among these comparisons, sensitivity estimates ranged from 58.3% (95% CI: 42.2%, 72.9%) to 100% (95% CI: 90.1%, 100%), while specificity estimates ranged from 59.0% (95% CI: 54.2%, 63.6%) to 100% (95% CI: 51.0%, 100%) (**Figure 2.3C**). One diagnostic test comparison (Pusterla 2023) evaluated the sensitivity and specificity of an enriched fecal PCR compared to another enriched fecal PCR; sensitivity was estimated at 92.6% (95% CI: 76.6%, 97.9%), and specificity was estimated at 98.1% (95% CI: 93.2%, 99.5%) (**Figure 2.3D**).

Publication bias

Funnel plots for diagnostic sensitivity and specificity are presented in **Figure 2.4A** and **Figure 2.4B**, respectively. Both funnel plots demonstrated asymmetry, with most diagnostic test comparisons falling in the top half of the plot for sensitivity (i.e., with a standard error < 0.075). Comparisons evaluating enriched fecal culture as the index test tended to lie in the center and left side of the sensitivity plot, consistent with a lower sensitivity, while those evaluating enriched

fecal PCR as the index test fell predominantly in the top right corner of the sensitivity plot, indicating a higher sensitivity. Cross-sectional diagnostic test comparisons were concentrated in approximately the top third of the sensitivity plot due to their smaller standard errors, while diagnostic test evaluations involving experimental inoculation of fecal samples, with larger standard errors, tended to fall more towards the center (**Figure 2.4A**). In the specificity plot, nearly all diagnostic test comparisons are in the top right corner of the specificity plot, indicating small standard errors coupled with high specificity (**Figure 2.4B**).

Discussion

Summary of evidence

The objective of this review was to identify, evaluate, and synthesize the evidence from diagnostic test assessments reporting the sensitivity and specificity of enriched fecal culture and PCR for the detection of *Salmonella* in equine feces. There was wide variability in study design and test methods that were reported across the included studies, precluding the generation of summary estimates of test performance. Additionally, insufficient reporting of study methods and study population demographics were common, hindering comprehensive assessments of the internal and external validity of the studies under evaluation. In general, estimates of the specificity of both enriched fecal culture and enriched fecal PCR tended to be higher and more homogenous than the estimates of the sensitivity of these tests, and sensitivity estimates of enriched fecal PCR were higher and more consistent than sensitivity estimates for enriched fecal culture. However, the heterogeneity and inconsistent reporting of test methods, as well as the lack of a consistent reference standard used across these comparisons, underscore the challenges of drawing conclusions about the performance of these tests based on this body of evidence.

Risk of bias within studies

Risk of bias in the Patient Selection category was classified as "low" for approximately half of all diagnostic test comparisons and "unclear" for the remaining half. However, this bias should be interpreted in the context of the study design for a given comparison. For example, risk of bias in the Patient Selection category was consistently rated as "low" for experimental studies involving experimental inoculation of equine feces with *Salmonella* in a laboratory setting. However, this was primarily because questions in the QUADAS-2 tool regarding patient enrollment and exclusion were not applicable to these studies. In contrast, for study designs in which Patient Selection bias was a concern, including cross-sectional diagnostic studies, field studies/outbreak investigations, and experimental studies involving experimental infection of horses with *Salmonella*, risk of bias in the Patient Selection category was classified as "unclear" among most test comparisons. This was predominantly due to the sampling method not being described in adequate detail to determine if a consecutive, random, or other method was used, as well as the inability to determine if horses were excluded based on factors likely associated with *Salmonella* shedding status. Incomplete reporting of these items makes it challenging to evaluate the external validity of these studies. For example, exclusion of horses based on symptoms associated with clinical salmonellosis, such as diarrhea, would likely result in only horses with subclinical salmonellosis being included in the study population. The distribution of disease severity in a population can impact the sensitivity and specificity of diagnostic tests.[58] However, the explicit reporting of exclusion criteria was rare among these studies, convoluting the assessment of the degree and directionality of this potential bias.

Risk of bias in the Index Test category was classified as "unclear" for most diagnostic test comparisons. This was primarily due to insufficient detail being provided to determine whether

investigators knew the results of the comparison test when the index test was performed. Knowledge of comparison test results may have influenced investigators' interpretation of the index test, potentially leading to overestimates of agreement between tests and inflated estimates of test sensitivity and specificity.[59] However, it is also possible that investigators were truly blinded to comparison test results in most studies, although this aspect of the study design was not reported.

Risk of bias in the Comparison Test category was classified as "low" for approximately half of all comparisons and "unclear" for the remaining half. The low-risk-of-bias comparisons were primarily from experimental studies. In these studies, the comparison test was the experimentally determined *Salmonella* status of the horses or fecal samples under investigation. However, for observational studies where the true *Salmonella* status of the study population was unknown, Comparison Test bias was classified as "unclear." Notably, in all but one of the diagnostic test comparisons from observational studies, there was insufficient detail provided to determine whether investigators knew the results of the index test when the comparison test was performed, which may have inflated the observed agreement between these tests.[59] Therefore, incomplete reporting of study methods was again an important barrier to the determination of risk of bias.

All but one diagnostic test comparisons were classified as "low" risk of bias in the Flow and Timing category. Therefore, across diagnostic test comparisons from both experimental and observational studies, Flow and Timing bias was not an important detractor from the overall quality of evidence. However, among the remaining categories, including Patient Selection, Index Test, and Reference Test, the determination of risk of bias within individual studies was made challenging by incomplete reporting of study methods. Collectively, this lack of reporting

reduces the quality of the body of evidence presented in this review, as it is impossible to determine the magnitude and directionality of the bias that may be present across these studies.

Directness/applicability

One-third of the diagnostic test comparisons included in this review were from benchtop experimental studies that involve the detection of *Salmonella* in experimentally inoculated feces rather than in feces from naturally or experimentally infected horses. Therefore, although these studies do provide preliminary evidence of the comparative performance of the tests under evaluation, they cannot be used as direct evidence of the clinical performance of enriched fecal culture or PCR in equine populations.

Among the observational studies and experimental studies involving experimental infection of horses with *Salmonella*, reporting of demographic variables was limited, making it challenging to ascertain the applicability of test sensitivity and specificity measurements from this review to desired target populations. Additionally, applicability may be limited by the country and clinical setting in which the included studies were performed. While diagnostic test comparisons performed in field, referral hospital, and research/teaching settings were represented in this review, nearly all of those performed in a referral hospital or research/teaching setting were conducted in the U.S., while those performed in field settings were primarily conducted in India and Brazil. Therefore, caution should be exercised in broadly generalizing findings across equine populations.

Indirectness is also a concern with regard to the tests under evaluation. There was wide variability in both the test methods employed within a given index test (e.g., sample weight; pre-enrichment, enrichment, and plating media; incubation time and temperature; PCR targets) and

in the comparator tests used across studies. Further, in several diagnostic test comparisons included in the review, multiple enrichment broths[52,60] or plating media[43,47,52,60-62] were used in parallel without an indication of which media produced positive test results. While these were considered a single test method for the purposes of data extraction, this incomplete reporting convolutes the interpretation of sensitivity and specificity estimates. The observed variability across test methods likely stems from the lack of standard methods for enriched culture and PCR for *Salmonella* detection in equine fecal samples and is therefore not necessarily a flaw of the studies themselves. However, factors such as sample weight and choice of enrichment media are known to impact the clinical performance of *Salmonella* detection methods in other species,[50,63,64] so the sensitivity and specificity of a single culture or PCR method cannot be generalized to all other methods. Therefore, the heterogeneity of the tests under evaluation prevents the generation of summary estimates of test performance and limits the applicability of the collective body of evidence to clinical or laboratory settings.

Inconsistency and imprecision

Sensitivity point estimates were quite variable across all diagnostic test comparisons; however, they were more consistent within a given type of comparison (e.g., among comparisons of enriched culture against enriched PCR, or enriched PCR against enriched culture), especially among observational studies. The use of different reference tests across these comparisons was likely a key contributor to the inconsistency in sensitivity estimates. However, there is no gold standard test for the detection of *Salmonella* in equine fecal samples. Accordingly, the use of an analytical method that does not require a gold standard reference test (i.e. a Bayesian latent class analysis) would be appropriate.[65] However, none of the studies in this review used this approach,

likely resulting in biased estimates of test performance that are not directly comparable to one another.

Confidence interval widths for sensitivity were also variable across diagnostic test comparisons, and while this variation could be explained by sample size among experimental studies, this was not the case among observational studies. This may be due to a limited number of *Salmonella*-positive individuals (as determined by the comparison test in a given study) being included in these study populations, leading to a small effective sample size for calculating sensitivity even when the total study population is relatively large. Calculating summary estimates across studies would help to overcome this limitation; however, the variability among study designs and test methods precludes such calculations in this review.

Across all diagnostic test comparisons included in the review, point estimates for specificity were quite consistent, with two outliers (Cohen 1996 and Ward 2005). However, confidence interval widths for specificity varied. Experimental studies tended to produce wider confidence intervals around specificity estimates, while the confidence intervals from observational studies were consistently narrow. This discrepancy is likely explained by sample size differences between these studies. Overall, the consistency across specificity estimates lends credence to the conclusions that both enriched culture and enriched PCR are highly specific for the detection of *Salmonella* in equine feces and that test specificity is less impacted by variations in enriched culture and enriched PCR methods than is test sensitivity.

Publication bias

Funnel plots for both sensitivity and specificity were asymmetric. However, factors other than publication bias may have contributed to this asymmetry. The wide range of estimates for

sensitivity of enriched fecal culture, including among larger studies with small standard errors, suggests true heterogeneity in these estimates rather than publication bias. This is likely explained by differences in test methods, study design, and comparison tests implemented across the studies included in this review. Sensitivity estimates for enriched fecal PCR were quite consistently high regardless of the magnitude of the standard errors; this may be due to underreporting of smaller studies in which test performance estimates are expected to be more variable. For specificity, although the asymmetry of the funnel plot could indicate selective reporting of studies estimating high test specificity, the variability in corresponding sensitivity estimates suggests that overreporting of high-performing tests is unlikely.

Limitations

In addition to the limitations of the studies included in this review, the limitations of the review itself must be considered. In an effort to capture the scope of the published literature on the performance of tests for the detection of *Salmonella* in equine feces, we purposefully utilized broad inclusion criteria. This approach did contribute to the heterogeneity of the studies ultimately included in the review and hindered the performance of a formal meta-analysis. However, it also allowed for a deeper exploration into the limitations of the existing body of evidence on the diagnosis of salmonellosis in horses. Still, this systematic review only captures the published literature on the performance of *Salmonella* diagnostic tests in horses, which may not be representative of all *Salmonella* detection methods used among clinical, reference, or commercial laboratories. Given the heterogeneity in methods demonstrated in this study alone, there may be far more variability that is not represented in this review. Finally, it should also be noted that several authors of this review were also authors of some of the included studies. To

avoid conflicts of interest, these authors were not permitted to screen or extract data from their own studies. Nevertheless, their participation in the review process may have biased the assessment of these publications.

Conclusions

The evidence from this review suggests that both enriched culture and enriched fecal PCR are both highly specific for the detection of *Salmonella* in equine feces, while culture is less sensitive than PCR. However, given the variability and incomplete reporting of test methods, as well as the diversity of study designs and comparison tests utilized in the included studies, data from the included studies were not synthesized to generate summary estimates of test performance. Therefore, this review emphasizes the need for increased standardization of diagnostic tests for equine salmonellosis; a comprehensive survey of test methods used across veterinary diagnostic laboratories, and an evaluation of their clinical performance, is warranted. Additionally, improved adherence to Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines in future evaluations of the performance of these tests would allow for improved comparisons across publications,[66] and the implementation of no-gold-standard analysis methods would facilitate the estimation of unbiased measures of test sensitivity and specificity.

Acknowledgements

Funding

None

Author Contributions

ECH, AMO, and BAB conceptualized the study and drafted the review protocol. All authors provided input and final approval of the protocol. ECH conducted the literature search. All authors participated in screening, data collection, and risk of bias assessment. ECH performed data analysis and drafted the manuscript, with oversight by BAB. All authors reviewed and provided approval of the final manuscript. All co-authors agreed that this work may be included in this dissertation.

Tables

Table 2.1: Electronic database search strategy to identify studies on the performance of *Salmonella* detection methods in horses.

Search Number	Search Parameter	Search Strings
1	Population	horse* OR equid* OR equine* OR equus OR mare* OR gelding* OR stallion* OR pony OR ponies OR foal*
2	Target condition	salmonell* OR enterica
3	Index tests – analytic methods	roc OR "roc curve*" OR "receiver operating characteristic*" OR auc OR "area under the curve"
4	Index tests – 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*
5	Index tests – test methods	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 OR rtPCR OR r-PCR OR rt-PCR OR qPCR OR q-PCR
6	Index tests	3 OR 4 OR 5
7	Exclude *Salmonella* serotype Abortusequi	abortusequi OR abortus-equi OR “abortus equi”
8	Final search	1 AND 2 AND 6 NOT 7

Table 2.2: Summary of characteristics of diagnostic test evaluations (N = 30) from included studies (N = 19).

Study	Comparison	Country	Study Design	Clinical Setting	N (Horses)	N (Samples)	Purpose of Sample Collection	Index test	Comparison test
Babu 2008	a	India	CSDS	Field	245	245	Research	PCR	Culture
Babu 2008	b	India	CSDS	Field	245	245	Research	Culture	Culture
Babu 2008	c	India	CSDS	Field	245	245	Research	Culture	Culture
Babu 2008	d	India	CSDS	Field	245	245	Research	Culture	Culture
Bohaychuk 2007	a	NR	CSDS	Field	NR	373	NR	PCR	Culture
Bohaychuk 2007	b	NR	Experimental (inoculation)	NR	NR	40	NR	Culture	Experimental inoculation
Bohaychuk 2007	c	NR	Experimental (inoculation)	NR	NR	40	NR	PCR	Experimental inoculation
Braga 2023	a	Brazil	CSDS	Field	200	200	Research	Culture	PCR
Braga 2023	b	Brazil	CSDS	Field	200	200	Research	Culture	PCR
Burgess 2014	a	USA	Experimental (inoculation)	Research/ teaching	5	137	Research	Culture	Experimental inoculation
Burgess 2014	b	USA	Experimental (inoculation)	Research/ teaching	5	137	Research	Culture	Experimental inoculation
Burgess 2014	c	USA	Experimental (inoculation)	Research/ teaching	5	137	Research	PCR	Experimental inoculation
Burgess 2015	a	USA	Experimental (inoculation)	Research/ teaching	5	50	Research	Culture	Experimental inoculation
Burgess 2015	b	USA	Experimental (inoculation)	Research/ teaching	5	50	Research	Culture	Experimental inoculation
Cohen 1994		USA	Experimental (inoculation)	NR	2	70	Research	Culture	Experimental inoculation
Cohen 1995	a	USA	Experimental (inoculation)	Research/ teaching	2	24	Research	Culture	Experimental inoculation
Cohen 1995	b	USA	Experimental (inoculation)	Research/ teaching	2	24	Research	PCR	Experimental inoculation
Cohen 1996		USA	CSDS	Referral hospital	262	434	Research; Clinical	PCR	Culture
Ekiri 2016		USA	CSDS	Referral hospital	93	343	Research; Surveillance	PCR	Culture
Fakour 2020		Iran	CSDS	NR	130	130	Research	Culture	PCR
Owen 1979		Canada	Experimental (infection)	Research/ teaching	19	19	Research	Culture	Experimental infection
Pusterla 2010		USA	CSDS	Referral hospital	911	911	Research; Surveillance; Clinical	PCR	Culture
Pusterla 2014	a	USA	CSDS	Referral hospital	NR	398	Surveillance	PCR	Culture
Pusterla 2014	b	USA	CSDS	Referral hospital	NR	279	Surveillance	PCR	Culture
Pusterla 2023		USA	CSDS	Referral hospital	NR	143	Surveillance	PCR	PCR
Ramin 2012		Iran	CSDS	NR	100	100	Research	PCR	Culture
Singh 2007		India	Field/Outbreak	Field	872	872	Research	Culture	PCR
Slovis 2014		USA	Field/Outbreak	Referral hospital; Field	88	88	Research	Culture	PCR
Stone 1994		USA	CSDS	NR	3	5	Clinical	PCR	Culture
Ward 2005		USA	CSDS	Referral hospital	116	873	Research	PCR	Culture

NR – not reported; CSDS – cross-sectional diagnostic study

Table 2.3: Culture methods reported among diagnostic test evaluations including enriched fecal culture as either the index or comparison test (N = 29).

Variable	Category	Frequency (%)
Pre-enrichment media	Buffered peptone water	13 (44.8%)
	None	16 (55.2%)
Enrichment media	Tetrathionate broth	17 (58.6%)
	Selenite broth	8 (27.6%)
	Rappaport-Vassiliadis broth	6 (20.7%)
	Salmonella selective enrichment broth	1 (3.4%)
Plating media	Hektoen Enteric	13 (44.8%)
	XLT4	9 (31.0%)
	Brilliant Green	12 (41.4%)
	MacConkey	4 (13.8%)
	Salmonella-Shigella agar	2 (6.9%)
	Rambach agar	2 (6.9%)

Table 2.4: PCR methods reported among diagnostic test evaluations including enriched fecal PCR as either the index or comparison test (N = 20).

Variable	Category	Frequency (%)
Pre-enrichment media	Buffered peptone water	4 (20.0%)
	Phosphate-buffer saline	2 (10.0%)
	None	12 (60.0%)
	Not reported	2 (10.0%)
Enrichment media	Tetrathionate broth	13 (65.0%)
	Selenite broth	7 (35.0%)
	Rappaport-Vassiliadis broth	2 (10.0%)
PCR Type	qPCR	11 (55.0%)
	Conventional (end-point)	8 (40.0%)
	Not reported	1 (5.0%)
PCR Manufacturer	In-house	17 (85.0%)
	Commercial kit	2 (10.0%)
	Not reported	1 (5.0%)
PCR Target(s)	*invA*	14 (70.0%)
	Histidine transport operon	5 (25.0%)
	invE	1 (5.0%)
	ttrC	1 (5.0%)
	Not reported	1 (5.0%)
Cycle threshold value[†]	35	1 (9.1%)
	37	1 (9.1%)
	Not reported	9 (81.8%)

[†]Among qPCR methods (N = 11)

Table 2.5: Risk of bias of diagnostic test evaluations (N = 30) from included studies (N = 19), as determined by modified QUADAS-2 tool.

Study	Comparison	Study Design	Index Test	Comparison Test	Bias Category			
					Patient Selection	Index Test	Comparison Test	Flow/Timing
Babu 2008	a	CSDS	PCR	Culture	Unclear	Unclear	Unclear	Low
Babu 2008	b	CSDS	Culture	Culture	Unclear	Unclear	Unclear	Low
Babu 2008	c	CSDS	Culture	Culture	Unclear	Unclear	Unclear	Low
Babu 2008	d	CSDS	Culture	Culture	Unclear	Unclear	Unclear	Low
Bohaychuk 2007	a	CSDS	PCR	Culture	Unclear	Unclear	Unclear	Low
Bohaychuk 2007	b	Experimental (inoculation)	Culture	Experimental inoculation	Low	Unclear	Low	Low
Bohaychuk 2007	c	Experimental (inoculation)	PCR	Experimental inoculation	Low	Unclear	Low	Low
Braga 2023	a	CSDS	Culture	PCR	Unclear	Unclear	Unclear	Low
Braga 2023	b	CSDS	Culture	PCR	Unclear	Unclear	Unclear	Low
Burgess 2014	a	Experimental (inoculation)	Culture	Experimental inoculation	Low	Low	Low	Low
Burgess 2014	b	Experimental (inoculation)	Culture	Experimental inoculation	Low	Low	Low	Low
Burgess 2014	c	Experimental (inoculation)	PCR	Experimental inoculation	Low	Low	Low	Low
Burgess 2015	a	Experimental (inoculation)	Culture	Experimental inoculation	Low	Low	Low	Low
Burgess 2015	b	Experimental (inoculation)	Culture	Experimental inoculation	Low	Unclear	Low	Low
Cohen 1994		Experimental (inoculation)	Culture	Experimental inoculation	Low	Unclear	Low	Low
Cohen 1995	a	Experimental (inoculation)	Culture	Experimental inoculation	Low	Unclear	Low	Low
Cohen 1995	b	Experimental (inoculation)	PCR	Experimental inoculation	Low	Unclear	Low	Low
Cohen 1996		CSDS	PCR	Culture	Unclear	Unclear	Unclear	Low
Ekiri 2016		CSDS	PCR	Culture	Unclear	Low	Low	Unclear
Fakour 2020		CSDS	Culture	PCR	Low	Unclear	Unclear	Low
Owen 1979		Experimental (infection)	Culture	Experimental infection	Low	Unclear	Low	Low
Pusterla 2010		CSDS	PCR	Culture	Low	Unclear	Unclear	Low
Pusterla 2014	a	CSDS	PCR	Culture	Unclear	Unclear	Unclear	Low
Pusterla 2014	b	CSDS	PCR	Culture	Unclear	Unclear	Unclear	Low
Pusterla 2023		CSDS	PCR	PCR	Unclear	Unclear	Unclear	Low
Ramin 2012		CSDS	PCR	Culture	Unclear	Unclear	Unclear	Low
Singh 2007		Field/Outbreak	Culture	PCR	Unclear	Unclear	Unclear	Low
Slovis 2014		Field/Outbreak	Culture	PCR	Low	Unclear	Unclear	Low
Stone 1994		CSDS	PCR	Culture	Unclear	Unclear	Unclear	Low
Ward 2005		CSDS	PCR	Culture	Unclear	Unclear	Unclear	Low

CSDS – cross-sectional diagnostic study

Figures

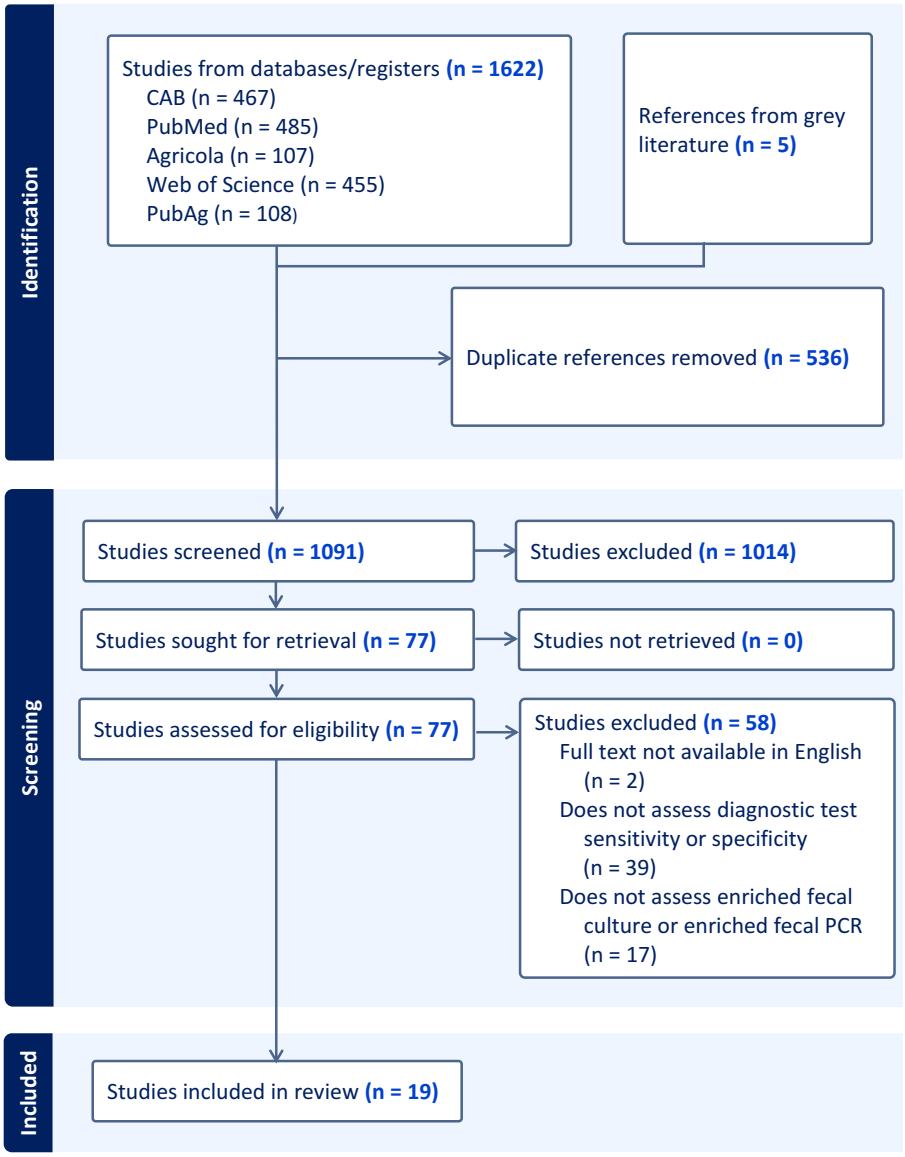


Figure 2.1: PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram of studies evaluated and included in the systematic review of sensitivity and specificity of *Salmonella* detection methods in equine feces.

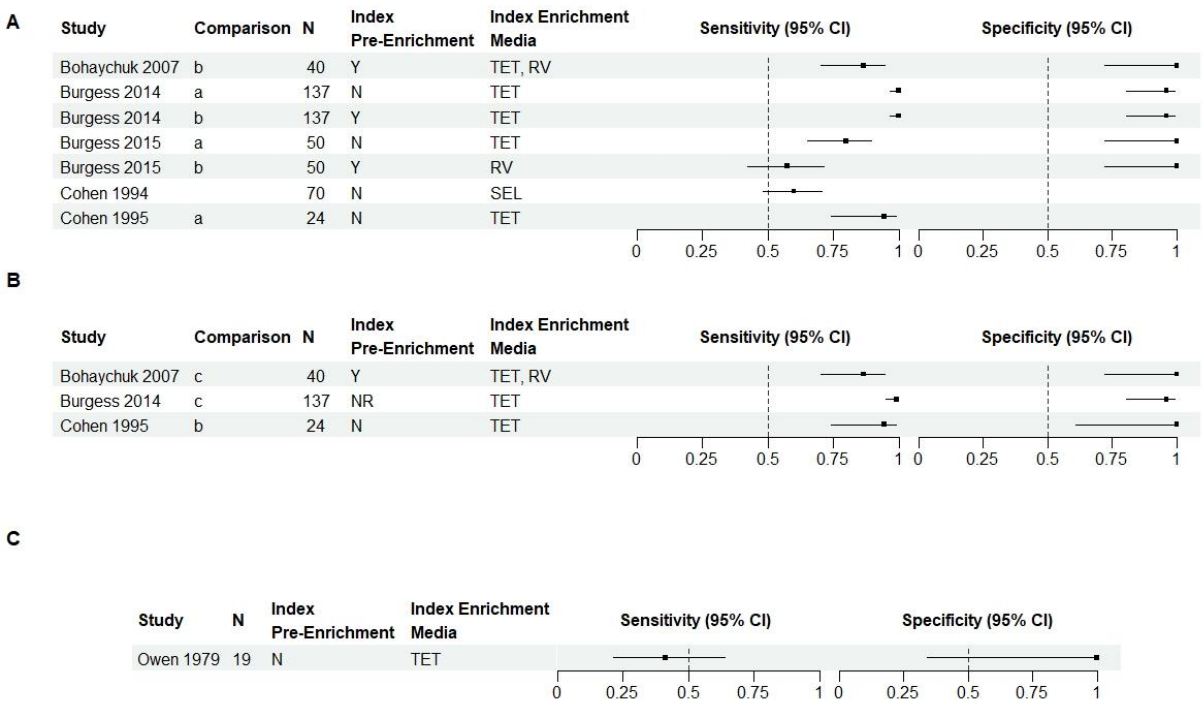


Figure 2.2: Forest plots of sensitivity and specificity estimates from experimental studies, including **A**) enriched fecal culture compared against experimental inoculation of fecal samples with *Salmonella*, **B**) enriched fecal PCR compared against experimental inoculation of fecal samples with *Salmonella*, and **C**) enriched fecal culture in horses experimentally infected with *Salmonella*. If multiple diagnostic test comparisons were included in a single study, each comparison is denoted with a lowercase letter in the "Comparison" column.

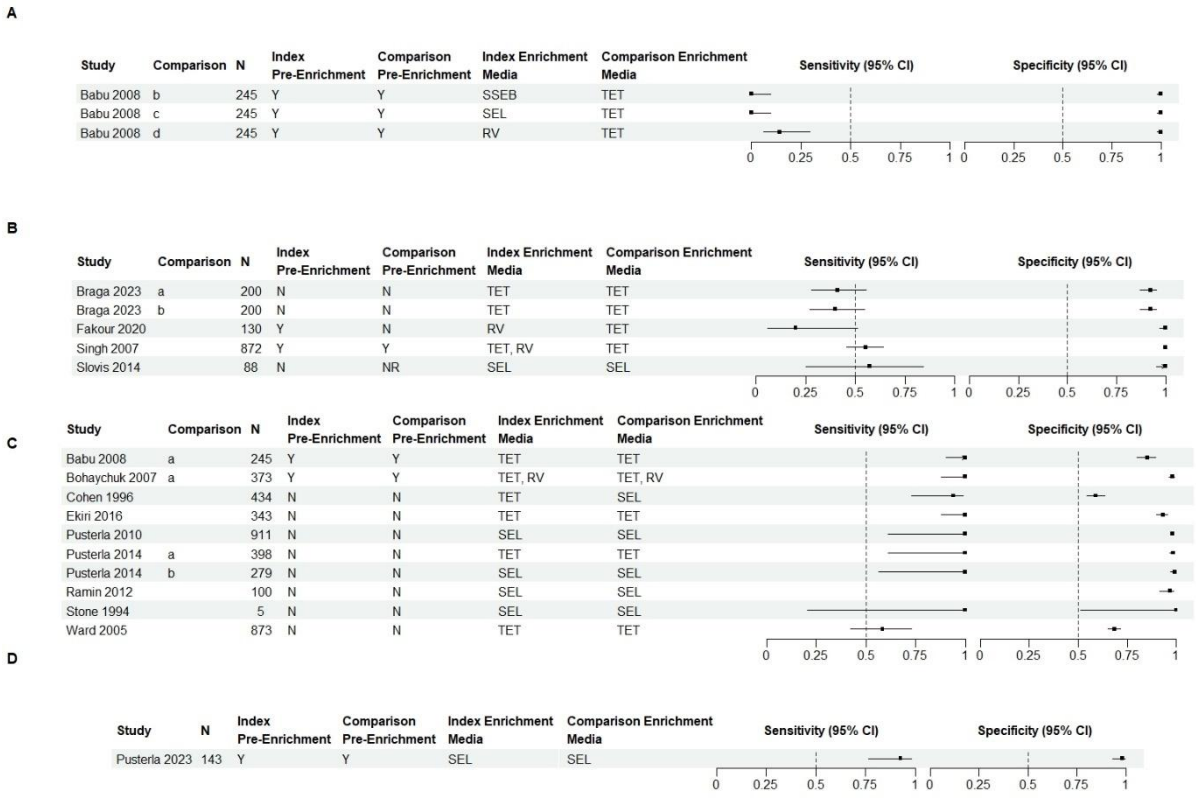


Figure 2.3: Forest plots of sensitivity and specificity estimates from experimental studies, including **A**) enriched fecal culture compared against enriched fecal culture, **B**) enriched fecal culture compared against enriched fecal PCR, **C**) enriched fecal PCR compared against enriched fecal culture, and **D**) enriched fecal PCR compared against enriched fecal PCR. If multiple diagnostic test comparisons were included in a single study, each comparison is denoted with a lowercase letter in the "Comparison" column.

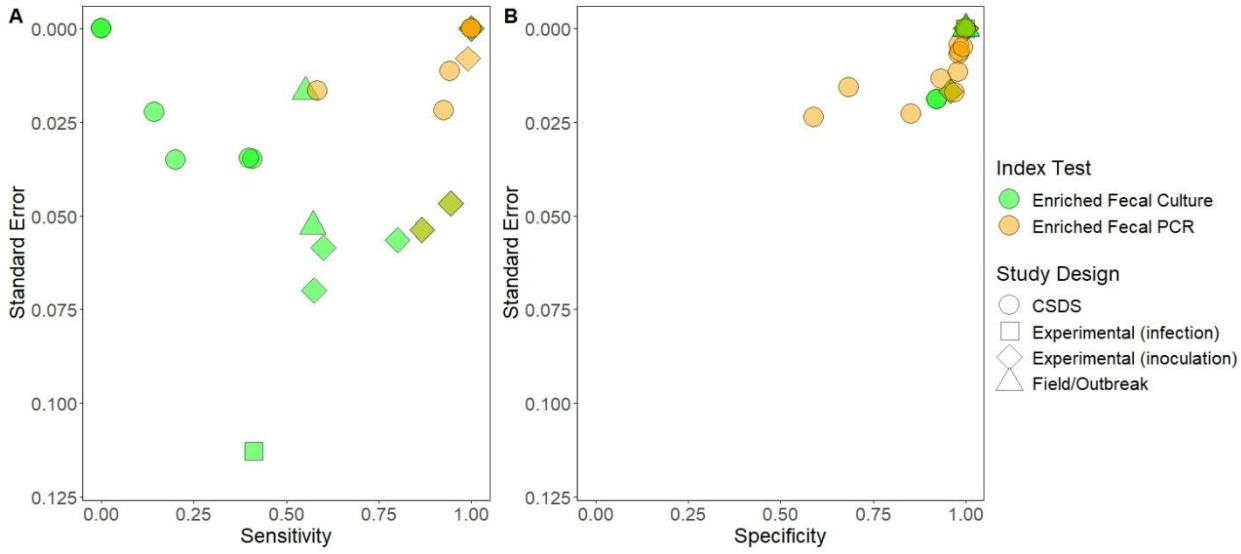


Figure 2.4 Funnel plots of estimates of the **A**) sensitivity and **B**) specificity of enriched fecal culture and enriched fecal PCR from included diagnostic test comparisons.

CHAPTER 3

BAYESIAN LATENT CLASS EVALUATION OF THE SENSITIVITY AND SPECIFICITY OF A LATERAL FLOW IMMUNOASSAY, POLYMERASE CHAIN REACTION, AND ENRICHED AEROBIC CULTURE FOR THE DETECTION OF *SALMONELLA ENTERICA* IN EQUINE FECES [b]

[b] Herring, E.C., Pabilonia, K.L., McConnico, R.S., Chapman, A.M., Velayudhan, B.T., Aceto, H.W., Slovis, N.M., Morley, P.S., and B.A. Burgess. To be submitted to *Preventive Veterinary Medicine.*

Abstract

Rapid, accurate detection of *Salmonella enterica* is critical to prevent transmission in equine facilities, but the sensitivity of currently available diagnostic tests is generally low to moderate on a per-sample basis. The Reveal® 2.0 rapid test, a lateral flow immunoassay (LFI), is a promising candidate for a point-of-care screening test. Therefore, we aimed to quantify the sensitivity and specificity of the LFI, as well as enriched culture and qPCR, using a Bayesian analytical method. Fecal samples were collected from three populations of horses with a high (N = 106), intermediate (N = 123), and low (N = 437) prevalence of *S. enterica*. Each fecal sample was subjected to four diagnostic tests (tetrathionate-enriched culture [TEC], selenite-enriched culture [SEC], qPCR, and the LFI). The sensitivity and specificity of each test – for both a single-sample and a two-test parallel testing strategy – were estimated using a Bayesian latent class model. The LFI was moderately sensitive (median [95% credible interval]: 59.8% [48.3%, 70.9%] and specific (68.2% [64.6%, 71.5%]) on a per-sample basis, while TEC and qPCR demonstrated superior sensitivity (66.1% [53.8%, 78.0%]; 87.5% [77.3%, 94.5%]) and specificity (97.2% [95.7%, 98.3%]; 97.1% [95.3%, 98.4%]), respectively. SEC was moderately sensitive (58.4% [47.7%, 69.0]) but more specific (89.8% [87.4%, 92.0%]). When used in a two-test parallel testing strategy, the sensitivity of the LFI increased to 83.9% [73.3%, 91.5%]. Therefore, when used in a two-test parallel testing strategy, the Reveal® 2.0 LFI is an affordable, convenient screening test that can aid in rapidly identifying *S. enterica*-infected horses.

Introduction

Salmonella enterica is a common and important cause of gastrointestinal illness in horses. Outbreaks often occur in settings where populations of horses are commingled, such as

veterinary hospitals, breeding farms, and boarding facilities, and may be characterized by high rates of morbidity and mortality.[12,13,27,80] However, these outbreaks are often facilitated by *S. enterica*-infected horses that shed the bacteria in the absence of clinical signs or with mild, nonspecific symptoms. This presentation is more common than clinical disease and can allow for widespread environmental contamination and disease transmission to occur before *S. enterica* is detected.[16,27]

Given the clinical impact of this pathogen and the financial burden of outbreak mitigation efforts, rapid, reliable detection of *S. enterica*-infected horses is a priority of equine infection control programs. However, these efforts are hindered by the limitations of the most commonly used *S. enterica* diagnostic tests, including culture and polymerase chain reaction (PCR). Infected horses tend to shed *S. enterica* intermittently and in low numbers, which limits the sensitivity of these tests on a per-sample basis.[39,41,42] To compensate for this, selective enrichment steps are usually incorporated into culture and PCR protocols, and multiple samples from a single horse are often tested and interpreted in parallel.[41,46] While these approaches do improve the overall sensitivity of the testing strategy, they also increase the costs and delay the results of these tests, hindering clinical decision-making and infection control efforts.

Collectively, these challenges highlight the need for a sensitive, cost-effective test that produces results quickly and does not require extensive technical expertise to perform. A test with these characteristics could be performed in-house at equine facilities if local regulations allow, facilitating the timely identification of infected horses and implementation of appropriate control measures. One candidate that may meet these criteria is the Reveal® 2.0 *Salmonella* rapid test (Neogen® Corporation, Lansing, MI), a commercially available LFI currently validated for *S. enterica* detection in food matrices, animal feed, and environmental samples.[81]

This LFI requires little technical expertise to perform and interpret and costs less than $15 per test. Further, preliminary evaluations of the Reveal® 2.0 LFI for the detection of *S. enterica* in experimentally inoculated equine fecal samples have demonstrated that it can detect as few as four cfu of *S. enterica* per gram of feces following overnight incubation in tetrathionate broth, indicating that it may have utility as a point-of-care screening test.[69,70] However, to generate reliable estimates of test sensitivity and specificity, the performance of the Reveal® 2.0 LFI must be evaluated using relevant clinical samples.

There is no gold standard test for the detection of *S. enterica* in equine fecal samples, and comparing the performance of the LFI against the performance of an imperfect reference test, such as culture or PCR, would result in biased estimates of test sensitivity and specificity. An alternative approach is to use a Bayesian latent class model, wherein two or more imperfect tests are compared in multiple populations of animals with different levels of disease prevalence.[65,82] The diagnostic test results in these populations are combined with prior knowledge about test performance to generate updated, posterior estimates of test sensitivity and specificity without needing to know the true disease status of each animal. Therefore, using this approach, in this study we aimed to calculate unbiased estimates of the sensitivity and specificity of the Reveal® 2.0 LFI, TEC, SEC, and qPCR for the detection of *S. enterica* in equine feces.

Methods

This study is reported according to the Standards for the Reporting of Diagnostic accuracy studies that use Bayesian Latent Class Models (STARD-BLCM; **Appendix 3A**).[83] Sample collection was approved by the Virginia-Polytechnic and State University Institutional

Animal Care and Use Committee (IACUC-15-031) and the University of Georgia College of Veterinary Medicine Clinical Research Committee (CR-485).

Study Design

Three populations of horses with different levels of *S. enterica* prevalence were prospectively enrolled in a study to evaluate the performance of four *S. enterica* diagnostic tests – the LFI, TEC, SEC, and qPCR. A fecal sample from each horse was subjected to testing with all four tests, and the sensitivity and specificity of each diagnostic test was evaluated using a Bayesian latent class model.

Participants

The total study population was comprised of 666 horses, which were stratified into three sub-populations based on their expected prevalence of *S. enterica* – high, intermediate, or low. The high-prevalence population included a convenience sample of horses (N= 106) enrolled in a longitudinal study on *S. enterica* shedding. These horses presented to a participating equine general practice or referral hospital in the U.S. between 2018 and 2020 and were selected for enrollment by a referring veterinarian after testing positive for *S. enterica* by either fecal culture or PCR. Horses in the intermediate- (N = 123) and low-prevalence (N = 437) populations were patients presenting to one of three equine referral hospitals in the northeastern and southern U.S. between 2013 and 2016. These horses were purposively selected by investigators and classified into intermediate- and low-prevalence populations based on the presence or absence of known risk factors for *S. enterica* shedding in horses. The intermediate-prevalence population included horses presenting for diarrhea, colitis, enteritis, or colic requiring surgical intervention; the low-

prevalence population included horses with any other presenting complaint and healthy companions to hospitalized horses.

Power analysis

A power analysis was performed to determine the precision with which the sensitivity and specificity of the LFI could be estimated given the number of available samples. The width of the 95% confidence intervals for the sensitivity and specificity of the LFI were calculated using the formulas defined by Buderer.[84] Because test sensitivity and specificity were unknown, both were assumed to be 50% to generate conservative estimates of precision. Sample size was set at 666, the type one error rate (α) was set at 0.05, and prevalence was estimated at 8% based on the proportion of positive qPCR results across all three populations of horses.

Sample collection

Fecal samples were collected from horses in the high-prevalence population on an approximately weekly basis while enrolled in a longitudinal study on *S. enterica* shedding. Approximately 15 g of freshly voided feces were collected with a clean, gloved hand by either hospital personnel or the horse owner, placed into a sterile specimen container, and shipped overnight on ice to the investigators for *S. enterica* testing. To avoid dependency between test results related to repeated measures, one fecal sample was randomly selected from each horse for inclusion in the current study. A single fecal sample was collected from each horse in the intermediate- and high-prevalence populations by a clinician at each participating equine hospital. Fecal samples were collected in the same manner as for the high-prevalence population

then either shipped overnight on ice to the investigators for *S. enterica* testing or processed for *S. enterica* testing at the hospital's in-house laboratory using the same testing protocols.

Test methods

Three-gram aliquots of each fecal sample were inoculated into 30 ml of tetrathionate broth (TET) supplemented with brilliant green and iodine (BD, Franklin Lakes, NJ) and 30 ml of selenite broth (SEL; BD, Franklin Lakes, NJ), respectively. TET was incubated for 18-24 hours at 43°C, then plated onto xylose lysine tergitol-4 (XLT4) agar (Hardy Diagnostics, Santa Maria, CA). SEL was incubated for 18-24 hours at 36°C, then plated onto Hektoen enteric (HE) agar (BD, Franklin Lakes, NJ). After incubation at 43°C and 36°C, respectively, for 18-24 hours, plates were inspected for black-centered colonies consistent with the morphology of *S. enterica.* Suspect colonies were sub-cultured onto trypticase soy agar (TSA) with 5% sheep blood and incubated for 18-24 hours at 43°C. *S. enterica* identity was confirmed by testing for agglutination using commercial polyvalent and O-group specific antisera.

A 200-µl aliquot of the TET broth culture was used to perform the LFI test per manufacturer's instructions. The test strip was placed into the enrichment media for 15 minutes at room temperature, then observed for the appearance of indicator lines in the test and control zones. A positive test was defined as having a test line at least as intense in color as the control line. A negative test was defined as the appearance of either no test line or a test line less intense in color than the control line. This interpretation was used to minimize false positive test results and to maintain consistency with previous applications of this test in equine fecal samples.[69,70]

An additional 1-ml aliquot of TET broth culture was frozen at -80°C to preserve for future qPCR testing; samples were preserved for up to three years before qPCR was performed.

At the time of testing, samples were thawed, and DNA was extracted from 250 µl of each sample using the PrepSEQ™ Nucleic Acid Extraction Kit (Applied Biosystems™, Waltham, MA) according to the manufacturer's instructions for automated DNA extraction with the KingFisher™ Flex-96 instrument (Thermo Scientific, Waltham, MA).[85] DNA was then processed for qPCR using the MicroSEQ™ *Salmonella* spp. Detection Kit (Applied Biosystems, Waltham, MA) according to the manufacturer's instructions.[85] Samples were amplified in a 7500 Fast thermal cycler (Applied Biosystems, Waltham, MA) for 2 min at 95°C and 40 cycles of 3 seconds at 95°C and 30 seconds at 60°C. In accordance with previous applications of this test to equine fecal samples, a cycle threshold (Ct) number ≤35 was considered a positive test result; a Ct number >35 indicated a negative test result.[69,70] This threshold was established prior to performance of qPCR. If PCR inhibition occurred, as evidenced by the lack of an internal positive control signal, DNA was diluted 1:10 with sterile nuclease-free water, and the qPCR assay was repeated. Investigators were not blinded to horses' clinical history during the performance of any tests under evaluation. Results of the four tests under evaluation were cross-tabulated within each population of horses.

Analysis

Definition of Infection

All four tests under evaluation detect the presence of *S. enterica* in an equine fecal sample, either via isolation of the organism, detection of *S. enterica* DNA, or reaction with a *S. enterica* antigen. Therefore, in this analysis, the latent infection status is defined as fecal shedding of *S. enterica*, demonstrated by identification of the organism in a fecal sample. This includes both clinical and subclinical shedding of *S. enterica*.

Conditional independence model

The sensitivity and specificity of TEC, SEC, qPCR, and the LFI were first estimated using a four-test, three-population Bayesian latent class model, under the assumption that the four tests were independent of one another, conditional on the true disease status of an individual (**Appendix 3B**). Posterior distributions of each test performance parameter were obtained using three Markov Chain Monte Carlo (MCMC) simulations, each run for 11,000 iterations with a 1000-iteration burn-in period. Each MCMC simulation was started from a different series of initial values. The Bayesian analysis was performed using the *R2jags* package (v0.7-1; JAGS v4.3.0) in R (v4.1.3).[86,87] Convergence and autocorrelation of Markov chains was evaluated using the *mcmcplots* package (v0.4.3).[88] Effective sample size was calculated using the *coda* package (v0.19-4).[89]

Conditional dependence between tests – correlation residual analysis

All tests evaluated in this study detect the presence of *S. enterica* in a sample, and further, three of these tests involve a selective enrichment step in TET. Therefore, because the tests rely on similar biological principles, the assumption of conditional independence between tests was unlikely to be met, necessitating the addition of covariance terms to the model. However, the addition of extraneous covariance terms would add unnecessary complexity to the model and potentially bias estimates of test sensitivity and specificity.[90] Therefore, a correlation residual analysis was performed to quantify the level of dependence between tests and determine which covariance terms should be added to the model. For each pair of tests in each population of horses, the correlation residual was defined as the difference between the observed and the

model-based correlation between tests. Within each population of horses, the correlation between each pair of tests (tests *A* and *B*) was calculated as

$$r_{AB} = \frac{P(T_A = 1, T_B = 1) - P(T_A = 1)P(T_B = 1)}{\sqrt{P(T_A = 1)\big(1 - P(T_A = 1)\big)P(T_B = 1)\big(1 - P(T_B = 1)\big)}} \quad (1)$$

where the binary test result $T = 1$ if the test result was positive, and $T = 0$ if the result was negative. The observed correlation between each pair of tests was calculated based on the cross-classified test results in each population, with $P(T_A = 1)$ and $P(T_B = 1)$ equal to the proportion of horses testing positive by tests *A* and *B*, respectively. The joint probability $[P(T_A = 1, T_B = 1)]$ for the observed correlation was calculated as the proportion of horses testing positive on both tests *A* and *B*. For the model-based correlation, $P(T_A = 1)$ and $P(T_B = 1)$ were calculated as

$$pSn_A + (1 - p)(1 - Sp_A) \quad (2)$$

and

$$pSn_B + (1 - p)(1 - Sp_B) \quad (3)$$

respectively, where p = disease prevalence in the population, and *Sn* and *Sp* are the sensitivity and specificity of the respective tests, estimated by the median of the posterior distribution generated by the conditional independence model for each of these parameters. The joint probability $[P(T_A = 1, T_B = 1)]$ for the model-based correlation was defined as

$$pSn_ASn_B + (1 - p)(1 - Sp_A)(1 - Sp_B) \quad (4)$$

.[90] For each pair of tests, the mean correlation residual across the three populations of horses was calculated, plotted, and evaluated for deviation from zero, indicating dependence between tests that was not sufficiently explained by the conditional independence model.

Conditional dependence model

Based on the results of the correlation residual analysis, covariance terms between the sensitivity and specificity of TEC and SEC were added to the four-test, three-population model to account for conditional dependence between these tests (**Appendix 3B**). Posterior distributions of model parameters were obtained using three MCMC simulations, each run for 101,000 iterations with a 1000-iteration burn-in period and starting from a different series of initial values. A second correlation residual analysis was performed as previously described, with one exception: the joint probability $[P(T_A = 1, T_B = 1)]$ for the model-based correlation was defined as

$$p(Sn_A Sn_B + covp) + (1 - p)((1 - Sp_A)(1 - Sp_B) + covn) \quad (5)$$

where *covp* and *covn* represent the covariances between tests given a disease-positive or disease-negative individual, respectively.[90] These values were estimated by the medians of the posterior distributions generated from the conditional dependence model.

Elicitation of informative priors

For each model, informative prior distributions for the sensitivity and specificity of each diagnostic test were obtained using expert opinion. An online survey (**Appendix 3C**) was sent to a panel of 11 individuals, identified by the investigators, with expertise in diagnostic testing and equine salmonellosis. The survey included questions about respondents' training and expertise, and for each diagnostic test under evaluation, respondents were asked to quantify the most likely value of the sensitivity and specificity of a test on a per-sample basis, as well as the lower and upper limits of the 95% confidence interval of their estimates. The mean of the estimates for the most likely value (mode) and lower 95% confidence interval limit were calculated and used to

generate a beta distribution for each test sensitivity and specificity parameter with the *epiR* package (v2.0.60).[91] Uniform priors were used for the prevalence parameter in each population of horses, as well as the covariance terms between the sensitivity and specificity of TEC and SEC. Prior distributions for the covariance terms were constrained between the limits proposed by Dendukuri and Joseph.[92]

Sensitivity analyses

Three sensitivity analyses were performed to evaluate the impact of the choice of informative priors on posterior estimates of test sensitivity and specificity. First (sensitivity analysis 1), the informative prior distributions were relaxed by widening the 95% confidence interval around the mode; for each test sensitivity and specificity parameter, the lower 95% confidence interval limit was set to the minimum of the lower 95% confidence interval limit provided by the expert panel. The mode was not changed from the original conditional dependence model. Because expert estimates of the 95% confidence intervals for the sensitivity of both qPCR and Reveal® 2.0 ranged from 0 to 100%, uniform (β [1,1]) prior distributions were used for these parameters. In sensitivity analysis 2, the mode of each informative prior distribution was decreased by 10%, and the 95% confidence intervals were not changed from the original conditional dependence model. Finally, in sensitivity analysis 3, uniform priors were utilized for all model parameters. To evaluate the assumption of constant sensitivity and specificity of tests across all populations, horses were randomly assigned to three new populations, and the main conditional dependence model, with the original informative prior distributions, was utilized to generate estimates of the sensitivity and specificity of the four tests under evaluation.

Parallel interpretation of diagnostic tests

To further assess the clinical utility of the four tests under evaluation, the sensitivity and specificity of using each test in a two-test parallel testing strategy was evaluated by adding the parallel test performance parameters to the conditional dependence model. The sensitivity of each two-test parallel testing strategy was estimated as

$$2Sn - Sn^2 \quad (6)$$

and specificity was estimated as

$$Sp^2 \quad (7)$$

where Sn and Sp were equal to the conditional dependence model-derived estimates of each test's sensitivity and specificity, respectively.[93] For each calculation, the same test was assumed to be used twice, with results interpreted in parallel. Using the median estimates of the sensitivity and specificity of each two-test parallel testing strategy, the cost of each testing strategy was then calculated for a population of 100 horses with a 10% prevalence of *S. enterica*. In each scenario, all 100 horses were assumed to have been tested once with the test under evaluation; horses that tested negative were then re-tested, while test-positive horses were accepted as positive and not re-tested. The cost of TEC, SEC, and qPCR were each assumed to be $45.00 based on the costs of *S. enterica* fecal culture and PCR in the University of Georgia Athens Veterinary Diagnostic Laboratory test catalog as of September 2024.[c,d] The total cost of the Reveal® 2.0 LFI was

[c] https://portal.vet.uga.edu/catalogItemDetails.zul?id=564&labId=1&max=30&offset=0&CatalogSearch=salmonella

[d] https://portal.vet.uga.edu/catalogItemDetails.zul?id=1817068&labId=1&max=30&offset=0&CatalogSearch=salmonella+pcr

estimated to be $15.00 based on the cost of the test ($14.20 per test),[e] tetrathionate broth base ($0.21 per 30 ml),[f] and iodine-iodide solution ($0.34 per 30 ml)[g] as of September 2024.

Results

Study population

Study population demographics are presented in **Table 3.1**. Among the total study population, the median age was 8 years (range: 0.02 – 28 years). Mares comprised the majority of the study population (383/666; 60.6%), and the most common breed was Thoroughbred (374/666; 59.3%).

Power analysis

With a sample size of 666 and *S. enterica* prevalence of 8%, the maximum 95% confidence interval widths for the sensitivity and specificity of the Reveal® 2.0 LFI were determined to be 0.13 and 0.04, respectively.

Diagnostic test results

Test results for TEC, SEC, qPCR, and the LFI in the high-, intermediate-, and low-prevalence populations of horses are cross-tabulated in **Table 3.2**.

[e] https://www.neogen.com/categories/microbiology/reveal-2-salmonella/
[f] https://www.neogen.com/categories/microbiology/tetrathionate-broth-base/?min=700003226
[g] https://hardydiagnostics.com/z139

Expert opinion survey

Of the 11 experts surveyed, nine (82%) responded. One survey response was incomplete, but the partial response was utilized when generating informative prior distributions. Respondent demographics are detailed in **Table 3.3**. Respondents' estimates of test sensitivity and specificity on a per-sample basis are provided in **Table 3.4**.

Conditional independence model

Results of the conditional independence model are presented in **Figure 3.1** and **Supplementary Table 3.1**. Of the four tests under evaluation, the estimated median sensitivity was highest for qPCR (87.2%; 95% credible interval (CI): [77.0%, 94.2%]) and lowest for the Reveal® 2.0 LFI (60.5%; 95% CI: [48.8%, 71.5%]). The estimated median specificity was highest for TEC (97.5%; 95% CI: [96.1%, 98.6%]) and lowest for the LFI (68.1%; [64.6%, 71.5%]).

Correlation residual analysis

Of the six pairwise correlation residuals (**Figure 3.2A**), the greatest deviation from zero was observed for TEC and SEC (0.29). Therefore, conditional dependence between these tests was accounted for by adding pairwise covariance terms to the original conditional independence model. The correlations residuals for TEC and qPCR (0.18), SEC and qPCR (0.21), and SEC and the LFI (0.11) also demonstrated potential conditional dependence between these pairs of tests. However, because it was not feasible to include covariance terms to account for these pairwise dependencies, in addition to the TEC/SEC dependency, all other pairs of tests were treated as conditionally independent in the updated model.

Conditional dependence model

Results of the main conditional dependence model and sensitivity analyses evaluating the impact of informative prior distributions are presented in **Figure 3.3A** and **Supplementary Table 3.2**. In the main model, the highest sensitivity was observed for qPCR (87.5%; 95% CI: [77.3%, 94.5%]), while SEC had the lowest sensitivity (58.4%; 95% CI: [47.7%, 69.0%]), followed by the Reveal® 2.0 LFI (59.8%; 95% CI: [48.3%, 70.9%]). Specificity was estimated to be highest for TEC (97.2%; 95% CI: [95.7%, 98.3%]) and lowest for the LFI (68.2%; 95% CI: [64.6%, 71.5%]). Model diagnostic plots confirmed convergence of the Markov chains and rapid reduction in autocorrelation between consecutive iterations for each parameter (**Appendix 3D**). The correlation residual analysis demonstrated a reduction in the correlation residual for TEC and SEC (0.10; **Figure 3.2B**) compared to that obtained from the conditional independence model. Correlation residuals for the remaining pairwise test comparisons deviated little from those obtained from the conditional independence model.

Sensitivity analysis

For sensitivity analysis 1 (relaxed prior distributions) and sensitivity analysis 3 (uniform prior distributions), credible intervals for test sensitivity estimates tended to increase in width compared to the main conditional dependence model. However, for sensitivity analysis 2 (decreased mode of prior distributions), credible intervals for test sensitivity estimates were narrower compared to the main model. For estimates of test specificity, credible intervals generated by all three sensitivity analyses remained more consistent in width compared to those generated by the main conditional dependence model (**Figure 3.3; Supplementary Table 3.2**). For sensitivity analysis 1, median posterior estimates of test sensitivity and specificity deviated

from the original model estimates by a mean of 1.5% (range: -2.4% to 7.5%) and 1.1% (range: -1.2% to 2.6%), respectively. For sensitivity analysis 3, median posterior estimates of test sensitivity and specificity deviated from the original model estimates by a mean of 0.1% (range: -4.5% to 5.6%) and 1.3% (range: -1.2% to 2.8%), respectively. Sensitivity analysis 2 demonstrated a greater impact on median estimates of test performance parameters; median posterior estimates of test sensitivity and specificity deviated from the main conditional dependence model by a mean of -11.2% (range: -14.1% to -9.1%) and -2.3% (range: -5.2% to -0.1%), respectively. In the model evaluating the assumption of constant test sensitivity and specificity across populations, median (95% credible interval) posterior estimates of the sensitivity of TEC, SEC, qPCR, and the LFI were 70.4% (57.2%, 82.1%), 60.4% (49.3%, 71.2%), 86.0% (75.0%, 93.6%), and 60.8% (49.1%, 71.8%), respectively. Posterior estimates of the specificity of these tests were 97.3% (95.8%, 98.4%), 90.0% (87.5%, 92.2%), 96.6% (94.9%, 98.0%), and 68.2% (64.7%, 71.5%), respectively**.**

Parallel interpretation of diagnostic tests

When used in a two-test parallel testing strategy, the Reveal® 2.0 LFI was estimated to have an overall sensitivity of 83.9% - a 40% increase from the estimated median sensitivity of the LFI on a per-sample basis (**Table 3.5**). While this was the lowest estimated sensitivity of all four parallel testing strategies under evaluation, the cost of using the LFI in a two-test parallel testing strategy in a theoretical population of 100 horses with a 10% prevalence of *S. enterica* ($2481.00) was substantially lower than the cost applying any of the other tests under evaluation in a similar strategy ($8324.10 – $8589.15).

Discussion

This study is the first evaluation of diagnostic test performance for the detection of *S. enterica* in equine fecal samples that employs a Bayesian latent class modeling approach. By using this method, we have overcome the key challenge of assessing the sensitivity and specificity of existing and novel diagnostic tests in the absence of a gold standard – an obstacle that has resulted in a lack of consensus regarding the reliability of available tests for equine *S. enterica* infections. In this study, this lack of consensus was confirmed by the wide variability in expert opinion regarding the performance of the tests under evaluation. However, with a four-test, three-population Bayesian latent class model, we generated unbiased estimates of the sensitivity and specificity of these tests. We have demonstrated that on a per-sample basis, the Reveal® 2.0 LFI is only moderately sensitive and specific for the detection of *S. enterica* in equine fecal samples, while qPCR was superior in terms of sensitivity, and TEC had the highest specificity of the four tests. However, when used in a two-test parallel testing strategy, the sensitivity of the LFI increases substantially, and its low cost and ease of use make it a good candidate for a point-of-care screening test for *S. enterica* in horses.

In this study, the survey of an expert panel regarding their opinions on the sensitivity and specificity of the tests under evaluation primarily served to provide informative prior distributions for the Bayesian latent class model. However, the results of this survey also provided valuable insight into the current understanding of diagnostic test performance for equine *S. enterica* infections among clinicians, epidemiologists, and laboratorians. For a single test, estimates of the most likely value of diagnostic sensitivity differed by as much as 65% between two experts, and for specificity, by as much as 69%. Wide variability in test performance estimates was observed not only for the LFI, but also for TEC, SEC, and qPCR –

three tests that are commonly used for the detection of *S. enterica* in horses [13,30,31,46,94]. This lack of consensus likely stems from the limitations of previous evaluations of tests for *S. enterica* in horses (e.g., comparison of an index test against an imperfect reference test and evaluation of diagnostic test performance in high-risk populations of horses), as well as the lack of standardization of test methods.[44] Collectively, these factors have resulted in inconsistent and biased estimates of test sensitivity and specificity. Therefore, some degree of disagreement among the expert panel was expected. However, the magnitude of the discrepancies underscores the notion that the lack of sound evidence regarding test performance is likely driving variability in the clinical interpretations of available tests for equine *S. enterica* infections, further highlighting the need for the current study.

The introduction of *S. enterica* into equine hospitals, breeding and boarding facilities, and competition venues is a critical biosecurity concern in these settings.[12,13,27] For this reason, one of the most crucial roles of diagnostic tests for *S. enterica* in horses is their application as screening tests in these facilities. In this study, we have demonstrated the potential utility of the Reveal® 2.0 LFI for this purpose. This test has several characteristics that make it an appealing candidate for a screening test – it is easy to perform, requiring no specialized training or equipment other than an incubator; it produces results quickly; and its cost is relatively low, at approximately $15.00 per test. For these reasons, this LFI could feasibly be performed in-house in most equine facilities if local regulations allow. In this study, the median sensitivity of the LFI was estimated at 59.8% on a per-sample basis, increasing to 83.9% if used in a parallel testing strategy, while median specificity was estimated at 68.2% and 46.4% for a single-sample or parallel testing strategy, respectively. While the high sensitivity of the parallel testing strategy is favorable for ensuring that *S. enterica*-infected horses are rapidly detected, this does come at the cost of a

reduction in specificity, potentially resulting in the implementation of enhanced biosecurity measures for horses that have falsely tested positive. In practice, two samples could be collected from horses upon arrival at a facility (e.g., at 12-hour intervals) and immediately processed for overnight enrichment and testing with the LFI. This approach would be expected to identify nearly 84% of truly *S. enterica*-positive horses within 36 hours of presentation. This is comparable to the expected turnaround time for qPCR, while enriched aerobic culture typically takes 48 hours at a minimum.[46,47] However, the ability to test samples with the LFI in-house offers a particular advantage for facilities without immediate access to a diagnostic laboratory, eliminating the need to ship samples for testing. If follow-up testing is desired, additional aliquots of enrichment broth can be used for culture or qPCR. Additionally, the low cost of the Reveal® 2.0 LFI in comparison to culture or qPCR substantially increases the cost-effectiveness of screening larger populations of horses. We have demonstrated that for a population of 100 horses with a 10% prevalence of *S. enterica*, testing in parallel with the LFI is approximately one-third of the cost of testing with enriched culture or qPCR, with only a 5% or 14% reduction in sensitivity compared to TEC or qPCR, respectively. Therefore, the application of the Reveal® 2.0 LFI as a *S. enterica* screening test is an economical option for equine biosecurity and infection control programs, which often rely on repeated testing of all horses admitted to the facility or a subset of horses with risk factors for salmonellosis (e.g., horses with gastrointestinal disease).[13,22,95]

Despite its utility as a point-of-care screening test, the Reveal® 2.0 LFI does not eliminate the need for other *S. enterica* diagnostic tests, including culture and qPCR. Isolates obtained from enriched culture are often further analyzed to characterize antimicrobial susceptibility and *S. enterica* serogroups, serotypes, and strains, which is crucial to inform

clinical decision-making and identify epidemiologic links between infected animals.[13,16,22] There is a great deal of variation in the reported culture methods utilized to isolate *S. enterica* from equine fecal samples; several types and combinations of enrichment media, plating media, and incubation times and temperatures have been used, with no consensus on the most sensitive or specific method.[44] In this study, we have provided reliable estimates of the performance of two commonly used culture methods – selective enrichment in tetrathionate broth followed by plating on XLT4 agar and selective enrichment in selenite broth followed by plating on HE agar.[31,78] We have demonstrated that the median estimates of both sensitivity and specificity are higher for the tetrathionate-enriched method (66.1% and 97.2%) compared to the selenite-enriched method (58.4% and 89.8%) on a per-sample basis. Therefore, the former method should be considered more reliable, while the latter is more likely to produce a higher proportion of both false-negative and false-positive results. While these two tests do not represent the full spectrum of culture methods used for *S. enterica* detection in horses, this comparison does highlight the impact of variability in test methods on the performance of these tests. Culture results from studies or laboratories using different enrichment or plating media should not be accepted as equivalent, and a movement towards standardization of culture methods across institutions would promote consistency in test interpretation and application. In addition to culture, we have evaluated the performance of a *S. enterica* qPCR assay, demonstrating that it is the most sensitive of the four tests under evaluation and also highly specific. Given the likelihood of this test to correctly classify both *S. enterica*-infected and non-infected horses, qPCR remains a reliable diagnostic test for horses suspected of shedding *S. enterica* based on clinical signs or screening test results. However, the relatively high cost and the technical expertise required to perform qPCR limit its utility and accessibility as a screening test. Additionally, since positive qPCR results do not

necessarily indicate the presence of viable organisms, these results should be interpreted with caution. Depending upon the goals of testing in a given scenario (e.g., clinical management of an individual horse versus preventing the introduction of *Salmonella* in a population of horses), either tetrathionate-enriched culture or qPCR may be considered as follow-up tests after screening with the Reveal® 2.0 LFI.

One assumption of the Hui and Walter latent class model, which provides the framework for the models employed in the current study, is that the tests under evaluation are independent of one another, conditional on the true disease status of an individual.[82] When two or more tests under evaluation rely on similar biological mechanisms to classify an individual as test-positive or -negative, the conditional independence assumption may not hold, resulting in an overestimation of test sensitivity or specificity.[92,96] In this study, three of the four tests under evaluation included a selective enrichment step in TET, and the two enriched culture methods under evaluation both capitalized on the ability of *S. enterica* to reduce tetrathionate or selenite, respectively.[97,98] These factors suggest that conditional independence cannot be assumed; however, accounting for dependence between pairs of tests that are truly independent can also result in biased estimates of sensitivity and specificity.[90] Therefore, we employed a correlation residual analysis to inform the inclusion of covariance terms for pairs of tests that demonstrated the strongest deviation from the conditional independence assumption.[90] With this method, we showed that the conditional independence model did not adequately account for the dependence between tetrathionate- and selenite-enriched culture, while the addition of covariance terms between these two tests in the conditional dependence model resulted in an improved model fit. The correlation residuals for other pairs of tests, including SEC/LFI, SEC/qPCR, and TEC/qPCR did also indicate, to a lesser degree, dependence between these tests. However, the complexity of

the four-test, three-population model used in this study precluded the inclusion of pairwise covariance terms for these pairs of tests. Therefore, there may be some correlation between these tests that was not accounted for in the main conditional dependence model, potentially resulting in biased estimates of test sensitivity and specificity. However, median sensitivity estimates for TEC and SEC only decreased by 3.1% and 3.0%, respectively, when covariance terms between these tests were added to the model, while median specificity estimates decreased by only 0.3% and 1.2%, respectively, with substantial overlap in the credible intervals of the estimates generated by each model. Therefore, while the assumption of conditional independence between the remaining tests may have resulted in overestimates of test sensitivity and specificity, we expect that the magnitude of this bias is relatively small.

Additional assumptions of the model used in this study include the assumption of constant sensitivity and specificity of the tests under evaluation across the populations to which the tests are applied, [92,99] as well as the assumption of varying disease prevalence between the populations under consideration.[82] We addressed the former assumption by randomly reassigning each horse to one of three populations, thereby ensuring that membership in a given population was unrelated to an individual's disease severity, which may impact test performance.[83,92] Median estimates of test sensitivity and specificity generated with the conditional dependence model using this approach were similar to those generated using the original high-, intermediate-, and low-*S. enterica*-prevalence populations, indicating that the assumption of constant sensitivity and specificity across populations was met. While the assumption of varying disease prevalence between populations was not formally tested, enrollment into each study population was based on either previous *S. enterica* diagnosis (high-prevalence) or the presence (intermediate-prevalence) or absence (low-prevalence) of well-established risk factors for equine *S. enterica*

shedding, such as diarrhea or colic requiring surgical intervention.[31,32,35,100] Further, the median prevalence estimates for these three populations generated by the conditional dependence model (20.4%, 8.1%, and 2.9%, respectively) were substantively different, with little overlap between their 95% credible intervals. These factors provide evidence that the assumption of varying disease prevalence across populations was met.

To ensure the robustness and reliability of the test sensitivity and specificity estimates generated by the conditional dependence model, we performed three sensitivity analyses, each of which evaluated the impact of the mode and/or width of the informative prior distributions.[101] The posterior estimates remained quite consistent with those obtained from the main model in sensitivity analysis 1, with more diffuse informative priors, and notably, in sensitivity analysis 3, with vague priors. These findings provide evidence that the estimates obtained from the main model are not unduly influenced by our choice of informative prior distributions; rather, they are well-aligned with estimates driven by the data alone. However, the leftward shift in the posterior estimates that was observed for sensitivity analysis 2, wherein the mode of each informative prior distribution was decreased, suggests that the estimates obtained by the main model are more sensitive to changes in the central tendency of the prior distributions than to changes in the width of the prior distributions

While the sensitivity analyses and tests of model assumptions performed in this study underscore the reliability of the model estimates of the sensitivity and specificity of the tests under evaluation, there are additional considerations regarding these tests that should be taken into account when applying them in a clinical setting. In the current study, the Reveal® 2.0 LFI was only considered to be positive when the test line was at least as intense in color as the control line, in accordance with initial evaluations of this test using equine fecal samples.[69,70]

Alternatively, this test could be considered positive if a test line is present, regardless of its intensity; this interpretation has been used in previous applications of the Reveal® 2.0 LFI for the detection of *S. enterica* in food and environmental samples.[81,102] Presumably, this approach would result in a higher sensitivity, likely at the cost of a lower specificity; however, test results using this interpretation were not available for analysis in this study. Additionally, clinicians using this test should recognize that it does not detect all *S. enterica* serotypes with equal reliability; in an evaluation of experimentally inoculated equine fecal samples, serotype Cerro (serogroup K) was poorly detected by the Reveal® 2.0 LFI.[69] Therefore, results of this test should be interpreted in the context of this limitation. Finally, given the variability in culture and PCR methods used to detect *S. enterica* in equine fecal samples, the estimates of test sensitivity and specificity generated in this study are not broadly applicable across all variations of these tests. Additional studies are needed to investigate the impact of factors such as enrichment media, plating media, and PCR target on test performance.

Conclusions

In summary, the Reveal® 2.0 LFI is a convenient, affordable rapid test for equine *S. enterica* infections that, especially when used in a two-test parallel testing strategy, can be used as a point-of-care screening test in equine hospitals, breeding and boarding facilities, and competition venues. However, TEC and qPCR are more sensitive and specific than the LFI and remain important tools for the diagnosis and management of *S. enterica* infections in horses.

Acknowledgements

Funding

This work was supported by the Grayson-Jockey Club Research Foundation; Boehringer-Ingelheim 2014 Advancement in Equine Research Award; the Colorado State University College Research Council; and the Foundation for the Horse. Sample collection was supported by the Morris Animal Foundation [D17EQ-304].

Author contributions

Conceptualization: PSM, BAB; Data curation: ECH, NMS, BAB; Formal analysis: ECH; Funding acquisition: ECH, PSM, BAB; Investigation: ECH, KLP, BAB; Methodology: ECH, PSM, BAB; Project administration: BAB; Resources: RSM, AMC, BTV, HWA, NMS; Supervision: PSM, BAB; Visualization: ECH; Writing – original draft: ECH; Writing – review and editing: ECH, KLP, RSM, AMC, BTV, HWA, NMS, PSM, BAB. All co-authors agreed that this work may be included in this dissertation.

Tables

Table 3.1: Demographic characteristics of the total study population and of horses in the high-, intermediate-, and low-prevalence populations.

Variable		**High-Prevalence (N = 106)**	**Intermediate-Prevalence (N = 123)**	**Low-Prevalence (N = 437)**	**Total Study Population (N = 666)**
Age (Years)	Median (Range)	11 (0.17 – 26) [N = 69]	5 (0.02 – 20) [N = 103]	8 (0.02 – 28) [N = 372]	8 (0.02 – 28) [N = 544]
Sex	Mare	32 (44.4%)	71 (57.7%)	280 (64.1%)	383 (60.6%)
	Gelding	33 (45.8%)	3 (2.4%)	20 (4.6%)	56 (8.9%)
	Stallion	4 (5.6%)	25 (20.3%)	51 (11.7%)	80 (12.7%)
	Male (unspecified)	0 (0%)	6 (4.9%)	57 (13.0%)	63 (10.0%)
	Unknown	3 (4.2%)	18 (14.6%)	29 (6.6%)	50 (7.9%)
Breed	Thoroughbred	15 (21.1%)	87 (70.7%)	272 (62.2%)	374 (59.3%)
	Quarter Horse	18 (25.4%)	3 (2.4%)	21 (4.8%)	42 (6.7%)
	Arabian/Arabian cross	4 (5.6%)	1 (0.8%)	6 (1.4%)	11 (1.7%)
	Warmblood	9 (12.7%)	2 (1.6%)	32 (7.3%)	43 (6.8%)
	Draft	7 (9.9%)	0 (0%)	3 (0.7%)	10 (1.6%)
	Standardbred	1 (1.4%)	1 (0.8%)	17 (3.9%)	19 (3.0%)
	Pony/Miniature	3 (4.2%)	0 (0%)	12 (2.7%)	15 (2.4%)
	Other	12 (16.9%)	5 (4.1%)	19 (4.3%)	36 (5.7%)
	Unknown	2 (2.8%)	24 (19.5%)	55 (12.6%)	81 (12.8%)

Table 3.2: Cross-tabulated diagnostic test results for tetrathionate-enriched culture (TEC), selenite-enriched culture (SEC), qPCR, and Reveal® 2.0 lateral flow immunoassay (LFI) among horses in the **(A)** high- (N=106), **(B)** intermediate- (N=123), and **(C)** low-prevalence (N=437) populations.

A	LFI+					LFI-					
qPCR+			qPCR-			qPCR+			qPCR-		
	TEC+	TEC-		TEC+	TEC-		TEC+	TEC-		TEC+	TEC-
SEC+	7	2	SEC+	0	0	SEC+	3	1	SEC+	0	1
SEC-	0	3	SEC-	0	24	SEC-	2	7	SEC-	0	56
B	LFI+					LFI-					
qPCR+			qPCR-			qPCR+			qPCR-		
	TEC+	TEC-		TEC+	TEC-		TEC+	TEC-		TEC+	TEC-
SEC+	3	1	SEC+	0	0	SEC+	1	0	SEC+	0	0
SEC-	1	2	SEC-	1	40	SEC-	2	0	SEC-	0	72
C	LFI+					LFI-					
qPCR+			qPCR-			qPCR+			qPCR-		
	TEC+	TEC-		TEC+	TEC-		TEC+	TEC-		TEC+	TEC-
SEC+	5	1	SEC+	1	25	SEC+	2	1	SEC+	1	17
SEC-	2	2	SEC-	1	112	SEC-	0	6	SEC-	0	261

Table 3.3: Demographic characteristics of expert panel surveyed to generate informative prior distributions (N = 9).

Variable	Category	Frequency (%)
Degrees held*	Doctor of Veterinary Medicine (DVM)	7 (77.8%)
	Master of Science (MS)	1 (11.1%)
	Doctor of Philosophy (PhD)	5 (55.6%)
	Other	1 (11.1%)
Board certifications*	Veterinary Internal Medicine	4 (44.4%)
	Veterinary Microbiology	1 (11.1%)
	Preventive Veterinary Medicine	1 (11.1%)
	Other	2 (22.2%)
	None	2 (22.2%)
Primary area of expertise	Epidemiology	3 (33.3%)
	Microbiology	3 (33.3%)
	Internal Medicine	3 (33.3%)

*Respondents were instructed to select all that apply

Table 3.4: Mean (range) of expert estimates of the mode and lower/upper 95% confidence interval (CI) limits of the diagnostic sensitivity and specificity (on a per-sample basis) of tetrathionate-enriched culture (TEC), selenite-enriched culture (SEC), qPCR, and Reveal® 2.0 lateral flow immunoassay (LFI).

Test	**N**	**Sensitivity**			**Specificity**		
		Mode	**Lower 95% CI Limit**	**Upper 95% CI Limit**	**Mode**	**Lower 95% CI Limit**	**Upper 95% CI Limit**
TEC	9	67.4 (30.0, 95.0)	49.4 (20.0, 90.0)	79.8 (50.0, 100)	81.3 (50.0, 99.0)	70.7 (40.0, 96.0)	88.9 (60.0, 100)
SEC	8	56.1 (30.0, 80.0)	41.9 (20.0, 75.0)	74.1 (50.0, 100)	78.1 (35.0, 98.0)	68.3 (30.0, 96.0)	84.4 (40.0, 100)
qPCR	8	79.4 (50.0, 95.0)	61.6 (0, 91.0)	93.5 (80.0, 100)	91.5 (75.0, 99.0)	82.8 (60.0, 96.0)	96.8 (85.0, 100)
LFI	8	56.7 (30.0, 83.5)	40.2 (0, 82.0)	74.1 (35.0, 100)	76.5 (30.0, 99.0)	67.0 (25.0, 96.0)	82.9 (35.0, 100)

Table 3.5: Estimated sensitivity and specificity (95% credible interval [CI]) of using tetrathionate-enriched culture (TEC), selenite-enriched culture (SEC), qPCR, or the Reveal® 2.0 lateral flow immunoassay (LFI) in a two-test parallel testing strategy to detect *S. enterica* in a theoretical population of 100 horses with a 10% prevalence of *S. enterica*.

Test	**Sensitivity of parallel interpretation (95% CI)**	**Specificity of parallel Interpretation (95% CI)**	**Cost per test**	**Total cost**
TEC	88.5% (78.7% - 95.1%)	94.5% (91.6% - 96.7%)	$45.00	$8589.15
SEC	82.7% (72.7% - 90.4%)	80.7% (76.3% - 84.7%)	$45.00	$8324.10
qPCR	98.4% (94.9% - 99.7%)	94.2% (90.9% - 96.9%)	$45.00	$8488.80
LFI	83.9% (73.3% - 91.5%)	46.4% (41.8% - 51.1%)	$15.00	$2481.00

Figures

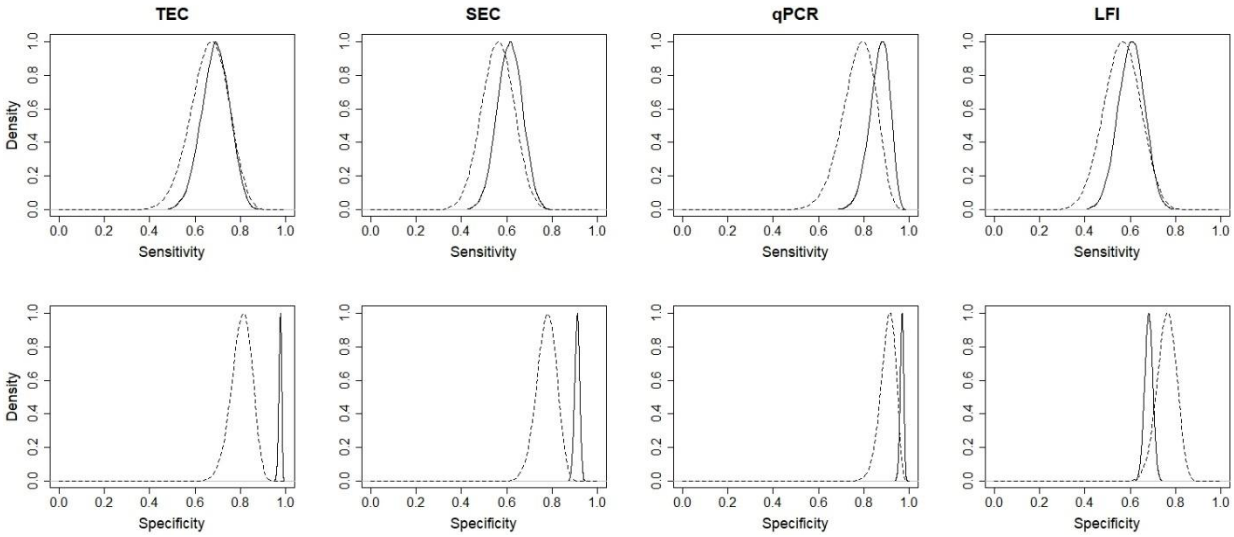


Figure 3.1: Probability density plots of prior (dashed lines) and posterior (solid lines) distributions of test sensitivity and specificity for tetrathionate-enriched culture (TEC), selenite-enriched culture (SEC), qPCR, and the Reveal® 2.0 lateral flow immunoassay (LFI), generated by a Bayesian latent class model under the assumption of conditional independence between tests.

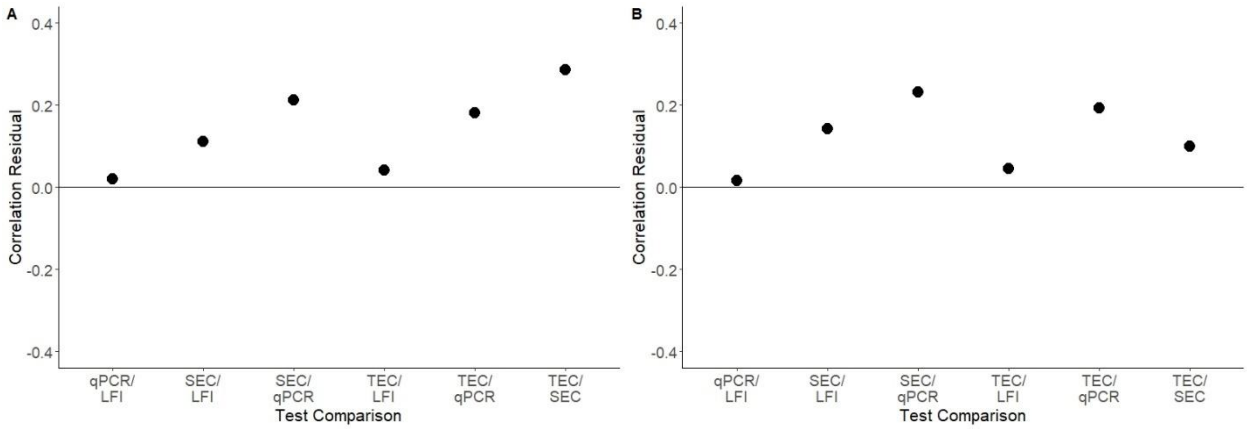
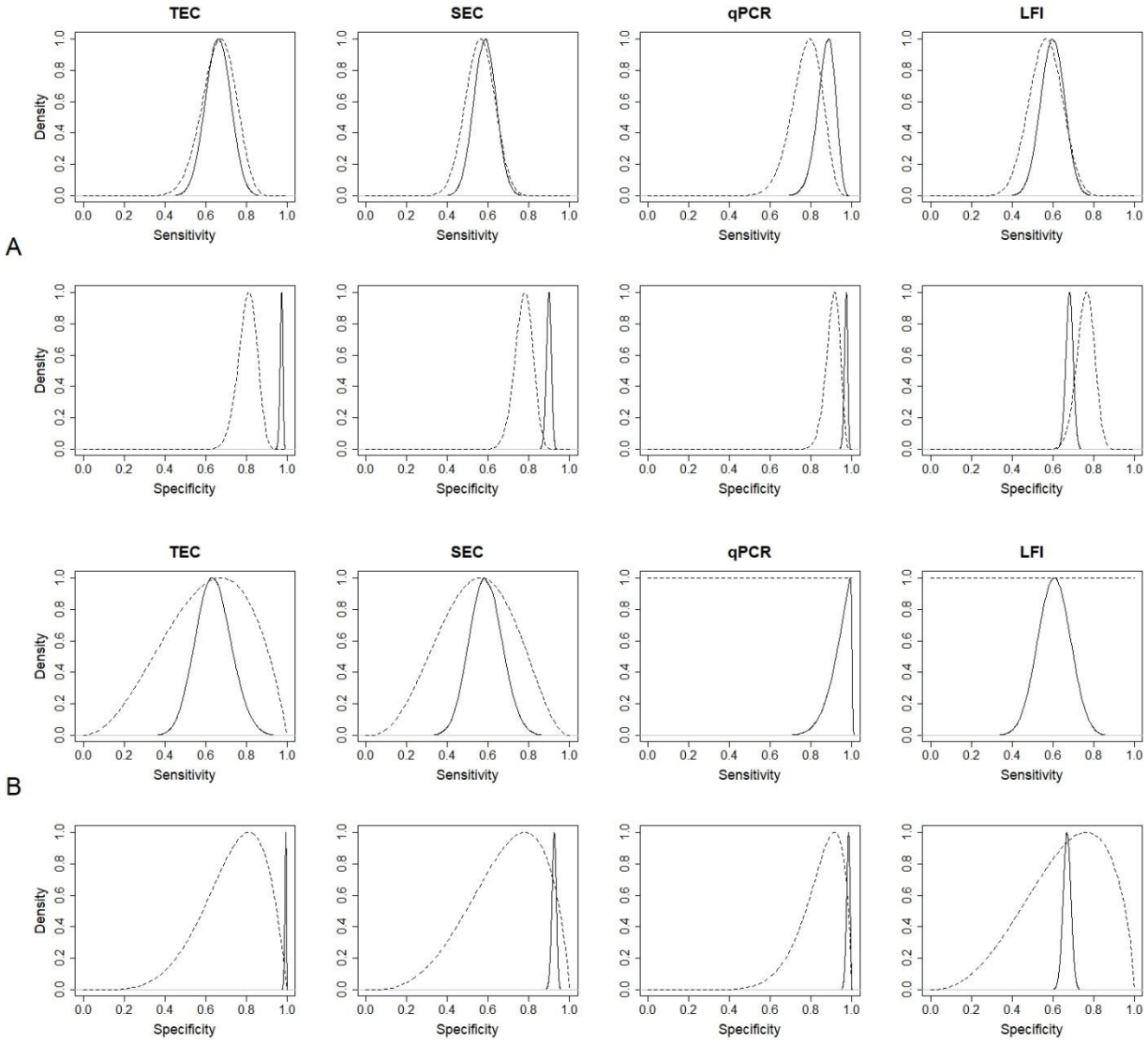


Figure 3.2: Correlation residual plot for the (**A**) conditional independence model and (**B**) conditional dependence model.


A
TEC
SEC
qPCR
LFI
Density
Sensitivity
Specificity
B
TEC
SEC
qPCR
LFI
Density
Sensitivity
Specificity

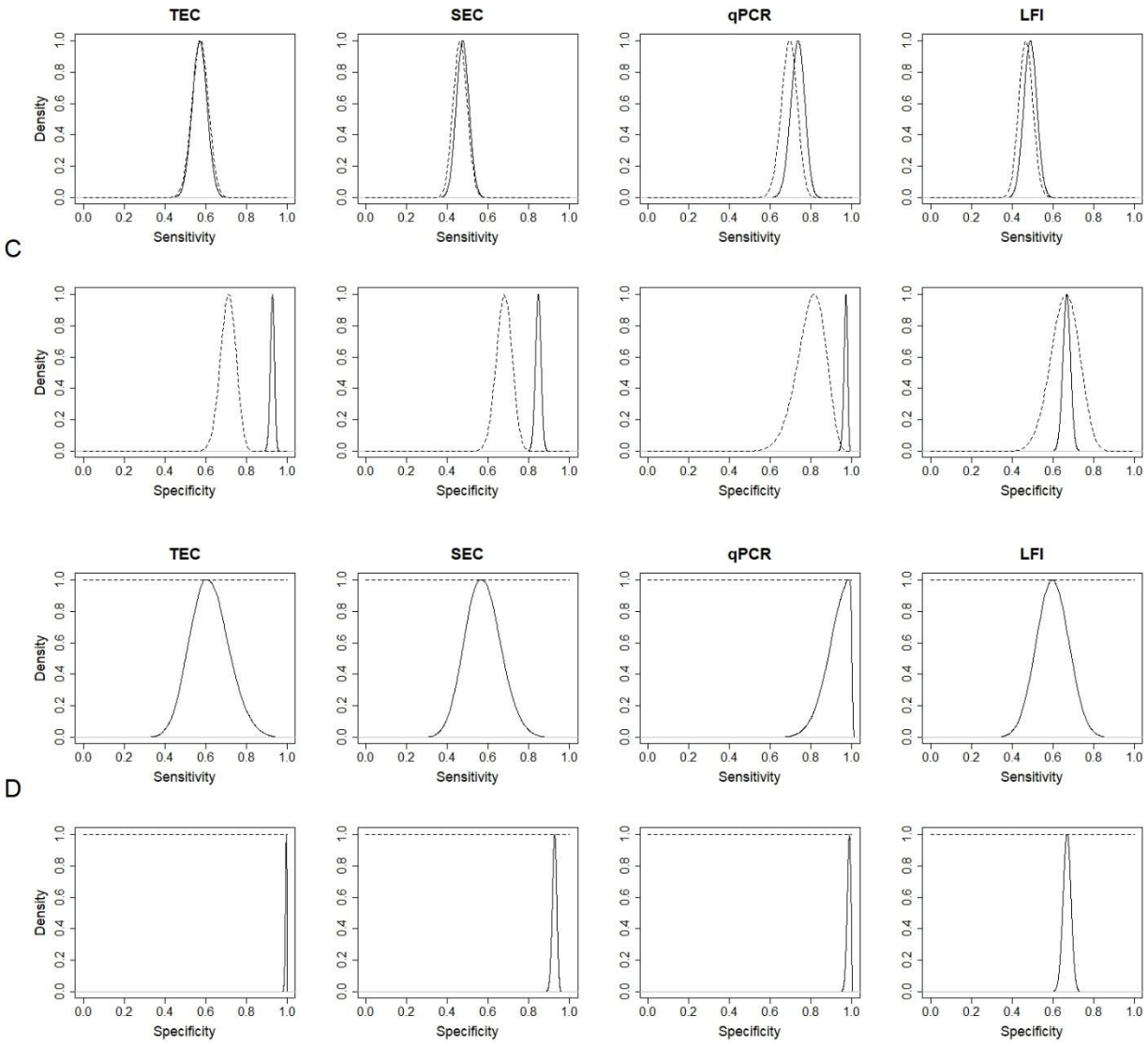


Figure 3.3: Probability density plots of prior (dashed lines) and posterior (solid lines) distributions of test sensitivity and specificity for tetrathionate-enriched culture (TEC), selenite-enriched culture (SEC), qPCR, and the Reveal® 2.0 lateral flow immunoassay (LFI), generated by (**A**) the main Bayesian latent class conditional dependence model, (**B**) sensitivity analysis 1, (**C**) sensitivity analysis 2, and (**D**) sensitivity analysis 3.

CHAPTER 4

THE DURATION OF FECAL *SALMONELLA* SHEDDING IN HORSES [h]

[h] Herring, E.C., Aceto, H.W., Barrell, E.A., McConnico, R.S., Slovis, N.M., Ryan, C.A., Morley, P.S., and B.A. Burgess. To be submitted to *Equine Veterinary Journal.*

Abstract

Salmonella enterica is an important cause of gastrointestinal illness and healthcare-associated infections in horses. However, the duration of fecal shedding of *Salmonella* in horses, a key aspect of the epidemiology of this disease that would inform appropriate management of *Salmonella*-positive horses, is not well described. We conducted a prospective, longitudinal study in order to characterize the duration of fecal *Salmonella* shedding among infected horses, identify clinical risk factors associated with fecal shedding duration, and evaluate the risk for adverse health effects in infected horses and their stablemates. Consecutive weekly fecal samples were collected from 135 *Salmonella*-positive horses positive over an 8-week study period. Fecal samples were cultured for *Salmonella*, and time-to-event analyses were performed to assess the duration of *Salmonella* shedding and risk factors that impact shedding duration. Frequency of adverse health outcomes among enrolled horses and stablemates were assessed via owner surveys. Horses shed *Salmonella* for a median of 13 days (95% CI: 9, 17). Horses with clinical and subclinical salmonellosis shed the bacteria for a median of 25 days (95%CI: 7, 158) and 11 days (95% CI: 7, 15), respectively. Diarrhea in the 48 hours prior to study enrollment and clinical involvement of any body system were associated with increased shedding duration. Loose feces was reported in 20.0% of enrolled *Salmonella*-positive horses, and *Salmonella* culture positivity was reported in 8.9% of stablemates of enrolled horses. Other adverse health outcomes were less frequently reported. Missing clinical data was common and may have resulted in some biased estimates. *Salmonella*-positive horses are expected to shed the bacteria in their feces for an average of approximately two weeks, but horses with clinical illness may shed *Salmonella* for a longer period of time. The risk of adverse health outcomes in stablemates of *Salmonella*-positive horses is low.

Introduction

Salmonella enterica is a common cause of healthcare-associated infections in horses;[12] outbreaks in veterinary hospitals are frequently characterized by widespread environmental contamination, facilitating nosocomial transmission.[13,16,132] Such outbreaks have resulted in severe disease and high case fatality rates among hospitalized horses, necessitating hospital closures and extensive mitigation efforts.[13,15] Risk factors for *Salmonella* shedding among horses in a hospital setting, such as systemic illness, abdominal surgery, and exposure to antimicrobial drugs, have been well characterized,[30-32,36] facilitating the implementation of appropriate surveillance and biosecurity measures to reduce the risk of transmission to other patients and personnel.

However, a key obstacle to the management of *Salmonella*-positive horses is subclinical fecal shedding of the bacteria; horses frequently shed *Salmonella* after resolution of clinical signs or in the absence of clinical disease altogether.[27,29,39] Therefore, infected horses often remain *Salmonella*-positive upon discharge from the hospital. Transmission of *Salmonella* to herd mates, as well as widespread environmental contamination, has been documented following the return of *Salmonella*-positive horses to their home farms.[22,133] These transmission events demonstrate the critical need to provide evidence-based guidance to owners about appropriate on-farm biosecurity measures and how long to implement them. To date, however, the duration of fecal shedding of *Salmonella* in infected horses has not been adequately characterized, hindering the provision of such guidelines.

In the few studies that have reported shedding duration, *Salmonella* has been isolated from the feces of most infected horses for two to six weeks from initial detection, although some horses continued to shed for longer periods of time.[29,39,42] However, these studies had key

limitations, such as being restricted to a single herd[29] or hospital,[39] limiting the potential to generalize study results across broader populations of horses in diverse environments, with varying clinical conditions, and shedding different *Salmonella* serotypes. Further, these studies varied in follow-up time and intervals between consecutive fecal samples, and their definitions of *Salmonella*-negativity (i.e., one negative fecal culture or multiple consecutive negative fecal cultures) were unclear. As such, their sensitivity in detecting true cessation of shedding may have been limited.

The lack of available evidence to inform the expected duration of shedding among *Salmonella*-positive horses hinders clinicians' ability to provide sound guidance to horse owners on how long to implement appropriate infection control measures. Therefore, the primary objectives of this study were to 1) characterize the duration of fecal *Salmonella* shedding among *Salmonella*-positive horses, 2) identify clinical factors associated with *Salmonella* shedding duration, and 3) evaluate the risk for adverse health effects in infected horses and their stablemates in their home environment.

Methods

Overview

A prospective longitudinal study was conducted to determine the duration of fecal shedding of *Salmonella enterica* among *Salmonella*-positive horses. Median duration of shedding among the study population and among subgroups of interest was determined using the Kaplan-Meier method. Cox proportional hazard modelling was used to determine the impact of clinical variables of interest on *Salmonella* shedding duration. The incidence of adverse health

outcomes among *Salmonella*-positive horses and their stablemates were evaluated by surveying owners and summarized using descriptive statistics.

Study population and enrollment

A convenience sample of horses was selected through a network of equine veterinary practices and referral hospitals in the U.S. from March 2018 to November 2020. All horses presenting to these institutions with at least one positive *Salmonella* fecal culture or PCR obtained during their hospitalization or clinical evaluation were eligible for inclusion in the study. Clinicians at participating institutions informed owners of the study, obtained consent to enroll, and completed an online enrollment survey (**Appendix 4A**), which provided the investigators with contact information for the owner and enrolling veterinarian, as well as clinical history information.

Clinical data collection

Clinical history detailed in the enrollment survey included age, sex, breed, use/occupation, date of hospital admission and discharge, history of culture-confirmed salmonellosis in the enrolled horse and on the farm, presenting complaint, body system(s) affected, level of care required during hospitalization, and class(es) of antimicrobials and gastroprotectants administered during hospitalization. Information on severity of systemic illness within the 48 hours prior to enrollment was also collected. Horses with minimal systemic illness included healthy companions to hospitalized horses, as well as horses with minor orthopedic, reproductive, or other non-systemic problems. Horses with moderate systemic illness included those with lacerations or recovering fractures, mild respiratory infections, fever of unknown

origin, and those recovering from more serious illness such as colic. Horses with major systemic illness included those with severe fractures, renal failure, liver failure, or peritonitis; severe gastrointestinal conditions such as colic, colitis, or enteritis; and severe respiratory conditions such as strangles, pleuritis, or pneumonia. Other information collected included incidence of diarrhea, fever (rectal temperature > 101.5°F), leukopenia (white blood cell count < 5000/µl); anesthesia or surgery; antimicrobial exposure (none, oral, parenteral, and/or topical/ophthalmic); and significant reduction in dietary intake in the 48 hours prior to enrollment.

Owners and/or enrolling clinicians (if the horse remained hospitalized at the time of sample collection) completed a weekly questionnaire on health outcomes in the enrolled horse and their stablemates throughout the duration of study participation, including incidence of colic episodes, soft or loose feces, hospitalization, and *Salmonella* culture positivity (**Appendix 4B**).

Sample collection

Upon enrollment, the first fecal sampling kit was shipped to owners. If horses remained hospitalized at the time of enrollment, fecal sampling kits were shipped to the enrolling institution for sample collection to be completed by the enrolling clinician until discharge. Each kit included written instructions for sample collection (**Appendix 4C**), a sample submission form (including dates of sample collection and a brief questionnaire on health outcomes in the culture-positive horse and their stablemates; **Appendix 4B**), and a one-page informational handout on equine salmonellosis (describing health risks to horses and humans, as well as appropriate biosecurity practices on-farm; **Appendix 4D**). Owners were directed to collect approximately three grams of feces (three one-gram fecal samples collected at 12-24-hour intervals) into a sterile fecal cup using disposable gloves, refrigerate the sample until shipment, and return the

sample and submission form to the University of Georgia (UGA) via overnight shipping on ice. Upon receipt of each sample at UGA, a new sampling kit was shipped to owners; this process was repeated until eight weekly fecal samples were collected. If horses continued to shed *Salmonella* at the end of the eight-week follow-up period, owners were given the option to continue participation in the study. If owners failed to return a fecal sample within one week of receiving a sampling kit, they were contacted with reminders a maximum of three times via phone and/or email.

Fecal Salmonella culture and characterization

Upon receipt, fecal samples were stored at 4°C until processing. Fecal balls were manually homogenized using a sterile cotton-tipped applicator, and 3 g of feces were inoculated into 30 ml of tetrathionate broth (BD, Franklin Lakes, NJ) supplemented with iodine and brilliant green (TET) and incubated at 43°C for 18-24 hours. TET culture broth was streaked for isolation onto xylose-lysine-tergitol 4 (XLT4; Hardy Diagnostics, Santa Maria, CA) agar plates and incubated at 43°C for 18-24 hours. Plates were observed for growth of black-centered colonies; if present, one colony was selected for sub-culture on tryptic soy agar (TSA) plates containing 5% sheep blood (Thermo Fisher Scientific Inc., Waltham, MA) and incubated at 43°C for 18-24 hours. Colonies were confirmed as *Salmonella* and serogroup was determined via agglutination testing with commercial polyvalent and O group-specific *Salmonella* antisera (BD, Franklin Lakes, NJ). Confirmed *Salmonella* isolates were further assessed for susceptibility to antimicrobial drugs via Kirby-Bauer disk diffusion. Three to five isolated colonies were selected from each TSA plate after incubation for 18-24 hours and inoculated into 1 mL of sterile saline using the BBL™ Prompt™ Inoculation System (BD, Franklin Lakes, NJ). A sterile cotton-tipped

swab was used to inoculate Mueller Hinton agar plates (Thermo Fisher Scientific Inc., Waltham, MA) with the suspension. Antimicrobial discs (BD, Franklin Lakes, NJ) for a standardized panel of antimicrobial drugs (AMD) – amikacin, ampicillin, cefazolin, cefotaxime, chloramphenicol, enrofloxacin, gentamicin, imipenem, tetracycline, ticarcillin-clavulanate, and trimethoprim-sulfamethoxazole – were applied to the agar surface, and plates were incubated at 35°C for 18-24 hours. Antimicrobial susceptibility was determined using a BIOMIC® V3 Microbiology System (Giles Scientific, Santa Barbara, CA). Breakpoints for interpretative categories (susceptible [S], intermediate [I], resistant [R]) were determined using Clinical & Laboratory Standards Institute (CLSI) guidelines.[134] All *Salmonella* isolates were also submitted to the United States Department of Agriculture (USDA) National Veterinary Services Laboratory (NVSL; Ames, IA) for serotype determination.

Data analysis

Study population demographics and clinical characteristics were summarized using descriptive statistics. Horses' geographic region was categorized based on U.S. census region of the enrolling institution.[135] Horses were classified as having clinical salmonellosis if they presented with or developed clinical symptoms consistent with salmonellosis (i.e., diarrhea and/or fever and leukopenia) during hospitalization or clinical evaluation by the enrolling veterinarian prior to study enrollment. Horses were classified as having subclinical salmonellosis if they did not develop these symptoms.

To determine the duration of *Salmonella* shedding among the study population, *Salmonella* shedding was considered to start on the date of study enrollment if an enrollment form was completed. If an enrollment form was not completed, *Salmonella* shedding was

considered to have begun one week prior to the collection date of the first fecal sample collected. If a sample collection date was not provided, *Salmonella* shedding was considered to start one week prior to the date that the first fecal sample was received. Horses were considered to have stopped shedding *Salmonella* when three consecutive negative *Salmonella* culture results were obtained. The date of shedding cessation was considered to be the date that the first culture-negative fecal sample was collected in the series of three consecutive culture-negative fecal samples. If a sample collection date was not provided, the sample was assumed to have been collected one day prior to the date of sample receipt. Horses were right-censored if three consecutive negative cultures were not obtained before they completed the study or were lost to follow-up. Censoring occurred on the date of sample collection for the last culture-positive fecal sample obtained. If a sample collection date was not provided, the sample was assumed to have been collected one day prior to the date of sample receipt. If no positive cultures were obtained, the horse was censored on the date of study enrollment.

The Kaplan-Meier method was used to evaluate the median duration of *Salmonella* shedding among the entire study population, and to compare the median duration of shedding between horses with clinical and subclinical salmonellosis; between horses with no AMD exposure during hospitalization and those that were treated with AMDs; and between horses with minimal, moderate, or major clinical illness.

The impact of clinical variables of interest on *Salmonella* shedding duration was evaluated using Cox proportional hazards models. Variables evaluated included age; sex; breed; clinical status (clinical or subclinical); any AMD exposure during hospitalization; exposure to aminoglycosides during hospitalization; exposure to beta-lactams during hospitalization; exposure to other AMDs during hospitalization; AMD exposure in the 48 hours prior to study

enrollment and route of administration; treatment with gastroprotectants during hospitalization; no body system affected (healthy); gastrointestinal system affected; severity of systemic illness in the 48 hours prior to study enrollment; incidence of diarrhea, fever, leukopenia, anaesthesia or surgery, and reduced dietary intake in the 48 hours prior to enrollment; *Salmonella* serotype; and *Salmonella* serogroup. The association of each of these variables with *Salmonella* shedding duration was evaluated in a univariable Cox proportion hazards model. In each model, farm identity was included as a random effect to account for clustering of horses from the same farm. A critical $\alpha \leq 0.25$ was used to screen variables for inclusion in the multivariable model.

To assess multicollinearity between variables associated with shedding duration in the univariable analysis, all independent variables were regressed on survival time, and variance inflation factors (VIF) were calculated. VIF values over 10 were considered evidence of collinearity; variables meeting this threshold were removed from the model in a stepwise fashion and not considered for inclusion in multivariable model selection.

The final multivariable model was generated using a backwards selection procedure. A critical $\alpha \leq 0.05$ was used to determine if each variable was retained in the final model. Confounding variables were identified by offering each excluded variable back to the model; if this resulted in a change in parameter estimates $\geq 20\%$, that variable was forced into the final model. First-order interaction terms were also offered to the final model; those with *P*-values $\leq$ 0.05 were retained. Hazard ratios and 95% confidence intervals (CIs) were calculated for each variable included in the final multivariable model. Model fit was assessed by evaluating Cox-Snell residuals, and the proportional hazards assumption was assessed by examining Schoenfeld residuals. The assumption that censoring was independent of shedding duration was evaluated by performing two sensitivity analyses – one in which censored horses were assumed to have

stopped shedding at the time of censoring, and one in which censored horses were assumed to have continued shedding until the longest observed shedding duration. The final model was refit under each of these conditions to assess the impact on model coefficients at each extreme.

The frequency of adverse outcomes among enrolled horses, including colic and loose feces, and the frequency of adverse outcomes among stablemates of enrolled horses, including *Salmonella* culture positivity, colic, loose feces, and hospitalization, as reported on sample submission forms, were summarized using descriptive statistics. All analyses were performed using SAS v9.4 (Cary, NC).

Results

Demographic and clinical characteristics of the study population

Between March 2018 and November 2020, 163 horses were initially enrolled in the study. Of these, 135 horses (82.8%) from 89 farms and 20 enrolling equine hospitals had at least one fecal sample submitted and were included in the final study population. An enrollment form was completed for 99 horses (73.3%). Demographic characteristics of the study population are detailed **Table 4.1**.

A total of 27 (20.0%) and 108 (80.0%) horses were determined to have clinical and subclinical salmonellosis, respectively. Of the 36 horses without a completed enrollment form, six (16.5%) had clinical salmonellosis and 30 (83.3%) had subclinical salmonellosis. Eighty-nine (65.9%) horses had been hospitalized prior to enrollment in the study, and 21 (15.6%) remained hospitalized at the time of enrollment. For 40 (29.6%) and 45 (33.3%) horses, information on previous and current hospitalization status was unknown. Among the 61 horses for which duration of hospitalization was known, median duration of hospitalization was 7 days (range: 2 -

23 days). Four (3.0%) and 23 (17.0%) horses had a previous history of culture-confirmed salmonellosis or were from a farm with a history of salmonellosis, respectively. This information was not reported for 47 (34.8%) and 58 (43.0%) horses, respectively. Presenting complaint included diarrhea/colitis/enteritis, colic, or fever in 16 (11.9%), 43 (31.9%), and 25 (18.5%) horses, respectively. Presenting complaint was unknown for 38 (28.1%) horses. The most common body system affected during hospitalization was gastrointestinal (67 horses; 49.6%), followed by musculoskeletal (8 horses; 5.9%) and respiratory (8 horses; 5.9%). No body system was affected in 22 horses (16.3%), and affected body system was not reported for 39 horses (28.9%). Severity of systemic illness was reported as healthy/minimal, moderate, or major for 53 (39.3%), 28 (20.7%), and 14 (10.4%) horses. Illness severity was not reported for 40 horses (29.6%). Diarrhea or soft fecal consistency, fever, leukopenia, or anaesthesia/surgery within the 48 hours prior to study enrollment were reported in 23 (17.0%), 23 (17.0%), 13 (9.6%), and 9 (6.7%) horses, respectively. This information was unknown for 43 (31.9%), 44 (32.6%), 73 (54.1%), and 36 (26.7%) horses, respectively.

Information on AMD exposure throughout hospitalization was reported for 94 horses (69.6%). Forty-eight horses (35.6%) received AMDs during hospitalization. The most commonly reported class of AMD administered was aminoglycosides (36 horses; 26.7%), followed by beta-lactams (32 horses; 23.7%). Information on AMD exposure in the 48 hours prior to study enrollment was reported for 97 horses (71.9%). Twenty-two horses (16.3%) received antimicrobial drugs during this period, including 1 (0.7%), 6 (4.4%), and 17 (12.6%) horses that received topical/ophthalmic, oral, and parenteral antimicrobials, respectively. Fifty-eight horses (43.0%) received gastroprotectants during hospitalization. Proton pump inhibitors (50 horses;

37.0%) and mucosal protectants (20 horses; 14.8%) were most commonly reported; information on gastroprotectant treatment was not available for 70 horses (51.9%).

Sampling and culture results

A total of 739 fecal samples were collected; 575 (77.8%) were *Salmonella* culture-negative, and 164 (22.2%) were *Salmonella* culture-positive. The median number of fecal samples submitted per horse was 5 (range: 1 - 21). Seventy-nine (58.5%) horses achieved *Salmonella*-negative status during the study period (i.e., three consecutive negative cultures were obtained). The remaining 56 horses (41.5%) either did not complete the study (i.e., lost to follow up before eight consecutive fecal samples were obtained; 48 horses [35.6%]) or did not reach *Salmonella*-negative status during the study period (8 horses [5.9%]). Ten horses (7.4%) resumed shedding after initially achieving *Salmonella*-negative status. Serogroup and serotype of *Salmonella* isolates identified in the study are presented in **Table 4.2**, and antimicrobial resistance profiles are presented in **Table 4.3**. Among the 164 *Salmonella*-positive fecal samples, the most commonly identified serotypes included Newport (36 [22%]), Typhimurium (24 [14.6%]), and Braenderup (21 [12.8%]). Seven horses (5.2%) shed more than one serotype throughout the study (i.e., different serotypes were identified from different samples from the same horse).

Duration of Salmonella shedding

The Kaplan-Meier median (95% CI) duration of *Salmonella* shedding overall was 13 days (9, 17) (**Figure 4.1A**). For horses with clinical and subclinical salmonellosis, the median duration of shedding was 25 days (7, 158) and 11 days (7, 15), respectively (**Figure 4.1B**). The

maximum shedding duration observed among subclinical horses was 111 days, while the maximum shedding duration for clinical horses was 180 days. Horses that received AMD treatment had a median shedding duration of 18 days (12, 28), while those with no AMD exposure had a median shedding duration of 8 days (5, 13), and those whose AMD exposure history was unknown had a median shedding duration of 10 days (7, 36) (**Figure 4.1C**). The median duration of *Salmonella* shedding for horses with minimal, moderate, and major systemic illness were 7 days (5, 14), 18 days (13, 57), and 19 days (5, 180), respectively, while those with unknown severity of systemic illness had a median shedding duration of 8.5 days (7, 22) (**Figure 4.1D**). Horses with missing enrollment data had a median shedding duration of 7 days (range: 0 - 35), while horses with a completed enrollment form had a median shedding duration of 14 days (range: 0 - 180).

Independent variables that passed screening for inclusion in the multivariable model included clinical status; AMD treatment during hospitalization (including any AMD, aminoglycoside, and other [non-aminoglycoside or -beta-lactam] treatment); oral AMD treatment in the 48 hours prior to enrollment; gastroprotectant treatment during hospitalization (including proton pump inhibitor or other [non-proton pump inhibitor or -mucosal protectant] gastroprotectant); body system affected during hospitalization (including no body system affected [healthy], gastrointestinal system affected, and other [non-gastrointestinal] system affected); level of systemic illness in the 48 hours prior to enrollment; incidence of diarrhea and fever in the 48 hours prior to enrollment; and *Salmonella* serotype (**Table 4.4**). Evaluation of multicollinearity resulted in the exclusion of the variables gastrointestinal system affected during hospitalization and any AMD treatment during hospitalization due to collinearity with no body system affected (healthy) and aminoglycoside treatment during hospitalization, respectively.

Because clinical status was an intervening variable (i.e., a horse's clinical status was determined by other variables considered for inclusion in the multivariable model, such as body system affected and level of systemic illness), clinical status was not included in the multivariable model building procedure.

Variables selected for inclusion in the final multivariable model included having no body system affected (healthy) and experiencing diarrhea in the 48 hours prior to enrollment. No confounding variables were identified, and the interaction term was not retained in the final model (**Table 4.5**). Controlling for the effect of experiencing diarrhea in the 48 hours prior to study enrollment, the hazard rate for healthy horses (i.e., those with no body system affected) was 3.21 (95% CI: 1.71, 6.02) times higher than that of horses experiencing disease (i.e., those with any body system affected). Controlling for the effect of having no body system affected, the hazard rate for horses experiencing diarrhea in the 48 hours prior to study enrollment was 0.47 (95% CI: 0.24, 0.92) times that of horses that did not experience diarrhea within 48 hours prior to study enrollment.

Sensitivity analyses demonstrated that violations of the assumption of independence between censoring and *Salmonella* shedding duration would result in more conservative (i.e., closer to the null) hazard ratio point estimates for both predictors retained in the final multivariable model (**Table 4.6**). At both extremes (i.e., assuming cessation of shedding at the time of censoring and assuming the maximum observed shedding duration among censored horses), diarrhea in the 48 hours prior to enrollment was no longer a statistically significant predictor of shedding duration (P = 0.09 and P = 0.11, respectively). However, 95% confidence intervals of hazard ratios obtained in both sensitivity analyses overlapped with those obtained in the original multivariable model.

Adverse outcomes among infected horses and their stablemates

The most commonly reported adverse outcome among enrolled horses was loose feces, with at least one instance of soft or loose feces reported for 27 horses (20.0%). Colic was reported less frequently, with at least one instance of colic reported for 12 horses (8.9%). Information about these outcomes was not reported for 31 horses (23.0%). The most commonly reported adverse outcome among stablemates of enrolled horses was *Salmonella* culture positivity, with at least one instance of culture positivity among stablemates reported for 12 enrolled horses (8.9%). Information about *Salmonella* culture positivity among stablemates was not reported for 39 enrolled horses (28.9%). Colic, loose feces, and hospitalization among stablemates were less common, with at least one instance of these events reported for 6 (4.4%), 8 (5.9%), and 7 (5.2%) enrolled horses, respectively. Information about these events among stablemates was not reported for 31 (23.0%), 31 (23.0%), and 32 (23.7%) of enrolled horses.

Discussion

In this study we have demonstrated that, on average, horses infected with *Salmonella* will shed the bacteria in their feces for 13 days, and horses experiencing clinical salmonellosis are expected to shed *Salmonella* for approximately two weeks longer than those with subclinical salmonellosis. However, increased duration of *Salmonella* shedding is not only associated with the incidence of diarrhea, a common manifestation of clinical salmonellosis, but also with the clinical involvement of any body system. While soft or loose feces were reported somewhat frequently among *Salmonella*-infected horses throughout the study period, adverse outcomes among stablemates were less common. The evidence provided here will aid in the appropriate

management of *Salmonella*-positive horses both in-hospital and on-farm to mitigate the risk of transmission in these settings.

The median duration of *Salmonella* shedding among horses with subclinical salmonellosis in this population, 11 days, was similar to the overall median duration of shedding at 13 days. In contrast, horses with clinical salmonellosis tended to shed the bacteria for more than twice as long, with a median shedding duration of 25 days. These estimates are comparable to at least one previous report of *Salmonella* shedding duration in horses; a population of 39 hospitalized horses evaluated for postoperative *Salmonella* shedding were found to shed the bacteria for 1-14 days after surgery.[39] However, the *Salmonella* status of these horses prior to surgery was not reported, so overall shedding duration may have been longer. Other studies have characterized longer shedding periods compared to our estimates. In a report of a *Salmonella* outbreak among a group of ponies, all were found to be fecal culture-negative six weeks following the outbreak and remained culture-negative for the following year.[29] Additionally, Palmer and Benson found that among a population of 81 horses recovering from clinical salmonellosis, 36% shed *Salmonella* for at least 30 days, with one horse continuing to shed for 300 days.[42] However, this study was not conducted or reported with rigorous methodology. Specifically, it is unclear if the horses in the study population were housed at the same or separate facilities, and while the follow-up period for some horses ended after five consecutive negative cultures were obtained, others continued to have fecal cultures performed even after this threshold was met. Therefore, shedding cessation was poorly defined, and it is possible that some horses were re-infected during the follow-up period, particularly if the study population was housed at a single location and the horses had direct or indirect contact with one another. Still, these studies, in addition to the current study, support the notion that *Salmonella*-infected horses

may continue to shed the bacteria for extended periods of time; the maximum shedding duration observed in the current study was 180 days.

This study is the first to provide insight into clinical risk factors that impact the duration of shedding of *Salmonella* in horses. Kaplan-Meier estimates of shedding duration stratified by clinical variables of interest, as well as univariable Cox proportional hazards models evaluating associations between clinical characteristics and duration of *Salmonella* shedding, identified several factors that may influence shedding duration. These included variables related to both severity and type of clinical illness, as well as drug exposure (AMDs or gastroprotectants) during hospitalization. However, in the final multivariable model, the strongest predictors of shedding duration were diarrhea in the 48 hours prior to study enrollment and the clinical involvement of any body system. This model demonstrated that the presence of diarrhea, a hallmark of clinical salmonellosis, increases the duration of *Salmonella* shedding in infected horses. However, duration of shedding also increases when any body system – not just the gastrointestinal system – is clinically affected. This was further supported by the Kaplan-Meier estimates of shedding duration among horses with different severity of systemic illness; while horses with minimal illness shed *Salmonella* for a median of 7 days, the median duration of shedding was longer for, but differed little between, horses with moderate or major illness, at 18 and 19 days, respectively. Therefore, it appears that the presence of any illness is a key driver of increased shedding duration, while the presence of diarrhea, specifically, is an additional risk factor. These factors likely account for the difference in duration of shedding observed between horses with clinical and subclinical salmonellosis. They may also explain the difference in shedding duration observed between horses with and without AMD exposure; that is, this difference may primarily

be driven by underlying illness that prompted antimicrobial treatment, rather than exposure to AMDs themselves.

While the mechanisms underlying the identified risk factors and *Salmonella* shedding duration are not fully explained by the current study, changes in the gastrointestinal microbiome may be involved. Horses with colitis, including colitis associated with *Salmonella* infection, were shown to have a distinct fecal microbiome composition and decreased fecal microbial alpha diversity compared to healthy horses.[136] Therefore, the gastrointestinal flora of horses experiencing diarrhea may have a diminished capacity to outcompete and exclude *Salmonella* organisms, allowing for persistent shedding. Other types of gastrointestinal and non-gastrointestinal illness, as well as factors such as treatment with AMDs, may similarly impact the gastrointestinal microbiome, so this mechanism may be involved in prolonging *Salmonella* shedding in non-diarrheic horses as well.[137-140]

In addition to characterizing *Salmonella* shedding duration, this study also evaluated the frequency of adverse health outcomes among enrolled *Salmonella*-positive horses and their stablemates. While soft or loose feces was a relatively common outcome among enrolled horses, occurring in 20% of the study population, the same was not true for stablemates of enrolled horses, with only 5.9% of stablemates reportedly experiencing this outcome. This finding aligns with the findings of Hartnack et al., who found that diarrhea occurred in approximately 20% of formerly hospitalized horses but only 6.7% of their stablemates.[95] In both this and an additional study, there was no association identified between *Salmonella* culture status of formerly hospitalized horses and the risk of diarrhea among their stablemates.[33,95] The most commonly reported adverse outcome among stablemates of enrolled horses was *Salmonella* culture positivity (reported in 8.9% of stablemates). Because we did not collect information on on-farm

infection control practices, we were unable to ascertain the impact of such practices on the likelihood of this outcome. However, reported adverse outcomes among stablemates of enrolled horses were uncommon overall.

While not defined objectives of this study, there were other notable findings. For example, 7.4% of enrolled horses shed *Salmonella* intermittently, defined here as resuming *Salmonella* shedding after three consecutive negative cultures were obtained. Intermittent shedding of *Salmonella* has been reported previously;[39,40] however, a lack of prospective, long-term studies of *Salmonella* shedding in a diverse cohort of horses has made it challenging to quantify how common this outcome is. We also demonstrated that 5.2% of horses shed more than one *Salmonella* serotype throughout the follow-up period. Multi-serotype equine *Salmonella* infections have been reported in numerous studies, usually as an uncommon, incidental finding.[27,38,39,94,103] In the current study, because we only chose a single colony for characterization in each culture-positive sample, we are unable to distinguish between true co-infection and sequential infection with different serotypes over time. However, these findings suggest that multi-serotype equine *Salmonella* infections may be more common than previously thought. Equine clinicians should be aware that horses may shed *Salmonella* even after several consecutive negative cultures have been obtained; therefore, continued surveillance testing and enhanced infection control practices may be warranted when managing previously *Salmonella*-positive horses, especially in a hospital setting. Additionally, the possibility of multi-serotype infections should be considered when investigating potential epidemiologic links between cases of equine salmonellosis.

While this study does provide crucial insight into the epidemiology of *Salmonella* infections in horses, it also has key limitations that must be taken into consideration. Complete

clinical and demographic data were missing for over 25% of the study population. However, among the horses with a missing enrollment form, the proportions of horses with clinical and subclinical salmonellosis were similar to proportions of these horses in the entire study population. Therefore, based on available data, missingness does not appear to be associated with clinical status. However, missingness may be associated with shedding duration, as the median estimate of shedding duration among horses with a completed enrollment form was twice that of horses with missing enrollment data. Therefore, comparisons of shedding duration based on clinical variables of interest, which excluded observations with missing enrollment data, may have overestimated the duration of *Salmonella* shedding. In addition to enrollment data, missing serotype data may have impacted study results. For horses that never had a positive *Salmonella* culture throughout the follow-up period, no serotype information was available. As such, horses that were lost to follow-up or experienced shedding cessation during the first week of the study were classified as having an "unknown" serotype, and these observations were excluded from the Cox proportional hazards model. Therefore, although there was no significant effect of serotype on *Salmonella* shedding duration identified in this study, it is possible that a true effect could not be ascertained due to missing data. In other species, including dairy cattle and swine, serotype identity was found to impact *Salmonella* shedding duration, so analogous effects may exist in horses.[141,142] Finally, on a per-sample basis, the sensitivity of *Salmonella* culture of equine fecal samples is approximately 44%, increasing to 82% if three samples are cultured and interpreted in parallel.[42] For this reason, we considered horses in this study to have stopped shedding *Salmonella* only after obtaining three consecutive negative cultures. While this approach did help to overcome the limited sensitivity of this test, decreasing the likelihood of incorrectly classifying horses as *Salmonella*-negative, it also contributed to loss to follow-up among the

study population. That is, if only one or two consecutive negative culture results were obtained, that individual was censored at the last known positive sample. The goal of this conservative approach was to prevent overestimation of shedding duration, and sensitivity analyses confirmed that this approach to censoring was unlikely to have resulted in drastically biased model estimates.

Conclusions

In this study, we have filled a crucial gap in our understanding of the epidemiology of *Salmonella* in horses, demonstrating that horses are likely to shed the bacteria in their feces for approximately two weeks. However, clinical illness increases the risk of prolonged shedding. Our findings also support previous evidence that the risk of adverse health outcomes among stablemates of *Salmonella*-infected horses is low. This information can be utilized by equine clinicians to provide evidence-based guidance to horse owners to improve the management of *Salmonella*-positive horses both in-hospital and on-farm.

Acknowledgements

Funding

This work was supported by the Morris Animal Foundation (Grant No. D17EQ-304) and the National Science Foundation (Grant No. DGE-1545433).

Author contributions

Conceptualization and supervision: BAB and PSM. Participant enrollment: BAB, HWA, EAB, RSM, CAR, NMS. Investigation and data curation: ECH, BAB. Analysis: ECH. Writing – original draft: ECH. Writing – review and editing: ECH, HWA, EAB, RSM, CAR, NMS, PSM, BAB. All co-authors agreed that this work may be included in this dissertation.

Tables

Table 4.1: Demographic characteristics of the study population (N = 135).

Variable	Category	Frequency (%)
Age (years)	<1	12 (8.9%)
	1 to <10	32 (23.7%)
	10 to <20	39 (28.9%)
	≥20	12 (8.9%)
	Unknown	40 (29.6%)
Sex	Mare	44 (32.6%)
	Gelding	46 (34.1%)
	Stallion	5 (3.7%)
	Unknown	40 (29.6%)
Breed	Quarter Horse	25 (18.5%)
	Thoroughbred	17 (12.6%)
	Draft	14 (10.4%)
	Warmblood	11 (8.2%)
	Other	30 (22.2%)
	Unknown	38 (28.2%)
Occupation*	Competition (English)	14 (10.4%)
	Competition (Western)	10 (7.4%)
	Pleasure	26 (19.3%)
	Breeding	14 (10.4%)
	None/in training	16 (11.9%)
	Other	22 (16.3%)
	Unknown	36 (26.7%)
Geographic region	South	57 (42.2%)
	West	39 (28.9%)
	Northeast	21 (15.6%)
	Midwest	18 (13.3%)

* More than one occupation could be selected

Table 4.2: Distribution of serogroups and serotypes among *Salmonella* isolates (N = 164).

Serogroup	**Serotype***	**Frequency (%)**
O:4 (B)	4, [5], 12:b:-	1 (0.6%)
	4, [5], 12:i:-	1 (0.6%)
	Reading	1 (0.6%)
	Typhimurium	24 (14.6%)
O:7 (C_1)	Bareilly	1 (0.6%)
	Braenderup	21 (12.8%)
	Hartford	1 (0.6%)
	Montevideo	1 (0.6%)
	Norwich	1 (0.6%)
	Ohio	3 (1.8%)
	Oranienburg	6 (3.7%)
	Thompson	8 (4.9%)
	Unknown	2 (1.2%)
O:8 (C_2)	Altona	2 (1.2%)
	Bovismorbificans	1 (0.6%)
	Kentucky	11 (6.7%)
	Litchfield	1 (0.6%)
	Muenchen	2 (1.2%)
	Newport	36 (22.0%)
	Unknown	2 (1.2%)
O:9 (D_1)	Javiana	14 (8.5%)
	Miami	1 (0.6%)
	Unknown	2 (1.2%)
O:3,10 (E_1)	Anatum	6 (3.7%)
	Anatum_var._15+	2 (1.2%)
	Give	5 (3.0%)
	London	1 (0.6%)
	Muenster	5 (3.0%)
O:6,14 (H)	Sundsvall	2 (1.2%)

*6 isolates were not submitted for serotyping

Table 4.3: Distribution of antimicrobial resistance among *Salmonella* isolates (N = 164).

Antimicrobial resistance*	**Frequency (%)**
Amikacin	163 (99.4%)
Ampicillin	41 (25.0%)
Cefazolin	162 (98.8%)
Cefotaxime	28 (17.1%)
Chloramphenicol	27 (16.5%)
Enrofloxacin	22 (13.4%)
Gentamicin	161 (98.2%)
Imipenem	0 (0%)
Tetracycline	21 (12.8%)
Ticarcillin-clavulanate	24 (14.6%)
Trimethoprim-sulfamethoxazole	38 (23.2%)

*1 isolate was not submitted for antimicrobial susceptibility testing

Table 4.4: Univariable Cox proportional hazards analysis of variables associated with duration of *Salmonella* shedding.

Variable	**Category**	**N**	**Hazard Ratio**	**95% CI**	***P*-value**
Age (years)	*continuous*	95	1.02	0.98-1.06	0.28
Sex	Gelding	46	Ref	Ref	0.39
	Mare	44	1.20	0.67-2.15	
	Stallion	5	0.48	0.10-2.30	
	Unknown	40	-	-	
Breed	Quarter Horse	25	Ref	Ref	0.31
	Thoroughbred	17	1.39	0.56-3.44	
	Draft	14	2.02	0.77-5.25	
	Warmblood	11	2.31	0.85-6.26	
	Other	30	1.84	0.85-3.99	
	Unknown	38	-	-	
Clinical status	Subclinical	108	Ref	Ref	0.01
	Clinical	27	0.43	0.22-0.82	
Any AMD during hospitalization	No	46	Ref	Ref	0.05
	Yes	48	0.59	0.34-1.05	
	Unknown	41	-	-	
Aminoglycoside during hospitalization	No	58	Ref	Ref	0.17
	Yes	36	0.69	0.39-1.23	
	Unknown	41	-	-	
Beta-lactam during hospitalization	No	62	Ref	Ref	0.28
	Yes	32	0.74	0.41-1.35	
	Unknown	41	-	-	
Other AMD during hospitalization	No	62	Ref	Ref	0.12
	Yes	31	0.65	0.36-1.18	
	Unknown	42	-	-	
Any AMD in 48 hrs prior to enrollment	No	75	Ref	Ref	0.39
	Yes	22	0.79	0.41-1.51	
	Unknown	38	-	-	
Oral AMD in 48 hrs prior to enrollment	No	91	Ref	Ref	0.21
	Yes	6	0.47	0.13-1.69	
	Unknown	38	-	-	
Parenteral AMD in 48 hrs prior to enrollment	No	80	Ref	Ref	0.80
	Yes	17	0.94	0.46-1.91	
	Unknown	38	-	-	
Any gastroprotectant during hospitalization	No	7	Ref	Ref	0.37
	Yes	58	0.71	0.22-2.31	
	Unknown	70	-	-	
Proton pump inhibitor during hospitalization	No	15	Ref	Ref	0.02
	Yes	50	0.45	0.22-0.93	
	Unknown	70	-	-	
Mucosal protectant during hospitalization	No	45	Ref	Ref	0.34
	Yes	20	0.72	0.33-1.58	

	Unknown	70	-	-	
Other gastroprotectant during hospitalization	No Yes Unknown	51 13 71	Ref 0.64 -	Ref 0.26-1.54 -	0.25
No body system affected (healthy)	No Yes Unknown	74 22 39	Ref 3.26 -	Ref 1.66-6.43 -	<0.001
Gastrointestinal system affected	No Yes Unknown	29 67 39	Ref 0.46 -	Ref 0.25-0.83 -	0.01
Other (non-gastrointestinal) body system affected	No Yes Unknown	71 24 40	Ref 0.69 -	Ref 0.36-1.35 -	0.23
Systemic illness in 48 hrs prior to enrollment	Minor Moderate Major Unknown	53 28 14 40	Ref 0.61 0.47 -	Ref 0.33-1.13 0.19-1.14 -	0.09
Diarrhea 48 hrs prior to enrollment	No Yes Unknown	69 23 43	Ref 0.48 -	Ref 0.23-1.01 -	0.04
Fever 48 hrs prior to enrollment	No Yes Unknown	68 23 44	Ref 0.54 -	Ref 0.25-1.14 -	0.08
Leukopenia 48 hrs prior to enrollment	No Yes Unknown	49 13 73	Ref 1.27 -	Ref 0.49-3.26 -	0.52
Anaesthesia/surgery 48 hrs prior to enrollment	No Yes Unknown	90 9 36	Ref 1.39 -	Ref 0.54-3.56 -	0.42
Reduced dietary intake 48 hrs prior to enrollment	No Yes Unknown	67 28 40	Ref 0.84 -	Ref 0.45-1.57 -	0.51
Salmonella serogroup	C2 B C1 D1 E H Unknown	18 8 15 3 9 2 80	Ref 0.88 0.84 0.21 1.50 0.38 -	Ref 0.25-3.13 0.25-2.76 0.02-1.92 0.44-5.15 0.04-4.10 -	0.41
Salmonella serotype	Newport Braenderup Javiana Typhimurium Other Unknown	11 5 3 6 30 80	Ref 0.49 0.17 0.57 1.30 -	Ref 0.09-2.80 0.02-1.97 0.11-2.87 0.39-4.36 -	0.19

Table 4.5: Final multivariable Cox proportional hazards model of factors associated with duration of *Salmonella* shedding.

Variable	**Category**	**N**	**Hazard Ratio**	**95% CI**	***P*-value**
No body system affected (healthy)	No Yes	70 20	Ref 3.21	Ref 1.71-6.02	<0.001
Diarrhea 48 hrs prior to enrollment	No Yes	68 22	Ref 0.47	Ref 0.24-0.92	0.03

Table 4.6: Estimated hazard ratios for Cox proportional hazards model of factors associated with duration of *Salmonella* shedding under different censoring assumptions.

Variable	**Category**	**Original hazard ratio (95% CI)**	**Hazard ratio (95% CI) assuming cessation of shedding at time of censoring**	**Hazard ratio (95% CI) assuming maximum observed shedding duration for censored horses**
No body system affected (healthy)	No Yes	Ref 3.21 (1.71-6.02)	Ref 2.07 (1.23-3.50)	Ref 2.56 (1.21-5.43)
Diarrhea 48 hrs prior to enrollment	No Yes	Ref 0.47 (0.24-0.92)	Ref 0.65 (0.40-1.07)	Ref 0.59 (0.29-1.19)

Figures

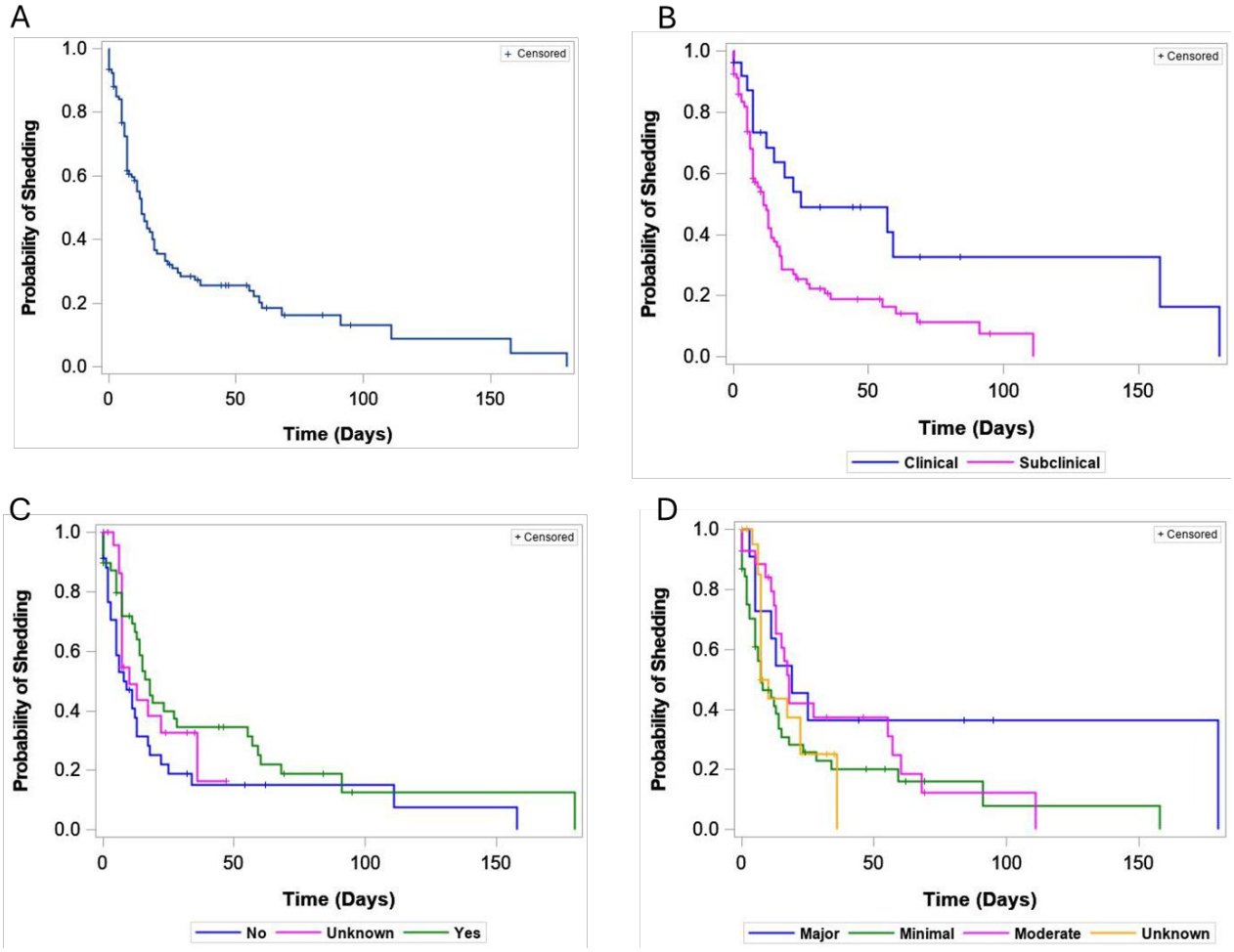


Figure 4.1: Kaplan-Meier survival curves for *Salmonella* shedding duration in the study population **A**) unstratified, **B**) stratified by clinical status, **C**) stratified by AMD exposure, and **D**) stratified by severity of systemic illness.

CHAPTER 5

THE FECAL MICROBIOME AND SUBCLINICAL SHEDDING OF *SALMONELLA ENTERICA* IN HORSES [i]

[i] Herring, E.H., Gaire, T.N., Brown, J.L., Groover, E.S., Noyes, N.R., and B.A. Burgess. To be submitted to *Journal of Veterinary Internal Medicine*.

Abstract

Subclinical shedding of *Salmonella* in horses is more common than clinical disease and poses challenges to infection control efforts in equine facilities. Known risk factors for *Salmonella* positivity suggest that disruptions of the gastrointestinal microbiome may play a role in fecal *Salmonella* shedding. Therefore, we aimed to characterize fecal taxonomic profiles of horses with subclinical salmonellosis and evaluate associations between shedding status and shifts in the fecal microbiome over time. Six adult horses from a resident herd at a veterinary teaching hospital with fecal culture-confirmed subclinical salmonellosis were enrolled. Samples from a prospective longitudinal study were selected for retrospective analysis. *Salmonella* fecal cultures were performed weekly for 8 weeks, and banked fecal samples (N = 48) were subjected to 16S rRNA amplicon sequencing. Fecal microbial communities were compared between periods of *Salmonella*-negativity and -positivity and between horses with different shedding patterns over time. Horses demonstrated 3 patterns of *Salmonella* shedding: short-term, intermittent, and prolonged. Phylum-level richness, Shannon index, and inverse Simpson index were greater during periods of *Salmonella*-negativity than -positivity ($P = 0.03$, $P = 0.02$, $P = 0.05$), but overall microbial community composition did not differ between periods of shedding and non-shedding ($P \geq 0.49$). While microbial community composition was not significantly different between short-term, intermittent, and prolonged shedders ($P \geq 0.38$), shedding pattern described more variability in microbial community composition at all taxonomic levels ($R^2 = 0.18 – 0.24$) than did *Salmonella*-positivity ($R^2 = 0.02 – 0.03$). While changes in fecal microbial community do not appear to be associated with *Salmonella* positivity on a per-sample basis, composition of the fecal microbiome may be associated with shedding patterns over time among subclinically infected horses.

Introduction

Salmonella enterica is among the most common causes of healthcare-associated infections in horses and is a challenge to infection control efforts in any setting where horses are commingled, including hospitals, competition venues, and breeding or boarding facilities.[12,27] *Salmonella* outbreaks in equine hospitals have frequently been characterized by severe disease and case fatality rates over 30%, often resulting in partial or complete facility closure.[15,24,103] More commonly, however, horses shed *Salmonella* subclinically, and they tend to do so intermittently.[39] A lack of discernible symptoms reduces clinical suspicion of infection, and the intermittent shedding of low numbers of bacteria limits the sensitivity of commonly used diagnostic tests for equine salmonellosis, including culture and polymerase chain reaction (PCR).[41,43] Together, these aspects of this disease make control and prevention efforts particularly challenging.

To aid in the development of targeted infection control practices, many studies have aimed to identify risk factors for *Salmonella* shedding among horses. Key risk factors identified to date include diarrhea,[31,100] antimicrobial drug exposure,[35] systemic illness,[31] recent abdominal surgery,[104] and increased duration of travel to the hospital.[10] Investigators have repeatedly hypothesized that these factors increase the likelihood of *Salmonella* shedding by altering the microbial community within the equine gastrointestinal tract.[35,100,104] These hypotheses have, in turn, spurred attempts to treat or prevent *Salmonella* shedding through manipulation of the gastrointestinal microbiome (i.e., through probiotic administration).[39,100,104] However, studies investigating the impact of probiotic treatment on *Salmonella* shedding in horses have failed to demonstrate significant effects.[39,100,104] A limited number of studies have shown differences in the fecal microbiome composition between *Salmonella*-infected and -noninfected horses, and

that changes in the fecal microbiome coincide with increased shedding.[105,106] However, the *Salmonella*-infected horses in these studies were also treated with antimicrobial drugs or experiencing clinical disease, so the impact of infection on the equine gastrointestinal microbiome, independent of these potentially confounding factors, remains undescribed.

Detailing the mechanistic pathways that may exist between *Salmonella* infection, the equine gastrointestinal microbiome, and known risk factors for *Salmonella* shedding in horses is a crucial step in gauging the utility of interventions for salmonellosis that aim to alter the microbiome, such as probiotics and fecal microbial transplants. Further, given the importance of subclinical shedding in facilitating the transmission of *Salmonella* among horses, characterizing the gastrointestinal microbiome of subclinically infected horses may provide important insight into predictors of shedding that could serve as potential targets for disease prevention and control efforts. Therefore, the objectives of this study were to characterize the fecal taxonomic profile of horses with subclinical salmonellosis and to identify associations between changes in the fecal microbiome and *Salmonella* shedding patterns among subclinically infected horses.

Methods

Overview

Fecal samples collected from *Salmonella*-positive horses enrolled in a prospective, longitudinal study were retrospectively analyzed to evaluate the association between fecal microbiome composition and *Salmonella* shedding status. Six horses with subclinical salmonellosis from a single herd were enrolled in a cohort study investigating *Salmonella* shedding duration from June to August 2019. Serial weekly fecal samples were collected from each horse for 8 weeks and cultured for *Salmonella*. Banked, frozen fecal samples from this

study were processed for 16S rRNA amplicon sequencing, and differences in fecal microbial community composition were assessed relative to *Salmonella* shedding status both within and between horses.

Study population

A group of 6 horses was selected for inclusion in the current study from a cohort of 163 *Salmonella*-positive horses enrolled in a prospective, longitudinal study investigating *Salmonella* shedding duration. Horses in this cohort were identified by clinicians at participating equine practices in the U.S. between March 2018 and December 2020 after testing positive for *Salmonella* by either fecal culture or PCR. Demographic information and clinical history were provided in an online survey completed by the enrolling clinician. Fecal samples were then collected weekly from enrolled horses for 8 weeks and submitted for enriched *Salmonella* culture. Six horses with subclinical salmonellosis housed at a single facility were purposively selected from this cohort for retrospective analysis of the fecal microbiome throughout the 8-week study period. These horses were chosen on the basis of shared management characteristics to limit the impact of variables that may confound the relationship between the fecal microbiome and *Salmonella* shedding (e.g., environment, diet, clinical symptoms of disease).

Fecal sample collection and Salmonella culture

Once per week throughout the 8-week study period from June to August 2019, the facility manager collected a fecal sample consisting of approximately 3 fecal balls rectally from each horse with a clean, gloved hand. The sample was placed into a sterile specimen container and shipped overnight on ice to the University of Georgia. Upon receipt, 3 g of each fecal sample

were processed for *Salmonella* culture, and up to 10 g of the remaining fecal sample were frozen at -80°C for future analyses. For *Salmonella* culture, 3 g of feces were inoculated into 30 ml of tetrathionate broth supplemented with brilliant green and iodine (BD, Franklin Lakes, NJ) and incubated for 18-24 h at 43°C. The tetrathionate-enriched fecal sample was then streaked for isolation onto xylose-lysine-tergitol 4 (XLT4) agar (Hardy Diagnostics, Santa Maria, CA) and incubated at 43°C for 18-24 h. XLT4 agar plates were inspected for colonies consistent with the appearance of *Salmonella* (black or black-centered colonies indicative of hydrogen sulfide production). Suspect colonies were sub-cultured onto trypticase soy agar with 5% sheep blood (TSA; BD, Franklin Lakes, NJ) and incubated for 18-24 hours at 43°C. Colonies were confirmed as *Salmonella* by testing for agglutination with commercial polyvalent and O-group specific antisera (BD, Franklin Lakes, NJ). All *Salmonella* isolates were then serotyped at the USDA National Veterinary Services Laboratory (NVSL, Ames, Iowa). Given the low sensitivity of culture for the detection of *Salmonella* in equine fecal samples on a per-sample basis,[7] horses were considered *Salmonella*-negative only after testing negative on 3 consecutive fecal samples. Horses were then classified into 3 categories based on their overall pattern of *Salmonella* shedding throughout the 8-week study period: short-term shedders were *Salmonella*-negative by week 3 and remained *Salmonella*-negative throughout the remainder of the study, intermittent shedders were initially *Salmonella*-negative by week 3 but resumed shedding before the end of the study, and prolonged shedders continued to shed *Salmonella* for ≥ 4 weeks of the study.

DNA extraction, library preparation, and sequencing

The 48 banked, frozen fecal samples (6 horses x 8 time points) were thawed at room temperature, and DNA was isolated from 0.2 g of each sample with the DNeasy PowerSoil Pro

Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. DNA concentration and quality were measured via spectrophotometry (Nanodrop, Thermo Scientific, Waltham, MA), and upon confirming a minimum concentration of 10 ng/μL and a 260/280 ratio of 1.8-2.0, DNA samples were shipped overnight on dry ice to Novogene (Sacramento, CA) for 16S rRNA amplicon library preparation and sequencing. A sample of the elution buffer was also included as a negative control. DNA concentration was confirmed using the Qubit 2.0 DNA HS Assay (Life Technologies, Grand Island, NY). PCR amplification of the 16S rRNA gene was performed using barcoded primers specific to the V3-V4 region (Forward primer: 5'-CCTAYGGGRBGCASCAG- 3'; Reverse primer: 5'- GGACTACNNGGGTATCTAAT -3'). PCR products were size selected using 2% agarose gel electrophoresis and quantified via real-time PCR. Equimolar amounts of PCR products were pooled, end-repaired, A-tailed, and ligated with Illumina adapters. Amplicon libraries were sequenced using paired-end 250-bp reads on an Illumina MiSeq (Illumina Inc., San Diego, CA).

Sequencing data processing

Amplicon primers were removed from raw reads using cutadapt (v1.18).[107] Reads were then processed using the DADA2 pipeline (v1.18.0) in R (v4.0.4).[16] Truncation lengths were set to 230 and 220 bp, and maximum expected error rates were set to 4 and 5 for forward and reverse reads, respectively. Trimmed and filtered reads were dereplicated and error-corrected, and forward and reverse reads were merged. Chimeras were removed, and an amplicon sequence variant (ASV) count table was generated. Taxonomy was assigned via alignment to the SILVA reference database (v138.1) and species-level annotations were added using the *addSpecies* function.[108] The ASV count and taxonomy tables were then imported into phyloseq (v1.38.0) for

microbiome analysis.[109] Potential contaminant ASVs were identified using the prevalence method in the decontam package (v1.14.0).[110]

Sequencing depth and count normalization

Univariable linear regression models were used to assess potential bias in sequencing depth by horse identity, *Salmonella* status, and *Salmonella* shedding pattern. The number of raw reads per sample was modeled as a function of each of these variables using the *lm* function. F-tests were performed for each model using the *anova* function, and 95% confidence intervals for model coefficients were calculated using the *confint* function. P-values ≤ 0.05 were considered statistically significant. ASVs with fewer than 5 total read counts were discarded, and these filtered ASV counts were normalized using cumulative sum scaling with the *cumNorm* function in the metagenomeSeq package (v1.36.0) to account for differences in sequencing depth across samples.[111] Normalized read counts were utilized for all downstream analyses.

Alpha diversity

Normalized counts were rounded up to the nearest integer for alpha diversity analysis. Alpha diversity indices, including richness, Shannon, and inverse Simpson, were measured at the genus, family, order, class, and phylum levels using the *estimate_richness* function in phyloseq.[109] Pielou's evenness was also calculated at each of these taxonomic levels using the *evenness* function in the microbiome package (v1.16.0).[112] Linear mixed-effects models were used to evaluate the impact of *Salmonella* shedding status (based on parallel interpretation of *Salmonella* culture results) on each alpha diversity index, as well as the impact of *Salmonella* shedding pattern (short-term, intermittent, or prolonged) on each alpha diversity index (*lmer*

function in lmerTest package [v3.1-3]).[113] In each model, horse identity was included as a random effect to account for repeated measures within horses. Partial F-tests (*anova* function) were used to determine whether each predictor significantly improved the fit of each model. Estimated marginal means were compared using the *lsmeans* function, with the Benjamini-Hochberg procedure used to adjust for multiple comparisons (*p.adjust* function). P-values ≤ 0.05 were considered statistically significant.

Beta diversity

Bray-Curtis distance matrices were calculated from normalized counts aggregated to the phylum, class, order, family, and genus levels using the *vegdist* function in the vegan package (v2.5-7),[114] then ordinated using non-metric multidimensional scaling (NMDS) with the *ordinate* function in phyloseq.[109] NMDS plots were visualized using the ggplot2 package (v3.4.2).[115] Homogeneity of group dispersions was tested using the *betadisper* and *permutest* functions in vegan.[114] Permutational analysis of variance (PERMANOVA) was performed using the *adonis2* function in vegan in order to evaluate the impact of *Salmonella* shedding status and *Salmonella* shedding pattern, respectively, on Bray-Curtis distances at each taxonomic level.[114] In all models, permutations were restricted within horse identity to account for repeated measures. P-values ≤ 0.05 were considered statistically significant. Hierarchical clustering among samples based on Bray-Curtis distances was visualized using Ward's agglomerative clustering (*hclust* function).

Differential abundance

At each taxonomic level, the relative abundance of each feature within a sample was calculated by dividing the counts attributed to that feature by the sum of all counts in the sample.

Relative abundance was visualized in stacked bar charts. Differentially abundant features between *Salmonella*-positive and -negative samples, and between samples from short-term, intermittent, and prolonged *Salmonella* shedders, were evaluated at each taxonomic level using generalized linear mixed models with the Maaslin2 package (v1.8.0).[116] All models included horse identity as a random effect to account for repeated measures, and the Benjamini-Hochberg method was used to control the false discovery rate. Benjamini-Hochberg-adjusted P-values ≤ 0.05 were considered statistically significant.

Results

Study population

Six adult horses from a herd used for teaching and research at a veterinary school in the southeastern U.S. were enrolled in the study. Two mares (33%) and 4 geldings (67%) were included, with a median age of 11.5 years (range: 6-15 years). Breeds included in the study population included Quarter Horse/Quarter Horse cross (3 [50%]), Thoroughbred (1 [17%]), Tennessee Walking Horse (1 [17%]), and Warmblood (1 [17%]). All horses were housed at a single facility within a three-mile radius, with two horses housed together in each of three pastures. Throughout the study period, all horses remained healthy, with no incidence of diarrhea, fever, hospitalization, or other illness. The horses were not treated with any antimicrobial drugs throughout the study period. Additionally, the horses' diets remained consistent throughout the study and included Bermuda/Bahia grass pasture, free choice bermudagrass hay, pelleted grain with 12% crude protein (Triumph® Active 12 Pellet, Nutrena®, Giddings, TX), and free choice trace minerals. In June 2019, fecal samples from all 6 horses were determined to be culture-positive for *Salmonella* as part of a surveillance program

conducted at the facility, at which time they were enrolled in a longitudinal study investigating *Salmonella* shedding duration until August 2019.

Culture results

Of the 48 fecal samples submitted throughout the study period, 6 samples from 4 horses were culture-positive for *Salmonella*. The remaining 2 horses remained culture-negative throughout the duration of the study. Of the 6 *Salmonella*-positive samples, 2 (33.3%) were detected in week 2 of the study, 1 (16.7%) was detected in week 3 of the study, 2 (33.3%) were detected in week 5 of the study, and 1 (16.7%) was detected in week 6 of the study. *Salmonella* serotypes identified within this study population included Muenchen (1 isolate) and Muenster (5 isolates). Two horses were classified as short-term *Salmonella* shedders, 2 were classified as prolonged *Salmonella* shedders, and 2 were classified as intermittent *Salmonella* shedders based on their overall pattern of shedding throughout the study (**Table 5.1**).

Sequencing results

A total of 7,231,815 raw paired reads (mean: 150,662.8; range: 78,942 - 229,997) were generated from 16S rRNA amplicon sequencing of the 48 samples. Sequencing of the negative control sample generated a total of 3,660 raw paired reads. Sequencing depth differed significantly by horse identity ($P < 0.001$) and *Salmonella* shedding pattern ($P < 0.001$) but was not significantly different between *Salmonella*-positive and -negative samples ($P = 0.06$; **Supplementary Table 5.1**). After quality filtering, merging of forward and reverse reads, and removal of chimeras, 2,429,638 total reads remained across all samples. No contaminant sequences were identified using the decontam package. A total of 99.7%, 99.7%, 99.0%, 96.6%,

72.6%, and 2.2% of reads were classified at the phylum, class, order, family, genus, and species levels. No further analyses were performed at the species level given the low rate of classification.

Alpha diversity

Results of mixed-effects linear regression analyses evaluating the associations between *Salmonella* shedding status (based on parallel interpretation of *Salmonella* culture results) and overall *Salmonella* shedding pattern (short-term, intermittent, or prolonged), respectively, with alpha diversity indices are shown in **Table 5.2**. Pairwise contrasts between estimated marginal means at the phylum, order, and family level are shown in **Figure 5.1**. At the phylum level, richness, Shannon index, and inverse Simpson index were higher among samples from *Salmonella*-negative horses compared to samples from *Salmonella*-positive horses ($P = 0.03$, $P = 0.01$, and $P = 0.05$). However, evenness was not associated with *Salmonella* status at the phylum level, and richness, evenness, Shannon index, and inverse Simpson index did not differ significantly between samples from *Salmonella*-negative and -positive horses at any other taxonomic level ($P > 0.05$). At the phylum, class, and genus levels, there was no association between *Salmonella* shedding pattern and any of the measured alpha diversity indices ($P > 0.05$). However, at the order level, richness was highest among samples from short-term shedders, intermediate among samples from intermittent shedders, and lowest among samples from prolonged shedders; all pairwise differences were statistically significant (short-term vs. intermittent: $P = 0.001$, short-term vs. prolonged: $P < 0.001$, intermittent vs. prolonged: $P = 0.05$). *Salmonella* shedding pattern was also significantly associated with Shannon index at the order level overall ($P = 0.05$). However, after adjusting for multiple comparisons, no pairwise

differences between short-term, intermittent or prolonged shedders were statistically significant ($P > 0.05$). *Salmonella* shedding pattern was not associated with evenness or inverse Simpson index at the order level ($P > 0.05$). At the family level, richness was higher among short-term shedders than prolonged shedders ($P = 0.05$) but did not differ between short-term and intermittent shedders ($P = 0.16$) or between intermittent and prolonged shedders ($P = 0.09$). Family-level evenness was higher among samples from intermittent shedders compared to those from short-term or prolonged shedders ($P < 0.001$, $P < 0.001$), but evenness did not differ significantly between samples from short-term and prolonged shedders ($P = 0.69$). Additionally, samples from horses with an intermittent *Salmonella* shedding pattern had a higher family-level Shannon index compared to samples from horses with a prolonged shedding pattern ($P = 0.05$). However, Shannon index did not differ significantly between samples from short-term and intermittent shedders ($P = 0.24$), or between samples from short-term and prolonged shedders at the family level ($P = 0.06$). While *Salmonella* shedding pattern was associated with inverse Simpson index at the family level overall ($P = 0.05$), no pairwise contrasts between short-term, intermittent, and prolonged shedders were significantly different ($P > 0.05$).

Beta diversity

There were no significant differences in group dispersions by *Salmonella* status or *Salmonella* shedding pattern at any taxonomic level (beta-dispersion, $P > 0.05$; **Table 5.3**). Neither *Salmonella* status nor *Salmonella* shedding pattern explained a significant amount of variation in microbial community composition at any taxonomic level (PERMANOVA, $P > 0.05$; **Table 5.3; Figure 5.2**). Although the PERMANOVA results were not statistically significant, R^2 values were consistently higher for *Salmonella* shedding pattern (0.18 – 0.24) compared to

Salmonella status (0.02 – 0.03) across all taxonomic levels (**Table 5.3**). Hierarchical clustering was largely driven by horse identity and did not appear to be associated with *Salmonella* positivity or negativity at any taxonomic level. At lower taxonomic levels (family and genus), samples from short-term and prolonged shedders, respectively, were more closely clustered than they were at higher taxonomic levels (**Figure 5.3**).

Differential abundance

Among all samples, the most abundant phyla included Firmicutes, Bacteroidota, Spirochaetota, Fibrobacterota, and Euryarchaeota, which together accounted for 94.0% of total sequence reads (**Figure 5.3A**). Clostridia, Bacteroidia, Spirochaetia, Negativicutes, and Fibrobacteria were the predominant classes across all samples, accounting for 88.2% of all reads. The most abundant orders detected among all samples were Bacteroidales, Lachnospirales, Oscillospirales, Spirochaetales, and Clostridiales, comprising 74.9% of all reads. *Lachnospiraceae*, *Prevotellaceae*, *Spirochaetaceae*, *Rikenellaceae*, and *Oscillospiraceae* were the most commonly identified families, accounting for 47.59% of all reads. The predominant genera across all samples included *Treponema*, a *Rikenellaceae* RC9 gut group, *Clostridium sensu stricto 1*, *Fibrobacter*, and an *Oscillospiraceae* NK4A214 group, comprising 35.87% of reads (**Figure 5.3B**). At the phylum level, samples from *Salmonella*-positive horses had a lower abundance of Euryarchaeota than did samples from *Salmonella*-negative horses ($P = 0.05$; **Supplementary Table 5.2**). However, there were no significant differences in feature abundance between samples from *Salmonella*-negative and -positive horses at the class, order, family, or genus levels (**Supplementary Tables 5.3 – 5.6**). At the class level, samples from horses with an intermittent *Salmonella* shedding pattern had a lower abundance of Alphaproteobacteria

compared to samples from short-term shedders (P = 0.02; **Supplementary Table 5.8A**). However, abundance of Alphaproteobacteria did not differ between samples from short-term and prolonged shedders (P = 0.60; **Supplementary Table 5.8A**) or between those from prolonged and intermittent shedders (P = 0.75; **Supplementary Table 5.8B**). At the family level, bacteria belonging to the family gir-aah93h0 were more abundant in samples from prolonged shedders compared to those from intermittent shedders (P = 0.001; **Supplementary Table 5.10B**), but the abundance of these organisms did not differ significantly between samples from short-term and prolonged shedders (P = 0.91; **Supplementary Table 5.10A**) or between samples from short-term and intermittent shedders (P = 0.07; **Supplementary Table 5.10A**). At the genus level, the abundance of *Mogibacterium* was lower among samples from prolonged *Salmonella* shedders compared to short-term shedders (P = 0.005; **Supplementary Table 5.11A**) but did not differ between samples from prolonged and intermittent shedders (P = 0.94; **Supplementary Table 5.11B**) or between samples from short-term and intermittent shedders (P = 0.18; **Supplementary Table 5.11A**). There were no differentially abundant features between short-term, prolonged, and intermittent *Salmonella* shedders at the phylum or order level.

Discussion

In this population of horses with subclinical salmonellosis, differences in the fecal microbial community between periods of *Salmonella* shedding and non-shedding, and between short-term, intermittent, and prolonged *Salmonella* shedders were subtle overall. While the *Salmonella* shedding status (positive vs. negative) of subclinically infected horses may be associated with the alpha diversity of the fecal microbiome, *Salmonella* positivity appeared to have little impact on, or be impacted by, overall fecal microbial community composition. In contrast, differences in microbial community composition between horses with a short-term,

intermittent, or prolonged pattern of *Salmonella* shedding over time were more distinct, although these differences were not statistically significant. Therefore, while it has long been hypothesized that shifts in the equine gastrointestinal microbiome instigate *Salmonella* shedding in horses, this study challenges this notion and instead suggests that among horses with subclinical salmonellosis, fecal microbial community composition remains quite consistent between periods of *Salmonella* shedding and non-shedding. However, the role of the gastrointestinal microbiome in shaping equine *Salmonella* shedding patterns over time warrants further investigation.

In a previous comparison of the microbial communities of healthy horses and horses with clinical salmonellosis, samples from *Salmonella*-positive horses were found to have a lower microbial richness and Shannon diversity index compared to healthy horses.[105] This is consistent with our findings at the phylum level, which demonstrate that richness, Shannon index, and inverse Simpson index are higher among horses experiencing periods of *Salmonella* negativity compared to those shedding *Salmonella*. However, we did not observe a difference in Pielou's evenness between samples from *Salmonella*-negative and -positive horses at the phylum level. Richness is a simple measure of the number of unique features in a community, while Pielou's evenness demonstrates how evenly different features are distributed in a population.[117,118] Both the Shannon and inverse Simpson diversity indices are compound measures of diversity that are influenced by both richness and evenness, with inverse Simpson being more strongly impacted by dominant (i.e., more abundant) features.[119] The observed changes in the 3 metrics that incorporate richness suggest that richness, rather than evenness, plays a more important role in driving differences in phylum-level diversity between periods of *Salmonella* negativity and positivity. We identified one phylum – Euryarchaeota – that was more abundant among samples from *Salmonella*-negative horses compared to those from *Salmonella*-positive horses. This

phylum may be driving the difference in richness between these groups; however, given the small sample size, the study may have been underpowered to detect other differentially abundant taxa. Contrary to our findings, Arnold et al. demonstrated an increase in Euryarchaeota among horses with *Salmonella* compared to healthy horses.[105] Therefore, the role of this phylum in the microbial ecology of equine salmonellosis warrants further characterization.

While the observed differences in diversity at the phylum level were statistically significant, the magnitude of these changes were small, and the clinical impact is unclear. Although Arnold et al. observed significant differences in alpha diversity between healthy horses and horses with clinical salmonellosis, they also identified more extreme differences in alpha diversity between healthy horses and horses with antimicrobial-associated diarrhea.[105] This suggests that changes in alpha diversity may be driven more by clinical disease than by *Salmonella* infection alone, while other studies have found no association between colitis and gastrointestinal microbial diversity in horses.[120,121] Therefore, there are not established thresholds of alpha diversity that distinguish between health and disease, and while our findings provide evidence that even subclinical *Salmonella* shedding may be linked to decreased gastrointestinal microbial diversity, whether this decrease in diversity is indicative of an unhealthy shift in microbiome composition remains uncertain. Additionally, similar associations between alpha diversity metrics and *Salmonella* shedding status were not detected at lower taxonomic levels, which is consistent with findings in other species, including cattle and swine.[122,123] Further, *Salmonella* shedding status had very little impact on overall microbial community composition at any taxonomic level, as demonstrated by ordination of Bray-Curtis distances, which showed nearly complete overlap between samples from *Salmonella*-negative and -positive horses, as well as visualization of hierarchical clustering, which did not demonstrate any distinct clustering

based on *Salmonella* status. Similarly, no differences in fecal microbial community composition were identified in a comparison of dairy cows with and without asymptomatic *Salmonella* infections.[124] Therefore, among horses with subclinical *Salmonella* infections the diversity, membership, and structure of the equine fecal microbiome appears to deviate very little overall between periods of *Salmonella* shedding and non-shedding, confirming findings in other species and challenging the often-proposed notion that *Salmonella* shedding in horses necessarily results from shifts in the gastrointestinal microbiome.[35,100,104]

There were 3 patterns of *Salmonella* shedding identified among the horses in this study – short-term, intermittent, and prolonged. These findings are consistent with previous reports of equine salmonellosis, which have demonstrated cessation of shedding within one week of infection,[125] shedding that persisted for months after infection,[38,126] and intermittent detection of *Salmonella* in feces over a period of time.[39,40] While the tendency for horses to shed *Salmonella* intermittently and/or for prolonged durations is a known challenge to infection control programs, the drivers of these shedding patterns have not previously been characterized.

Here, we have provided preliminary evidence that horses' *Salmonella* shedding pattern over time may be associated with the composition of the gastrointestinal microbiome. Although there were few statistically significant associations of alpha diversity indices with *Salmonella* shedding pattern, and these only occurred at the order and family levels, fecal microbial diversity, in terms of richness, evenness, and compound diversity indices, was consistently lowest among the prolonged shedders compared to short-term or intermittent shedders. This may be indicative of a dysbiosis that either results from, or allows for, the persistence of *Salmonella* within the gastrointestinal tract of prolonged shedders, while a more diverse microbiome facilitates the long-term or intermittent competitive exclusion of *Salmonella* among the short-

term and intermittent shedders, respectively. Our findings are similar to observations in pigs experimentally infected with *S.* Typhimurium; while fecal microbial alpha diversity decreased post-infection compared to pre-infection among pigs experiencing higher levels of *Salmonella* shedding, similar changes in diversity were not observed among pigs shedding lower levels of *Salmonella*, suggesting that gastrointestinal microbial diversity is more strongly associated with the type of shedding than with the presence or absence of *Salmonella* alone.[127] However, a second study found no association between alpha diversity and *Salmonella* shedding pattern in naturally infected pigs.[128]

Visualization of the beta diversity of these samples, both through ordination and hierarchical clustering, demonstrated more marked clustering by *Salmonella* shedding pattern compared to *Salmonella* positivity or negativity, and the clustering became increasingly distinct at lower taxonomic levels. While the PERMANOVA did not reveal statistically significant differences in microbial community composition between short-term, intermittent, and prolonged shedders, shedding pattern explained 18-24% of variation in microbial community composition across all taxonomic levels, compared to only 2-3% that was explained by *Salmonella* status (positive vs. negative). This suggests that among horses with subclinical *Salmonella* infections, the composition of the gastrointestinal microbiome is more strongly associated with the overall course of *Salmonella* shedding over time than with *Salmonella* positivity or negativity at a single point in time. However, the directionality of this relationship cannot be determined from our observations alone. In swine, two studies found that pigs shedding *Salmonella* more frequently or in higher amounts had distinct fecal microbial communities compared to lower-level or non-shedders.[127,129] In one of these studies, differences in microbial community composition between high shedders and low shedders preceded experimental infection with *S.* Typhimurium,

suggesting that the gastrointestinal microbiome does drive the observed shedding phenotype.[127] However, a third study found no relationship between shedding pattern and beta diversity of the fecal microbiome in swine.[128]

While this research does provide novel insights into the ecology and epidemiology of *Salmonella* infections in horses, the limitations of this study should be taken into consideration when interpreting our findings. The study population is a small, homogeneous group which was selected purposefully to limit the impact of confounding variables, such as environment and diet, on the associations of interest between *Salmonella* shedding and the fecal microbiome. However, because these horses were housed at a single facility and managed in a nearly identical fashion, our ability to extrapolate the results of this study to other equine populations is somewhat limited. Further, only two *Salmonella* serotypes – Muenchen and Muenster – were identified among this study population. A wide variety of *Salmonella* serotypes can infect horses,[130] and in swine, infection with different *Salmonella* serotypes was found to drive distinct gastrointestinal microbiome profiles.[131] Therefore, our findings may not be representative of subclinical *Salmonella* infections across all serotypes. Additionally, given the limited sample size, this study may have been underpowered to detect true differences between samples from *Salmonella*-negative and -positive horses, or between samples from short-term, intermittent, and prolonged shedders. Therefore, comparisons that are not statistically significant in this study may still be biologically relevant. Finally, the design of this study limits our ability to describe causal relationships between asymptomatic equine *Salmonella* infections and changes in the fecal microbiome. These horses were known to be *Salmonella* culture-positive prior to enrollment in the study, so it is not possible to ascertain if the composition of their gastrointestinal microbiome prior to infection impacted the course of *Salmonella* shedding throughout the study, or

alternatively, if the infection itself caused alterations in the microbial ecology of the gastrointestinal tract. Additionally, because samples were collected on a weekly basis, there may be changes in the microbiome that occurred between sampling timepoints that could not be appreciated; finer temporal resolution (e.g., daily sampling) in future studies may offer improved insight into the microbial ecology of equine *Salmonella* infections over time. Further, there were no *Salmonella*-negative horses included in the study population; the inclusion of farm-matched healthy controls would allow us to more definitively determine if changes in the equine fecal microbiome are truly associated with *Salmonella* shedding, or if they are a result of some other unmeasured factor. Collectively, these limitations suggest that the results of this study should primarily be considered hypothesis-generating and should be replicated in a larger, more diverse study population before being used to guide clinical decision-making.

Conclusions

In summary, we found that the composition of the fecal microbiome among horses with subclinical salmonellosis remains quite consistent during periods of *Salmonella* shedding and non-shedding. However, horses with different patterns of *Salmonella*-shedding over time appear to have more distinct microbiome profiles from one another. Therefore, while gastrointestinal microbial community composition may not be a useful predictor of *Salmonella* negativity or positivity on a per-sample basis, it could provide valuable insight into the expected course of *Salmonella* shedding over time.

Acknowledgements

Funding

This material is based upon work supported by the National Science Foundation under Grant No. DGE-1545433. Funding for sample collection and *Salmonella* culture was provided by Morris Animal Foundation (Grant number: D17EQ-307)

Author Contributions

Conceptualization: BAB, ECH. Supervision: BAB. Participant enrollment and sample collection: JLB, ESG. Investigation and data curation: ECH. Analysis: ECH, TNG, NRN. Writing – original draft: ECH. Writing – review and editing: ECH, TNG, JLB, ESG, NRN, BAB. All co-authors agreed that this work may be included in this dissertation.

Tables

Table 5.1: (**A**) *Salmonella* culture results for weekly fecal samples submitted over 8-week study period. (**B**) Interpretation of *Salmonella* culture results in parallel (i.e., classification of horses as *Salmonella*-negative after three consecutive negative cultures obtained) and associated *Salmonella* shedding pattern over time (short-term, prolonged, or intermittent).

Horse identity	**A**	***Salmonella* culture results**								***Salmonella* shedding pattern**	**B**	**Parallel interpretation of *Salmonella* culture results**							
	Week										**Week**								
	0*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		**0***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	+	-	-	-	-	-	-	-	-	Short-term	+	+	+	-	-	-	-	-	-
2	+	-	-	-	-	+	-	-	-	Intermittent	+	+	+	-	-	+	+	+	-
3	+	-	+	-	-	-	-	-	-	Prolonged	+	+	+	+	+	-	-	-	-
4	+	-	+	+	-	+	-	-	-	Prolonged	+	+	+	+	+	+	+	+	-
5	+	-	-	-	-	-	-	-	-	Short-term	+	+	+	-	-	-	-	-	-
6	+	-	-	-	-	-	+	-	-	Intermittent	+	+	+	-	-	-	+	+	+

*Week 0 culture results were obtained prior to study enrollment

Table 5.2: Mixed-effects linear regression analysis of associations of *Salmonella* shedding status (based on parallel interpretation of *Salmonella* culture results) and *Salmonella* shedding pattern with richness, Pielou's evenness, Shannon index, and inverse Simpson index at the phylum, class, order, family, and genus levels.

Taxonomic Level	Variable	Category	N	Richness			Pielou's Evenness			Shannon Index			Inverse Simpson Index		
				Coefficient	95% CI	*P*-value	Coefficient	95% CI	*P*-value	Coefficient	95% CI	*P*-value	Coefficient	95% CI	*P*-value
Phylum	*Salmonella* status	negative	23	ref	ref	0.03	ref	ref	0.46	ref	ref	0.02	ref	ref	0.05
		positive	25	-1.02	(-1.93, -0.15)		-0.01	(-0.02, 0.01)		-0.05	(-0.08, -0.01)		-0.10	(-0.19, 0.00)	
	Salmonella shedding pattern	short-term	16	ref	ref	0.08	ref	ref	0.80	ref	ref	0.21	ref	ref	0.87
		intermittent	16	-0.50	(-1.76, 0.76)		0.01	(-0.01, 0.02)		0.00	(-0.06, 0.06)		-0.01	(-0.20, 0.18)	
		prolonged	16	-2.63	(-3.89, -1.36)		0.00	(-0.01, 0.02)		-0.07	(-0.13, -0.01)		-0.06	(-0.25, 0.13)	
Class	*Salmonella* status	negative	23	ref	ref	0.19	ref	ref	0.68	ref	ref	0.16	ref	ref	0.45
		positive	25	-0.86	(-2.17, 0.40)		0.00	(-0.01, 0.01)		-0.03	(-0.06, 0.01)		-0.05	(-0.18, 0.08)	
	Salmonella shedding pattern	short-term	16	ref	ref	0.12	ref	ref	0.81	ref	ref	0.38	ref	ref	0.89
		intermittent	16	-1.31	(-3.17, 0.55)		0.01	(-0.01, 0.03)		-0.01	(-0.10, 0.09)		0.00	(-0.38, 0.37)	
		prolonged	16	-3.44	(-5.30, -1.58)		0.00	(-0.02, 0.02)		-0.08	(-0.17, 0.01)		-0.10	(-0.48, 0.28)	
Order	*Salmonella* status	negative	23	ref	ref	0.84	ref	ref	0.79	ref	ref	0.80	ref	ref	0.21
		positive	25	-0.17	(-1.96, 1.45)		0.00	(-0.01, 0.01)		0.00	(-0.04, 0.03)		0.12	(-0.07, 0.30)	
	Salmonella shedding pattern	short-term	16	ref	ref	<0.001	ref	ref	0.08	ref	ref	0.05	ref	ref	0.13
		intermittent	16	-3.38	(-5.15, -1.60)		0.02	(0.01, 0.03)		0.02	(-0.03, 0.07)		0.15	(-0.29, 0.59)	
		prolonged	16	-5.25	(-7.02, -3.48)		-0.01	(-0.02, 0.01)		-0.11	(-0.17, -0.06)		-0.59	(-1.03, -0.15)	

Taxonomic Level	Variable	Category	N	Richness			Pielou's Evenness			Shannon Index			Inverse Simpson Index		
Family	*Salmonella* status	negative	23	ref	ref	0.11	ref	ref	0.94	ref	ref	0.33	ref	ref	0.36
		positive	25	-1.93	(-4.36, 0.38)		0.00	(-0.01, 0.01)		-0.02	(-0.06, 0.02)		-0.34	(-1.07, 0.39)	
	Salmonella shedding pattern	short-term	16	ref	ref	0.04	ref	ref	<0.001	ref	ref	0.04	ref	ref	0.05
		intermittent	16	-3.13	(-5.76, -0.49)		0.02	(0.01, 0.03)		0.05	(-0.01, 0.11)		1.48	(0.33, 2.63)	
		prolonged	16	-8.13	(-10.76, -5.49)		0.00	(-0.01, 0.01)		-0.12	(-0.18, -0.06)		-1.50	(-2.65, -0.35)	
Genus	*Salmonella* status	negative	23	ref	ref	0.72	ref	ref	0.65	ref	ref	0.86	ref	ref	0.94
		positive	25	-0.71	(-4.79, 3.06)		0.00	(-0.01, 0.01)		0.00	(-0.04, 0.05)		-0.05	(-1.24, 1.09)	
	Salmonella shedding pattern	short-term	16	ref	ref	0.13	ref	ref	0.93	ref	ref	0.59	ref	ref	0.81
		intermittent	16	-10.06	(-16.74, -3.39)		0.01	(-0.02, 0.03)		-0.07	(-0.23, 0.08)		-0.67	(-5.48, 4.15)	
		prolonged	16	-10.75	(-17.42, -4.08)		0.00	(-0.02, 0.02)		-0.10	(-0.25, 0.05)		-1.96	(-6.77, 2.86)	

Table 5.3: Effects of *Salmonella* status and *Salmonella* shedding pattern on beta diversity (Bray-Curtis distance) of equine fecal microbial communities based on permutational multivariate analysis of variance (PERMANOVA).

Taxonomic level	Variable	PERMANOVA			Beta-dispersion
		R^2	F	*P*	*P*
Phylum	*Salmonella* status	0.02	0.84	0.76	0.12
	Salmonella shedding pattern	0.24	7.14	0.38	0.82
Class	*Salmonella* status	0.02	1.12	0.64	0.26
	Salmonella shedding pattern	0.21	5.93	0.80	0.28
Order	*Salmonella* status	0.02	0.99	0.69	0.71
	Salmonella shedding pattern	0.18	4.84	0.59	0.96
Family	*Salmonella* status	0.03	1.39	0.50	0.54
	Salmonella shedding pattern	0.23	6.72	0.61	0.09
Genus	*Salmonella* status	0.03	1.33	0.49	0.24
	Salmonella shedding pattern	0.24	7.18	0.36	0.21

Figures

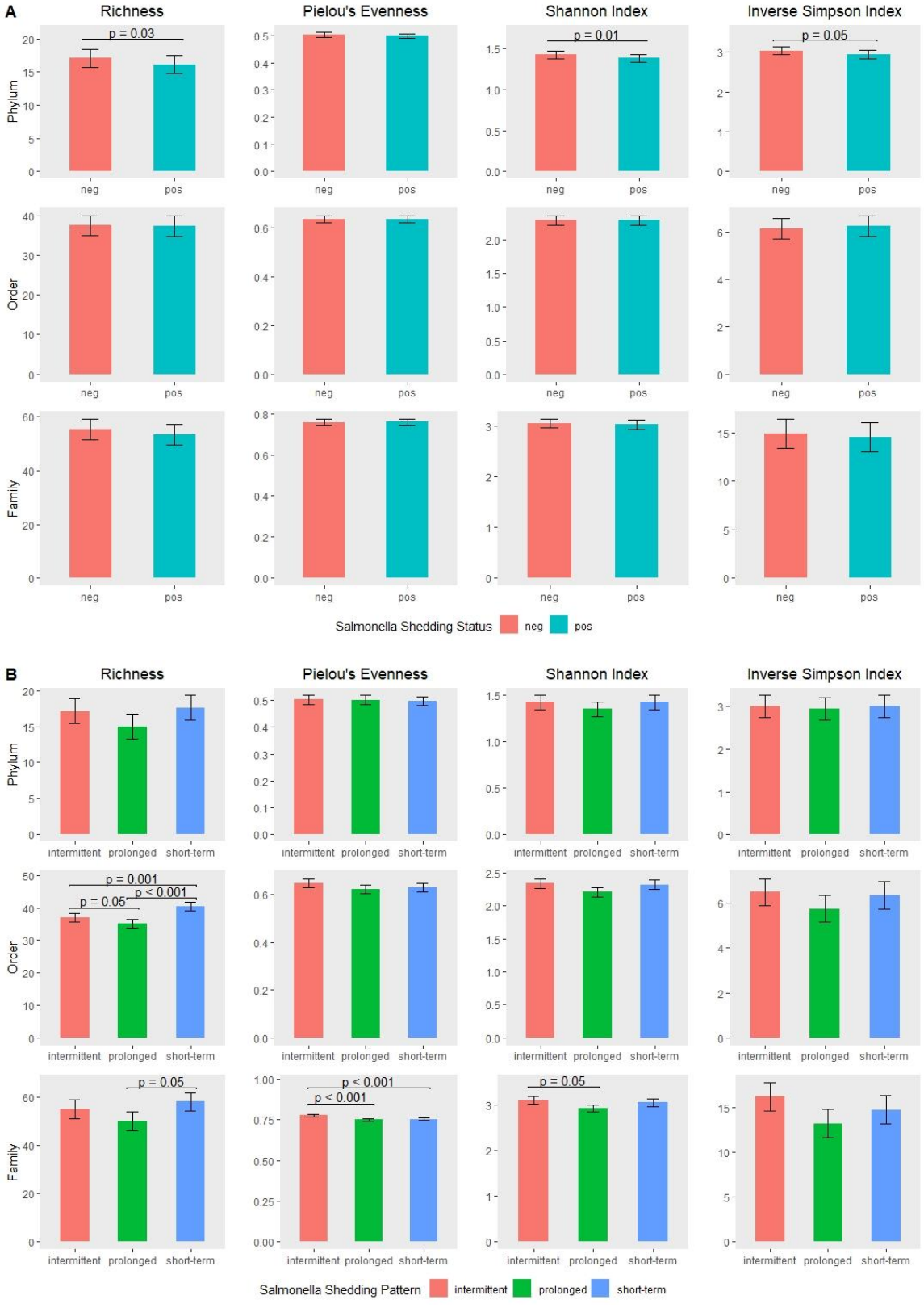

A
Richness
Pielou's Evenness
Shannon Index
Inverse Simpson Index
p = 0.03
p = 0.01
p = 0.05
Phylum
Order
Family
neg
pos
Salmonella Shedding Status
B
intermittent
prolonged
short-term
p = 0.001
p < 0.001
p = 0.05
Salmonella Shedding Pattern

Figure 5.1: Bar plots of marginal means of alpha diversity indices (richness, Pielou's evenness, Shannon index, and inverse Simpson index) by **(A)** *Salmonella* shedding status and **(B)** *Salmonella* shedding pattern at the phylum, order, and family levels. Error bars represent 95% confidence intervals of marginal means. P-values are displayed for comparisons with statistically significant differences in marginal means ($P < 0.05$).

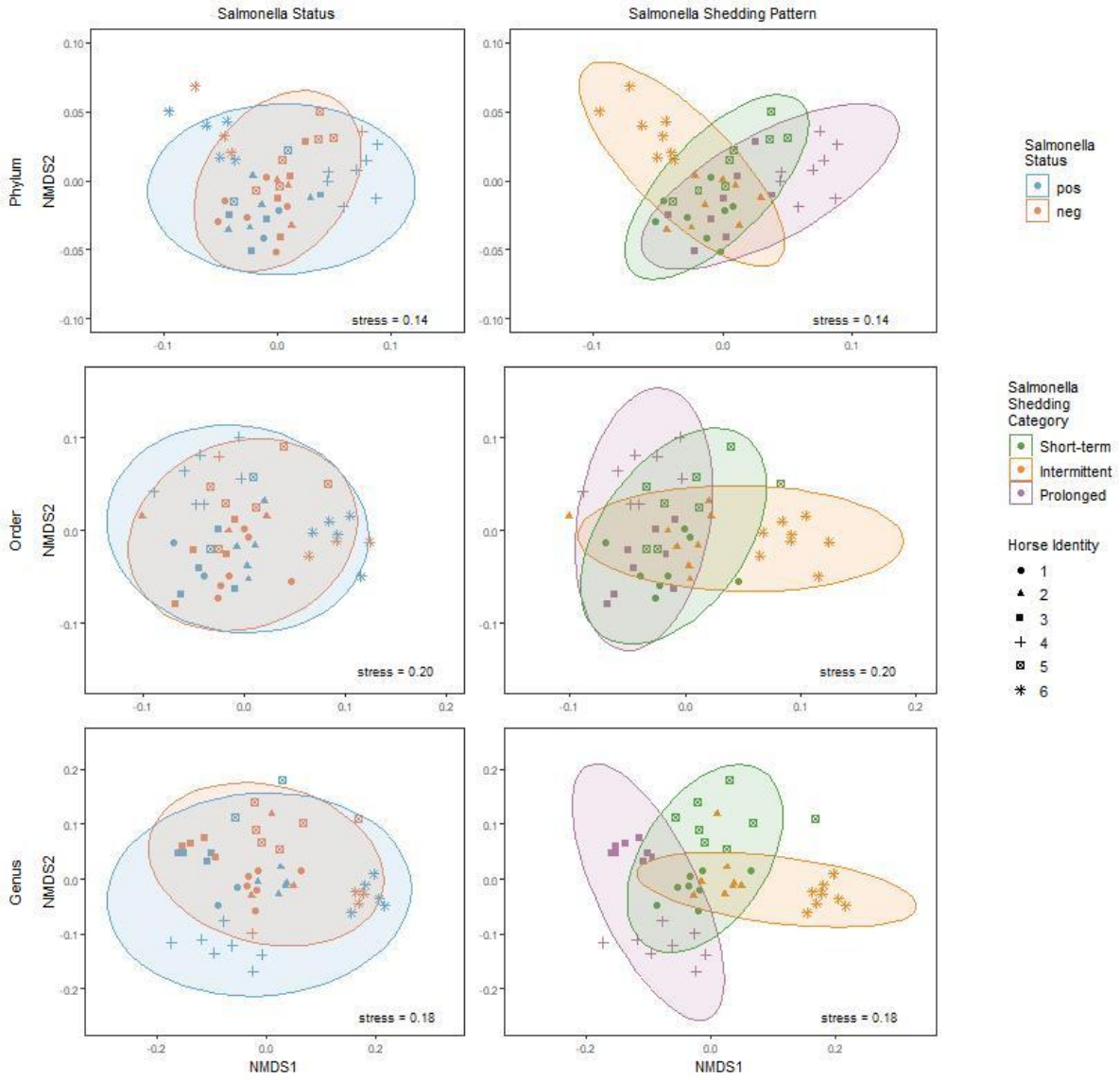
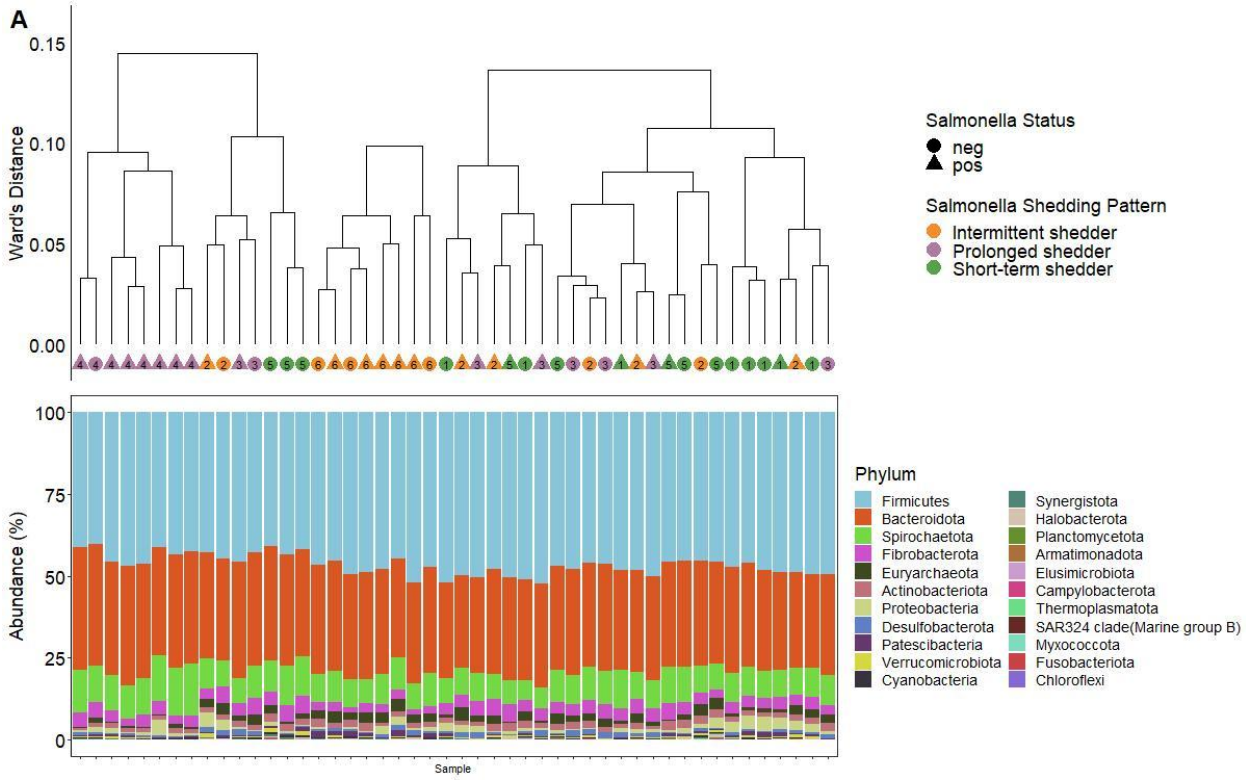


Figure 5.2: Non-metric multidimensional scaling (NMDS) ordination plots of Bray-Curtis distances for *Salmonella* shedding status and *Salmonella* shedding pattern at the phylum, order, and genus levels. Ellipses represent 95% confidence intervals of the group means.


A
Ward's Distance
0.15
0.10
0.05
0.00
Salmonella Status
neg
pos
Salmonella Shedding Pattern
Intermittent shedder
Prolonged shedder
Short-term shedder
Abundance (%)
100
75
50
25
0
Sample
Phylum
Firmicutes
Bacteroidota
Spirochaetota
Fibrobacterota
Euryarchaeota
Actinobacteriota
Proteobacteria
Desulfobacterota
Patescibacteria
Verrucomicrobiota
Cyanobacteria
Synergistota
Halobacterota
Planctomycetota
Armatimonadota
Elusimicrobiota
Campylobacterota
Thermoplasmatota
SAR324 clade(Marine group B)
Myxococcota
Fusobacteriota
Chloroflexi

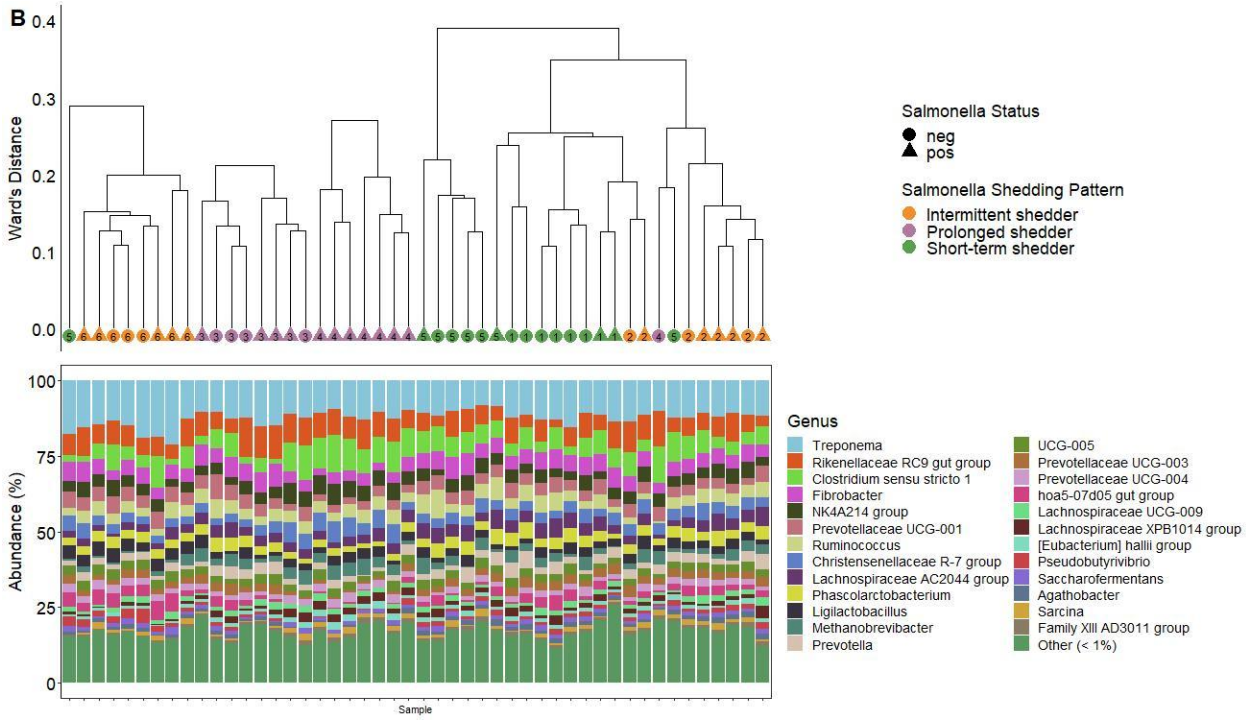

B
Ward's Distance
0.4
0.3
0.2
0.1
0.0
Salmonella Status
neg
pos
Salmonella Shedding Pattern
Intermittent shedder
Prolonged shedder
Short-term shedder
Abundance (%)
100
75
50
25
0
Sample
Genus
Treponema
Rikenellaceae RC9 gut group
Clostridium sensu stricto 1
Fibrobacter
NK4A214 group
Prevotellaceae UCG-001
Ruminococcus
Christensenellaceae R-7 group
Lachnospiraceae AC2044 group
Phascolarctobacterium
Ligilactobacillus
Methanobrevibacter
Prevotella
UCG-005
Prevotellaceae UCG-003
Prevotellaceae UCG-004
hoa5-07d05 gut group
Lachnospiraceae UCG-009
Lachnospiraceae XPB1014 group
[Eubacterium] hallii group
Pseudobutyrivibrio
Saccharofermentans
Agathobacter
Sarcina
Family XIII AD3011 group
Other (< 1%)

Figure 5.3: Hierarchical clustering (Ward's distance) based on beta diversity (Bray-Curtis distance) of microbial communities from horses with subclinical *Salmonella* infections, and stacked bar plots demonstrating the relative abundance of microbial taxa in each sample at the **(A)** phylum and **(B)** genus level. Numbers (1-6) within the colored shapes associated with each sample represent horse identity.

CHAPTER 6

CONCLUSIONS

Salmonella infections in horses are not a novel challenge. Outbreaks of *Salmonella* among equine populations have plagued veterinary hospitals and other equine facilities for decades, spurring numerous investigations into risk factors for infection, and prompting the development of tailored biosecurity and infection control practices. However, in spite of these efforts, prevention of *Salmonella* infections in these facilities remains a challenge today. This body of work aimed to address two primary facets of this challenge – barriers to reliable detection of *Salmonella* in horses and a limited understanding of key components of the epidemiology of this disease.

In chapter two, we explored the current landscape of diagnostic testing for equine salmonellosis. Through a systematic review of the literature, we demonstrated that there is wide variability among enriched culture and PCR methods used to detect *Salmonella* in equine fecal samples. While this has been acknowledged previously,[44] this study was the first to quantify and detail the number and types of diagnostic test methods used across studies. This variability, in addition to incomplete reporting of study methods, hindered the estimation of summary estimates of the sensitivity and specificity of these tests. Therefore, while this review did provide an overview of the performance of fecal culture and PCR for the detection of *Salmonella* in horses, more importantly, it serves as a call to action for future investigations. That is, investigators should adhere to rigorous standards for reporting test methods, study population demographics, and approaches to data analysis to facilitate future comparisons across studies and allow clinicians and laboratorians to better understand the applicability of a given study to the equine

populations they serve. Additionally, standardization of diagnostic tests for equine salmonellosis would improve our ability to adopt clinical recommendations based on the demonstrated reliability of these tests.

To develop recommendations on the application of these tests, however, we must first generate reliable estimates of test performance – a need directly addressed by chapter three, an assessment of the clinical performance of a lateral flow immunoassay, culture, and PCR for the detection of *Salmonella* in equine feces. In this study, we not only demonstrated the utility of a novel screening test for equine salmonellosis, but also provided estimates of the sensitivity and specificity of a PCR assay and two culture methods for *Salmonella* detection in horses. Notably, by using a Bayesian latent class analysis, we generated these estimates in a manner that overcomes a key challenge of diagnostic test assessments for *Salmonella* in horses – the lack of a gold standard test. Through our analysis, we demonstrated that the PCR assay under evaluation is both highly sensitive and specific, while among culture methods, tetrathionate-enriched culture was superior to selenite-enriched culture in terms of both sensitivity and specificity. Additionally, the Reveal® 2.0 lateral flow immunoassay was moderately sensitive and specific, and its sensitivity was improved when used in a parallel testing strategy. Given the rapid turnaround time afforded by this test, it could be a valuable screening tool for equine hospitals and other facilities.

In chapters four and five, we took a step back from the diagnosis of equine salmonellosis to address remaining gaps in our understanding of the epidemiology of this disease. To date, the duration of shedding of *Salmonella* in infected horses has been poorly described, making it challenging for clinicians to provide evidence-based guidance to horse owners regarding appropriate management of these horses upon return to their home facility. We demonstrated

that, on average, horses shed *Salmonella* for approximately two weeks. However, horses with clinical salmonellosis tend to shed the bacteria longer than those with subclinical salmonellosis. Additionally, increased shedding duration was not only associated with classic clinical signs of salmonellosis (i.e., diarrhea), but also with the clinical involvement of any body system. Therefore, equine clinicians should be aware and advise clients of the risk of prolonged *Salmonella* shedding among any horses experiencing signs of disease.

This investigation provided crucial insight into a poorly understood aspect of equine salmonellosis, and the final study in this body of work, chapter five, continued this endeavor by exploring an often-discussed but infrequently studied facet of this disease – the relationship between *Salmonella* shedding and the equine gastrointestinal microbiome. By studying a population of horses with subclinical *Salmonella* infections managed under nearly identical conditions, we were able to demonstrate that while there were no drastic differences between or changes in the fecal microbiome of these horses over time, consistent, subtle differences were observed between horses with different *Salmonella* shedding patterns over time (i.e., short-term, intermittent, and prolonged). While this was a small study, so our ability to generalize these findings to other equine populations is limited, we have provided preliminary evidence that in horses with subclinical salmonellosis, the composition of the gastrointestinal microbiome may be more closely associated with overall shedding pattern than with *Salmonella* positivity or negativity at a given point in time. However, the directionality of this relationship is unclear and should be addressed by future research.

In summary, this collection of research tackles several lingering challenges to understanding and managing *Salmonella enterica* in equine populations. We have demonstrated the utility of new and existing diagnostic tests for *Salmonella* detection, characterized key

aspects of the natural history and epidemiology of *Salmonella* infections, and opened the door for continued exploration into the microbial ecology of salmonellosis in horses. Taken together, this body of research serves as both a toolkit and a springboard for improved understanding and management of this pathogen in equine populations.

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APPENDIX 1: SUPPLEMENTARY TABLES

Supplementary Table 2.1: Serotypes identified by at least one test among diagnostic test comparisons included in the review, as reported in the publication (N = 30).

Study	Comparison	Serotypes
Babu 2008	a	4,5,12,27: r,i: 1,5 Drogana Lagos Kottbus Bovismorbificans Dumfries Tshiongwe I. 3, 10, 15: r:- I. 6,7: y: l,z_{28} *S. enterica* ssp *salamae* 6,7:g t: z_{42}
Babu 2008	b	4,5,12,27: r,i: 1,5 Drogana Lagos Kottbus Bovismorbificans Dumfries Tshiongwe I. 3, 10, 15: r:- I. 6,7: y: l,z_{28} *S. enterica* ssp *salamae* 6,7:g t: z_{42}
Babu 2008	c	4,5,12,27: r,i: 1,5 Drogana Lagos Kottbus Bovismorbificans Dumfries Tshiongwe I. 3, 10, 15: r:- I. 6,7: y: l,z_{28} *S. enterica* ssp *salamae* 6,7:g t: z_{42}
Babu 2008	d	4,5,12,27: r,i: 1,5 Drogana Lagos Kottbus Bovismorbificans Dumfries Tshiongwe I. 3, 10, 15: r:- I. 6,7: y: l,z_{28} *S. enterica* ssp *salamae* 6,7:g t: z_{42}
Bohaychuk 2007	a	NR
Bohaychuk 2007	b	Typhimurium Rubislaw
Bohaychuk 2007	c	Typhimurium Rubislaw

Braga 2023	a	Infantis Minnesota I.4,5,12:i:- Anatum Cerro Oranienburg Braenderup Give Newport IIIb 61:c:z35 I.:O9:-:1,5 I.4,12:d:- I.6.8:-:-
Braga 2023	b	Infantis Minnesota I.4,5,12:i:- Anatum Cerro Oranienburg Braenderup Give Newport IIIb 61:c:z35 I.:O9:-:1,5 I.4,12:d:- I.6.8:-:-
Burgess 2014	a	Typhimurium Typhimurium var. 5 Mbandaka Montevideo Muenchen Newport Kentucky Meleagridis Muenster Cerro
Burgess 2014	b	Typhimurium Typhimurium var. 5 Mbandaka Montevideo Muenchen Newport Kentucky Meleagridis Muenster Cerro
Burgess 2014	c	Typhimurium Typhimurium var. 5 Mbandaka Montevideo Muenchen

		Newport Kentucky Meleagridis Muenster Cerro
Burgess 2015	a	Typhimurium
Burgess 2015	b	Typhimurium
Cohen 1994	*NA*	Enteritidis Anatum Derby Heidelberg Newport Typhimurium
Cohen 1995	a	Enteritidis
Cohen 1995	b	Enteritidis
Cohen 1996	*NA*	NR
Ekiri 2016	*NA*	Give_var._15+,34+ Newport IV Rough O: autoagglutinate Inverness Montevideo Saintpaul Unknown Gaminara Anatum Rubislaw Tallahassee Memphis Derby
Fakour 2020	*NA*	Typhimurium
Owen 1979	*NA*	Typhimurium
Pusterla 2010	*NA*	Newport Braenderup Cerro Poona
Pusterla 2014	a	NR
Pusterla 2014	b	NR
Pusterla 2023	*NA*	NR
Ramin 2012	*NA*	NR
Singh 2007	*NA*	Paratyphi B var Java I. 4,5,12,27 : r,I : 1,5 Drogana Newpor Saintpaul Lagos Typhimurium Kottbus Bovismorbificans Dumfries Tshiongwe Weltevreden (monophasic)

		S. enterica ssp *salamae* II: 6, 7: g,t: z_{42}
Slovis 2014	*NA*	Typhimurium
Stone 1994	*NA*	Havana
Ward 2005	*NA*	NR

NR = not reported

Supplementary Table 3.1: Median and 95% credible interval (CI) of posterior estimates of test sensitivity (Sn) and specificity (Sp) for tetrathionate-enriched culture (TEC), selenite-enriched culture (SEC), qPCR, and the Reveal® 2.0 lateral flow immunoassay (LFI), and *S. enterica* prevalence estimates for the high-, low-, and intermediate-prevalence populations, obtained from a Bayesian latent class model with the specified prior distributions under the assumption of conditional independence between tests.

Parameter	Prior distribution	Median (%)	95% CI (%)	Effective sample size
TEC Sn	β (21.3, 10.8)	69.2	56.6, 80.6	30,000
TEC Sp	β (59.0, 14.3)	97.5	96.1, 98.6	30,000
SEC Sn	β (26.6, 20.9)	61.4	50.3, 71.8	3700
SEC Sp	β (67.8, 19.7)	91.0	88.7, 93.0	13,000
qPCR Sn	β (24.2, 7.0)	87.2	77.0, 94.2	7600
qPCR Sp	β (67.4, 7.2)	96.7	95.0, 98.1	11,000
LFI Sn	β (20.7, 16.1)	60.5	48.8, 71.5	6600
LFI Sp	β (73.5, 23.3)	68.1	64.6, 71.5	25,000
Prevalence (High)	β (1, 1)	18.5	11.0, 27.4	30,000
Prevalence (Intermediate)	β (1, 1)	7.6	3.6, 13.6	30,000
Prevalence (Low)	β (1, 1)	3.0	1.5, 5.1	30,000

Supplementary Table 3.2: Summary results of conditional dependence Bayesian latent class models estimating the sensitivity (Sn) and specificity (Sp) of tetrathionate-enriched culture (TEC), selenite-enriched culture (SEC), qPCR, and the Reveal® 2.0 lateral flow immunoassay (LFI); prevalence of *Salmonella* in the low-, intermediate-, and high-prevalence populations; and the negative (CovN) and positive (CovP) covariance between TEC and SEC, with sensitivity analyses evaluating the impact of informative prior distributions.

Parameter	Model															
	Conditional dependence model				Sensitivity analysis 1 (Relaxed prior distributions)				Sensitivity analysis 2 (Decreased mode of prior distributions)				Sensitivity analysis 3 (Uniform prior distributions)			
	Prior distribution	Median	95% CI	Effective sample size	Prior distribution	Median	95% CI	Effective sample size	Prior distribution	Median	95% CI	Effective sample size	Prior distribution	Median	95% CI	Effective sample size
TEC Sn	β (21.3, 10.8)	66.1%	53.8%, 78.0%	150,000	β (3.1, 2.0)	63.7%	47.4%, 81.4%	190,000	β (87.4, 65.1)	57.0%	50.0%, 64.1%	300,000	β (1, 1)	61.6%	44.7%, 81.3%	36,000
TEC Sp	β (59.0, 14.3)	97.2%	95.7%, 98.3%	100,000	β (5.8, 2.1)	99.2%	98.2%, 99.8%	13,000	β (100.0, 40.8)	92.8%	90.6%, 94.6%	160,000	β (1, 1)	99.5%	98.6%, 100.0%	30,000
SEC Sn	β (26.6, 20.9)	58.4%	47.7%, 69.0%	93,000	β (3.8, 3.2)	58.8%	43.7%, 81.4%	230,000	β (100.0, 115.8)	47.5%	41.5%, 53.6%	98,000	β (1, 1)	57.3%	41.5%, 75.3%	67,000
SEC Sp	β (67.8, 19.7)	89.8%	87.4%, 92.0%	150,000	β (4.1, 1.9)	92.4%	90.1%, 94.3%	59,000	β (100, 47.4)	84.6%	81.9%, 87.2%	71,000	β (1, 1)	92.6%	90.4%, 94.5%	300,000
qPCR Sn	β (24.2, 7.0)	87.5%	77.3%, 94.5%	190,000	β (1, 1)	95.0%	80.7%, 99.8%	74,000	β (100, 44.7)	73.4%	66.5%, 79.7%	300,000	β (1, 1)	93.3%	78.1%, 99.7%	260,000
qPCR Sp	β (67.4, 7.2)	97.1%	95.3%, 98.4%	160,000	β (9.9, 1.8)	98.2%	96.2%, 99.5%	230,000	β (26.2, 6.7)	96.9%	95.0%, 98.4%	300,000	β (1, 1)	98.5%	96.5%, 99.8%	100,000
LFI Sn	β (20.7, 16.1)	59.8%	48.3%, 70.9%	110,000	β (1, 1)	60.6%	45.1%, 75.8%	140,000	β (100.0, 114.0)	48.9%	42.7%, 55.1%	82,000	β (1, 1)	59.8%	44.6%, 75.1%	50,000
LFI Sp	β (73.5, 23.3)	68.2%	64.6%, 71.5%	300,000	β (3.4, 1.7)	66.9%	63.2%, 70.6%	300,000	β (29.8, 15.5)	66.8%	63.1%, 70.3%	300,000	β (1, 1)	66.9%	63.1%, 70.6%	290,000
Prev (High)	β (1, 1)	20.4%	12.4%, 29.9%	300,000	β (1, 1)	21.3%	13.0%, 30.9%	300,000	β (1, 1)	23.1%	14.3%, 33.2%	300,000	β (1, 1)	22.0%	13.3%, 31.6%	48,000
Prev (Inter)	β (1, 1)	8.1%	3.9%, 14.3%	290,000	β (1, 1)	8.3%	4.1%, 14.4%	140,000	β (1, 1)	8.8%	4.0%, 15.5%	75,000	β (1, 1)	8.6%	4.3%, 14.7%	300,000
Prev (Low)	β (1, 1)	2.9%	1.4%, 5.1%	300,000	β (1, 1)	3.3%	1.6%, 5.7%	300,000	β (1, 1)	3.0%	1.2%, 5.5%	300,000	β (1, 1)	3.6%	1.8%, 6.3%	290,000
CovN	Unif (a, b)*	0.015	0.003, 0.030	58,000	Unif (a, b)*	0.003	0.00002, 0.01	20,000	Unif (a, b)*	0.052	0.032, 0.070	92,000	Unif (a, b)*	0.002	-0.00004, 0.008	31,000
CovP	Unif (c, d)†	0.082	-0.003, 0.151	220,000	Unif (c, d)†	0.086	-0.0008, 0.153	300,000	Unif (c, d)†	0.109	0.031, 0.170	190,000	Unif (c, d)†	0.091	0.002, 0.156	72,000

*$a = (1 - Sn_{TEC})*(Sn_{SEC} - 1)$; $b = \min(Sn_{TEC}, Sn_{SEC}) - (Sn_{TEC} * Sn_{SEC})$

†$c = (Sp_{TEC} - 1)*(1 - Sp_{SEC})$; $d = \min(Sp_{TEC}, Sp_{SEC}) - (Sp_{TEC} * Sp_{SEC})$

Supplementary Table 5.1: Results of univariable linear regression analysis of associations with sequencing depth.

Variable	**Category**	**Coefficient**	**95% CI**	**P-value**
Horse identity	1	Reference		< 0.001
	2	-23075	(-45041.34, -1109.41)	
	3	-58422	(-80387.47, -36455.53)	
	4	-65613	(-87578.47, -43646.53)	
	5	1355	(-20611.09, 23320.84)	
	6	-40070	(-62035.59, -18103.66)	
Salmonella status	Positive	Reference		0.06
	Negative	-18183	(-37118.89, 753.03)	
Salmonella shedding pattern	Intermittent	Reference		< 0.001
	Short-term	32250	(16767.29, 47732.59)	
	Prolonged	-30445	(-45927.15, -14961.85)	

Supplementary Table 5.2: Results of generalized linear mixed models evaluating phylum-level differential abundance by *Salmonella* shedding status.

Feature	Value	Coefficient	Standard Error	P-value (unadjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Euryarchaeota	pos	-0.70	0.21	0.002	0.045	48	48
Actinobacteriota	pos	-0.23	0.11	0.036	0.185	48	48
Firmicutes	pos	0.06	0.03	0.029	0.185	48	48
Planctomycetota	pos	-0.58	0.28	0.044	0.185	48	36
SAR324.clade.Marine.group.B.	pos	-0.39	0.17	0.026	0.185	48	6
Halobacterota	pos	-0.58	0.29	0.054	0.188	48	37
Armatimonadota	pos	0.67	0.40	0.099	0.210	48	37
Bacteroidota	pos	-0.04	0.02	0.103	0.210	48	48
Cyanobacteria	pos	-0.65	0.36	0.081	0.210	48	48
Myxococcota	pos	-0.36	0.19	0.073	0.210	48	13
Patescibacteria	pos	-0.52	0.32	0.110	0.210	48	48
Elusimicrobiota	pos	-1.04	0.73	0.160	0.280	48	30
Campylobacterota	pos	-0.64	0.47	0.182	0.294	48	29
Synergistota	pos	0.28	0.28	0.324	0.486	48	47
Desulfobacterota	pos	0.19	0.21	0.379	0.531	48	48
Chloroflexi	pos	-0.17	0.22	0.435	0.571	48	11
Fibrobacterota	pos	0.02	0.10	0.802	0.935	48	48
Spirochaetota	pos	-0.02	0.07	0.770	0.935	48	48
Proteobacteria	pos	0.01	0.36	0.982	0.982	48	48
Thermoplasmatota	pos	-0.02	0.43	0.968	0.982	48	19
Verrucomicrobiota	pos	-0.05	0.35	0.889	0.982	48	48

Supplementary Table 5.3: Results of generalized linear mixed models evaluating class-level differential abundance by *Salmonella* shedding status.

Feature	Value	Coefficient	Standard Error	P-value (unadjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Methanobacteria	pos	-0.70	0.21	0.002	0.067	48	48
Bacilli	pos	0.38	0.14	0.009	0.139	48	48
Coriobacteriia	pos	-0.25	0.10	0.020	0.176	48	48
Gracilibacteria	pos	-0.84	0.36	0.023	0.176	48	46
vadinHA49	pos	-0.58	0.28	0.044	0.273	48	36
Methanomicrobia	pos	-0.58	0.29	0.054	0.277	48	37
Myxococcia	pos	-0.36	0.19	0.073	0.284	48	13
Elusimicrobia	pos	-1.09	0.59	0.070	0.284	48	15
Bacteroidia	pos	-0.04	0.02	0.103	0.321	48	48
Vampirivibrionia	pos	-0.64	0.39	0.103	0.321	48	47
Clostridia	pos	0.05	0.03	0.145	0.401	48	48
MVP.15	pos	-0.61	0.42	0.155	0.401	48	43
Campylobacteria	pos	-0.64	0.47	0.182	0.434	48	29
Synergistia	pos	0.28	0.28	0.324	0.696	48	47
Endomicrobia	pos	-0.32	0.33	0.337	0.696	48	25
Desulfovibrionia	pos	-0.21	0.38	0.595	0.837	48	46
Alphaproteobacteria	pos	-0.22	0.30	0.469	0.837	48	48
Desulfotomaculia	pos	0.21	0.33	0.530	0.837	48	15
Syntrophomonadia	pos	-0.12	0.24	0.621	0.837	48	17
Anaerolineae	pos	-0.17	0.22	0.435	0.837	48	11
Cyanobacteriia	pos	-0.17	0.28	0.549	0.837	48	7
Actinobacteria	pos	-0.25	0.48	0.608	0.837	48	36
Kiritimatiellae	pos	0.11	0.16	0.509	0.837	48	6
Fibrobacteria	pos	0.02	0.10	0.802	0.956	48	48
Verrucomicrobiae	pos	-0.07	0.35	0.833	0.956	48	48
Desulfuromonadia	pos	0.09	0.28	0.758	0.956	48	47
Saccharimonadia	pos	0.12	0.52	0.820	0.956	48	45
Gammaproteobacteria	pos	-0.02	0.43	0.967	0.968	48	48
Spirochaetia	pos	0.00	0.07	0.966	0.968	48	48
Negativicutes	pos	-0.01	0.06	0.901	0.968	48	48
Thermoplasmata	pos	-0.02	0.43	0.968	0.968	48	19

Supplementary Table 5.4: Results of generalized linear mixed models evaluating order-level differential abundance by *Salmonella* shedding status.

Feature	Value	Coefficient	Standard Error	P-value (unadjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Lactobacillales	pos	0.42	0.13	0.003	0.072	48	48
Methanobacteriales	pos	-0.70	0.21	0.002	0.072	48	48
Coriobacteriales	pos	-0.25	0.10	0.020	0.301	48	48
Absconditabacteriales..SR1.	pos	-0.84	0.36	0.023	0.301	48	46
Paenibacillales	pos	0.30	0.14	0.039	0.384	48	6
Corynebacteriales	pos	-0.70	0.34	0.044	0.384	48	17
Methanomicrobiales	pos	-0.58	0.29	0.054	0.388	48	37
PB19	pos	0.41	0.21	0.060	0.388	48	16
Myxococcales	pos	-0.36	0.19	0.073	0.388	48	13
Elusimicrobiales	pos	-1.09	0.59	0.070	0.388	48	15
Bacteroidales	pos	-0.04	0.02	0.110	0.430	48	48
Christensenellales	pos	-0.15	0.09	0.100	0.430	48	48
Gastranaerophilales	pos	-0.64	0.39	0.103	0.430	48	47
Burkholderiales	pos	-0.63	0.39	0.114	0.430	48	39
Micrococcales	pos	0.66	0.45	0.145	0.512	48	17
Campylobacterales	pos	-0.64	0.47	0.182	0.536	48	29
Peptococcales	pos	0.57	0.41	0.174	0.536	48	32
Pedosphaerales	pos	-0.40	0.30	0.182	0.536	48	10
Clostridia.UCG.014	pos	-0.48	0.40	0.235	0.655	48	40
Lachnospirales	pos	0.08	0.07	0.252	0.667	48	48
Clostridiales	pos	0.20	0.20	0.329	0.714	48	48
Synergistales	pos	0.28	0.28	0.324	0.714	48	47
Rhodospirillales	pos	-0.51	0.52	0.336	0.714	48	37
Endomicrobiales	pos	-0.32	0.33	0.337	0.714	48	25
Propionibacteriales	pos	0.20	0.20	0.316	0.714	48	6
Erysipelotrichales	pos	-0.21	0.23	0.367	0.721	48	48
Izemoplasmatales	pos	0.37	0.40	0.366	0.721	48	42
Anaerolineales	pos	-0.17	0.22	0.435	0.823	48	11
Oscillospirales	pos	0.03	0.05	0.533	0.839	48	48
Bacillales	pos	0.28	0.41	0.501	0.839	48	47
Eubacteriales	pos	0.20	0.33	0.554	0.839	48	47
Desulfotomaculales	pos	0.21	0.33	0.530	0.839	48	15
Clostridia.vadinBB60.group	pos	0.27	0.41	0.509	0.839	48	42

Chloroplast	pos	-0.17	0.28	0.549	0.839	48	7
WCHB1.41	pos	0.11	0.16	0.509	0.839	48	6
Veillonellales.Selenomonadales	pos	-0.05	0.09	0.594	0.853	48	48
Desulfovibrionales	pos	-0.21	0.38	0.595	0.853	48	46
Syntrophomonadales	pos	-0.12	0.24	0.621	0.866	48	17
Fibrobacterales	pos	0.02	0.10	0.802	0.945	48	48
Bradymonadales	pos	0.08	0.28	0.784	0.945	48	47
Saccharimonadales	pos	0.12	0.52	0.820	0.945	48	45
Rickettsiales	pos	-0.15	0.59	0.804	0.945	48	32
Acholeplasmatales	pos	-0.17	0.46	0.711	0.945	48	40
Mycoplasmatales	pos	0.09	0.34	0.791	0.945	48	14
Monoglobales	pos	-0.13	0.40	0.755	0.945	48	30
Actinomycetales	pos	-0.10	0.31	0.756	0.945	48	7
Peptostreptococcales.Tissierellales	pos	0.01	0.08	0.856	0.952	48	48
Verrucomicrobiales	pos	-0.06	0.35	0.862	0.952	48	48
Enterobacterales	pos	-0.01	0.48	0.983	0.983	48	48
Spirochaetales	pos	0.00	0.07	0.966	0.983	48	48
Acidaminococcales	pos	0.00	0.09	0.963	0.983	48	48
Rhizobiales	pos	0.03	0.30	0.915	0.983	48	10
Methanomassiliicoccales	pos	-0.02	0.43	0.968	0.983	48	19

Supplementary Table 5.5: Results of generalized linear mixed models evaluating family-level differential abundance by *Salmonella* shedding status.

Feature	Value	Coefficient	Standard Error	P-value (unadjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Lactobacillaceae	pos	0.39	0.12	0.002	0.084	48	48
Methanobacteriaceae	pos	-0.70	0.21	0.002	0.084	48	48
Prevotellaceae	pos	-0.11	0.04	0.006	0.156	48	48
Methanocorpusculaceae	pos	-0.58	0.29	0.054	0.519	48	37
gir.aah93h0	pos	-0.84	0.41	0.045	0.519	48	29
M2PB4.65.termite.group	pos	-0.88	0.43	0.046	0.519	48	34
Myxococcaceae	pos	-0.36	0.19	0.073	0.519	48	13
Oxalobacteraceae	pos	-0.65	0.33	0.050	0.519	48	28
Paenibacillaceae	pos	0.30	0.14	0.039	0.519	48	6
Elusimicrobiaceae	pos	-1.09	0.59	0.070	0.519	48	15
Mycobacteriaceae	pos	-0.26	0.14	0.070	0.519	48	6
Christensenellaceae	pos	-0.15	0.09	0.100	0.639	48	48
Micrococcaceae	pos	0.70	0.43	0.109	0.639	48	16
Porphyromonadaceae	pos	-0.39	0.24	0.115	0.639	48	7
Lachnospiraceae	pos	0.09	0.07	0.205	0.652	48	48
Bacteroidales.BS11.gut.group	pos	-0.35	0.24	0.155	0.652	48	48
UCG.010	pos	0.18	0.12	0.133	0.652	48	48
Marinifilaceae	pos	-0.29	0.21	0.176	0.652	48	39
Coriobacteriales.Incertae.Sedis	pos	-0.62	0.47	0.188	0.652	48	44
Defluviitaleaceae	pos	-0.61	0.48	0.211	0.652	48	41
Muribaculaceae	pos	-0.67	0.53	0.214	0.652	48	37
Campylobacteraceae	pos	-0.64	0.47	0.182	0.652	48	29
CAP.aah99b04	pos	-0.39	0.28	0.174	0.652	48	17
Nocardiaceae	pos	-0.35	0.28	0.217	0.652	48	12
Peptococcaceae	pos	0.57	0.41	0.174	0.652	48	32
Pedosphaeraceae	pos	-0.40	0.30	0.182	0.652	48	10
Anaerofustaceae	pos	-0.48	0.42	0.252	0.728	48	34
Clostridiaceae	pos	0.20	0.20	0.329	0.773	48	48
Hungateiclostridiaceae	pos	-0.16	0.15	0.311	0.773	48	48
Bacteroidetes.BD2.2	pos	-0.31	0.30	0.295	0.773	48	36
Planococcaceae	pos	0.44	0.43	0.313	0.773	48	45
Synergistaceae	pos	0.28	0.28	0.324	0.773	48	47
Endomicrobiaceae	pos	-0.32	0.33	0.337	0.773	48	25
Nocardioidaceae	pos	0.20	0.20	0.316	0.773	48	6
Eggerthellaceae	pos	-0.09	0.10	0.370	0.802	48	48

Butyricicoccaceae	pos	0.36	0.40	0.368	0.802	48	32
Peptostreptococcaceae	pos	0.31	0.36	0.396	0.836	48	39
Paludibacteraceae	pos	-0.34	0.41	0.421	0.865	48	48
Anaerolineaceae	pos	-0.17	0.22	0.435	0.870	48	11
Streptococcaceae	pos	0.38	0.54	0.482	0.940	48	37
p.251.o5	pos	0.00	0.12	0.978	0.978	48	48
Rikenellaceae	pos	0.01	0.06	0.856	0.978	48	48
Oscillospiraceae	pos	0.00	0.06	0.950	0.978	48	48
Succinivibrionaceae	pos	0.20	0.64	0.759	0.978	48	46
Bacteroidales.UCG.001	pos	-0.07	0.18	0.708	0.978	48	48
Spirochaetaceae	pos	0.00	0.07	0.966	0.978	48	48
F082	pos	-0.02	0.08	0.770	0.978	48	48
Ruminococcaceae	pos	0.06	0.14	0.661	0.978	48	48
Anaerovoracaceae	pos	-0.02	0.08	0.795	0.978	48	48
Fibrobacteraceae	pos	0.02	0.10	0.802	0.978	48	48
Selenomonadaceae	pos	-0.05	0.09	0.562	0.978	48	48
Acidaminococcaceae	pos	0.00	0.09	0.963	0.978	48	48
X.Eubacterium..coprostanoligenes.group	pos	0.02	0.10	0.809	0.978	48	48
Dysgonomonadaceae	pos	-0.05	0.29	0.859	0.978	48	20
Akkermansiaceae	pos	-0.06	0.35	0.862	0.978	48	48
Bacteroidales.RF16.group	pos	-0.08	0.16	0.623	0.978	48	48
Bacillaceae	pos	0.15	0.37	0.691	0.978	48	47
Saccharimonadaceae	pos	0.24	0.54	0.661	0.978	48	44
Desulfovibrionaceae	pos	-0.21	0.38	0.595	0.978	48	46
Pasteurellaceae	pos	0.02	0.38	0.968	0.978	48	34
Eubacteriaceae	pos	0.19	0.39	0.620	0.978	48	45
Marinilabiliaceae	pos	-0.01	0.10	0.948	0.978	48	8
Atopobiaceae	pos	0.11	0.28	0.696	0.978	48	16
Enterobacteriaceae	pos	0.04	0.43	0.920	0.978	48	13
Erysipelatoclostridiaceae	pos	0.04	0.36	0.909	0.978	48	47
Erysipelotrichaceae	pos	-0.23	0.42	0.593	0.978	48	38
Desulfurisporaceae	pos	0.21	0.33	0.530	0.978	48	15
Acholeplasmataceae	pos	-0.17	0.46	0.711	0.978	48	40
Mycoplasmataceae	pos	0.09	0.34	0.791	0.978	48	14
Monoglobaceae	pos	-0.13	0.40	0.755	0.978	48	30
Syntrophomonadaceae	pos	-0.12	0.24	0.621	0.978	48	17
Methanomethylophilaceae	pos	-0.02	0.43	0.968	0.978	48	19
Ethanoligenenaceae	pos	0.20	0.32	0.530	0.978	48	13
Actinomycetaceae	pos	-0.10	0.31	0.756	0.978	48	7
Beijerinckiaceae	pos	-0.04	0.22	0.862	0.978	48	8
Sutterellaceae	pos	-0.25	0.42	0.560	0.978	48	16

Cellulomonadaceae	pos	0.06	0.14	0.646	0.978	48	5
Mitochondria	pos	-0.02	0.19	0.926	0.978	48	8

Supplementary Table 5.6: Results of generalized linear mixed models evaluating genus-level differential abundance by *Salmonella* shedding status.

Feature	Value	Coefficient	Standard Error	P-value (unadjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Ligilactobacillus	pos	0.34	0.12	0.006	0.157	48	48
Prevotella	pos	-0.39	0.13	0.004	0.157	48	48
Methanobrevibacter	pos	-0.70	0.21	0.002	0.157	48	48
Lactococcus	pos	0.87	0.31	0.007	0.157	48	9
Selenomonas	pos	-0.76	0.26	0.006	0.157	48	11
Butyrivibrio	pos	0.49	0.17	0.007	0.157	48	11
Prevotellaceae.UCG.003	pos	-0.22	0.10	0.043	0.511	48	48
Prevotellaceae.UCG.004	pos	-0.16	0.08	0.071	0.511	48	48
Alloprevotella	pos	-0.55	0.29	0.067	0.511	48	48
Methanocorpusculum	pos	-0.58	0.29	0.054	0.511	48	37
dgA.11.gut.group	pos	0.72	0.38	0.066	0.511	48	45
Lachnoclostridium	pos	0.45	0.24	0.066	0.511	48	37
Parvibacter	pos	-0.42	0.22	0.063	0.511	48	25
Weissella	pos	0.83	0.41	0.051	0.511	48	11
X.Eubacterium..nodatum.group	pos	-0.62	0.33	0.066	0.511	48	22
Paenibacillus	pos	0.30	0.14	0.039	0.511	48	6
Elusimicrobium	pos	-1.09	0.59	0.070	0.511	48	15
Aggregicoccus	pos	-0.34	0.17	0.055	0.511	48	5
Mycobacterium	pos	-0.26	0.14	0.070	0.511	48	6
Family.XIII.UCG.001	pos	-0.15	0.09	0.083	0.563	48	48
M2PT2.76.termite.group	pos	-0.75	0.43	0.086	0.563	48	21
Christensenellaceae.R.7.group	pos	-0.15	0.09	0.104	0.620	48	48
possible.genus.Sk018	pos	0.42	0.26	0.117	0.620	48	37
Lachnospiraceae.NK4A136.group	pos	0.52	0.32	0.114	0.620	48	25
Mailhella	pos	-0.76	0.48	0.118	0.620	48	34
Porphyromonas	pos	-0.39	0.24	0.115	0.620	48	7
Lactobacillus	pos	0.76	0.49	0.128	0.650	48	24
Frisingicoccus	pos	0.44	0.29	0.138	0.676	48	44
Saccharofermentans	pos	-0.23	0.17	0.179	0.756	48	48
Defluviitaleaceae.UCG.011	pos	-0.61	0.48	0.211	0.756	48	41
FD2005	pos	0.36	0.28	0.210	0.756	48	24
Campylobacter	pos	-0.64	0.47	0.182	0.756	48	29
Denitrobacterium	pos	-0.39	0.29	0.188	0.756	48	12
Rhodococcus	pos	-0.35	0.28	0.217	0.756	48	12
Arthrobacter	pos	0.60	0.43	0.167	0.756	48	15
Roseburia	pos	-0.42	0.34	0.221	0.756	48	17

DEV114	pos	-0.40	0.30	0.182	0.756	48	10
Aeromicrobium	pos	0.23	0.18	0.208	0.756	48	5
Lachnospiraceae.UCG.008	pos	0.49	0.36	0.174	0.756	48	18
Howardella	pos	-0.22	0.18	0.217	0.756	48	7
Family.XIII.AD3011.group	pos	-0.12	0.10	0.242	0.810	48	48
Anaerofustis	pos	-0.48	0.42	0.252	0.822	48	34
Kurthia	pos	0.37	0.32	0.259	0.826	48	28
Anaerovorax	pos	0.16	0.14	0.267	0.830	48	48
Clostridium.sensu.stricto.1	pos	0.19	0.21	0.361	0.890	48	48
Quinella	pos	-0.10	0.11	0.346	0.890	48	48
Terrisporobacter	pos	0.31	0.36	0.396	0.890	48	39
Prevotellaceae.Ga6A1.group	pos	0.31	0.35	0.379	0.890	48	42
Rummeliibacillus	pos	0.29	0.34	0.388	0.890	48	35
Acetitomaculum	pos	0.42	0.46	0.368	0.890	48	31
UCG.002	pos	0.24	0.23	0.302	0.890	48	48
UCG.009	pos	0.42	0.42	0.323	0.890	48	31
Ruminiclostridium	pos	0.37	0.39	0.348	0.890	48	28
Oscillibacter	pos	-0.41	0.41	0.324	0.890	48	17
Pyramidobacter	pos	-0.42	0.45	0.357	0.890	48	26
Catenisphaera	pos	-0.50	0.47	0.293	0.890	48	27
Endomicrobium	pos	-0.32	0.33	0.337	0.890	48	25
Shuttleworthia	pos	-0.24	0.24	0.334	0.890	48	10
Lachnospiraceae.UCG.006	pos	-0.43	0.50	0.393	0.890	48	20
Candidatus.Methanomethylophilus	pos	-0.20	0.22	0.374	0.890	48	9
X.Eubacterium..saphenum.group	pos	0.27	0.28	0.334	0.890	48	11
DNF00809	pos	-0.17	0.22	0.422	0.931	48	34
Synergistes	pos	0.31	0.39	0.428	0.931	48	29
Flexilinea	pos	-0.17	0.22	0.435	0.931	48	11
NK4A214.group	pos	-0.06	0.08	0.454	0.957	48	48
Pseudobutyrivibrio	pos	0.03	0.17	0.838	0.967	48	46
Rikenellaceae.RC9.gut.group	pos	0.00	0.08	0.953	0.967	48	48
Sarcina	pos	0.11	0.27	0.688	0.967	48	47
UCG.005	pos	-0.06	0.09	0.498	0.967	48	48
Lachnospiraceae.AC2044.group	pos	0.09	0.14	0.508	0.967	48	48
X.Eubacterium..hallii.group	pos	-0.06	0.10	0.539	0.967	48	48
Treponema	pos	0.01	0.07	0.945	0.967	48	48
Ruminococcus	pos	0.11	0.17	0.516	0.967	48	48
Fibrobacter	pos	0.02	0.10	0.802	0.967	48	48
Prevotellaceae.UCG.001	pos	0.06	0.09	0.513	0.967	48	48
Succinivibrio	pos	-0.03	0.59	0.955	0.967	48	27
Phascolarctobacterium	pos	0.00	0.09	0.963	0.967	48	48

Supplementary Table 5.7: Results of generalized linear mixed models evaluating phylum-level differential abundance by *Salmonella* shedding pattern with **(A)** short-term shedders as the reference level and **(B)** prolonged shedders as the reference level.

A Feature	Value	Coefficient	Standard Error	P-value (un-adjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Firmicutes	Prolonged shedder	-0.13	0.13	0.363	0.811	48	48
Euryarchaeota	Intermittent shedder	1.29	0.70	0.163	0.811	48	48
Actinobacteriota	Prolonged shedder	-0.53	0.29	0.164	0.811	48	48
Verrucomicrobiota	Prolonged shedder	-1.07	0.84	0.293	0.811	48	48
Desulfobacterota	Intermittent shedder	1.29	0.98	0.277	0.811	48	48
Desulfobacterota	Prolonged shedder	0.99	0.98	0.384	0.811	48	48
Halobacterota	Prolonged shedder	-3.43	1.38	0.088	0.811	48	37
Patescibacteria	Prolonged shedder	-1.38	1.04	0.275	0.811	48	48
Cyanobacteria	Prolonged shedder	-0.48	0.46	0.294	0.811	48	48
Proteobacteria	Intermittent shedder	-0.67	0.67	0.388	0.811	48	48
Proteobacteria	Prolonged shedder	-1.07	0.67	0.207	0.811	48	48
Myxococcota	Intermittent shedder	1.55	1.52	0.384	0.811	48	13
Campylobacterota	Intermittent shedder	-1.50	1.47	0.382	0.811	48	29
Campylobacterota	Prolonged shedder	-2.26	1.47	0.222	0.811	48	29
Synergistota	Prolonged shedder	-0.87	0.79	0.351	0.811	48	47
Planctomycetota	Prolonged shedder	-1.35	0.86	0.214	0.811	48	36
Armatimonadota	Intermittent shedder	-0.93	0.54	0.184	0.811	48	37
Armatimonadota	Prolonged shedder	-1.68	0.54	0.053	0.811	48	37
Thermoplasmatota	Intermittent shedder	-0.51	0.53	0.405	0.811	48	19
Thermoplasmatota	Prolonged shedder	-1.06	0.53	0.138	0.811	48	19
Elusimicrobiota	Intermittent shedder	-1.46	1.30	0.342	0.811	48	30
Fibrobacterota	Intermittent shedder	-0.27	0.31	0.441	0.842	48	48
Bacteroidota	Intermittent shedder	0.08	0.10	0.472	0.861	48	48
Firmicutes	Intermittent shedder	0.09	0.13	0.541	0.909	48	48
Chloroflexi	Intermittent shedder	-0.37	0.52	0.529	0.909	48	11
Euryarchaeota	Prolonged shedder	-0.43	0.70	0.585	0.918	48	48
Verrucomicrobiota	Intermittent shedder	-0.50	0.84	0.590	0.918	48	48
SAR324.clade.Marine.group.B.	Prolonged shedder	-0.15	0.28	0.631	0.946	48	6
Fibrobacterota	Prolonged shedder	-0.14	0.31	0.669	0.969	48	48
Spirochaetota	Prolonged shedder	0.06	0.23	0.809	0.981	48	48
Actinobacteriota	Intermittent shedder	-0.03	0.29	0.921	0.981	48	48
Halobacterota	Intermittent shedder	-0.40	1.38	0.793	0.981	48	37
Patescibacteria	Intermittent shedder	-0.15	1.04	0.891	0.981	48	48

Cyanobacteria	Intermittent shedder	0.13	0.46	0.781	0.981	48	48
Myxococcota	Prolonged shedder	-0.24	1.52	0.884	0.981	48	13
Synergistota	Intermittent shedder	-0.14	0.79	0.867	0.981	48	47
Planctomycetota	Intermittent shedder	-0.09	0.86	0.923	0.981	48	36
Elusimicrobiota	Prolonged shedder	-0.12	1.30	0.934	0.981	48	30
Chloroflexi	Prolonged shedder	0.12	0.52	0.834	0.981	48	11
SAR324.clade.Marine. group.B.	Intermittent shedder	0.10	0.28	0.753	0.981	48	6
Bacteroidota	Prolonged shedder	0.00	0.10	0.970	0.989	48	48
Spirochaetota	Intermittent shedder	0.00	0.23	0.989	0.989	48	48

B	**Feature**	**Value**	**Coefficient**	**Standard Error**	**P-value (un-adjusted)**	**P-value (BH-adjusted)**	**N**	**N (non-zero counts)**
Firmicutes		Short-term shedder	0.13	0.13	0.363	0.672	48	48
Firmicutes		Intermittent shedder	0.22	0.13	0.177	0.672	48	48
Euryarchaeota		Intermittent shedder	1.72	0.70	0.092	0.672	48	48
Actinobacteriota		Short-term shedder	0.53	0.29	0.164	0.672	48	48
Actinobacteriota		Intermittent shedder	0.50	0.29	0.182	0.672	48	48
Verrucomicrobiota		Short-term shedder	1.07	0.84	0.293	0.672	48	48
Desulfobacterota		Short-term shedder	-0.99	0.98	0.384	0.672	48	48
Halobacterota		Short-term shedder	3.43	1.38	0.088	0.672	48	37
Halobacterota		Intermittent shedder	3.04	1.38	0.115	0.672	48	37
Patescibacteria		Short-term shedder	1.38	1.04	0.275	0.672	48	48
Patescibacteria		Intermittent shedder	1.23	1.04	0.322	0.672	48	48
Cyanobacteria		Short-term shedder	0.48	0.46	0.294	0.672	48	48
Cyanobacteria		Intermittent shedder	0.61	0.46	0.186	0.672	48	48
Proteobacteria		Short-term shedder	1.07	0.67	0.207	0.672	48	48
Myxococcota		Intermittent shedder	1.79	1.52	0.324	0.672	48	13
Campylobacterota		Short-term shedder	2.26	1.47	0.222	0.672	48	29
Synergistota		Short-term shedder	0.87	0.79	0.351	0.672	48	47
Planctomycetota		Short-term shedder	1.35	0.86	0.214	0.672	48	36
Planctomycetota		Intermittent shedder	1.26	0.86	0.238	0.672	48	36
Armatimonadota		Short-term shedder	1.68	0.54	0.053	0.672	48	37
Armatimonadota		Intermittent shedder	0.75	0.54	0.258	0.672	48	37
Thermoplasmatota		Short-term shedder	1.06	0.53	0.138	0.672	48	19
Thermoplasmatota		Intermittent shedder	0.55	0.53	0.374	0.672	48	19
Elusimicrobiota		Intermittent shedder	-1.34	1.30	0.376	0.672	48	30
Synergistota		Intermittent shedder	0.73	0.79	0.426	0.688	48	47
Chloroflexi		Intermittent shedder	-0.48	0.52	0.417	0.688	48	11
SAR324.clade.Marine. group.B.		Intermittent shedder	0.24	0.28	0.445	0.691	48	6
Bacteroidota		Intermittent shedder	0.07	0.10	0.492	0.738	48	48

Verrucomicrobiota	Intermittent shedder	0.56	0.84	0.550	0.797	48	48
Euryarchaeota	Short-term shedder	0.43	0.70	0.585	0.803	48	48
Proteobacteria	Intermittent shedder	0.40	0.67	0.593	0.803	48	48
Campylobacterota	Intermittent shedder	0.76	1.47	0.642	0.817	48	29
SAR324.clade.Marine.group.B.	Short-term shedder	0.15	0.28	0.631	0.817	48	6
Fibrobacterota	Short-term shedder	0.14	0.31	0.669	0.827	48	48
Fibrobacterota	Intermittent shedder	-0.13	0.31	0.707	0.848	48	48
Spirochaetota	Short-term shedder	-0.06	0.23	0.809	0.894	48	48
Spirochaetota	Intermittent shedder	-0.06	0.23	0.799	0.894	48	48
Desulfobacterota	Intermittent shedder	0.30	0.98	0.778	0.894	48	48
Chloroflexi	Short-term shedder	-0.12	0.52	0.834	0.898	48	11
Myxococcota	Short-term shedder	0.24	1.52	0.884	0.928	48	13
Elusimicrobiota	Short-term shedder	0.12	1.30	0.934	0.957	48	30
Bacteroidota	Short-term shedder	0.00	0.10	0.970	0.970	48	48

Supplementary Table 5.8: Results of generalized linear mixed models evaluating class-level differential abundance by *Salmonella* shedding pattern with **(A)** short-term shedders as the reference level and **(B)** prolonged shedders as the reference level.

A	Feature	Value	Coefficient	Standard Error	P-value (un-adjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Alphaproteobacteria		Intermittent shedder	-1.30	0.34	0.000	0.022	48	48
Alphaproteobacteria		Prolonged shedder	-0.76	0.34	0.029	0.598	48	48
Kiritimatiellae		Intermittent shedder	-0.44	0.18	0.020	0.598	48	6
Clostridia		Prolonged shedder	-0.15	0.18	0.472	0.935	48	48
Bacilli		Intermittent shedder	0.47	0.44	0.363	0.935	48	48
Bacteroidia		Intermittent shedder	0.08	0.10	0.472	0.935	48	48
Methanobacteria		Intermittent shedder	1.29	0.70	0.163	0.935	48	48
Gammaproteobacteria		Intermittent shedder	-0.69	0.78	0.442	0.935	48	48
Gammaproteobacteria		Prolonged shedder	-1.11	0.78	0.249	0.935	48	48
Fibrobacteria		Intermittent shedder	-0.27	0.31	0.441	0.935	48	48
Negativicutes		Intermittent shedder	-0.44	0.29	0.224	0.935	48	48
Coriobacteriia		Prolonged shedder	-0.36	0.19	0.155	0.935	48	48
MVP.15		Prolonged shedder	-2.09	2.24	0.421	0.935	48	43
Verrucomicrobiae		Prolonged shedder	-1.05	0.85	0.302	0.935	48	48
Desulfuromonadia		Intermittent shedder	1.90	1.51	0.298	0.935	48	47
Methanomicrobia		Prolonged shedder	-3.43	1.38	0.088	0.935	48	37
Gracilibacteria		Intermittent shedder	-1.27	1.58	0.483	0.935	48	46
Saccharimonadia		Prolonged shedder	-2.16	1.06	0.135	0.935	48	45
Vampirivibrionia		Prolonged shedder	-0.50	0.48	0.303	0.935	48	47
Myxococcia		Intermittent shedder	1.55	1.52	0.384	0.935	48	13
Campylobacteria		Intermittent shedder	-1.50	1.47	0.382	0.935	48	29
Campylobacteria		Prolonged shedder	-2.26	1.47	0.222	0.935	48	29
Synergistia		Prolonged shedder	-0.87	0.79	0.351	0.935	48	47
vadinHA49		Prolonged shedder	-1.35	0.86	0.214	0.935	48	36
Endomicrobia		Intermittent shedder	-0.67	0.39	0.092	0.935	48	25
Thermoplasmata		Intermittent shedder	-0.51	0.53	0.405	0.935	48	19
Thermoplasmata		Prolonged shedder	-1.06	0.53	0.138	0.935	48	19
Elusimicrobia		Intermittent shedder	-1.46	1.45	0.388	0.935	48	15
Cyanobacteriia		Intermittent shedder	-0.39	0.40	0.401	0.935	48	7
Cyanobacteriia		Prolonged shedder	-0.58	0.40	0.242	0.935	48	7
Actinobacteria		Prolonged shedder	-2.72	1.76	0.221	0.935	48	36
Kiritimatiellae		Prolonged shedder	-0.31	0.18	0.103	0.935	48	6

Clostridia	Intermittent shedder	0.09	0.18	0.660	0.945	48	48
Bacilli	Prolonged shedder	-0.14	0.44	0.776	0.945	48	48
Methanobacteria	Prolonged shedder	-0.43	0.70	0.585	0.945	48	48
Spirochaetia	Intermittent shedder	-0.03	0.20	0.899	0.945	48	48
Spirochaetia	Prolonged shedder	0.10	0.20	0.666	0.945	48	48
Fibrobacteria	Prolonged shedder	-0.14	0.31	0.669	0.945	48	48
Negativicutes	Prolonged shedder	-0.04	0.29	0.893	0.945	48	48
Coriobacteriia	Intermittent shedder	0.06	0.19	0.785	0.945	48	48
MVP.15	Intermittent shedder	0.77	2.24	0.755	0.945	48	43
Verrucomicrobiae	Intermittent shedder	-0.47	0.85	0.617	0.945	48	48
Desulfuromonadia	Prolonged shedder	1.15	1.51	0.503	0.945	48	47
Methanomicrobia	Intermittent shedder	-0.40	1.38	0.793	0.945	48	37
Gracilibacteria	Prolonged shedder	-0.52	1.58	0.764	0.945	48	46
Saccharimonadia	Intermittent shedder	0.59	1.06	0.620	0.945	48	45
Vampirivibrionia	Intermittent shedder	0.09	0.48	0.849	0.945	48	47
Desulfovibrionia	Intermittent shedder	-0.51	1.54	0.760	0.945	48	46
Desulfovibrionia	Prolonged shedder	0.66	1.54	0.698	0.945	48	46
Myxococcia	Prolonged shedder	-0.24	1.52	0.884	0.945	48	13
Synergistia	Intermittent shedder	-0.14	0.79	0.867	0.945	48	47
Desulfotomaculia	Intermittent shedder	0.50	1.06	0.667	0.945	48	15
Syntrophomonadia	Intermittent shedder	-0.12	0.65	0.865	0.945	48	17
Syntrophomonadia	Prolonged shedder	0.23	0.65	0.750	0.945	48	17
Endomicrobia	Prolonged shedder	-0.10	0.39	0.805	0.945	48	25
Elusimicrobia	Prolonged shedder	-0.96	1.45	0.556	0.945	48	15
Anaerolineae	Intermittent shedder	-0.37	0.52	0.529	0.945	48	11
Anaerolineae	Prolonged shedder	0.12	0.52	0.834	0.945	48	11
Actinobacteria	Intermittent shedder	-0.70	1.76	0.718	0.945	48	36
vadinHA49	Intermittent shedder	-0.09	0.86	0.923	0.954	48	36
Bacteroidia	Prolonged shedder	0.00	0.10	0.970	0.970	48	48
Desulfotomaculia	Prolonged shedder	-0.06	1.06	0.957	0.970	48	15

B	Feature	Value	Coefficient	Standard Error	P-value (un-adjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Clostridia		Intermittent shedder	0.24	0.18	0.282	0.751	48	48
Bacilli		Intermittent shedder	0.61	0.44	0.261	0.751	48	48
Methanobacteria		Intermittent shedder	1.72	0.70	0.092	0.751	48	48
Gammaproteobacteria		Short-term shedder	1.11	0.78	0.249	0.751	48	48
Negativicutes		Intermittent shedder	-0.40	0.29	0.261	0.751	48	48

Coriobacteriia	Short-term shedder	0.36	0.19	0.155	0.751	48	48
Coriobacteriia	Intermittent shedder	0.41	0.19	0.116	0.751	48	48
MVP.15	Intermittent shedder	2.85	2.24	0.293	0.751	48	43
Verrucomicrobiae	Short-term shedder	1.05	0.85	0.302	0.751	48	48
Methanomicrobia	Short-term shedder	3.43	1.38	0.088	0.751	48	37
Methanomicrobia	Intermittent shedder	3.04	1.38	0.115	0.751	48	37
Saccharimonadia	Short-term shedder	2.16	1.06	0.135	0.751	48	45
Saccharimonadia	Intermittent shedder	2.74	1.06	0.082	0.751	48	45
Vampirivibrionia	Short-term shedder	0.50	0.48	0.303	0.751	48	47
Vampirivibrionia	Intermittent shedder	0.60	0.48	0.224	0.751	48	47
Alphaproteobacteria	Short-term shedder	0.76	0.34	0.029	0.751	48	48
Alphaproteobacteria	Intermittent shedder	-0.54	0.34	0.114	0.751	48	48
Campylobacteria	Short-term shedder	2.26	1.47	0.222	0.751	48	29
vadinHA49	Short-term shedder	1.35	0.86	0.214	0.751	48	36
vadinHA49	Intermittent shedder	1.26	0.86	0.238	0.751	48	36
Endomicrobia	Intermittent shedder	-0.57	0.39	0.148	0.751	48	25
Thermoplasmata	Short-term shedder	1.06	0.53	0.138	0.751	48	19
Cyanobacteriia	Short-term shedder	0.58	0.40	0.242	0.751	48	7
Actinobacteria	Short-term shedder	2.72	1.76	0.221	0.751	48	36
Kiritimatiellae	Short-term shedder	0.31	0.18	0.103	0.751	48	6
Myxococcia	Intermittent shedder	1.79	1.52	0.324	0.770	48	13
Actinobacteria	Intermittent shedder	2.02	1.76	0.335	0.770	48	36
Synergistia	Short-term shedder	0.87	0.79	0.351	0.778	48	47
Thermoplasmata	Intermittent shedder	0.55	0.53	0.374	0.799	48	19
MVP.15	Short-term shedder	2.09	2.24	0.421	0.825	48	43
Synergistia	Intermittent shedder	0.73	0.79	0.426	0.825	48	47
Anaerolineae	Intermittent shedder	-0.48	0.52	0.417	0.825	48	11
Clostridia	Short-term shedder	0.15	0.18	0.472	0.831	48	48
Bacteroidia	Intermittent shedder	0.07	0.10	0.492	0.831	48	48
Methanobacteria	Short-term shedder	0.43	0.70	0.585	0.831	48	48
Gammaproteobacteria	Intermittent shedder	0.42	0.78	0.624	0.831	48	48
Spirochaetia	Short-term shedder	-0.10	0.20	0.666	0.831	48	48
Spirochaetia	Intermittent shedder	-0.13	0.20	0.582	0.831	48	48
Fibrobacteria	Short-term shedder	0.14	0.31	0.669	0.831	48	48
Verrucomicrobiae	Intermittent shedder	0.58	0.85	0.542	0.831	48	48
Desulfuromonadia	Short-term shedder	-1.15	1.51	0.503	0.831	48	47
Desulfuromonadia	Intermittent shedder	0.75	1.51	0.653	0.831	48	47
Gracilibacteria	Intermittent shedder	-0.74	1.58	0.670	0.831	48	46

Desulfovibrionia	Intermittent shedder	-1.17	1.54	0.501	0.831	48	46
Campylobacteria	Intermittent shedder	0.76	1.47	0.642	0.831	48	29
Desulfotomaculi a	Intermittent shedder	0.57	1.06	0.631	0.831	48	15
Syntrophomonad ia	Intermittent shedder	-0.35	0.65	0.630	0.831	48	17
Elusimicrobia	Short-term shedder	0.96	1.45	0.556	0.831	48	15
Cyanobacteriia	Intermittent shedder	0.19	0.40	0.666	0.831	48	7
Kiritimatiellae	Intermittent shedder	-0.14	0.18	0.465	0.831	48	6
Fibrobacteria	Intermittent shedder	-0.13	0.31	0.707	0.842	48	48
Desulfovibrionia	Short-term shedder	-0.66	1.54	0.698	0.842	48	46
Bacilli	Short-term shedder	0.14	0.44	0.776	0.859	48	48
Gracilibacteria	Short-term shedder	0.52	1.58	0.764	0.859	48	46
Syntrophomonad ia	Short-term shedder	-0.23	0.65	0.750	0.859	48	17
Elusimicrobia	Intermittent shedder	-0.50	1.45	0.752	0.859	48	15
Endomicrobia	Short-term shedder	0.10	0.39	0.805	0.876	48	25
Anaerolineae	Short-term shedder	-0.12	0.52	0.834	0.891	48	11
Negativicutes	Short-term shedder	0.04	0.29	0.893	0.923	48	48
Myxococcia	Short-term shedder	0.24	1.52	0.884	0.923	48	13
Bacteroidia	Short-term shedder	0.00	0.10	0.970	0.970	48	48
Desulfotomaculi a	Short-term shedder	0.06	1.06	0.957	0.970	48	15

Supplementary Table 5.9: Results of generalized linear mixed models evaluating order-level differential abundance by *Salmonella* shedding pattern with **(A)** short-term shedders as the reference level and **(B)** prolonged shedders as the reference level.

A Feature	Value	Coefficient	Standard Error	P-value (un-adjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Rhodospirillales	Prolonged shedder	-2.06	0.59	0.001	0.094	48	37
Monoglobales	Intermittent shedder	-1.49	0.45	0.002	0.094	48	30
Actinomycetales	Prolonged shedder	-0.82	0.36	0.027	0.705	48	7
WCHB1.41	Intermittent shedder	-0.44	0.18	0.020	0.705	48	6
Methanobacteriales	Intermittent shedder	1.29	0.70	0.163	0.865	48	48
Oscillospirales	Intermittent shedder	0.13	0.06	0.147	0.865	48	48
Veillonellales.Selenomonadales	Intermittent shedder	-0.54	0.26	0.128	0.865	48	48
Coriobacteriales	Prolonged shedder	-0.36	0.19	0.155	0.865	48	48
Bacillales	Intermittent shedder	1.46	0.64	0.106	0.865	48	47
Methanomicrobiales	Prolonged shedder	-3.43	1.38	0.088	0.865	48	37
Saccharimonadales	Prolonged shedder	-2.16	1.06	0.135	0.865	48	45
Rhizobiales	Intermittent shedder	-0.85	0.40	0.123	0.865	48	10
Rhizobiales	Prolonged shedder	-0.75	0.40	0.156	0.865	48	10
Rickettsiales	Intermittent shedder	-2.91	1.57	0.160	0.865	48	32
Pedosphaerales	Intermittent shedder	-1.81	0.65	0.069	0.865	48	10
Pedosphaerales	Prolonged shedder	-1.81	0.65	0.069	0.865	48	10
Endomicrobiales	Intermittent shedder	-0.67	0.39	0.092	0.865	48	25
Methanomassiliicoccales	Prolonged shedder	-1.06	0.53	0.138	0.865	48	19
Actinomycetales	Intermittent shedder	-0.53	0.36	0.145	0.865	48	7
WCHB1.41	Prolonged shedder	-0.31	0.18	0.103	0.865	48	6
Burkholderiales	Intermittent shedder	-0.99	0.56	0.175	0.885	48	39
Oscillospirales	Prolonged shedder	-0.09	0.06	0.256	0.915	48	48
Enterobacterales	Prolonged shedder	-1.20	0.81	0.236	0.915	48	48
Peptostreptococcales.Tissierellales	Prolonged shedder	-0.44	0.27	0.208	0.915	48	48
Christensenellales	Intermittent shedder	0.37	0.30	0.311	0.915	48	48
Christensenellales	Prolonged shedder	-0.38	0.30	0.301	0.915	48	48
Bacillales	Prolonged shedder	0.80	0.64	0.300	0.915	48	47
Bradymonadales	Intermittent shedder	2.01	1.40	0.248	0.915	48	47
Gastranaerophilales	Prolonged shedder	-0.50	0.48	0.303	0.915	48	47
Clostridia.UCG.014	Prolonged shedder	-1.41	1.15	0.307	0.915	48	40
Campylobacterales	Prolonged shedder	-2.26	1.47	0.222	0.915	48	29
Erysipelotrichales	Intermittent shedder	0.31	0.28	0.273	0.915	48	48
Micrococcales	Prolonged shedder	-1.19	0.78	0.225	0.915	48	17

Izemoplasmatales	Intermittent shedder	1.54	1.23	0.300	0.915	48	42
Propionibacteriales	Prolonged shedder	-0.48	0.35	0.266	0.915	48	6
Chloroplast	Prolonged shedder	-0.58	0.40	0.242	0.915	48	7
Clostridiales	Intermittent shedder	0.43	0.54	0.483	0.921	48	48
Lactobacillales	Intermittent shedder	0.39	0.56	0.533	0.921	48	48
Bacteroidales	Intermittent shedder	0.08	0.10	0.499	0.921	48	48
Enterobacterales	Intermittent shedder	-0.76	0.81	0.421	0.921	48	48
Peptostreptococcales.Tissierellales	Intermittent shedder	-0.18	0.27	0.554	0.921	48	48
Fibrobacterales	Intermittent shedder	-0.27	0.31	0.441	0.921	48	48
Veillonellales.Selenomonadales	Prolonged shedder	-0.18	0.26	0.528	0.921	48	48
Acidaminococcales	Intermittent shedder	-0.37	0.38	0.402	0.921	48	48
Verrucomicrobiales	Prolonged shedder	-0.98	0.84	0.328	0.921	48	48
Bradymonadales	Prolonged shedder	1.18	1.40	0.463	0.921	48	47
Absconditabacteriales..SR1.	Intermittent shedder	-1.27	1.58	0.483	0.921	48	46
Eubacteriales	Prolonged shedder	-0.71	0.94	0.509	0.921	48	47
Clostridia.UCG.014	Intermittent shedder	0.85	1.15	0.515	0.921	48	40
PB19	Intermittent shedder	-1.55	2.01	0.496	0.921	48	16
Myxococcales	Intermittent shedder	1.55	1.52	0.384	0.921	48	13
Campylobacterales	Intermittent shedder	-1.50	1.47	0.382	0.921	48	29
Synergistales	Prolonged shedder	-0.87	0.79	0.351	0.921	48	47
Paenibacillales	Intermittent shedder	0.30	0.33	0.424	0.921	48	6
Paenibacillales	Prolonged shedder	0.26	0.33	0.480	0.921	48	6
Acholeplasmatales	Intermittent shedder	-0.99	0.93	0.362	0.921	48	40
Mycoplasmatales	Prolonged shedder	-0.75	0.82	0.431	0.921	48	14
Propionibacteriales	Intermittent shedder	-0.24	0.35	0.551	0.921	48	6
Methanomassiliicoccales	Intermittent shedder	-0.51	0.53	0.405	0.921	48	19
Elusimicrobiales	Intermittent shedder	-1.46	1.45	0.388	0.921	48	15
Elusimicrobiales	Prolonged shedder	-0.96	1.45	0.556	0.921	48	15
Anaerolineales	Intermittent shedder	-0.37	0.52	0.529	0.921	48	11
Chloroplast	Intermittent shedder	-0.39	0.40	0.401	0.921	48	7
Corynebacteriales	Intermittent shedder	0.81	1.01	0.482	0.921	48	17
Methanobacteriales	Prolonged shedder	-0.43	0.70	0.585	0.927	48	48
Micrococcales	Intermittent shedder	0.47	0.78	0.586	0.927	48	17
Monoglobales	Prolonged shedder	-0.25	0.45	0.584	0.927	48	30
Clostridiales	Prolonged shedder	-0.23	0.54	0.702	0.950	48	48
Lactobacillales	Prolonged shedder	-0.21	0.56	0.732	0.950	48	48
Lachnospirales	Intermittent shedder	-0.07	0.23	0.773	0.950	48	48
Lachnospirales	Prolonged shedder	-0.06	0.23	0.821	0.950	48	48
Spirochaetales	Intermittent shedder	-0.03	0.20	0.899	0.950	48	48
Spirochaetales	Prolonged shedder	0.10	0.20	0.666	0.950	48	48

Fibrobacterales	Prolonged shedder	-0.14	0.31	0.669	0.950	48	48
Coriobacteriales	Intermittent shedder	0.06	0.19	0.785	0.950	48	48
Verrucomicrobiales	Intermittent shedder	-0.40	0.84	0.668	0.950	48	48
Methanomicrobiales	Intermittent shedder	-0.40	1.38	0.793	0.950	48	37
Absconditabacteriales..SR1.	Prolonged shedder	-0.52	1.58	0.764	0.950	48	46
Saccharimonadales	Intermittent shedder	0.59	1.06	0.620	0.950	48	45
Gastranaerophilales	Intermittent shedder	0.09	0.48	0.849	0.950	48	47
Desulfovibrionales	Intermittent shedder	-0.51	1.54	0.760	0.950	48	46
Desulfovibrionales	Prolonged shedder	0.66	1.54	0.698	0.950	48	46
Eubacteriales	Intermittent shedder	-0.30	0.94	0.768	0.950	48	47
PB19	Prolonged shedder	0.36	2.01	0.870	0.950	48	16
Myxococcales	Prolonged shedder	-0.24	1.52	0.884	0.950	48	13
Synergistales	Intermittent shedder	-0.14	0.79	0.867	0.950	48	47
Erysipelotrichales	Prolonged shedder	-0.06	0.28	0.824	0.950	48	48
Rhodospirillales	Intermittent shedder	-0.22	0.59	0.714	0.950	48	37
Rickettsiales	Prolonged shedder	0.65	1.57	0.705	0.950	48	32
Izemoplasmatales	Prolonged shedder	0.28	1.23	0.836	0.950	48	42
Desulfotomaculales	Intermittent shedder	0.50	1.06	0.667	0.950	48	15
Acholeplasmatales	Prolonged shedder	-0.14	0.93	0.891	0.950	48	40
Mycoplasmatales	Intermittent shedder	-0.11	0.82	0.905	0.950	48	14
Clostridia.vadinBB60.group	Intermittent shedder	0.28	0.80	0.748	0.950	48	42
Clostridia.vadinBB60.group	Prolonged shedder	-0.20	0.80	0.815	0.950	48	42
Peptococcales	Intermittent shedder	0.60	1.26	0.666	0.950	48	32
Peptococcales	Prolonged shedder	-0.55	1.26	0.691	0.950	48	32
Syntrophomonadales	Intermittent shedder	-0.12	0.65	0.865	0.950	48	17
Syntrophomonadales	Prolonged shedder	0.23	0.65	0.750	0.950	48	17
Endomicrobiales	Prolonged shedder	-0.10	0.39	0.805	0.950	48	25
Anaerolineales	Prolonged shedder	0.12	0.52	0.834	0.950	48	11
Bacteroidales	Prolonged shedder	-0.01	0.10	0.934	0.953	48	48
Acidaminococcales	Prolonged shedder	0.03	0.38	0.934	0.953	48	48
Corynebacteriales	Prolonged shedder	0.09	1.01	0.935	0.953	48	17
Desulfotomaculales	Prolonged shedder	-0.06	1.06	0.957	0.966	48	15
Burkholderiales	Prolonged shedder	0.00	0.56	0.999	0.999	48	39

B	Feature	Value	Coefficient	Standard Error	P-value (un-adjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Rhodospirillales		Short-term shedder	2.06	0.59	0.001	0.110	48	37
Rhodospirillales		Intermittent shedder	1.84	0.59	0.003	0.158	48	37
Monoglobales		Intermittent shedder	-1.24	0.45	0.008	0.285	48	30

Actinomycetales	Short-term shedder	0.82	0.36	0.027	0.705	48	7
Methanobacteriales	Intermittent shedder	1.72	0.70	0.092	0.786	48	48
Oscillospirales	Intermittent shedder	0.22	0.06	0.044	0.786	48	48
Christensenellales	Intermittent shedder	0.75	0.30	0.091	0.786	48	48
Coriobacteriales	Short-term shedder	0.36	0.19	0.155	0.786	48	48
Coriobacteriales	Intermittent shedder	0.41	0.19	0.116	0.786	48	48
Methanomicrobiales	Short-term shedder	3.43	1.38	0.088	0.786	48	37
Methanomicrobiales	Intermittent shedder	3.04	1.38	0.115	0.786	48	37
Saccharimonadales	Short-term shedder	2.16	1.06	0.135	0.786	48	45
Saccharimonadales	Intermittent shedder	2.74	1.06	0.082	0.786	48	45
Rhizobiales	Short-term shedder	0.75	0.40	0.156	0.786	48	10
Clostridia.UCG.014	Intermittent shedder	2.26	1.15	0.144	0.786	48	40
Rickettsiales	Intermittent shedder	-3.56	1.57	0.107	0.786	48	32
Micrococcales	Intermittent shedder	1.66	0.78	0.123	0.786	48	17
Pedosphaerales	Short-term shedder	1.81	0.65	0.069	0.786	48	10
Endomicrobiales	Intermittent shedder	-0.57	0.39	0.148	0.786	48	25
Methanomassiliicoccales	Short-term shedder	1.06	0.53	0.138	0.786	48	19
WCHB1.41	Short-term shedder	0.31	0.18	0.103	0.786	48	6
Burkholderiales	Intermittent shedder	-0.99	0.56	0.175	0.843	48	39
Clostridiales	Intermittent shedder	0.66	0.54	0.309	0.861	48	48
Lactobacillales	Intermittent shedder	0.60	0.56	0.360	0.861	48	48
Oscillospirales	Short-term shedder	0.09	0.06	0.256	0.861	48	48
Enterobacterales	Short-term shedder	1.20	0.81	0.236	0.861	48	48
Peptostreptococcales.Tissierellales	Short-term shedder	0.44	0.27	0.208	0.861	48	48
Peptostreptococcales.Tissierellales	Intermittent shedder	0.26	0.27	0.419	0.861	48	48
Christensenellales	Short-term shedder	0.38	0.30	0.301	0.861	48	48
Veillonellales.Selenomonadales	Intermittent shedder	-0.36	0.26	0.261	0.861	48	48
Acidaminococcales	Intermittent shedder	-0.41	0.38	0.365	0.861	48	48
Verrucomicrobiales	Short-term shedder	0.98	0.84	0.328	0.861	48	48
Bacillales	Short-term shedder	-0.80	0.64	0.300	0.861	48	47
Bacillales	Intermittent shedder	0.67	0.64	0.374	0.861	48	47
Gastranaerophilales	Short-term shedder	0.50	0.48	0.303	0.861	48	47
Gastranaerophilales	Intermittent shedder	0.60	0.48	0.224	0.861	48	47
Clostridia.UCG.014	Short-term shedder	1.41	1.15	0.307	0.861	48	40
PB19	Intermittent shedder	-1.91	2.01	0.412	0.861	48	16
Myxococcales	Intermittent shedder	1.79	1.52	0.324	0.861	48	13
Campylobacterales	Short-term shedder	2.26	1.47	0.222	0.861	48	29
Synergistales	Short-term shedder	0.87	0.79	0.351	0.861	48	47
Synergistales	Intermittent shedder	0.73	0.79	0.426	0.861	48	47
Erysipelotrichales	Intermittent shedder	0.37	0.28	0.189	0.861	48	48

Micrococcales	Short-term shedder	1.19	0.78	0.225	0.861	48	17
Izemoplasmatales	Intermittent shedder	1.26	1.23	0.381	0.861	48	42
Acholeplasmatales	Intermittent shedder	-0.86	0.93	0.424	0.861	48	40
Mycoplasmatales	Short-term shedder	0.75	0.82	0.431	0.861	48	14
Peptococcales	Intermittent shedder	1.16	1.26	0.428	0.861	48	32
Propionibacteriales	Short-term shedder	0.48	0.35	0.266	0.861	48	6
Methanomassiliicoccales	Intermittent shedder	0.55	0.53	0.374	0.861	48	19
Actinomycetales	Intermittent shedder	0.29	0.36	0.423	0.861	48	7
Anaerolineales	Intermittent shedder	-0.48	0.52	0.417	0.861	48	11
Chloroplast	Short-term shedder	0.58	0.40	0.242	0.861	48	7
Bacteroidales	Intermittent shedder	0.09	0.10	0.455	0.881	48	48
Bradymonadales	Short-term shedder	-1.18	1.40	0.463	0.881	48	47
WCHB1.41	Intermittent shedder	-0.14	0.18	0.465	0.881	48	6
Clostridiales	Short-term shedder	0.23	0.54	0.702	0.892	48	48
Methanobacteriales	Short-term shedder	0.43	0.70	0.585	0.892	48	48
Enterobacterales	Intermittent shedder	0.45	0.81	0.621	0.892	48	48
Spirochaetales	Short-term shedder	-0.10	0.20	0.666	0.892	48	48
Spirochaetales	Intermittent shedder	-0.13	0.20	0.582	0.892	48	48
Fibrobacterales	Short-term shedder	0.14	0.31	0.669	0.892	48	48
Fibrobacterales	Intermittent shedder	-0.13	0.31	0.707	0.892	48	48
Veillonellales.Selenomonadales	Short-term shedder	0.18	0.26	0.528	0.892	48	48
Verrucomicrobiales	Intermittent shedder	0.58	0.84	0.538	0.892	48	48
Bradymonadales	Intermittent shedder	0.83	1.40	0.595	0.892	48	47
Absconditabacteriales..SR1.	Intermittent shedder	-0.74	1.58	0.670	0.892	48	46
Desulfovibrionales	Short-term shedder	-0.66	1.54	0.698	0.892	48	46
Desulfovibrionales	Intermittent shedder	-1.17	1.54	0.501	0.892	48	46
Eubacteriales	Short-term shedder	0.71	0.94	0.509	0.892	48	47
Eubacteriales	Intermittent shedder	0.40	0.94	0.699	0.892	48	47
Campylobacterales	Intermittent shedder	0.76	1.47	0.642	0.892	48	29
Rickettsiales	Short-term shedder	-0.65	1.57	0.705	0.892	48	32
Paenibacillales	Short-term shedder	-0.26	0.33	0.480	0.892	48	6
Desulfotomaculales	Intermittent shedder	0.57	1.06	0.631	0.892	48	15
Mycoplasmatales	Intermittent shedder	0.64	0.82	0.493	0.892	48	14
Clostridia.vadinBB60.group	Intermittent shedder	0.49	0.80	0.586	0.892	48	42
Monoglobales	Short-term shedder	0.25	0.45	0.584	0.892	48	30
Peptococcales	Short-term shedder	0.55	1.26	0.691	0.892	48	32
Syntrophomonadales	Intermittent shedder	-0.35	0.65	0.630	0.892	48	17
Propionibacteriales	Intermittent shedder	0.24	0.35	0.537	0.892	48	6
Elusimicrobiales	Short-term shedder	0.96	1.45	0.556	0.892	48	15
Chloroplast	Intermittent shedder	0.19	0.40	0.666	0.892	48	7

Corynebacteriales	Intermittent shedder	0.72	1.01	0.528	0.892	48	17
Lactobacillales	Short-term shedder	0.21	0.56	0.732	0.913	48	48
Syntrophomonadales	Short-term shedder	-0.23	0.65	0.750	0.916	48	17
Elusimicrobiales	Intermittent shedder	-0.50	1.45	0.752	0.916	48	15
Absconditabacteriales..SR1.	Short-term shedder	0.52	1.58	0.764	0.920	48	46
Lachnospirales	Short-term shedder	0.06	0.23	0.821	0.932	48	48
Rhizobiales	Intermittent shedder	-0.10	0.40	0.822	0.932	48	10
Erysipelotrichales	Short-term shedder	0.06	0.28	0.824	0.932	48	48
Izemoplasmatales	Short-term shedder	-0.28	1.23	0.836	0.932	48	42
Clostridia.vadinBB60.group	Short-term shedder	0.20	0.80	0.815	0.932	48	42
Endomicrobiales	Short-term shedder	0.10	0.39	0.805	0.932	48	25
Anaerolineales	Short-term shedder	-0.12	0.52	0.834	0.932	48	11
PB19	Short-term shedder	-0.36	2.01	0.870	0.960	48	16
Myxococcales	Short-term shedder	0.24	1.52	0.884	0.964	48	13
Acholeplasmatales	Short-term shedder	0.14	0.93	0.891	0.964	48	40
Bacteroidales	Short-term shedder	0.01	0.10	0.934	0.972	48	48
Acidaminococcales	Short-term shedder	-0.03	0.38	0.934	0.972	48	48
Paenibacillales	Intermittent shedder	0.04	0.33	0.912	0.972	48	6
Corynebacteriales	Short-term shedder	-0.09	1.01	0.935	0.972	48	17
Lachnospirales	Intermittent shedder	-0.02	0.23	0.950	0.975	48	48
Desulfotomaculales	Short-term shedder	0.06	1.06	0.957	0.975	48	15
Burkholderiales	Short-term shedder	0.00	0.56	0.999	1.000	48	39
Pedosphaerales	Intermittent shedder	0.00	0.65	1.000	1.000	48	10

Supplementary Table 5.10: Results of generalized linear mixed models evaluating family-level differential abundance by *Salmonella* shedding pattern with **(A)** short-term shedders as the reference level and **(B)** prolonged shedders as the reference level.

A	Feature	Value	Coefficient	Standard Error	P-value (un-adjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
gir.aah93h0		Intermittent shedder	-1.74	0.46	0.000	0.073	48	29
Bacteroidales.BS11.gut.group		Prolonged shedder	-0.97	0.28	0.001	0.078	48	48
Monoglobaceae		Intermittent shedder	-1.49	0.45	0.002	0.089	48	30
Beijerinckiaceae		Prolonged shedder	-0.81	0.25	0.002	0.089	48	8
Atopobiaceae		Intermittent shedder	-0.82	0.32	0.013	0.357	48	16
Beijerinckiaceae		Intermittent shedder	-0.64	0.25	0.014	0.357	48	8
Actinomycetaceae		Prolonged shedder	-0.82	0.36	0.027	0.593	48	7
Clostridiaceae		Intermittent shedder	0.43	0.54	0.483	0.908	48	48
Lactobacillaceae		Intermittent shedder	0.39	0.56	0.537	0.908	48	48
p.251.o5		Intermittent shedder	-0.81	0.56	0.245	0.908	48	48
p.251.o5		Prolonged shedder	0.53	0.56	0.412	0.908	48	48
Prevotellaceae		Intermittent shedder	-0.21	0.22	0.413	0.908	48	48
Methanobacteriaceae		Intermittent shedder	1.29	0.70	0.163	0.908	48	48
Methanobacteriaceae		Prolonged shedder	-0.43	0.70	0.585	0.908	48	48
Oscillospiraceae		Prolonged shedder	-0.31	0.16	0.156	0.908	48	48
Succinivibrionaceae		Prolonged shedder	-1.97	1.29	0.224	0.908	48	46
Bacteroidales.UCG.001		Intermittent shedder	1.05	1.19	0.442	0.908	48	48
Bacteroidales.UCG.001		Prolonged shedder	-0.82	1.19	0.541	0.908	48	48
F082		Intermittent shedder	0.44	0.55	0.476	0.908	48	48
F082		Prolonged shedder	-0.39	0.55	0.527	0.908	48	48
Bacteroidales.BS11.gut.group		Intermittent shedder	-0.54	0.28	0.056	0.908	48	48
Ruminococcaceae		Intermittent shedder	0.23	0.38	0.596	0.908	48	48
Ruminococcaceae		Prolonged shedder	0.34	0.38	0.444	0.908	48	48
Anaerovoracaceae		Intermittent shedder	-0.14	0.22	0.580	0.908	48	48
Anaerovoracaceae		Prolonged shedder	-0.41	0.22	0.162	0.908	48	48
Fibrobacteraceae		Intermittent shedder	-0.27	0.31	0.441	0.908	48	48
Christensenellaceae		Intermittent shedder	0.37	0.30	0.311	0.908	48	48
Christensenellaceae		Prolonged shedder	-0.38	0.30	0.301	0.908	48	48
Selenomonadaceae		Intermittent shedder	-0.54	0.26	0.129	0.908	48	48
Selenomonadaceae		Prolonged shedder	-0.17	0.26	0.549	0.908	48	48
Hungateiclostridiaceae		Intermittent shedder	0.79	0.48	0.201	0.908	48	48
Hungateiclostridiaceae		Prolonged shedder	0.60	0.48	0.301	0.908	48	48
Eggerthellaceae		Intermittent shedder	-0.23	0.38	0.587	0.908	48	48

Eggerthellaceae	Prolonged shedder	-0.43	0.38	0.340	0.908	48	48
Acidaminococcaceae	Intermittent shedder	-0.37	0.38	0.402	0.908	48	48
UCG.010	Intermittent shedder	0.25	0.31	0.470	0.908	48	48
UCG.010	Prolonged shedder	-0.35	0.31	0.332	0.908	48	48
Peptostreptococcaceae	Intermittent shedder	-1.14	1.95	0.599	0.908	48	39
Peptostreptococcaceae	Prolonged shedder	-1.76	1.95	0.434	0.908	48	39
X.Eubacterium..coprostanoligenes.group	Intermittent shedder	-0.38	0.33	0.326	0.908	48	48
X.Eubacterium..coprostanoligenes.group	Prolonged shedder	-0.55	0.33	0.193	0.908	48	48
Paludibacteraceae	Prolonged shedder	-1.65	1.48	0.346	0.908	48	48
Dysgonomonadaceae	Intermittent shedder	3.93	1.91	0.132	0.908	48	20
Akkermansiaceae	Prolonged shedder	-0.98	0.84	0.328	0.908	48	48
Bacteroidetes.BD2.2	Intermittent shedder	1.99	2.72	0.517	0.908	48	36
Planococcaceae	Intermittent shedder	1.42	0.65	0.118	0.908	48	45
Planococcaceae	Prolonged shedder	0.88	0.65	0.272	0.908	48	45
Coriobacteriales.Incertae.Sedis	Intermittent shedder	2.05	1.13	0.167	0.908	48	44
Methanocorpusculaceae	Prolonged shedder	-3.43	1.38	0.088	0.908	48	37
Bacillaceae	Intermittent shedder	1.02	0.55	0.162	0.908	48	47
Saccharimonadaceae	Prolonged shedder	-2.25	1.04	0.119	0.908	48	44
Streptococcaceae	Prolonged shedder	-1.51	1.48	0.382	0.908	48	37
Pasteurellaceae	Intermittent shedder	-1.53	1.50	0.383	0.908	48	34
Eubacteriaceae	Intermittent shedder	-0.52	0.77	0.549	0.908	48	45
Eubacteriaceae	Prolonged shedder	-0.49	0.77	0.569	0.908	48	45
Defluviitaleaceae	Intermittent shedder	-0.91	1.40	0.561	0.908	48	41
Defluviitaleaceae	Prolonged shedder	-0.91	1.40	0.563	0.908	48	41
Muribaculaceae	Intermittent shedder	-1.69	2.61	0.563	0.908	48	37
Anaerofustaceae	Prolonged shedder	-1.18	1.53	0.496	0.908	48	34
Marinilabiliaceae	Intermittent shedder	1.06	0.87	0.308	0.908	48	8
gir.aah93h0	Prolonged shedder	0.56	0.46	0.230	0.908	48	29
M2PB4.65.termite.group	Prolonged shedder	-2.56	2.28	0.343	0.908	48	34
Butyricicoccaceae	Intermittent shedder	-1.19	1.59	0.506	0.908	48	32
Myxococcaceae	Intermittent shedder	1.55	1.52	0.384	0.908	48	13
Atopobiaceae	Prolonged shedder	-0.40	0.32	0.217	0.908	48	16
Enterobacteriaceae	Prolonged shedder	-0.62	0.67	0.418	0.908	48	13
Campylobacteraceae	Intermittent shedder	-1.50	1.47	0.382	0.908	48	29
Campylobacteraceae	Prolonged shedder	-2.26	1.47	0.222	0.908	48	29
CAP.aah99b04	Prolonged shedder	-1.37	1.83	0.510	0.908	48	17
Synergistaceae	Prolonged shedder	-0.87	0.79	0.351	0.908	48	47
Erysipelatoclostridiaceae	Intermittent shedder	1.01	1.13	0.438	0.908	48	47
Erysipelatoclostridiaceae	Prolonged shedder	0.71	1.13	0.577	0.908	48	47
Nocardiaceae	Intermittent shedder	1.07	0.85	0.296	0.908	48	12
Erysipelotrichaceae	Prolonged shedder	-1.13	1.22	0.423	0.908	48	38

Micrococcaceae	Intermittent shedder	0.52	0.79	0.557	0.908	48	16
Micrococcaceae	Prolonged shedder	-1.06	0.79	0.275	0.908	48	16
Oxalobacteraceae	Prolonged shedder	0.41	0.65	0.569	0.908	48	28
Paenibacillaceae	Intermittent shedder	0.30	0.33	0.424	0.908	48	6
Paenibacillaceae	Prolonged shedder	0.26	0.33	0.480	0.908	48	6
Acholeplasmataceae	Intermittent shedder	-0.99	0.93	0.362	0.908	48	40
Mycoplasmataceae	Prolonged shedder	-0.75	0.82	0.431	0.908	48	14
Monoglobaceae	Prolonged shedder	-0.25	0.45	0.584	0.908	48	30
Pedosphaeraceae	Intermittent shedder	-1.81	0.65	0.069	0.908	48	10
Pedosphaeraceae	Prolonged shedder	-1.81	0.65	0.069	0.908	48	10
Endomicrobiaceae	Intermittent shedder	-0.67	0.39	0.092	0.908	48	25
Nocardioidaceae	Intermittent shedder	-0.24	0.35	0.551	0.908	48	6
Nocardioidaceae	Prolonged shedder	-0.48	0.35	0.266	0.908	48	6
Methanomethylophilaceae	Intermittent shedder	-0.51	0.53	0.405	0.908	48	19
Methanomethylophilaceae	Prolonged shedder	-1.06	0.53	0.138	0.908	48	19
Elusimicrobiaceae	Intermittent shedder	-1.46	1.45	0.388	0.908	48	15
Elusimicrobiaceae	Prolonged shedder	-0.96	1.45	0.556	0.908	48	15
Porphyromonadaceae	Intermittent shedder	-0.30	0.45	0.556	0.908	48	7
Porphyromonadaceae	Prolonged shedder	-0.38	0.45	0.455	0.908	48	7
Ethanoligenenaceae	Intermittent shedder	0.66	0.46	0.248	0.908	48	13
Ethanoligenenaceae	Prolonged shedder	-0.29	0.46	0.570	0.908	48	13
Actinomycetaceae	Intermittent shedder	-0.53	0.36	0.145	0.908	48	7
Sutterellaceae	Intermittent shedder	-0.70	0.69	0.381	0.908	48	16
Sutterellaceae	Prolonged shedder	-0.71	0.69	0.376	0.908	48	16
Mycobacteriaceae	Intermittent shedder	-0.24	0.33	0.517	0.908	48	6
Cellulomonadaceae	Intermittent shedder	-0.17	0.28	0.588	0.908	48	5
Cellulomonadaceae	Prolonged shedder	-0.31	0.28	0.347	0.908	48	5
Anaerolineaceae	Intermittent shedder	-0.37	0.52	0.529	0.908	48	11
Mitochondria	Intermittent shedder	-0.26	0.33	0.490	0.908	48	8
Saccharimonadaceae	Intermittent shedder	0.59	1.04	0.613	0.911	48	44
Streptococcaceae	Intermittent shedder	0.83	1.48	0.612	0.911	48	37
Oscillospiraceae	Intermittent shedder	0.08	0.16	0.646	0.916	48	48
Succinivibrionaceae	Intermittent shedder	-0.63	1.29	0.657	0.916	48	46
Spirochaetaceae	Prolonged shedder	0.10	0.20	0.666	0.916	48	48
Fibrobacteraceae	Prolonged shedder	-0.14	0.31	0.669	0.916	48	48
Marinifilaceae	Intermittent shedder	0.91	1.85	0.655	0.916	48	39
Akkermansiaceae	Intermittent shedder	-0.40	0.84	0.668	0.916	48	48
CAP.aah99b04	Intermittent shedder	0.88	1.83	0.665	0.916	48	17
Desulfurisporaceae	Intermittent shedder	0.50	1.06	0.667	0.916	48	15
Peptococcaceae	Intermittent shedder	0.60	1.26	0.666	0.916	48	32
Clostridiaceae	Prolonged shedder	-0.23	0.54	0.702	0.920	48	48

Rikenellaceae	Prolonged shedder	-0.11	0.24	0.678	0.920	48	48
Desulfovibrionaceae	Prolonged shedder	0.66	1.54	0.698	0.920	48	46
Enterobacteriaceae	Intermittent shedder	-0.29	0.67	0.688	0.920	48	13
Peptococcaceae	Prolonged shedder	-0.55	1.26	0.691	0.920	48	32
Marinifilaceae	Prolonged shedder	-0.69	1.85	0.732	0.921	48	39
Dysgonomonadaceae	Prolonged shedder	0.78	1.91	0.711	0.921	48	20
Bacillaceae	Prolonged shedder	0.21	0.55	0.732	0.921	48	47
M2PB4.65.termite.group	Intermittent shedder	-0.89	2.28	0.721	0.921	48	34
Oxalobacteraceae	Intermittent shedder	-0.24	0.65	0.732	0.921	48	28
Bacteroidales.RF16.group	Prolonged shedder	-0.36	1.09	0.760	0.933	48	48
Desulfovibrionaceae	Intermittent shedder	-0.51	1.54	0.760	0.933	48	46
Syntrophomonadaceae	Prolonged shedder	0.23	0.65	0.750	0.933	48	17
Lactobacillaceae	Prolonged shedder	-0.13	0.56	0.829	0.948	48	48
Lachnospiraceae	Intermittent shedder	-0.07	0.24	0.806	0.948	48	48
Lachnospiraceae	Prolonged shedder	-0.05	0.24	0.848	0.948	48	48
Spirochaetaceae	Intermittent shedder	-0.03	0.20	0.899	0.948	48	48
Paludibacteraceae	Intermittent shedder	-0.23	1.48	0.886	0.948	48	48
Bacteroidetes.BD2.2	Prolonged shedder	0.42	2.72	0.888	0.948	48	36
Bacteroidales.RF16.group	Intermittent shedder	0.22	1.09	0.849	0.948	48	48
Methanocorpusculaceae	Intermittent shedder	-0.40	1.38	0.793	0.948	48	37
Pasteurellaceae	Prolonged shedder	-0.21	1.50	0.897	0.948	48	34
Muribaculaceae	Prolonged shedder	0.51	2.61	0.859	0.948	48	37
Anaerofustaceae	Intermittent shedder	0.40	1.53	0.809	0.948	48	34
Myxococcaceae	Prolonged shedder	-0.24	1.52	0.884	0.948	48	13
Synergistaceae	Intermittent shedder	-0.14	0.79	0.867	0.948	48	47
Nocardiaceae	Prolonged shedder	-0.22	0.85	0.809	0.948	48	12
Erysipelotrichaceae	Intermittent shedder	-0.18	1.22	0.893	0.948	48	38
Acholeplasmataceae	Prolonged shedder	-0.14	0.93	0.891	0.948	48	40
Mycoplasmataceae	Intermittent shedder	-0.11	0.82	0.905	0.948	48	14
Syntrophomonadaceae	Intermittent shedder	-0.12	0.65	0.865	0.948	48	17
Endomicrobiaceae	Prolonged shedder	-0.10	0.39	0.805	0.948	48	25
Mycobacteriaceae	Prolonged shedder	0.08	0.33	0.820	0.948	48	6
Anaerolineaceae	Prolonged shedder	0.12	0.52	0.834	0.948	48	11
Mitochondria	Prolonged shedder	-0.04	0.33	0.903	0.948	48	8
Coriobacteriales.Incertae.Sedis	Prolonged shedder	-0.11	1.13	0.926	0.963	48	44
Acidaminococcaceae	Prolonged shedder	0.03	0.38	0.934	0.965	48	48
Desulfurisporaceae	Prolonged shedder	-0.06	1.06	0.957	0.982	48	15
Rikenellaceae	Intermittent shedder	0.01	0.24	0.972	0.991	48	48
Butyricicoccaceae	Prolonged shedder	-0.04	1.59	0.982	0.994	48	32
Prevotellaceae	Prolonged shedder	0.00	0.22	0.996	1.000	48	48
Marinilabiliaceae	Prolonged shedder	0.00	0.87	1.000	1.000	48	8

B	Feature	Value	Coefficient	Standard Error	P-value (un-adjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
gir.aah93h0		Intermittent shedder	-2.31	0.46	0.000	0.001	48	29
Bacteroidales.BS11.gut.group		Short-term shedder	0.97	0.28	0.001	0.078	48	48
Beijerinckiaceae		Short-term shedder	0.81	0.25	0.002	0.119	48	8
Monoglobaceae		Intermittent shedder	-1.24	0.45	0.008	0.315	48	30
Actinomycetaceae		Short-term shedder	0.82	0.36	0.027	0.830	48	7
Clostridiaceae		Intermittent shedder	0.66	0.54	0.309	0.877	48	48
Lactobacillaceae		Intermittent shedder	0.52	0.56	0.420	0.877	48	48
p.251.o5		Short-term shedder	-0.53	0.56	0.412	0.877	48	48
p.251.o5		Intermittent shedder	-1.34	0.56	0.096	0.877	48	48
Prevotellaceae		Intermittent shedder	-0.20	0.22	0.416	0.877	48	48
Methanobacteriaceae		Short-term shedder	0.43	0.70	0.585	0.877	48	48
Methanobacteriaceae		Intermittent shedder	1.72	0.70	0.092	0.877	48	48
Oscillospiraceae		Short-term shedder	0.31	0.16	0.156	0.877	48	48
Oscillospiraceae		Intermittent shedder	0.39	0.16	0.096	0.877	48	48
Succinivibrionaceae		Short-term shedder	1.97	1.29	0.224	0.877	48	46
Succinivibrionaceae		Intermittent shedder	1.33	1.29	0.376	0.877	48	46
Bacteroidales.UCG.001		Short-term shedder	0.82	1.19	0.541	0.877	48	48
Bacteroidales.UCG.001		Intermittent shedder	1.88	1.19	0.214	0.877	48	48
Spirochaetaceae		Intermittent shedder	-0.13	0.20	0.582	0.877	48	48
F082		Short-term shedder	0.39	0.55	0.527	0.877	48	48
F082		Intermittent shedder	0.83	0.55	0.224	0.877	48	48
Bacteroidales.BS11.gut.group		Intermittent shedder	0.43	0.28	0.126	0.877	48	48
Ruminococcaceae		Short-term shedder	-0.34	0.38	0.444	0.877	48	48
Anaerovoracaceae		Short-term shedder	0.41	0.22	0.162	0.877	48	48
Anaerovoracaceae		Intermittent shedder	0.27	0.22	0.308	0.877	48	48
Christensenellaceae		Short-term shedder	0.38	0.30	0.301	0.877	48	48
Christensenellaceae		Intermittent shedder	0.75	0.30	0.091	0.877	48	48
Selenomonadaceae		Short-term shedder	0.17	0.26	0.549	0.877	48	48
Selenomonadaceae		Intermittent shedder	-0.36	0.26	0.254	0.877	48	48
Hungateiclostridiaceae		Short-term shedder	-0.60	0.48	0.301	0.877	48	48
Eggerthellaceae		Short-term shedder	0.43	0.38	0.340	0.877	48	48
Acidaminococcaceae		Intermittent shedder	-0.41	0.38	0.365	0.877	48	48
UCG.010		Short-term shedder	0.35	0.31	0.332	0.877	48	48
UCG.010		Intermittent shedder	0.61	0.31	0.142	0.877	48	48
Marinifilaceae		Intermittent shedder	1.61	1.85	0.448	0.877	48	39
Peptostreptococcaceae		Short-term shedder	1.76	1.95	0.434	0.877	48	39
X.Eubacterium..coprostanoligenes.group		Short-term shedder	0.55	0.33	0.193	0.877	48	48
Paludibacteraceae		Short-term shedder	1.65	1.48	0.346	0.877	48	48
Paludibacteraceae		Intermittent shedder	1.42	1.48	0.408	0.877	48	48
Dysgonomonadaceae		Intermittent shedder	3.15	1.91	0.199	0.877	48	20

Akkermansiaceae	Short-term shedder	0.98	0.84	0.328	0.877	48	48
Akkermansiaceae	Intermittent shedder	0.58	0.84	0.538	0.877	48	48
Planococcaceae	Short-term shedder	-0.88	0.65	0.272	0.877	48	45
Planococcaceae	Intermittent shedder	0.54	0.65	0.466	0.877	48	45
Coriobacteriales.Incertae.Sedis	Intermittent shedder	2.17	1.13	0.151	0.877	48	44
Methanocorpusculaceae	Short-term shedder	3.43	1.38	0.088	0.877	48	37
Methanocorpusculaceae	Intermittent shedder	3.04	1.38	0.115	0.877	48	37
Bacillaceae	Intermittent shedder	0.81	0.55	0.238	0.877	48	47
Saccharimonadaceae	Short-term shedder	2.25	1.04	0.119	0.877	48	44
Saccharimonadaceae	Intermittent shedder	2.84	1.04	0.072	0.877	48	44
Streptococcaceae	Short-term shedder	1.51	1.48	0.382	0.877	48	37
Streptococcaceae	Intermittent shedder	2.34	1.48	0.211	0.877	48	37
Desulfovibrionaceae	Intermittent shedder	-1.17	1.54	0.501	0.877	48	46
Pasteurellaceae	Intermittent shedder	-1.32	1.50	0.444	0.877	48	34
Eubacteriaceae	Short-term shedder	0.49	0.77	0.569	0.877	48	45
Defluviitaleaceae	Short-term shedder	0.91	1.40	0.563	0.877	48	41
Muribaculaceae	Intermittent shedder	-2.20	2.61	0.461	0.877	48	37
Anaerofustaceae	Short-term shedder	1.18	1.53	0.496	0.877	48	34
Anaerofustaceae	Intermittent shedder	1.59	1.53	0.376	0.877	48	34
Marinilabiliaceae	Intermittent shedder	1.06	0.87	0.308	0.877	48	8
gir.aah93h0	Short-term shedder	-0.56	0.46	0.230	0.877	48	29
M2PB4.65.termite.group	Short-term shedder	2.56	2.28	0.343	0.877	48	34
M2PB4.65.termite.group	Intermittent shedder	1.66	2.28	0.518	0.877	48	34
Butyricicoccaceae	Intermittent shedder	-1.15	1.59	0.519	0.877	48	32
Myxococcaceae	Intermittent shedder	1.79	1.52	0.324	0.877	48	13
Atopobiaceae	Short-term shedder	0.40	0.32	0.217	0.877	48	16
Atopobiaceae	Intermittent shedder	-0.42	0.32	0.188	0.877	48	16
Enterobacteriaceae	Short-term shedder	0.62	0.67	0.418	0.877	48	13
Campylobacteraceae	Short-term shedder	2.26	1.47	0.222	0.877	48	29
CAP.aah99b04	Short-term shedder	1.37	1.83	0.510	0.877	48	17
CAP.aah99b04	Intermittent shedder	2.25	1.83	0.308	0.877	48	17
Synergistaceae	Short-term shedder	0.87	0.79	0.351	0.877	48	47
Synergistaceae	Intermittent shedder	0.73	0.79	0.426	0.877	48	47
Erysipelatoclostridiaceae	Short-term shedder	-0.71	1.13	0.577	0.877	48	47
Nocardiaceae	Intermittent shedder	1.30	0.85	0.224	0.877	48	12
Erysipelotrichaceae	Short-term shedder	1.13	1.22	0.423	0.877	48	38
Erysipelotrichaceae	Intermittent shedder	0.95	1.22	0.492	0.877	48	38
Micrococcaceae	Short-term shedder	1.06	0.79	0.275	0.877	48	16
Micrococcaceae	Intermittent shedder	1.58	0.79	0.141	0.877	48	16
Oxalobacteraceae	Short-term shedder	-0.41	0.65	0.569	0.877	48	28
Oxalobacteraceae	Intermittent shedder	-0.66	0.65	0.386	0.877	48	28

Paenibacillaceae	Short-term shedder	-0.26	0.33	0.480	0.877	48	6
Acholeplasmataceae	Intermittent shedder	-0.86	0.93	0.424	0.877	48	40
Mycoplasmataceae	Short-term shedder	0.75	0.82	0.431	0.877	48	14
Mycoplasmataceae	Intermittent shedder	0.64	0.82	0.493	0.877	48	14
Monoglobaceae	Short-term shedder	0.25	0.45	0.584	0.877	48	30
Peptococcaceae	Intermittent shedder	1.16	1.26	0.428	0.877	48	32
Pedosphaeraceae	Short-term shedder	1.81	0.65	0.069	0.877	48	10
Endomicrobiaceae	Intermittent shedder	-0.57	0.39	0.148	0.877	48	25
Nocardioidaceae	Short-term shedder	0.48	0.35	0.266	0.877	48	6
Nocardioidaceae	Intermittent shedder	0.24	0.35	0.537	0.877	48	6
Methanomethylophilaceae	Short-term shedder	1.06	0.53	0.138	0.877	48	19
Methanomethylophilaceae	Intermittent shedder	0.55	0.53	0.374	0.877	48	19
Elusimicrobiaceae	Short-term shedder	0.96	1.45	0.556	0.877	48	15
Porphyromonadaceae	Short-term shedder	0.38	0.45	0.455	0.877	48	7
Ethanoligenenaceae	Short-term shedder	0.29	0.46	0.570	0.877	48	13
Ethanoligenenaceae	Intermittent shedder	0.95	0.46	0.131	0.877	48	13
Actinomycetaceae	Intermittent shedder	0.29	0.36	0.423	0.877	48	7
Beijerinckiaceae	Intermittent shedder	0.17	0.25	0.507	0.877	48	8
Sutterellaceae	Short-term shedder	0.71	0.69	0.376	0.877	48	16
Mycobacteriaceae	Intermittent shedder	-0.32	0.33	0.399	0.877	48	6
Cellulomonadaceae	Short-term shedder	0.31	0.28	0.347	0.877	48	5
Anaerolineaceae	Intermittent shedder	-0.48	0.52	0.417	0.877	48	11
Mitochondria	Intermittent shedder	-0.21	0.33	0.561	0.877	48	8
Rikenellaceae	Intermittent shedder	0.12	0.24	0.654	0.896	48	48
Eggerthellaceae	Intermittent shedder	0.20	0.38	0.635	0.896	48	48
X.Eubacterium..coprostanoligenes.group	Intermittent shedder	0.16	0.33	0.649	0.896	48	48
Bacteroidetes.BD2.2	Intermittent shedder	1.57	2.72	0.603	0.896	48	36
Bacteroidales.RF16.group	Intermittent shedder	0.59	1.09	0.625	0.896	48	48
Enterobacteriaceae	Intermittent shedder	0.33	0.67	0.655	0.896	48	13
Campylobacteraceae	Intermittent shedder	0.76	1.47	0.642	0.896	48	29
Desulfurisporaceae	Intermittent shedder	0.57	1.06	0.631	0.896	48	15
Syntrophomonadaceae	Intermittent shedder	-0.35	0.65	0.630	0.896	48	17
Cellulomonadaceae	Intermittent shedder	0.14	0.28	0.646	0.896	48	5
Spirochaetaceae	Short-term shedder	-0.10	0.20	0.666	0.900	48	48
Fibrobacteraceae	Short-term shedder	0.14	0.31	0.669	0.900	48	48
Rikenellaceae	Short-term shedder	0.11	0.24	0.678	0.904	48	48
Clostridiaceae	Short-term shedder	0.23	0.54	0.702	0.909	48	48
Fibrobacteraceae	Intermittent shedder	-0.13	0.31	0.707	0.909	48	48
Dysgonomonadaceae	Short-term shedder	-0.78	1.91	0.711	0.909	48	20
Desulfovibrionaceae	Short-term shedder	-0.66	1.54	0.698	0.909	48	46
Peptococcaceae	Short-term shedder	0.55	1.26	0.691	0.909	48	32

Hungateiclostridiaceae	Intermittent shedder	0.19	0.48	0.725	0.914	48	48
Marinifilaceae	Short-term shedder	0.69	1.85	0.732	0.914	48	39
Bacillaceae	Short-term shedder	-0.21	0.55	0.732	0.914	48	47
Syntrophomonadaceae	Short-term shedder	-0.23	0.65	0.750	0.923	48	17
Elusimicrobiaceae	Intermittent shedder	-0.50	1.45	0.752	0.923	48	15
Bacteroidales.RF16.group	Short-term shedder	0.36	1.09	0.760	0.926	48	48
Peptostreptococcaceae	Intermittent shedder	0.61	1.95	0.773	0.935	48	39
Ruminococcaceae	Intermittent shedder	-0.11	0.38	0.791	0.949	48	48
Erysipelatoclostridiaceae	Intermittent shedder	0.31	1.13	0.805	0.949	48	47
Nocardiaceae	Short-term shedder	0.22	0.85	0.809	0.949	48	12
Endomicrobiaceae	Short-term shedder	0.10	0.39	0.805	0.949	48	25
Mycobacteriaceae	Short-term shedder	-0.08	0.33	0.820	0.954	48	6
Lactobacillaceae	Short-term shedder	0.13	0.56	0.829	0.956	48	48
Anaerolineaceae	Short-term shedder	-0.12	0.52	0.834	0.956	48	11
Lachnospiraceae	Short-term shedder	0.05	0.24	0.848	0.964	48	48
Muribaculaceae	Short-term shedder	-0.51	2.61	0.859	0.964	48	37
Porphyromonadaceae	Intermittent shedder	0.09	0.45	0.858	0.964	48	7
Bacteroidetes.BD2.2	Short-term shedder	-0.42	2.72	0.888	0.978	48	36
Pasteurellaceae	Short-term shedder	0.21	1.50	0.897	0.978	48	34
Myxococcaceae	Short-term shedder	0.24	1.52	0.884	0.978	48	13
Acholeplasmataceae	Short-term shedder	0.14	0.93	0.891	0.978	48	40
Mitochondria	Short-term shedder	0.04	0.33	0.903	0.978	48	8
Paenibacillaceae	Intermittent shedder	0.04	0.33	0.912	0.981	48	6
Coriobacteriales.Incertae.Sedis	Short-term shedder	0.11	1.13	0.926	0.989	48	44
Acidaminococcaceae	Short-term shedder	-0.03	0.38	0.934	0.991	48	48
Prevotellaceae	Short-term shedder	0.00	0.22	0.996	1.000	48	48
Lachnospiraceae	Intermittent shedder	-0.01	0.24	0.957	1.000	48	48
Eubacteriaceae	Intermittent shedder	-0.03	0.77	0.974	1.000	48	45
Defluviitaleaceae	Intermittent shedder	-0.01	1.40	0.997	1.000	48	41
Marinilabiliaceae	Short-term shedder	0.00	0.87	1.000	1.000	48	8
Butyricicoccaceae	Short-term shedder	0.04	1.59	0.982	1.000	48	32
Desulfurisporaceae	Short-term shedder	0.06	1.06	0.957	1.000	48	15
Pedosphaeraceae	Intermittent shedder	0.00	0.65	1.000	1.000	48	10
Sutterellaceae	Intermittent shedder	0.01	0.69	0.991	1.000	48	16

Supplementary Table 5.11: Results of generalized linear mixed models evaluating genus-level differential abundance by *Salmonella* shedding pattern with **(A)** short-term shedders as the reference level and **(B)** prolonged shedders as the reference level.

A Feature	Value	Coefficient	Standard Error	P-value (unadjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Mogibacterium	Prolonged shedder	-0.70	0.15	0.000	0.005	48	48
Monoglobus	Intermittent shedder	-1.49	0.45	0.002	0.121	48	30
T2WK15B57	Intermittent shedder	-1.23	0.37	0.002	0.121	48	6
T2WK15B57	Prolonged shedder	-1.23	0.37	0.002	0.121	48	6
Methylobacterium.Methylorubrum	Prolonged shedder	-0.81	0.25	0.002	0.125	48	8
Mogibacterium	Intermittent shedder	-0.44	0.15	0.004	0.182	48	48
Blautia	Intermittent shedder	-0.67	0.22	0.005	0.182	48	48
Blautia	Prolonged shedder	-0.61	0.22	0.009	0.313	48	48
Lachnospiraceae.UCG.007	Intermittent shedder	1.03	0.38	0.010	0.313	48	16
Methylobacterium.Methylorubrum	Intermittent shedder	-0.64	0.25	0.014	0.376	48	8
Arcanobacterium	Prolonged shedder	-0.82	0.36	0.027	0.663	48	7
Denitrobacterium	Intermittent shedder	1.73	0.44	0.029	0.672	48	12
Clostridium.sensu.stricto.1	Intermittent shedder	0.44	0.58	0.508	0.963	48	48
Ligilactobacillus	Intermittent shedder	0.35	0.50	0.534	0.963	48	48
Prevotella	Intermittent shedder	-0.97	0.48	0.136	0.963	48	48
Prevotella	Prolonged shedder	-0.25	0.48	0.638	0.963	48	48
Pseudobutyrivibrio	Intermittent shedder	-1.74	1.44	0.315	0.963	48	46
Pseudobutyrivibrio	Prolonged shedder	0.60	1.44	0.705	0.963	48	46
Methanobrevibacter	Intermittent shedder	1.29	0.70	0.163	0.963	48	48
Methanobrevibacter	Prolonged shedder	-0.43	0.70	0.585	0.963	48	48
Rikenellaceae.RC9.gut.group	Prolonged shedder	-0.63	0.41	0.221	0.963	48	48
Sarcina	Intermittent shedder	0.59	0.62	0.412	0.963	48	47
Sarcina	Prolonged shedder	-0.85	0.62	0.263	0.963	48	47
X.Eubacterium..hallii.group	Intermittent shedder	0.43	0.48	0.440	0.963	48	48
X.Eubacterium..hallii.group	Prolonged shedder	-0.40	0.48	0.469	0.963	48	48
Treponema	Prolonged shedder	0.10	0.20	0.659	0.963	48	48
Ruminococcus	Intermittent shedder	0.36	0.46	0.497	0.963	48	48
Ruminococcus	Prolonged shedder	0.48	0.46	0.377	0.963	48	48
Family.XIII.UCG.001	Intermittent shedder	0.11	0.13	0.467	0.963	48	48
Fibrobacter	Intermittent shedder	-0.27	0.31	0.441	0.963	48	48
Fibrobacter	Prolonged shedder	-0.14	0.31	0.669	0.963	48	48
Christensenellaceae.R.7.group	Intermittent shedder	0.37	0.31	0.308	0.963	48	48
Christensenellaceae.R.7.group	Prolonged shedder	-0.37	0.31	0.309	0.963	48	48

Quinella	Intermittent shedder	-1.40	0.91	0.222	0.963	48	48
Prevotellaceae.UCG.003	Intermittent shedder	-0.88	0.60	0.241	0.963	48	48
Saccharofermentans	Intermittent shedder	0.93	0.44	0.125	0.963	48	48
Saccharofermentans	Prolonged shedder	0.76	0.44	0.184	0.963	48	48
Prevotellaceae.UCG.001	Prolonged shedder	0.39	0.23	0.195	0.963	48	48
NK4A214.group	Intermittent shedder	0.30	0.25	0.319	0.963	48	48
NK4A214.group	Prolonged shedder	-0.41	0.25	0.208	0.963	48	48
Succinivibrio	Intermittent shedder	1.08	2.07	0.639	0.963	48	27
Succinivibrio	Prolonged shedder	-2.24	2.07	0.358	0.963	48	27
Prevotellaceae.UCG.004	Intermittent shedder	0.50	0.31	0.211	0.963	48	48
Frisingicoccus	Intermittent shedder	1.57	1.41	0.347	0.963	48	44
Phascolarctobacterium	Intermittent shedder	-0.37	0.38	0.402	0.963	48	48
Terrisporobacter	Intermittent shedder	-1.14	1.95	0.599	0.963	48	39
Terrisporobacter	Prolonged shedder	-1.76	1.95	0.434	0.963	48	39
possible.genus.Sk018	Prolonged shedder	0.96	1.20	0.483	0.963	48	37
hoa5.07d05.gut.group	Intermittent shedder	-0.96	1.04	0.425	0.963	48	48
hoa5.07d05.gut.group	Prolonged shedder	1.32	1.04	0.293	0.963	48	48
Lachnospiraceae.UCG.009	Intermittent shedder	0.19	0.19	0.317	0.963	48	48
Lachnospiraceae.UCG.009	Prolonged shedder	-0.07	0.19	0.722	0.963	48	48
Alloprevotella	Prolonged shedder	-0.51	0.97	0.639	0.963	48	48
Cellulosilyticum	Intermittent shedder	0.57	1.45	0.719	0.963	48	46
Cellulosilyticum	Prolonged shedder	-1.37	1.45	0.414	0.963	48	46
Family.XIII.AD3011.group	Prolonged shedder	-0.69	0.43	0.209	0.963	48	48
Anaerovibrio	Intermittent shedder	-1.57	0.94	0.195	0.963	48	45
Anaerosporobacter	Prolonged shedder	-0.71	1.04	0.545	0.963	48	48
Prevotellaceae.Ga6A1.group	Intermittent shedder	-2.37	1.28	0.160	0.963	48	42
Agathobacter	Intermittent shedder	-1.22	0.97	0.298	0.963	48	47
Akkermansia	Intermittent shedder	-0.40	0.84	0.668	0.963	48	48
Akkermansia	Prolonged shedder	-0.98	0.84	0.328	0.963	48	48
Rummeliibacillus	Intermittent shedder	2.76	0.92	0.058	0.963	48	35
Rummeliibacillus	Prolonged shedder	1.19	0.92	0.288	0.963	48	35
Anaerovorax	Prolonged shedder	-0.36	0.46	0.491	0.963	48	48
Lachnospiraceae.XPB1014.group	Intermittent shedder	0.47	1.14	0.705	0.963	48	48
Phoenicibacter	Intermittent shedder	2.32	1.61	0.247	0.963	48	39
Phoenicibacter	Prolonged shedder	-1.58	1.61	0.400	0.963	48	39
Acetitomaculum	Intermittent shedder	-2.37	1.42	0.194	0.963	48	31
Acetitomaculum	Prolonged shedder	1.36	1.42	0.408	0.963	48	31
UCG.002	Intermittent shedder	-0.84	0.82	0.381	0.963	48	48
UCG.002	Prolonged shedder	-0.82	0.82	0.393	0.963	48	48
Methanocorpusculum	Prolonged shedder	-3.43	1.38	0.088	0.963	48	37
Bacillus	Intermittent shedder	0.99	0.54	0.160	0.963	48	47

Bacillus	Prolonged shedder	0.21	0.54	0.724	0.963	48	47
Marvinbryantia	Intermittent shedder	-0.80	0.34	0.101	0.963	48	48
Marvinbryantia	Prolonged shedder	-0.64	0.34	0.158	0.963	48	48
Candidatus.Saccharimonas	Intermittent shedder	0.59	1.04	0.613	0.963	48	44
Candidatus.Saccharimonas	Prolonged shedder	-2.25	1.04	0.119	0.963	48	44
Streptococcus	Intermittent shedder	0.87	1.16	0.506	0.963	48	35
Streptococcus	Prolonged shedder	-1.71	1.16	0.236	0.963	48	35
Schwartzia	Intermittent shedder	-0.77	1.95	0.721	0.963	48	31
Schwartzia	Prolonged shedder	-1.01	1.95	0.639	0.963	48	31
dgA.11.gut.group	Intermittent shedder	0.93	1.18	0.488	0.963	48	45
dgA.11.gut.group	Prolonged shedder	1.49	1.18	0.297	0.963	48	45
Lactobacillus	Intermittent shedder	1.42	0.99	0.246	0.963	48	24
Lactobacillus	Prolonged shedder	-1.02	0.99	0.376	0.963	48	24
Desulfovibrio	Prolonged shedder	0.82	1.50	0.625	0.963	48	44
Lachnoclostridium	Intermittent shedder	-0.78	1.00	0.495	0.963	48	37
Lachnoclostridium	Prolonged shedder	0.69	1.00	0.541	0.963	48	37
Seminibacterium	Intermittent shedder	-1.51	1.50	0.390	0.963	48	34
Lachnospiraceae.NK4A136.group	Intermittent shedder	-0.85	1.54	0.619	0.963	48	25
Eubacterium	Intermittent shedder	-0.52	0.77	0.549	0.963	48	45
Eubacterium	Prolonged shedder	-0.49	0.77	0.569	0.963	48	45
X.Eubacterium..ruminantium.group	Intermittent shedder	-2.51	2.69	0.421	0.963	48	33
Solibacillus	Prolonged shedder	0.63	0.97	0.564	0.963	48	32
Defluviitaleaceae.UCG.011	Intermittent shedder	-0.91	1.40	0.561	0.963	48	41
Defluviitaleaceae.UCG.011	Prolonged shedder	-0.91	1.40	0.563	0.963	48	41
Coprococcus	Intermittent shedder	-1.56	1.31	0.318	0.963	48	30
Coprococcus	Prolonged shedder	-1.24	1.31	0.415	0.963	48	30
Candidatus.Soleaferrea	Intermittent shedder	-1.21	0.71	0.188	0.963	48	43
Candidatus.Soleaferrea	Prolonged shedder	-1.90	0.71	0.076	0.963	48	43
Parvibacter	Intermittent shedder	-1.09	1.01	0.361	0.963	48	25
FD2005	Prolonged shedder	1.15	1.24	0.424	0.963	48	24
Oribacterium	Prolonged shedder	-1.05	1.58	0.553	0.963	48	43
Papillibacter	Intermittent shedder	0.48	1.18	0.711	0.963	48	32
Anaerofustis	Prolonged shedder	-1.18	1.53	0.496	0.963	48	34
Mailhella	Intermittent shedder	-0.64	1.49	0.696	0.963	48	34
Mailhella	Prolonged shedder	0.90	1.49	0.587	0.963	48	34
Weissella	Intermittent shedder	0.91	0.51	0.084	0.963	48	11
DNF00809	Prolonged shedder	0.40	1.03	0.724	0.963	48	34
UCG.009	Intermittent shedder	-1.07	1.58	0.545	0.963	48	31
Escherichia.Shigella	Intermittent shedder	-0.37	0.60	0.578	0.963	48	10
Escherichia.Shigella	Prolonged shedder	-0.89	0.60	0.233	0.963	48	10
Campylobacter	Intermittent shedder	-1.50	1.47	0.382	0.963	48	29

Campylobacter	Prolonged shedder	-2.26	1.47	0.222	0.963	48	29
Colidextribacter	Intermittent shedder	1.72	1.46	0.324	0.963	48	10
Synergistes	Intermittent shedder	-1.33	1.44	0.424	0.963	48	29
Synergistes	Prolonged shedder	-2.61	1.44	0.169	0.963	48	29
XBB1006	Intermittent shedder	-1.31	0.93	0.255	0.963	48	19
XBB1006	Prolonged shedder	-0.42	0.93	0.681	0.963	48	19
Ruminiclostridium	Intermittent shedder	-1.30	1.37	0.410	0.963	48	28
Ruminiclostridium	Prolonged shedder	-1.44	1.37	0.369	0.963	48	28
UCG.004	Intermittent shedder	1.22	1.73	0.530	0.963	48	41
Oscillibacter	Intermittent shedder	-1.43	0.56	0.083	0.963	48	17
Oscillibacter	Prolonged shedder	-0.47	0.56	0.464	0.963	48	17
Lysinibacillus	Intermittent shedder	0.21	0.34	0.533	0.963	48	7
Denitrobacterium	Prolonged shedder	0.22	0.44	0.658	0.963	48	12
Rhodococcus	Intermittent shedder	1.07	0.85	0.296	0.963	48	12
Pyramidobacter	Intermittent shedder	-1.50	1.12	0.275	0.963	48	26
Arthrobacter	Intermittent shedder	0.62	0.87	0.529	0.963	48	15
Arthrobacter	Prolonged shedder	-0.96	0.87	0.351	0.963	48	15
Selenomonas	Intermittent shedder	-1.42	0.91	0.216	0.963	48	11
Selenomonas	Prolonged shedder	-0.96	0.91	0.367	0.963	48	11
Butyrivibrio	Intermittent shedder	-1.02	0.91	0.345	0.963	48	11
Butyrivibrio	Prolonged shedder	-0.56	0.91	0.582	0.963	48	11
X.Eubacterium..nodatum.group	Intermittent shedder	-0.60	0.60	0.387	0.963	48	22
X.Eubacterium..nodatum.group	Prolonged shedder	0.40	0.60	0.549	0.963	48	22
Erysipelatoclostridium	Intermittent shedder	-0.94	1.58	0.595	0.963	48	14
Erysipelatoclostridium	Prolonged shedder	0.65	1.58	0.706	0.963	48	14
Paenibacillus	Intermittent shedder	0.30	0.33	0.424	0.963	48	6
Paenibacillus	Prolonged shedder	0.26	0.33	0.480	0.963	48	6
Desulfurispora	Intermittent shedder	0.50	1.06	0.667	0.963	48	15
Anaeroplasma	Intermittent shedder	-0.77	0.94	0.471	0.963	48	39
M2PT2.76.termite.group	Intermittent shedder	1.46	1.10	0.278	0.963	48	21
Mycoplasma	Prolonged shedder	-0.75	0.82	0.431	0.963	48	14
Enterorhabdus	Intermittent shedder	0.27	0.60	0.680	0.963	48	7
Enterorhabdus	Prolonged shedder	-0.30	0.60	0.651	0.963	48	7
Monoglobus	Prolonged shedder	-0.25	0.45	0.584	0.963	48	30
Lachnospiraceae.ND3007.group	Intermittent shedder	0.48	0.84	0.610	0.963	48	26
Lachnospiraceae.ND3007.group	Prolonged shedder	1.25	0.84	0.232	0.963	48	26
Roseburia	Intermittent shedder	-1.14	0.74	0.219	0.963	48	17
Roseburia	Prolonged shedder	-0.65	0.74	0.440	0.963	48	17
DEV114	Intermittent shedder	-1.81	0.65	0.069	0.963	48	10
DEV114	Prolonged shedder	-1.81	0.65	0.069	0.963	48	10

Endomicrobium	Intermittent shedder	-0.67	0.39	0.092	0.963	48	25
Aeromicrobium	Intermittent shedder	-0.16	0.36	0.686	0.963	48	5
Aeromicrobium	Prolonged shedder	-0.38	0.36	0.367	0.963	48	5
Lachnospiraceae.UCG.008	Intermittent shedder	-0.54	1.17	0.676	0.963	48	18
Shuttleworthia	Intermittent shedder	-0.33	0.30	0.270	0.963	48	10
Shuttleworthia	Prolonged shedder	-0.35	0.30	0.244	0.963	48	10
Oscillospira	Intermittent shedder	-0.70	0.57	0.308	0.963	48	5
Oscillospira	Prolonged shedder	-0.70	0.57	0.308	0.963	48	5
Kurthia	Intermittent shedder	2.24	0.77	0.063	0.963	48	28
Kurthia	Prolonged shedder	1.02	0.77	0.280	0.963	48	28
Elusimicrobium	Intermittent shedder	-1.46	1.45	0.388	0.963	48	15
Elusimicrobium	Prolonged shedder	-0.96	1.45	0.556	0.963	48	15
Lachnospiraceae.UCG.006	Intermittent shedder	0.43	1.01	0.700	0.963	48	20
Lachnospiraceae.UCG.006	Prolonged shedder	-0.71	1.01	0.535	0.963	48	20
X.Eubacterium..siraeum.group	Intermittent shedder	-0.28	0.56	0.646	0.963	48	7
X.Eubacterium..siraeum.group	Prolonged shedder	0.38	0.56	0.544	0.963	48	7
Porphyromonas	Intermittent shedder	-0.30	0.45	0.556	0.963	48	7
Porphyromonas	Prolonged shedder	-0.38	0.45	0.455	0.963	48	7
Aggregicoccus	Intermittent shedder	-0.33	0.35	0.412	0.963	48	5
Aggregicoccus	Prolonged shedder	-0.24	0.35	0.534	0.963	48	5
Incertae.Sedis_1	Intermittent shedder	0.66	0.46	0.248	0.963	48	13
Incertae.Sedis_1	Prolonged shedder	-0.29	0.46	0.570	0.963	48	13
Succinivibrionaceae.UCG.002	Prolonged shedder	0.41	0.52	0.480	0.963	48	10
Arcanobacterium	Intermittent shedder	-0.53	0.36	0.145	0.963	48	7
Ruminobacter	Intermittent shedder	-1.24	0.39	0.052	0.963	48	12
Ruminobacter	Prolonged shedder	-0.56	0.39	0.251	0.963	48	12
Sediminispirochaeta	Intermittent shedder	-0.36	0.91	0.717	0.963	48	16
Sediminispirochaeta	Prolonged shedder	-0.54	0.91	0.597	0.963	48	16
UCG.007	Intermittent shedder	-0.16	0.21	0.451	0.963	48	5
Candidatus.Methanomethylophilus	Intermittent shedder	-0.47	0.45	0.374	0.963	48	9
Sutterella	Intermittent shedder	-0.70	0.69	0.381	0.963	48	16
Sutterella	Prolonged shedder	-0.71	0.69	0.376	0.963	48	16
Pygmaiobacter	Intermittent shedder	-0.23	0.53	0.698	0.963	48	7
Pygmaiobacter	Prolonged shedder	0.51	0.53	0.408	0.963	48	7
Mycobacterium	Intermittent shedder	-0.24	0.33	0.517	0.963	48	6
Cellulomonas	Intermittent shedder	-0.17	0.28	0.588	0.963	48	5
Cellulomonas	Prolonged shedder	-0.31	0.28	0.347	0.963	48	5
Flexilinea	Intermittent shedder	-0.37	0.52	0.529	0.963	48	11
Fretibacterium	Intermittent shedder	0.39	0.71	0.625	0.963	48	17
Howardella	Intermittent shedder	0.18	0.26	0.549	0.963	48	7

Howardella	Prolonged shedder	-0.12	0.26	0.683	0.963	48	7
X.Eubacterium..saphenum.group	Intermittent shedder	0.79	0.69	0.336	0.963	48	11
Mucinivorans	Prolonged shedder	-0.31	0.30	0.302	0.963	48	9
Jonquetella	Intermittent shedder	-0.28	0.48	0.603	0.963	48	6
Jonquetella	Prolonged shedder	0.29	0.48	0.590	0.963	48	6
Incertae.Sedis_2	Intermittent shedder	-0.18	0.46	0.727	0.963	48	10
V9D2013.group	Intermittent shedder	0.18	0.24	0.442	0.963	48	6
V9D2013.group	Prolonged shedder	-0.25	0.24	0.296	0.963	48	6
Alistipes	Intermittent shedder	-0.69	0.31	0.114	0.963	48	6
Alistipes	Prolonged shedder	-0.69	0.31	0.114	0.963	48	6
NED5E9	Intermittent shedder	-0.16	0.34	0.662	0.963	48	7
Prevotella_9	Prolonged shedder	0.37	0.43	0.459	0.963	48	5
Rikenellaceae.RC9.gut.group	Intermittent shedder	0.15	0.41	0.742	0.973	48	48
M2PT2.76.termite.group	Prolonged shedder	-0.40	1.10	0.741	0.973	48	21
UCG.005	Prolonged shedder	-0.05	0.18	0.809	0.978	48	48
Prevotellaceae.UCG.003	Prolonged shedder	-0.16	0.60	0.802	0.978	48	48
possible.genus.Sk018	Intermittent shedder	-0.41	1.20	0.755	0.978	48	37
Anaerovibrio	Prolonged shedder	-0.27	0.94	0.796	0.978	48	45
Lachnospiraceae.XPB1014.group	Prolonged shedder	-0.31	1.14	0.802	0.978	48	48
Methanocorpusculum	Intermittent shedder	-0.40	1.38	0.793	0.978	48	37
Oribacterium	Intermittent shedder	0.42	1.58	0.807	0.978	48	43
Anaerofustis	Intermittent shedder	0.40	1.53	0.809	0.978	48	34
Weissella	Prolonged shedder	0.15	0.51	0.772	0.978	48	11
Lactococcus	Prolonged shedder	-0.13	0.49	0.803	0.978	48	9
Lysinibacillus	Prolonged shedder	-0.09	0.34	0.780	0.978	48	7
Rhodococcus	Prolonged shedder	-0.22	0.85	0.809	0.978	48	12
Catenisphaera	Prolonged shedder	-0.38	1.38	0.801	0.978	48	27
Pelospora	Prolonged shedder	0.23	0.71	0.770	0.978	48	14
Endomicrobium	Prolonged shedder	-0.10	0.39	0.805	0.978	48	25
Succinivibrionaceae.UCG.002	Intermittent shedder	0.13	0.52	0.811	0.978	48	10
Fretibacterium	Prolonged shedder	0.23	0.71	0.770	0.978	48	17
Prevotella_9	Intermittent shedder	-0.14	0.43	0.762	0.978	48	5
Clostridium.sensu.stricto.1	Prolonged shedder	-0.12	0.58	0.844	0.980	48	48
Agathobacter	Prolonged shedder	-0.21	0.97	0.840	0.980	48	47
Anaerovorax	Intermittent shedder	-0.10	0.46	0.838	0.980	48	48
UCG.004	Prolonged shedder	-0.37	1.73	0.846	0.980	48	41
Catenisphaera	Intermittent shedder	-0.29	1.38	0.847	0.980	48	27
Pelospora	Intermittent shedder	-0.15	0.71	0.848	0.980	48	14
UCG.007	Prolonged shedder	0.04	0.21	0.836	0.980	48	5
Mycobacterium	Prolonged shedder	0.08	0.33	0.820	0.980	48	6

Flexilinea	Prolonged shedder	0.12	0.52	0.834	0.980	48	11
X.Eubacterium..saphenum.group	Prolonged shedder	0.17	0.69	0.820	0.980	48	11
UCG.005	Intermittent shedder	0.03	0.18	0.861	0.981	48	48
Prevotellaceae.UCG.001	Intermittent shedder	-0.04	0.23	0.861	0.981	48	48
Prevotellaceae.UCG.004	Prolonged shedder	-0.06	0.31	0.866	0.981	48	48
Lachnospiraceae.NK4A136.group	Prolonged shedder	0.29	1.54	0.862	0.981	48	25
Lachnospiraceae.UCG.008	Prolonged shedder	0.21	1.17	0.866	0.981	48	18
Lachnospiraceae.AC2044.group	Intermittent shedder	0.09	0.56	0.880	0.983	48	48
Treponema	Intermittent shedder	-0.03	0.20	0.886	0.983	48	48
Family.XIII.UCG.001	Prolonged shedder	-0.02	0.13	0.874	0.983	48	48
X.Eubacterium..ruminantium.group	Prolonged shedder	-0.43	2.69	0.883	0.983	48	33
Pyramidobacter	Prolonged shedder	0.18	1.12	0.882	0.983	48	26
Lachnospiraceae.AC2044.group	Prolonged shedder	-0.07	0.56	0.907	0.985	48	48
Quinella	Prolonged shedder	-0.11	0.91	0.910	0.985	48	48
Family.XIII.AD3011.group	Intermittent shedder	-0.06	0.43	0.894	0.985	48	48
Seminibacterium	Prolonged shedder	-0.18	1.50	0.911	0.985	48	34
Parvibacter	Prolonged shedder	-0.14	1.01	0.897	0.985	48	25
Mycoplasma	Intermittent shedder	-0.11	0.82	0.905	0.985	48	14
Candidatus.Methanomethylophilus	Prolonged shedder	-0.05	0.45	0.913	0.985	48	9
Ligilactobacillus	Prolonged shedder	-0.04	0.50	0.937	0.987	48	48
Phascolarctobacterium	Prolonged shedder	0.03	0.38	0.934	0.987	48	48
Prevotellaceae.Ga6A1.group	Prolonged shedder	-0.12	1.28	0.931	0.987	48	42
DNF00809	Intermittent shedder	-0.08	1.03	0.940	0.987	48	34
Anaeroplasma	Prolonged shedder	0.09	0.94	0.931	0.987	48	39
Mucinivorans	Intermittent shedder	0.03	0.30	0.925	0.987	48	9
NED5E9	Prolonged shedder	-0.03	0.34	0.929	0.987	48	7
Desulfovibrio	Intermittent shedder	0.06	1.50	0.971	0.991	48	44
Solibacillus	Intermittent shedder	0.04	0.97	0.973	0.991	48	32
FD2005	Intermittent shedder	0.07	1.24	0.956	0.991	48	24
Papillibacter	Prolonged shedder	0.07	1.18	0.959	0.991	48	32
UCG.009	Prolonged shedder	0.06	1.58	0.973	0.991	48	31
Colidextribacter	Prolonged shedder	0.08	1.46	0.960	0.991	48	10
Desulfurispora	Prolonged shedder	-0.06	1.06	0.957	0.991	48	15
Incertae.Sedis_2	Prolonged shedder	-0.02	0.46	0.972	0.991	48	10
Lactococcus	Intermittent shedder	-0.01	0.49	0.978	0.992	48	9
Frisingicoccus	Prolonged shedder	0.03	1.41	0.986	0.997	48	44
Alloprevotella	Intermittent shedder	-0.01	0.97	0.992	0.999	48	48
Anaerosporobacter	Intermittent shedder	0.00	1.04	1.000	1.000	48	48
Lachnospiraceae.UCG.007	Prolonged shedder	0.00	0.38	1.000	1.000	48	16

B	Feature	Value	Coefficient	Standard Error	P-value (un-adjusted)	P-value (BH-adjusted)	N	N (non-zero counts)
Mogibacterium		Short-term shedder	0.70	0.15	0.000	0.005	48	48
Methylobacterium.Methylorubrum		Short-term shedder	0.81	0.25	0.002	0.209	48	8
T2WK15B57		Short-term shedder	1.23	0.37	0.002	0.209	48	6
Blautia		Short-term shedder	0.61	0.22	0.009	0.469	48	48
Lachnospiraceae.UCG.007		Intermittent shedder	1.03	0.38	0.010	0.469	48	16
Monoglobus		Intermittent shedder	-1.24	0.45	0.008	0.469	48	30
Clostridium.sensu.stricto.1		Intermittent shedder	0.56	0.58	0.406	0.935	48	48
Prevotella		Intermittent shedder	-0.72	0.48	0.230	0.935	48	48
Pseudobutyrivibrio		Intermittent shedder	-2.34	1.44	0.203	0.935	48	46
Methanobrevibacter		Intermittent shedder	1.72	0.70	0.092	0.935	48	48
Rikenellaceae.RC9.gut.group		Short-term shedder	0.63	0.41	0.221	0.935	48	48
Rikenellaceae.RC9.gut.group		Intermittent shedder	0.77	0.41	0.153	0.935	48	48
Sarcina		Short-term shedder	0.85	0.62	0.263	0.935	48	47
Sarcina		Intermittent shedder	1.44	0.62	0.103	0.935	48	47
X.Eubacterium..hallii.group		Intermittent shedder	0.83	0.48	0.185	0.935	48	48
Ruminococcus		Short-term shedder	-0.48	0.46	0.377	0.935	48	48
Family.XIII.UCG.001		Intermittent shedder	0.13	0.13	0.390	0.935	48	48
Christensenellaceae.R.7.group		Short-term shedder	0.37	0.31	0.309	0.935	48	48
Christensenellaceae.R.7.group		Intermittent shedder	0.75	0.31	0.092	0.935	48	48
Quinella		Intermittent shedder	-1.29	0.91	0.253	0.935	48	48
Prevotellaceae.UCG.003		Intermittent shedder	-0.71	0.60	0.322	0.935	48	48
Saccharofermentans		Short-term shedder	-0.76	0.44	0.184	0.935	48	48
Prevotellaceae.UCG.001		Short-term shedder	-0.39	0.23	0.195	0.935	48	48
Prevotellaceae.UCG.001		Intermittent shedder	-0.43	0.23	0.161	0.935	48	48
NK4A214.group		Short-term shedder	0.41	0.25	0.208	0.935	48	48
NK4A214.group		Intermittent shedder	0.71	0.25	0.068	0.935	48	48
Succinivibrio		Short-term shedder	2.24	2.07	0.358	0.935	48	27
Succinivibrio		Intermittent shedder	3.32	2.07	0.207	0.935	48	27
Prevotellaceae.UCG.004		Intermittent shedder	0.56	0.31	0.175	0.935	48	48
Frisingicoccus		Intermittent shedder	1.54	1.41	0.355	0.935	48	44
Phascolarctobacterium		Intermittent shedder	-0.41	0.38	0.365	0.935	48	48
Mogibacterium		Intermittent shedder	0.26	0.15	0.085	0.935	48	48
Terrisporobacter		Short-term shedder	1.76	1.95	0.434	0.935	48	39
possible.genus.Sk018		Intermittent shedder	-1.37	1.20	0.337	0.935	48	37
hoa5.07d05.gut.group		Short-term shedder	-1.32	1.04	0.293	0.935	48	48
hoa5.07d05.gut.group		Intermittent shedder	-2.28	1.04	0.116	0.935	48	48
Lachnospiraceae.UCG.009		Intermittent shedder	0.26	0.19	0.178	0.935	48	48
Cellulosilyticum		Short-term shedder	1.37	1.45	0.414	0.935	48	46
Cellulosilyticum		Intermittent shedder	1.94	1.45	0.272	0.935	48	46

Family.XIII.AD3011.group	Short-term shedder	0.69	0.43	0.209	0.935	48	48
Family.XIII.AD3011.group	Intermittent shedder	0.62	0.43	0.243	0.935	48	48
Anaerovibrio	Intermittent shedder	-1.31	0.94	0.261	0.935	48	45
Prevotellaceae.Ga6A1.group	Intermittent shedder	-2.25	1.28	0.176	0.935	48	42
Agathobacter	Intermittent shedder	-1.01	0.97	0.376	0.935	48	47
Akkermansia	Short-term shedder	0.98	0.84	0.328	0.935	48	48
Rummeliibacillus	Short-term shedder	-1.19	0.92	0.288	0.935	48	35
Rummeliibacillus	Intermittent shedder	1.57	0.92	0.188	0.935	48	35
Phoenicibacter	Short-term shedder	1.58	1.61	0.400	0.935	48	39
Phoenicibacter	Intermittent shedder	3.90	1.61	0.095	0.935	48	39
Acetitomaculum	Short-term shedder	-1.36	1.42	0.408	0.935	48	31
Acetitomaculum	Intermittent shedder	-3.73	1.42	0.078	0.935	48	31
UCG.002	Short-term shedder	0.82	0.82	0.393	0.935	48	48
Methanocorpusculum	Short-term shedder	3.43	1.38	0.088	0.935	48	37
Methanocorpusculum	Intermittent shedder	3.04	1.38	0.115	0.935	48	37
Bacillus	Intermittent shedder	0.79	0.54	0.238	0.935	48	47
Marvinbryantia	Short-term shedder	0.64	0.34	0.158	0.935	48	48
Candidatus.Saccharimonas	Short-term shedder	2.25	1.04	0.119	0.935	48	44
Candidatus.Saccharimonas	Intermittent shedder	2.84	1.04	0.072	0.935	48	44
Streptococcus	Short-term shedder	1.71	1.16	0.236	0.935	48	35
Streptococcus	Intermittent shedder	2.58	1.16	0.112	0.935	48	35
dgA.11.gut.group	Short-term shedder	-1.49	1.18	0.297	0.935	48	45
Lactobacillus	Short-term shedder	1.02	0.99	0.376	0.935	48	24
Lactobacillus	Intermittent shedder	2.44	0.99	0.090	0.935	48	24
Lachnoclostridium	Intermittent shedder	-1.47	1.00	0.240	0.935	48	37
Seminibacterium	Intermittent shedder	-1.32	1.50	0.444	0.935	48	34
Coprococcus	Short-term shedder	1.24	1.31	0.415	0.935	48	30
Candidatus.Soleaferrea	Short-term shedder	1.90	0.71	0.076	0.935	48	43
Candidatus.Soleaferrea	Intermittent shedder	0.69	0.71	0.405	0.935	48	43
Parvibacter	Intermittent shedder	-0.95	1.01	0.418	0.935	48	25
FD2005	Short-term shedder	-1.15	1.24	0.424	0.935	48	24
Oribacterium	Intermittent shedder	1.47	1.58	0.420	0.935	48	43
Anaerofustis	Intermittent shedder	1.59	1.53	0.376	0.935	48	34
Mailhella	Intermittent shedder	-1.54	1.49	0.376	0.935	48	34
Weissella	Intermittent shedder	0.76	0.51	0.148	0.935	48	11
Escherichia.Shigella	Short-term shedder	0.89	0.60	0.233	0.935	48	10
Campylobacter	Short-term shedder	2.26	1.47	0.222	0.935	48	29
Colidextribacter	Intermittent shedder	1.65	1.46	0.343	0.935	48	10
Synergistes	Short-term shedder	2.61	1.44	0.169	0.935	48	29
Synergistes	Intermittent shedder	1.28	1.44	0.442	0.935	48	29
XBB1006	Intermittent shedder	-0.88	0.93	0.412	0.935	48	19

Ruminiclostridium	Short-term shedder	1.44	1.37	0.369	0.935	48	28
UCG.004	Intermittent shedder	1.59	1.73	0.425	0.935	48	41
Oscillibacter	Intermittent shedder	-0.96	0.56	0.183	0.935	48	17
Lysinibacillus	Intermittent shedder	0.31	0.34	0.368	0.935	48	7
Denitrobacterium	Intermittent shedder	1.52	0.44	0.041	0.935	48	12
Rhodococcus	Intermittent shedder	1.30	0.85	0.224	0.935	48	12
Pyramidobacter	Intermittent shedder	-1.68	1.12	0.232	0.935	48	26
Arthrobacter	Short-term shedder	0.96	0.87	0.351	0.935	48	15
Arthrobacter	Intermittent shedder	1.58	0.87	0.168	0.935	48	15
Selenomonas	Short-term shedder	0.96	0.91	0.367	0.935	48	11
X.Eubacterium..nodatum.group	Intermittent shedder	-1.01	0.60	0.191	0.935	48	22
Erysipelatoclostridium	Intermittent shedder	-1.59	1.58	0.388	0.935	48	14
Anaeroplasma	Intermittent shedder	-0.86	0.94	0.427	0.935	48	39
M2PT2.76.termite.group	Intermittent shedder	1.86	1.10	0.191	0.935	48	21
Mycoplasma	Short-term shedder	0.75	0.82	0.431	0.935	48	14
Enterorhabdus	Intermittent shedder	0.58	0.60	0.410	0.935	48	7
Lachnospiraceae.ND3007.group	Short-term shedder	-1.25	0.84	0.232	0.935	48	26
Lachnospiraceae.ND3007.group	Intermittent shedder	-0.78	0.84	0.423	0.935	48	26
Roseburia	Short-term shedder	0.65	0.74	0.440	0.935	48	17
DEV114	Short-term shedder	1.81	0.65	0.069	0.935	48	10
Endomicrobium	Intermittent shedder	-0.57	0.39	0.148	0.935	48	25
Aeromicrobium	Short-term shedder	0.38	0.36	0.367	0.935	48	5
Shuttleworthia	Short-term shedder	0.35	0.30	0.244	0.935	48	10
Oscillospira	Short-term shedder	0.70	0.57	0.308	0.935	48	5
Kurthia	Short-term shedder	-1.02	0.77	0.280	0.935	48	28
Kurthia	Intermittent shedder	1.22	0.77	0.212	0.935	48	28
Lachnospiraceae.UCG.006	Intermittent shedder	1.14	1.01	0.343	0.935	48	20
X.Eubacterium..siraeum.group	Intermittent shedder	-0.67	0.56	0.319	0.935	48	7
Incertae.Sedis_1	Intermittent shedder	0.95	0.46	0.131	0.935	48	13
Arcanobacterium	Short-term shedder	0.82	0.36	0.027	0.935	48	7
Arcanobacterium	Intermittent shedder	0.29	0.36	0.423	0.935	48	7
Ruminobacter	Short-term shedder	0.56	0.39	0.251	0.935	48	12
Ruminobacter	Intermittent shedder	-0.68	0.39	0.184	0.935	48	12
UCG.007	Intermittent shedder	-0.20	0.21	0.338	0.935	48	5
Candidatus.Methanomethylophilus	Intermittent shedder	-0.42	0.45	0.424	0.935	48	9
Sutterella	Short-term shedder	0.71	0.69	0.376	0.935	48	16
Pygmaiobacter	Short-term shedder	-0.51	0.53	0.408	0.935	48	7
Pygmaiobacter	Intermittent shedder	-0.74	0.53	0.259	0.935	48	7
Mycobacterium	Intermittent shedder	-0.32	0.33	0.399	0.935	48	6
Cellulomonas	Short-term shedder	0.31	0.28	0.347	0.935	48	5
Flexilinea	Intermittent shedder	-0.48	0.52	0.417	0.935	48	11

Howardella	Intermittent shedder	0.29	0.26	0.343	0.935	48	7
X.Eubacterium..saphenum.group	Intermittent shedder	0.62	0.69	0.437	0.935	48	11
Mucinivorans	Short-term shedder	0.31	0.30	0.302	0.935	48	9
Mucinivorans	Intermittent shedder	0.34	0.30	0.261	0.935	48	9
Jonquetella	Intermittent shedder	-0.57	0.48	0.322	0.935	48	6
V9D2013.group	Short-term shedder	0.25	0.24	0.296	0.935	48	6
V9D2013.group	Intermittent shedder	0.43	0.24	0.073	0.935	48	6
Alistipes	Short-term shedder	0.69	0.31	0.114	0.935	48	6
Prevotella_9	Intermittent shedder	-0.51	0.43	0.323	0.935	48	5
FD2005	Intermittent shedder	-1.07	1.24	0.452	0.937	48	24
Escherichia.Shigella	Intermittent shedder	0.52	0.60	0.450	0.937	48	10
Porphyromonas	Short-term shedder	0.38	0.45	0.455	0.938	48	7
Prevotella_9	Short-term shedder	-0.37	0.43	0.459	0.939	48	5
Ligilactobacillus	Intermittent shedder	0.39	0.50	0.489	0.940	48	48
Methanobrevibacter	Short-term shedder	0.43	0.70	0.585	0.940	48	48
X.Eubacterium..hallii.group	Short-term shedder	0.40	0.48	0.469	0.940	48	48
Treponema	Intermittent shedder	-0.13	0.20	0.566	0.940	48	48
possible.genus.Sk018	Short-term shedder	-0.96	1.20	0.483	0.940	48	37
Anaerosporobacter	Short-term shedder	0.71	1.04	0.545	0.940	48	48
Anaerosporobacter	Intermittent shedder	0.71	1.04	0.545	0.940	48	48
Akkermansia	Intermittent shedder	0.58	0.84	0.538	0.940	48	48
Anaerovorax	Short-term shedder	0.36	0.46	0.491	0.940	48	48
Lachnospiraceae.XPB1014.group	Intermittent shedder	0.78	1.14	0.540	0.940	48	48
Lachnoclostridium	Short-term shedder	-0.69	1.00	0.541	0.940	48	37
Lachnospiraceae.NK4A136.group	Intermittent shedder	-1.14	1.54	0.512	0.940	48	25
Eubacterium	Short-term shedder	0.49	0.77	0.569	0.940	48	45
X.Eubacterium..ruminantium.group	Intermittent shedder	-2.08	2.69	0.497	0.940	48	33
Solibacillus	Short-term shedder	-0.63	0.97	0.564	0.940	48	32
Solibacillus	Intermittent shedder	-0.59	0.97	0.585	0.940	48	32
Defluviitaleaceae.UCG.011	Short-term shedder	0.91	1.40	0.563	0.940	48	41
Oribacterium	Short-term shedder	1.05	1.58	0.553	0.940	48	43
Anaerofustis	Short-term shedder	1.18	1.53	0.496	0.940	48	34
Mailhella	Short-term shedder	-0.90	1.49	0.587	0.940	48	34
UCG.009	Intermittent shedder	-1.13	1.58	0.525	0.940	48	31
Oscillibacter	Short-term shedder	0.47	0.56	0.464	0.940	48	17
Butyrivibrio	Short-term shedder	0.56	0.91	0.582	0.940	48	11
X.Eubacterium..nodatum.group	Short-term shedder	-0.40	0.60	0.549	0.940	48	22
Paenibacillus	Short-term shedder	-0.26	0.33	0.480	0.940	48	6
Mycoplasma	Intermittent shedder	0.64	0.82	0.493	0.940	48	14
Monoglobus	Short-term shedder	0.25	0.45	0.584	0.940	48	30

Roseburia	Intermittent shedder	-0.49	0.74	0.556	0.940	48	17
Aeromicrobium	Intermittent shedder	0.22	0.36	0.583	0.940	48	5
Lachnospiraceae.UCG.008	Intermittent shedder	-0.76	1.17	0.565	0.940	48	18
Elusimicrobium	Short-term shedder	0.96	1.45	0.556	0.940	48	15
Lachnospiraceae.UCG.006	Short-term shedder	0.71	1.01	0.535	0.940	48	20
X.Eubacterium..siraeum.group	Short-term shedder	-0.38	0.56	0.544	0.940	48	7
Aggregicoccus	Short-term shedder	0.24	0.35	0.534	0.940	48	5
Incertae.Sedis_1	Short-term shedder	0.29	0.46	0.570	0.940	48	13
Succinivibrionaceae.UCG.002	Short-term shedder	-0.41	0.52	0.480	0.940	48	10
Methylobacterium.Methylorubrum	Intermittent shedder	0.17	0.25	0.507	0.940	48	8
Jonquetella	Short-term shedder	-0.29	0.48	0.590	0.940	48	6
Sediminispirochaeta	Short-term shedder	0.54	0.91	0.597	0.946	48	16
Prevotella	Short-term shedder	0.25	0.48	0.638	0.947	48	48
Treponema	Short-term shedder	-0.10	0.20	0.659	0.947	48	48
Fibrobacter	Short-term shedder	0.14	0.31	0.669	0.947	48	48
Alloprevotella	Short-term shedder	0.51	0.97	0.639	0.947	48	48
Alloprevotella	Intermittent shedder	0.50	0.97	0.646	0.947	48	48
Anaerovorax	Intermittent shedder	0.26	0.46	0.615	0.947	48	48
Marvinbryantia	Intermittent shedder	-0.16	0.34	0.668	0.947	48	48
Schwartzia	Short-term shedder	1.01	1.95	0.639	0.947	48	31
dgA.11.gut.group	Intermittent shedder	-0.56	1.18	0.671	0.947	48	45
Desulfovibrio	Short-term shedder	-0.82	1.50	0.625	0.947	48	44
Desulfovibrio	Intermittent shedder	-0.76	1.50	0.649	0.947	48	44
DNF00809	Intermittent shedder	-0.48	1.03	0.670	0.947	48	34
Campylobacter	Intermittent shedder	0.76	1.47	0.642	0.947	48	29
Denitrobacterium	Short-term shedder	-0.22	0.44	0.658	0.947	48	12
Selenomonas	Intermittent shedder	-0.46	0.91	0.649	0.947	48	11
Butyrivibrio	Intermittent shedder	-0.46	0.91	0.650	0.947	48	11
Desulfurispora	Intermittent shedder	0.57	1.06	0.631	0.947	48	15
Enterorhabdus	Short-term shedder	0.30	0.60	0.651	0.947	48	7
Pelospora	Intermittent shedder	-0.37	0.71	0.634	0.947	48	14
Succinivibrionaceae.UCG.002	Intermittent shedder	-0.28	0.52	0.625	0.947	48	10
Cellulomonas	Intermittent shedder	0.14	0.28	0.646	0.947	48	5
UCG.005	Intermittent shedder	0.08	0.18	0.680	0.949	48	48
XBB1006	Short-term shedder	0.42	0.93	0.681	0.949	48	19
Howardella	Short-term shedder	0.12	0.26	0.683	0.949	48	7
Pseudobutyrivibrio	Short-term shedder	-0.60	1.44	0.705	0.968	48	46
Fibrobacter	Intermittent shedder	-0.13	0.31	0.707	0.968	48	48
Erysipelatoclostridium	Short-term shedder	-0.65	1.58	0.706	0.968	48	14
Saccharofermentans	Intermittent shedder	0.17	0.44	0.722	0.970	48	48
Lachnospiraceae.UCG.009	Short-term shedder	0.07	0.19	0.722	0.970	48	48

Bacillus	Short-term shedder	-0.21	0.54	0.724	0.970	48	47
DNF00809	Short-term shedder	-0.40	1.03	0.724	0.970	48	34
NED5E9	Intermittent shedder	-0.13	0.34	0.725	0.970	48	7
UCG.005	Short-term shedder	0.05	0.18	0.809	0.981	48	48
Lachnospiraceae.AC2044.group	Intermittent shedder	0.16	0.56	0.790	0.981	48	48
Ruminococcus	Intermittent shedder	-0.12	0.46	0.808	0.981	48	48
Prevotellaceae.UCG.003	Short-term shedder	0.16	0.60	0.802	0.981	48	48
Terrisporobacter	Intermittent shedder	0.61	1.95	0.773	0.981	48	39
Anaerovibrio	Short-term shedder	0.27	0.94	0.796	0.981	48	45
Lachnospiraceae.XPB1014.group	Short-term shedder	0.31	1.14	0.802	0.981	48	48
Blautia	Intermittent shedder	-0.06	0.22	0.799	0.981	48	48
Coprococcus	Intermittent shedder	-0.33	1.31	0.819	0.981	48	30
Papillibacter	Intermittent shedder	0.42	1.18	0.748	0.981	48	32
Weissella	Short-term shedder	-0.15	0.51	0.772	0.981	48	11
Lactococcus	Short-term shedder	0.13	0.49	0.803	0.981	48	9
Lysinibacillus	Short-term shedder	0.09	0.34	0.780	0.981	48	7
Rhodococcus	Short-term shedder	0.22	0.85	0.809	0.981	48	12
Catenisphaera	Short-term shedder	0.38	1.38	0.801	0.981	48	27
M2PT2.76.termite.group	Short-term shedder	0.40	1.10	0.741	0.981	48	21
Pelospora	Short-term shedder	-0.23	0.71	0.770	0.981	48	14
Endomicrobium	Short-term shedder	0.10	0.39	0.805	0.981	48	25
Elusimicrobium	Intermittent shedder	-0.50	1.45	0.752	0.981	48	15
Aggregicoccus	Intermittent shedder	-0.09	0.35	0.818	0.981	48	5
Mycobacterium	Short-term shedder	-0.08	0.33	0.820	0.981	48	6
Fretibacterium	Short-term shedder	-0.23	0.71	0.770	0.981	48	17
X.Eubacterium..saphenum.group	Short-term shedder	-0.17	0.69	0.820	0.981	48	11
Incertae.Sedis_2	Intermittent shedder	-0.16	0.46	0.753	0.981	48	10
Clostridium.sensu.stricto.1	Short-term shedder	0.12	0.58	0.844	0.982	48	48
Agathobacter	Short-term shedder	0.21	0.97	0.840	0.982	48	47
Lactococcus	Intermittent shedder	0.12	0.49	0.824	0.982	48	9
UCG.004	Short-term shedder	0.37	1.73	0.846	0.982	48	41
UCG.007	Short-term shedder	-0.04	0.21	0.836	0.982	48	5
Flexilinea	Short-term shedder	-0.12	0.52	0.834	0.982	48	11
Fretibacterium	Intermittent shedder	0.16	0.71	0.837	0.982	48	17
Prevotellaceae.UCG.004	Short-term shedder	0.06	0.31	0.866	0.985	48	48
Lachnospiraceae.NK4A136.group	Short-term shedder	-0.29	1.54	0.862	0.985	48	25
Lachnospiraceae.UCG.008	Short-term shedder	-0.21	1.17	0.866	0.985	48	18
Porphyromonas	Intermittent shedder	0.09	0.45	0.858	0.985	48	7
Sediminispirochaeta	Intermittent shedder	0.17	0.91	0.861	0.985	48	16
Family.XIII.UCG.001	Short-term shedder	0.02	0.13	0.874	0.989	48	48

X.Eubacterium..ruminantium.group	Short-term shedder	0.43	2.69	0.883	0.992	48	33
Pyramidobacter	Short-term shedder	-0.18	1.12	0.882	0.992	48	26
Lachnospiraceae.AC2044.group	Short-term shedder	0.07	0.56	0.907	0.997	48	48
Quinella	Short-term shedder	0.11	0.91	0.910	0.997	48	48
Schwartzia	Intermittent shedder	0.25	1.95	0.907	0.997	48	31
Seminibacterium	Short-term shedder	0.18	1.50	0.911	0.997	48	34
Parvibacter	Short-term shedder	0.14	1.01	0.897	0.997	48	25
Paenibacillus	Intermittent shedder	0.04	0.33	0.912	0.997	48	6
Candidatus.Methanomethylophilus	Short-term shedder	0.05	0.45	0.913	0.997	48	9
Ligilactobacillus	Short-term shedder	0.04	0.50	0.937	0.999	48	48
Phascolarctobacterium	Short-term shedder	-0.03	0.38	0.934	0.999	48	48
Prevotellaceae.Ga6A1.group	Short-term shedder	0.12	1.28	0.931	0.999	48	42
Ruminiclostridium	Intermittent shedder	0.14	1.37	0.927	0.999	48	28
Anaeroplasma	Short-term shedder	-0.09	0.94	0.931	0.999	48	39
NED5E9	Short-term shedder	0.03	0.34	0.929	0.999	48	7
Frisingicoccus	Short-term shedder	-0.03	1.41	0.986	1.000	48	44
UCG.002	Intermittent shedder	-0.02	0.82	0.979	1.000	48	48
Eubacterium	Intermittent shedder	-0.03	0.77	0.974	1.000	48	45
Defluviitaleaceae.UCG.011	Intermittent shedder	-0.01	1.40	0.997	1.000	48	41
Lachnospiraceae.UCG.007	Short-term shedder	0.00	0.38	1.000	1.000	48	16
Papillibacter	Short-term shedder	-0.07	1.18	0.959	1.000	48	32
UCG.009	Short-term shedder	-0.06	1.58	0.973	1.000	48	31
Colidextribacter	Short-term shedder	-0.08	1.46	0.960	1.000	48	10
Catenisphaera	Intermittent shedder	0.09	1.38	0.952	1.000	48	27
Desulfurispora	Short-term shedder	0.06	1.06	0.957	1.000	48	15
DEV114	Intermittent shedder	0.00	0.65	1.000	1.000	48	10
Shuttleworthia	Intermittent shedder	0.02	0.30	0.950	1.000	48	10
Oscillospira	Intermittent shedder	0.00	0.57	1.000	1.000	48	5
T2WK15B57	Intermittent shedder	0.00	0.37	1.000	1.000	48	6
Sutterella	Intermittent shedder	0.01	0.69	0.991	1.000	48	16
Incertae.Sedis_2	Short-term shedder	0.02	0.46	0.972	1.000	48	10
Alistipes	Intermittent shedder	0.00	0.31	1.000	1.000	48	6

APPENDIX 2A: SYSTEMATIC REVIEW PROTOCOL

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

Authors and Contributions:

Emily C. Herring[1], Helen W. Aceto[2], Paul S. Morley[3], Annette M. O'Connor[4], Nicola Pusterla[5], Shelley C. Rankin[6], Brandy A. Burgess[1*]

[1] Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA

[2] Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA

[3] Veterinary Education, Research, and Outreach (VERO) Program, Texas A&M University/West Texas A&M University, Canyon, TX

[4] Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI

[5] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA

[6] Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

*Corresponding author. Email address: brandy.burgess@uga.edu

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.

Amendments: This review is not an amendment of a previously published protocol.

Support: This material is based upon work supported by the National Science Foundation under Grant No. DGE-1545433.

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 and/or samples 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. If age questions are not applicable (e.g., experimental inoculation of fecal samples), write NA. 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, interquartile range, minimum, or maximum age), in the first row, report 12.50 for mean, 3.15 for standard deviation, and NR for median, standard error, interquartile range, 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, standard error, interquartile range; 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 males, please report proportions of castrated/intact males if provided in the study. Otherwise, report as "Male (unspecified)." If sex is not applicable (e.g., experimental inoculation of fecal samples), write NA. 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 - Proportion male (unspecified) - 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. If disease type is not applicable (e.g., experimental inoculation of fecal samples), write NA. 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)

Category	Variable	Definition/Levels
		- 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. If hospitalization is not applicable (e.g., experimental inoculation of fecal samples), write NA. For example, if 50% of the population was hospitalized, report 0.50. If there are 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. If survival is not applicable (e.g., experimental inoculation of fecal samples), write NA. 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

Category	Variable	Definition/Levels
		- 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.*
	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

Category	Variable	Definition/Levels
		- 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
	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 qPCR 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

Category	Variable	Definition/Levels
	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:

	Risk of Bias			
	Signaling question	**Yes or N/A**	**No**	**Unclear**
Domain: Patient selection	Was a consecutive or random sample of patients enrolled?	Sampling method is explicitly described as either consecutive or random OR patient enrollment is not applicable (e.g., benchtop study with experimental inoculation of feces)	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 OR the study was experimental	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 OR exclusion of horses is not applicable (e.g., benchtop study with experimental inoculation of feces)	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
		Risk level		

	Could the selection of patients have introduced bias?	**Low** Answers to two or more "Patient selection" signaling questions are "Yes" or "N/A"	**High** Answers to two or more "Patient selection" signaling questions are "No"	**Unclear** 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 or N/A**	**No**	**Unclear**
	Were the index tests results interpreted without knowledge of the results of the reference/ comparison test?	Index test was performed prior to the reference/comparison test OR investigators were blinded to results of the reference/comparison test when index test was performed	Investigators knew the results of the reference/ comparison test when the index test was performed	Insufficient detail is provided to determine whether 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 OR no threshold was used	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** Answers to two "Index test" signaling questions are "Yes" or "N/A"	**High** Answers to two "Index test" signaling questions are "No"	**Unclear** 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 or N/A**	**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	The results of the reference/ comparison test are unlikely to demonstrate the true *Salmonella* shedding	Insufficient detail is provided to determine whether the results of the reference/

		tested in this study population	status of the horses tested in this study population	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 OR the reference/comparison was experimental infection/inoculation	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/ comparison test, its conduct, or its interpretation have introduced bias?	**Risk level**		
		Low	**High**	**Unclear**
		Answers to two "Reference/ comparison test" signaling questions are "Yes" or "N/A"	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 or N/A**	**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) OR the reference/comparison was experimental infection/inoculation	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 OR the reference/comparison	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/

		was experimental infection/inoculation		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 OR the reference/comparison was experimental infection/inoculation	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" or "N/A"	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]

References

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APPENDIX 2B: PRISMA-DTA CHECKLIST

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
TITLE / ABSTRACT			
Title	1	Identify the report as a systematic review (+/- meta-analysis) of diagnostic test accuracy (DTA) studies.	8
Abstract	2	Abstract: See PRISMA-DTA for abstracts.	9
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	10-11
Clinical role of index test	D1	State the scientific and clinical background, including the intended use and clinical role of the index test, and if applicable, the rationale for minimally acceptable test accuracy (or minimum difference in accuracy for comparative design).	10
Objectives	4	Provide an explicit statement of question(s) being addressed in terms of participants, index test(s), and target condition(s).	11
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	11
Eligibility criteria	6	Specify study characteristics (participants, setting, index test(s), reference standard(s), target condition(s), and study design) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	11-12
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	12
Search	8	Present full search strategies for all electronic databases and other sources searched, including any limits used, such that they could be repeated.	12
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	13
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	13-14
Definitions for data extraction	11	Provide definitions used in data extraction and classifications of target condition(s), index test(s), reference standard(s) and other characteristics (e.g. study design, clinical setting).	14-15
Risk of bias and applicability	12	Describe methods used for assessing risk of bias in individual studies and concerns regarding the applicability to the review question.	15-16
Diagnostic accuracy measures	13	State the principal diagnostic accuracy measure(s) reported (e.g. sensitivity, specificity) and state the unit of assessment (e.g. per-patient, per-lesion).	16
Synthesis of results	14	Describe methods of handling data, combining results of studies and describing variability between studies. This could include, but is not limited to: a) handling of multiple definitions of target condition. b) handling of multiple thresholds of test positivity, c) handling multiple index test readers, d) handling of indeterminate test results, e) grouping and comparing tests, f) handling of different reference standards	16-17

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
Meta-analysis	D2	Report the statistical methods used for meta-analyses, if performed.	17
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	17
RESULTS			
Study selection	17	Provide numbers of studies screened, assessed for eligibility, included in the review (and included in meta-analysis, if applicable) with reasons for exclusions at each stage, ideally with a flow diagram.	17-18
Study characteristics	18	For each included study provide citations and present key characteristics including: a) participant characteristics (presentation, prior testing), b) clinical setting, c) study design, d) target condition definition, e) index test, f) reference standard, g) sample size, h) funding sources	18-22
Risk of bias and applicability	19	Present evaluation of risk of bias and concerns regarding applicability for each study.	22
Results of individual studies	20	For each analysis in each study (e.g. unique combination of index test, reference standard, and positivity threshold) report 2x2 data (TP, FP, FN, TN) with estimates of diagnostic accuracy and confidence intervals, ideally with a forest or receiver operator characteristic (ROC) plot.	22-24
Synthesis of results	21	Describe test accuracy, including variability; if meta-analysis was done, include results and confidence intervals.	40-41
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression; analysis of index test: failure rates, proportion of inconclusive results, adverse events).	24-25
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence.	25
Limitations	25	Discuss limitations from included studies (e.g. risk of bias and concerns regarding applicability) and from the review process (e.g. incomplete retrieval of identified research).	31-32
Conclusions	26	Provide a general interpretation of the results in the context of other evidence. Discuss implications for future research and clinical practice (e.g. the intended use and clinical role of the index test).	32
FUNDING			
Funding	27	For the systematic review, describe the sources of funding and other support and the role of the funders.	32

APPENDIX 3A: STARD-BLCM CHECKLIST

SECTION	TOPIC	ITEM	DESCRIPTION	REPORTED ON PAGE #
Title/Abstract/ Keywords		1	Identification as a study of diagnostic accuracy, using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC) and Bayesian latent class models	43
Abstract		2	Structured summary of study design, methods, results, and conclusions	44
Introduction		3	Scientific and clinical background, including the intended use and clinical role of the tests under evaluation	44-46
		4	Study objectives and hypotheses, such as estimation of diagnostic accuracy of the tests for a defined purpose through BLCM	46
Methods	Study Design	5	Whether data collection was planned before the tests were performed (prospective study) or after (retrospective study)	47
	Participants	6	Eligibility criteria and description of the source population	47-48
		7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	47-48
		8	Where and when potentially eligible participants were identified (setting, location, and dates)	47-48
		9	Whether participants formed a consecutive, random or convenience series	47
	Test Methods	10	Description of the tests under evaluation, in sufficient detail to allow replication, and/or cite references	49-50
		11	Rationale for choosing the tests under evaluation in relation to their purpose	44-46
		12	Rationale for test positivity cut-offs or result categories of the tests under evaluation, distinguishing pre-specified from exploratory	49-50
		13	Whether clinical information was available to the performers or readers of the tests under evaluation	50
	Analysis	14a	BLCM model for estimating measures of diagnostic accuracy	51-53
		14b	Definition and rationale of prior information and sensitivity analysis	53-55
		15	How indeterminate results of the tests under evaluation were handled	N/A

		16	How missing data of the tests under evaluation were handled	N/A
		17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	54
		18	Intended sample size and how it was determined	48
Results	Participants	19	Flow of participants, using a diagram	N/A
		20	Baseline demographic and clinical characteristics of participant	56, 69
		21	Not applicable: the distribution of the targeted conditions is unknown, hence the use of BLCM	N/A
		22	Time interval and any clinical interventions between the tests under evaluation	N/A
	Test Results	23	Cross tabulation of the tests' results (or for continuous tests results their distribution by infection stage)	70
		24	Estimates of diagnostic accuracy under alternative prior specification and their precision (such as 95% credible/probability intervals)	72, 76-77
		25	Report any adverse events from performing the of the tests under evaluation	N/A
Discussion		26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	60-67
		27	Implications for practice, including the intended use and clinical role of the tests under evaluation in relevant settings (clinical, research, surveillance etc.)	60-67
Other information		28	Registration number and name of registry	N/A
		29	Where the full study protocol can be accessed	N/A
		30	Sources of funding and other support; role of funders	67-68

APPENDIX 3B: BAYESIAN LATENT CLASS MODELS

```
TestA <- "TETXLT4"
TestB <- "SELHE"
TestC <- "PCR"
TestD <- "LFI"


# Priors - use informative priors for test Sn and Sp, and
uninformative priors for prevalence ----------------------------
-----------

# Culture (TET-XLT4) sensitivity ---------------------------------
--------------------------
# Sensitivity of TET-XLT4:  Mode=0.674, and we are 97.5% sure
>0.494
Se.TETXLT4 <- epi.betabuster(mode=0.674, conf=0.975,
greaterthan=T, x=0.494)
# Check values for Se
Se.TETXLT4$shape1                #View the a shape parameter
Se.TETXLT4$shape2                #View the b shape parameter
#plot the Se prior distribution
curve(dbeta(x, shape1=Se.TETXLT4$shape1,
shape2=Se.TETXLT4$shape2), from=0, to=1,
      main="Original prior for culture (TET-XLT4) sensitivity",
xlab = "Proportion", ylab = "Density", lwd = 5)

# Culture (TET-XLT4) specificity ---------------------------------
--------------------------
# Specificity of TET-XLT4:  Mode=0.813, and we are 97.5% sure
>0.707
Sp.TETXLT4 <- epi.betabuster(mode=0.813, conf=0.975,
greaterthan=T, x=0.707)
# Check values for Sp
Sp.TETXLT4$shape1                #View the a shape parameter
Sp.TETXLT4$shape2                #View the b shape parameter
#plot the Sp prior distribution
curve(dbeta(x, shape1=Sp.TETXLT4$shape1,
shape2=Sp.TETXLT4$shape2), from=0, to=1,
      main="Original prior distribution for  culture (TET-XLT4)
specificity", xlab = "Proportion", ylab = "Density", lwd = 5)

# Culture (SEL-HE) sensitivity -----------------------------------
-------------------------
# Sensitivity of SEL-HE:  Mode=0.563, and we are 97.5% sure
>0.419
Se.SELHE <- epi.betabuster(mode=0.563, conf=0.975,
greaterthan=T, x=0.419)
# Check values for Se
```

```
Se.SELHE$shape1                #View the a shape parameter
Se.SELHE$shape2                #View the b shape parameter
#plot the Se prior distribution
curve(dbeta(x, shape1=Se.SELHE$shape1, shape2=Se.SELHE$shape2),
from=0, to=1,
      main="Original prior distribution for culture (SEL-HE)
sensitivity", xlab = "Proportion", ylab = "Density", lwd = 5)

# Culture (SEL-HE) specificity ------------------------------
------------------------
# Specificity of SEL-HE:  Mode=0.781, and we are 97.5% sure
>0.682
Sp.SELHE <- epi.betabuster(mode=0.781, conf=0.975,
greaterthan=T, x=0.682)
# Check values for Sp
Sp.SELHE$shape1                #View the a shape parameter
Sp.SELHE$shape2                #View the b shape parameter
#plot the Sp prior distribution
curve(dbeta(x, shape1=Sp.SELHE$shape1, shape2=Sp.SELHE$shape2),
from=0, to=1,
      main="Original prior distribution for  culture (SEL-HE)
specificity", xlab = "Proportion", ylab = "Density", lwd = 5)

# PCR sensitivity -------------------------------------------
------------
# Sensitivity of PCR:  Mode=0.794, and we are 97.5% sure >0.616
Se.PCR <- epi.betabuster(mode=0.794, conf=0.975, greaterthan=T,
x=0.616)
# Check values for Se
Se.PCR$shape1                #View the a shape parameter
Se.PCR$shape2                #View the b shape parameter
#plot the Se prior distribution
curve(dbeta(x, shape1=Se.PCR$shape1, shape2=Se.PCR$shape2),
from=0, to=1,
      main="Original prior distribution for PCR sensitivity",
xlab = "Proportion", ylab = "Density", lwd = 5)

# PCR specificity -------------------------------------------
------------
# Specificity of PCR:  Mode=0.915, and we are 97.5% sure >0.828
Sp.PCR <- epi.betabuster(mode=0.915, conf=0.975, greaterthan=T,
x=0.828)
# Check values for Sp
Sp.PCR$shape1                #View the a shape parameter
Sp.PCR$shape2                #View the b shape parameter
#plot the Se prior distribution
curve(dbeta(x, shape1=Sp.PCR$shape1, shape2=Sp.PCR$shape2),
from=0, to=1,
      main="Original prior for PCR specificity", xlab =
"Proportion", ylab = "Density", lwd = 5)
```

```
# LFI (Rapid Test) sensitivity ---------------------------------
------------------------
# Sensitivity of LFI:  Mode=0.567, and we are 97.5% sure >0.403
Se.LFI <- epi.betabuster(mode=0.567, conf=0.975, greaterthan=T,
x=0.403)
# Check values for Se
Se.LFI$shape1                #View the a shape parameter
Se.LFI$shape2                #View the b shape parameter
#plot the Se prior distribution
curve(dbeta(x, shape1=Se.LFI$shape1, shape2=Se.LFI$shape2),
from=0, to=1,
      main="Original prior for LFI sensitivity", xlab =
"Proportion", ylab = "Density", lwd = 5)

# LFI (Rapid Test) specificity ---------------------------------
------------------------
# Specificity of LFI:  Mode=0.765, and we are 97.5% sure >0.670
Sp.LFI <- epi.betabuster(mode=0.765, conf=0.975, greaterthan=T,
x=0.670)
# Check values for Sp
Sp.LFI$shape1                #View the a shape parameter
Sp.LFI$shape2                #View the b shape parameter
#plot the Se prior distribution
curve(dbeta(x, shape1=Sp.LFI$shape1, shape2=Sp.LFI$shape2),
from=0, to=1,
      main="Original prior for LFI specificity", xlab =
"Proportion", ylab = "Density", lwd = 5)


# Specify priors

Prev1.shapea <- 1         #a shape parameter for Prev in
population 1
Prev1.shapeb <- 1         #b shape parameter for Prev in
population 1

Prev2.shapea <- 1         #a shape parameter for Prev in
population 2
Prev2.shapeb <- 1         #b shape parameter for Prev in
population 2

Prev3.shapea <- 1         #a shape parameter for Prev in
population 3
Prev3.shapeb <- 1         #b shape parameter for Prev in
population 3

Se.TestA.shapea <- Se.TETXLT4$shape1      #a shape parameter for
Se of TestA
```

```
Se.TestA.shapeb <- Se.TETXLT4$shape2      #b shape parameter for
Se of TestA
Sp.TestA.shapea <- Sp.TETXLT4$shape1      #a shape parameter for
Sp of TestA
Sp.TestA.shapeb <- Sp.TETXLT4$shape2      #b shape parameter for
Sp of TestA

Se.TestB.shapea <- Se.SELHE$shape1      #a shape parameter for
Se of TestB
Se.TestB.shapeb <- Se.SELHE$shape2      #b shape parameter for
Se of TestB
Sp.TestB.shapea <- Sp.SELHE$shape1      #a shape parameter for
Sp of TestB
Sp.TestB.shapeb <- Sp.SELHE$shape2      #a shape parameter for
Sp of TestB

Se.TestC.shapea <- Se.PCR$shape1     #a shape parameter for Se
of TestC
Se.TestC.shapeb <- Se.PCR$shape2     #b shape parameter for Se
of TestC
Sp.TestC.shapea <- Sp.PCR$shape1     #a shape parameter for Sp
of TestC
Sp.TestC.shapeb <- Sp.PCR$shape2     #b shape parameter for Sp
of TestC

Se.TestD.shapea <- Se.LFI$shape1     #a shape parameter for Se
of TestD
Se.TestD.shapeb <- Se.LFI$shape2     #b shape parameter for Se
of TestD
Sp.TestD.shapea <- Sp.LFI$shape1     #a shape parameter for Sp
of TestD
Sp.TestD.shapeb <- Sp.LFI$shape2     #b shape parameter for Sp
of TestD
```

CONDITIONAL INDEPENDENCE MODEL

```
# Create the JAGS text file -----------------------------------
----------
rapidtest_4test_3pop_indep <- paste0("model{

#=== LIKELIHOOD ===#

  #=== POPULATION 1 ===#
  Pop1[1:16] ~ dmulti(p1[1:16], ",nPop1,")
  p1[1] <- Prev1*Se_", TestA, "*Se_", TestB, "*Se_", TestC,
"*Se_", TestD, " + (1-Prev1)*(1-Sp_", TestA, ")*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p1[2] <- Prev1*Se_", TestA, "*(1-Se_", TestB, ")*Se_", TestC,
"*Se_", TestD, " + (1-Prev1)*(1-Sp_", TestA, ")*Sp_", TestB,
"*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p1[3] <- Prev1*(1-Se_", TestA, ")*Se_", TestB, "*Se_", TestC,
"*Se_", TestD, " + (1-Prev1)*Sp_", TestA, "*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p1[4] <- Prev1*(1-Se_", TestA, ")*(1-Se_", TestB, ")*Se_",
TestC, "*Se_", TestD," + (1-Prev1)*Sp_", TestA, "*Sp_", TestB,
"*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p1[5] <- Prev1*Se_", TestA, "*Se_", TestB, "*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev1)*(1-Sp_", TestA, ")*(1-Sp_",
TestB, ")*Sp_", TestC,"*(1-Sp_", TestD,")
  p1[6] <- Prev1*Se_", TestA, "*(1-Se_", TestB, ")*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev1)*(1-Sp_", TestA, ")*Sp_",
TestB, "*Sp_", TestC,"*(1-Sp_", TestD,")
  p1[7] <- Prev1*(1-Se_", TestA, ")*Se_", TestB, "*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev1)*Sp_", TestA, "*(1-Sp_",
TestB, ")*Sp_", TestC,"*(1-Sp_", TestD,")
  p1[8] <- Prev1*(1-Se_", TestA, ")*(1-Se_", TestB, ")*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev1)*Sp_", TestA, "*Sp_", TestB,
"*Sp_", TestC,"*(1-Sp_", TestD,")
  p1[9] <- Prev1*Se_", TestA, "*Se_", TestB, "*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev1)*(1-Sp_", TestA, ")*(1-Sp_",
TestB, ")*(1-Sp_", TestC,")*Sp_", TestD,"
  p1[10] <- Prev1*Se_", TestA, "*(1-Se_", TestB, ")*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev1)*(1-Sp_", TestA, ")*Sp_", TestB,
"*(1-Sp_", TestC,")*Sp_", TestD,"
  p1[11] <- Prev1*(1-Se_", TestA, ")*Se_", TestB, "*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev1)*Sp_", TestA, "*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*Sp_", TestD,"
  p1[12] <- Prev1*(1-Se_", TestA, ")*(1-Se_", TestB, ")*Se_",
TestC, "*(1-Se_", TestD,") + (1-Prev1)*Sp_", TestA, "*Sp_",
TestB, "*(1-Sp_", TestC,")*Sp_", TestD,"
  p1[13] <- Prev1*Se_", TestA, "*Se_", TestB, "*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev1)*(1-Sp_", TestA, ")*(1-
Sp_", TestB, ")*Sp_", TestC,"*Sp_", TestD,"
```

```
  p1[14] <- Prev1*Se_", TestA, "*(1-Se_", TestB, ")*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev1)*(1-Sp_", TestA, ")*Sp_",
TestB, "*Sp_", TestC,"*Sp_", TestD,"
  p1[15] <- Prev1*(1-Se_", TestA, ")*Se_", TestB, "*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev1)*Sp_", TestA, "*(1-Sp_",
TestB, ")*Sp_", TestC,"*Sp_", TestD,"
  p1[16] <- Prev1*(1-Se_", TestA, ")*(1-Se_", TestB, ")*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev1)*Sp_", TestA, "*Sp_",
TestB, "*Sp_", TestC,"*Sp_", TestD,"

  #=== POPULATION 2 ===#
  Pop2[1:16] ~ dmulti(p2[1:16], ",nPop2,")
  p2[1] <- Prev2*Se_", TestA, "*Se_", TestB, "*Se_", TestC,
"*Se_", TestD, " + (1-Prev2)*(1-Sp_", TestA, ")*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p2[2] <- Prev2*Se_", TestA, "*(1-Se_", TestB, ")*Se_", TestC,
"*Se_", TestD, " + (1-Prev2)*(1-Sp_", TestA, ")*Sp_", TestB,
"*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p2[3] <- Prev2*(1-Se_", TestA, ")*Se_", TestB, "*Se_", TestC,
"*Se_", TestD, " + (1-Prev2)*Sp_", TestA, "*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p2[4] <- Prev2*(1-Se_", TestA, ")*(1-Se_", TestB, ")*Se_",
TestC, "*Se_", TestD," + (1-Prev2)*Sp_", TestA, "*Sp_", TestB,
"*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p2[5] <- Prev2*Se_", TestA, "*Se_", TestB, "*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev2)*(1-Sp_", TestA, ")*(1-Sp_",
TestB, ")*Sp_", TestC,"*(1-Sp_", TestD,")
  p2[6] <- Prev2*Se_", TestA, "*(1-Se_", TestB, ")*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev2)*(1-Sp_", TestA, ")*Sp_",
TestB, "*Sp_", TestC,"*(1-Sp_", TestD,")
  p2[7] <- Prev2*(1-Se_", TestA, ")*Se_", TestB, "*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev2)*Sp_", TestA, "*(1-Sp_",
TestB, ")*Sp_", TestC,"*(1-Sp_", TestD,")
  p2[8] <- Prev2*(1-Se_", TestA, ")*(1-Se_", TestB, ")*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev2)*Sp_", TestA, "*Sp_", TestB,
"*Sp_", TestC,"*(1-Sp_", TestD,")
  p2[9] <- Prev2*Se_", TestA, "*Se_", TestB, "*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev2)*(1-Sp_", TestA, ")*(1-Sp_",
TestB, ")*(1-Sp_", TestC,")*Sp_", TestD,"
  p2[10] <- Prev2*Se_", TestA, "*(1-Se_", TestB, ")*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev2)*(1-Sp_", TestA, ")*Sp_", TestB,
"*(1-Sp_", TestC,")*Sp_", TestD,"
  p2[11] <- Prev2*(1-Se_", TestA, ")*Se_", TestB, "*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev2)*Sp_", TestA, "*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*Sp_", TestD,"
  p2[12] <- Prev2*(1-Se_", TestA, ")*(1-Se_", TestB, ")*Se_",
TestC, "*(1-Se_", TestD,") + (1-Prev2)*Sp_", TestA, "*Sp_",
TestB, "*(1-Sp_", TestC,")*Sp_", TestD,"
```

```
  p2[13] <- Prev2*Se_", TestA, "*Se_", TestB, "*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev2)*(1-Sp_", TestA, ")*(1-
Sp_", TestB, ")*Sp_", TestC,"*Sp_", TestD,"
  p2[14] <- Prev2*Se_", TestA, "*(1-Se_", TestB, ")*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev2)*(1-Sp_", TestA, ")*Sp_",
TestB, "*Sp_", TestC,"*Sp_", TestD,"
  p2[15] <- Prev2*(1-Se_", TestA, ")*Se_", TestB, "*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev2)*Sp_", TestA, "*(1-Sp_",
TestB, ")*Sp_", TestC,"*Sp_", TestD,"
  p2[16] <- Prev2*(1-Se_", TestA, ")*(1-Se_", TestB, ")*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev2)*Sp_", TestA, "*Sp_",
TestB, "*Sp_", TestC,"*Sp_", TestD,"

   #=== POPULATION 3 ===#
  Pop3[1:16] ~ dmulti(p3[1:16], ",nPop3,")
  p3[1] <- Prev3*Se_", TestA, "*Se_", TestB, "*Se_", TestC,
"*Se_", TestD, " + (1-Prev3)*(1-Sp_", TestA, ")*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p3[2] <- Prev3*Se_", TestA, "*(1-Se_", TestB, ")*Se_", TestC,
"*Se_", TestD, " + (1-Prev3)*(1-Sp_", TestA, ")*Sp_", TestB,
"*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p3[3] <- Prev3*(1-Se_", TestA, ")*Se_", TestB, "*Se_", TestC,
"*Se_", TestD, " + (1-Prev3)*Sp_", TestA, "*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p3[4] <- Prev3*(1-Se_", TestA, ")*(1-Se_", TestB, ")*Se_",
TestC, "*Se_", TestD," + (1-Prev3)*Sp_", TestA, "*Sp_", TestB,
"*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p3[5] <- Prev3*Se_", TestA, "*Se_", TestB, "*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev3)*(1-Sp_", TestA, ")*(1-Sp_",
TestB, ")*Sp_", TestC,"*(1-Sp_", TestD,")
  p3[6] <- Prev3*Se_", TestA, "*(1-Se_", TestB, ")*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev3)*(1-Sp_", TestA, ")*Sp_",
TestB, "*Sp_", TestC,"*(1-Sp_", TestD,")
  p3[7] <- Prev3*(1-Se_", TestA, ")*Se_", TestB, "*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev3)*Sp_", TestA, "*(1-Sp_",
TestB, ")*Sp_", TestC,"*(1-Sp_", TestD,")
  p3[8] <- Prev3*(1-Se_", TestA, ")*(1-Se_", TestB, ")*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev3)*Sp_", TestA, "*Sp_", TestB,
"*Sp_", TestC,"*(1-Sp_", TestD,")
  p3[9] <- Prev3*Se_", TestA, "*Se_", TestB, "*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev3)*(1-Sp_", TestA, ")*(1-Sp_",
TestB, ")*(1-Sp_", TestC,")*Sp_", TestD,"
  p3[10] <- Prev3*Se_", TestA, "*(1-Se_", TestB, ")*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev3)*(1-Sp_", TestA, ")*Sp_", TestB,
"*(1-Sp_", TestC,")*Sp_", TestD,"
  p3[11] <- Prev3*(1-Se_", TestA, ")*Se_", TestB, "*Se_", TestC,
"*(1-Se_", TestD,") + (1-Prev3)*Sp_", TestA, "*(1-Sp_", TestB,
")*(1-Sp_", TestC,")*Sp_", TestD,"
```

```
  p3[12] <- Prev3*(1-Se_", TestA, ")*(1-Se_", TestB, ")*Se_",
TestC, "*(1-Se_", TestD,") + (1-Prev3)*Sp_", TestA, "*Sp_",
TestB, "*(1-Sp_", TestC,")*Sp_", TestD,"
  p3[13] <- Prev3*Se_", TestA, "*Se_", TestB, "*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev3)*(1-Sp_", TestA, ")*(1-
Sp_", TestB, ")*Sp_", TestC,"*Sp_", TestD,"
  p3[14] <- Prev3*Se_", TestA, "*(1-Se_", TestB, ")*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev3)*(1-Sp_", TestA, ")*Sp_",
TestB, "*Sp_", TestC,"*Sp_", TestD,"
  p3[15] <- Prev3*(1-Se_", TestA, ")*Se_", TestB, "*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev3)*Sp_", TestA, "*(1-Sp_",
TestB, ")*Sp_", TestC,"*Sp_", TestD,"
  p3[16] <- Prev3*(1-Se_", TestA, ")*(1-Se_", TestB, ")*(1-Se_",
TestC,")*(1-Se_", TestD,") + (1-Prev3)*Sp_", TestA, "*Sp_",
TestB, "*Sp_", TestC,"*Sp_", TestD,"

#=== PRIOR  ===#

  Prev1 ~ dbeta(",Prev1.shapea,", ",Prev1.shapeb,")     ## Prior
for Prevalence in population 1
  Prev2 ~ dbeta(",Prev2.shapea,", ",Prev2.shapeb,")     ## Prior
for Prevalence in population 2
  Prev3 ~ dbeta(",Prev3.shapea,", ",Prev3.shapeb,")     ## Prior
for Prevalence in population 3
  Se_", TestA, " ~ dbeta(",Se.TestA.shapea,",
",Se.TestA.shapeb,")  ## Prior for Se of Test A
  Sp_", TestA, " ~ dbeta(",Sp.TestA.shapea,",
",Sp.TestA.shapeb,")  ## Prior for Sp of Test A
  Se_", TestB, " ~ dbeta(",Se.TestB.shapea,",
",Se.TestB.shapeb,")  ## Prior for Se of Test B
  Sp_", TestB, " ~ dbeta(",Sp.TestB.shapea,",
",Sp.TestB.shapeb,")  ## Prior for Sp of Test B
  Se_", TestC, " ~ dbeta(",Se.TestC.shapea,",
",Se.TestC.shapeb,")  ## Prior for Se of Test C
  Sp_", TestC, " ~ dbeta(",Sp.TestC.shapea,",
",Sp.TestC.shapeb,")  ## Prior for Sp of Test C
  Se_", TestD, " ~ dbeta(",Se.TestD.shapea,",
",Se.TestD.shapeb,")  ## Prior for Se of Test D
  Sp_", TestD, " ~ dbeta(",Sp.TestD.shapea,",
",Sp.TestD.shapeb,")  ## Prior for Sp of Test D

}")

# write as a text (.txt) file ----------------------------------
------------
write.table(rapidtest_4test_3pop_indep,
            file="rapidtest_4test_3pop_indep.txt",
            quote=FALSE,
            sep="",
            row.names=FALSE,
```

```
            col.names=FALSE)

# Initialize values for Prev parameters, and the Ses and Sps of
the 3 tests for the 3 chains ---------------------------------
-----------
inits <- list(list(Prev1=0.05,
                   Prev2=0.15,
                   Prev3=0.30,
                   Se_TETXLT4=0.85,
                   Sp_TETXLT4=0.95,
                   Se_SELHE=0.75,
                   Sp_SELHE=0.95,
                   Se_PCR=0.90,
                   Sp_PCR=0.95,
                   Se_LFI=0.70,
                   Sp_LFI=0.60),

              list(Prev1=0.15,
                   Prev2=0.25,
                   Prev3=0.50,
                   Se_TETXLT4=0.95,
                   Sp_TETXLT4=0.99,
                   Se_SELHE=0.85,
                   Sp_SELHE=0.99,
                   Se_PCR=0.95,
                   Sp_PCR=0.99,
                   Se_LFI=0.85,
                   Sp_LFI=0.70),

              list(Prev1=0.01,
                   Prev2=0.10,
                   Prev3=0.10,
                   Se_TETXLT4=0.75,
                   Sp_TETXLT4=0.75,
                   Se_SELHE=0.65,
                   Sp_SELHE=0.75,
                   Se_PCR=0.80,
                   Sp_PCR=0.80,
                   Se_LFI=0.45,
                   Sp_LFI=0.45)
)

# Run the Bayesian model ---------------------------------------
-------
set.seed(123)
bug.out <- jags(data=datalist,
                  model.file="rapidtest_4test_3pop_indep.txt",
                  parameters.to.save=c("Prev1", "Prev2",
"Prev3", "Se_TETXLT4", "Sp_TETXLT4", "Se_SELHE", "Sp_SELHE",
"Se_PCR", "Sp_PCR", "Se_LFI", "Sp_LFI"),
```

```
n.chains=3,
inits=inits,
n.iter=11000,
n.burnin=1000,
n.thin=1,
DIC=FALSE)
```

CONDITIONAL DEPENDENCE MODEL

```
# Create the JAGS text file -----------------------------------
----------
rapidtest_4test_3pop_dep <- paste0("model{

#=== LIKELIHOOD ===#

  #=== POPULATION 1 ===#
  Pop1[1:16] ~ dmulti(p1[1:16], ",nPop1,")
  p1[1] <- Prev1*(Se_", TestA, "*Se_", TestB, " + covp)*Se_",
TestC, "*Se_", TestD, " + (1-Prev1)*((1-Sp_", TestA, ")*(1-Sp_",
TestB, ") + covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p1[2] <- Prev1*(Se_", TestA, "*(1-Se_", TestB, ") -
covp)*Se_", TestC, "*Se_", TestD, " + (1-Prev1)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p1[3] <- Prev1*((1-Se_", TestA, ")*Se_", TestB, " -
covp)*Se_", TestC, "*Se_", TestD, " + (1-Prev1)*(Sp_", TestA,
"*(1-Sp_", TestB, ") - covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p1[4] <- Prev1*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*Se_", TestC, "*Se_", TestD," + (1-Prev1)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p1[5] <- Prev1*(Se_", TestA, "*Se_", TestB, " + covp)*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev1)*((1-Sp_", TestA, ")*(1-Sp_",
TestB, ") + covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p1[6] <- Prev1*(Se_", TestA, "*(1-Se_", TestB, ") - covp)*(1-
Se_", TestC,")*Se_", TestD," + (1-Prev1)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p1[7] <- Prev1*((1-Se_", TestA, ")*Se_", TestB, " - covp)*(1-
Se_", TestC,")*Se_", TestD," + (1-Prev1)*(Sp_", TestA, "*(1-
Sp_", TestB, ") - covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p1[8] <- Prev1*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*(1-Se_", TestC,")*Se_", TestD," + (1-Prev1)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p1[9] <- Prev1*(Se_", TestA, "*Se_", TestB, " + covp)*Se_",
TestC, "*(1-Se_", TestD,") + (1-Prev1)*((1-Sp_", TestA, ")*(1-
Sp_", TestB, ") + covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p1[10] <- Prev1*(Se_", TestA, "*(1-Se_", TestB, ") -
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev1)*((1-Sp_",
TestA, ")*Sp_", TestB, " - covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p1[11] <- Prev1*((1-Se_", TestA, ")*Se_", TestB, " -
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev1)*(Sp_", TestA,
"*(1-Sp_", TestB, ") - covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p1[12] <- Prev1*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev1)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p1[13] <- Prev1*(Se_", TestA, "*Se_", TestB, " + covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev1)*((1-Sp_", TestA,
")*(1-Sp_", TestB, ") + covn)*Sp_", TestC,"*Sp_", TestD,"
```

```
  p1[14] <- Prev1*(Se_", TestA, "*(1-Se_", TestB, ") - covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev1)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*Sp_", TestC,"*Sp_", TestD,"
  p1[15] <- Prev1*((1-Se_", TestA, ")*Se_", TestB, " - covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev1)*(Sp_", TestA, "*(1-
Sp_", TestB, ") - covn)*Sp_", TestC,"*Sp_", TestD,"
  p1[16] <- Prev1*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*(1-Se_", TestC,")*(1-Se_", TestD,") + (1-Prev1)*(Sp_",
TestA, "*Sp_", TestB, " + covn)*Sp_", TestC,"*Sp_", TestD,"

  #=== POPULATION 2 ===#
  Pop2[1:16] ~ dmulti(p2[1:16], ",nPop2,")
  p2[1] <- Prev2*(Se_", TestA, "*Se_", TestB, " + covp)*Se_",
TestC, "*Se_", TestD, " + (1-Prev2)*((1-Sp_", TestA, ")*(1-Sp_",
TestB, ") + covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p2[2] <- Prev2*(Se_", TestA, "*(1-Se_", TestB, ") -
covp)*Se_", TestC, "*Se_", TestD, " + (1-Prev2)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p2[3] <- Prev2*((1-Se_", TestA, ")*Se_", TestB, " -
covp)*Se_", TestC, "*Se_", TestD, " + (1-Prev2)*(Sp_", TestA,
"*(1-Sp_", TestB, ") - covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p2[4] <- Prev2*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*Se_", TestC, "*Se_", TestD," + (1-Prev2)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p2[5] <- Prev2*(Se_", TestA, "*Se_", TestB, " + covp)*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev2)*((1-Sp_", TestA, ")*(1-Sp_",
TestB, ") + covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p2[6] <- Prev2*(Se_", TestA, "*(1-Se_", TestB, ") - covp)*(1-
Se_", TestC,")*Se_", TestD," + (1-Prev2)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p2[7] <- Prev2*((1-Se_", TestA, ")*Se_", TestB, " - covp)*(1-
Se_", TestC,")*Se_", TestD," + (1-Prev2)*(Sp_", TestA, "*(1-
Sp_", TestB, ") - covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p2[8] <- Prev2*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*(1-Se_", TestC,")*Se_", TestD," + (1-Prev2)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p2[9] <- Prev2*(Se_", TestA, "*Se_", TestB, " + covp)*Se_",
TestC, "*(1-Se_", TestD,") + (1-Prev2)*((1-Sp_", TestA, ")*(1-
Sp_", TestB, ") + covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p2[10] <- Prev2*(Se_", TestA, "*(1-Se_", TestB, ") -
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev2)*((1-Sp_",
TestA, ")*Sp_", TestB, " - covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p2[11] <- Prev2*((1-Se_", TestA, ")*Se_", TestB, " -
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev2)*(Sp_", TestA,
"*(1-Sp_", TestB, ") - covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p2[12] <- Prev2*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev2)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*(1-Sp_", TestC,")*Sp_", TestD,"
```

```
  p2[13] <- Prev2*(Se_", TestA, "*Se_", TestB, " + covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev2)*((1-Sp_", TestA,
")*(1-Sp_", TestB, ") + covn)*Sp_", TestC,"*Sp_", TestD,"
  p2[14] <- Prev2*(Se_", TestA, "*(1-Se_", TestB, ") - covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev2)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*Sp_", TestC,"*Sp_", TestD,"
  p2[15] <- Prev2*((1-Se_", TestA, ")*Se_", TestB, " - covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev2)*(Sp_", TestA, "*(1-
Sp_", TestB, ") - covn)*Sp_", TestC,"*Sp_", TestD,"
  p2[16] <- Prev2*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*(1-Se_", TestC,")*(1-Se_", TestD,") + (1-Prev2)*(Sp_",
TestA, "*Sp_", TestB, " + covn)*Sp_", TestC,"*Sp_", TestD,"

   #=== POPULATION 3 ===#
  Pop3[1:16] ~ dmulti(p3[1:16], ",nPop3,")
  p3[1] <- Prev3*(Se_", TestA, "*Se_", TestB, " + covp)*Se_",
TestC, "*Se_", TestD, " + (1-Prev3)*((1-Sp_", TestA, ")*(1-Sp_",
TestB, ") + covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p3[2] <- Prev3*(Se_", TestA, "*(1-Se_", TestB, ") -
covp)*Se_", TestC, "*Se_", TestD, " + (1-Prev3)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p3[3] <- Prev3*((1-Se_", TestA, ")*Se_", TestB, " -
covp)*Se_", TestC, "*Se_", TestD, " + (1-Prev3)*(Sp_", TestA,
"*(1-Sp_", TestB, ") - covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p3[4] <- Prev3*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*Se_", TestC, "*Se_", TestD," + (1-Prev3)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*(1-Sp_", TestC,")*(1-Sp_", TestD,")
  p3[5] <- Prev3*(Se_", TestA, "*Se_", TestB, " + covp)*(1-Se_",
TestC,")*Se_", TestD," + (1-Prev3)*((1-Sp_", TestA, ")*(1-Sp_",
TestB, ") + covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p3[6] <- Prev3*(Se_", TestA, "*(1-Se_", TestB, ") - covp)*(1-
Se_", TestC,")*Se_", TestD," + (1-Prev3)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p3[7] <- Prev3*((1-Se_", TestA, ")*Se_", TestB, " - covp)*(1-
Se_", TestC,")*Se_", TestD," + (1-Prev3)*(Sp_", TestA, "*(1-
Sp_", TestB, ") - covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p3[8] <- Prev3*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*(1-Se_", TestC,")*Se_", TestD," + (1-Prev3)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*Sp_", TestC,"*(1-Sp_", TestD,")
  p3[9] <- Prev3*(Se_", TestA, "*Se_", TestB, " + covp)*Se_",
TestC, "*(1-Se_", TestD,") + (1-Prev3)*((1-Sp_", TestA, ")*(1-
Sp_", TestB, ") + covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p3[10] <- Prev3*(Se_", TestA, "*(1-Se_", TestB, ") -
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev3)*((1-Sp_",
TestA, ")*Sp_", TestB, " - covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p3[11] <- Prev3*((1-Se_", TestA, ")*Se_", TestB, " -
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev3)*(Sp_", TestA,
"*(1-Sp_", TestB, ") - covn)*(1-Sp_", TestC,")*Sp_", TestD,"
```

```
  p3[12] <- Prev3*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*Se_", TestC, "*(1-Se_", TestD,") + (1-Prev3)*(Sp_", TestA,
"*Sp_", TestB, " + covn)*(1-Sp_", TestC,")*Sp_", TestD,"
  p3[13] <- Prev3*(Se_", TestA, "*Se_", TestB, " + covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev3)*((1-Sp_", TestA,
")*(1-Sp_", TestB, ") + covn)*Sp_", TestC,"*Sp_", TestD,"
  p3[14] <- Prev3*(Se_", TestA, "*(1-Se_", TestB, ") - covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev3)*((1-Sp_", TestA,
")*Sp_", TestB, " - covn)*Sp_", TestC,"*Sp_", TestD,"
  p3[15] <- Prev3*((1-Se_", TestA, ")*Se_", TestB, " - covp)*(1-
Se_", TestC,")*(1-Se_", TestD,") + (1-Prev3)*(Sp_", TestA, "*(1-
Sp_", TestB, ") - covn)*Sp_", TestC,"*Sp_", TestD,"
  p3[16] <- Prev3*((1-Se_", TestA, ")*(1-Se_", TestB, ") +
covp)*(1-Se_", TestC,")*(1-Se_", TestD,") + (1-Prev3)*(Sp_",
TestA, "*Sp_", TestB, " + covn)*Sp_", TestC,"*Sp_", TestD,"

#=== PRIOR  ===#

  Prev1 ~ dbeta(",Prev1.shapea,", ",Prev1.shapeb,")     ## Prior
for Prevalence in population 1
  Prev2 ~ dbeta(",Prev2.shapea,", ",Prev2.shapeb,")     ## Prior
for Prevalence in population 2
  Prev3 ~ dbeta(",Prev3.shapea,", ",Prev3.shapeb,")     ## Prior
for Prevalence in population 3
  Se_", TestA, " ~ dbeta(",Se.TestA.shapea,",
",Se.TestA.shapeb,")  ## Prior for Se of Test A
  Sp_", TestA, " ~ dbeta(",Sp.TestA.shapea,",
",Sp.TestA.shapeb,")  ## Prior for Sp of Test A
  Se_", TestB, " ~ dbeta(",Se.TestB.shapea,",
",Se.TestB.shapeb,")  ## Prior for Se of Test B
  Sp_", TestB, " ~ dbeta(",Sp.TestB.shapea,",
",Sp.TestB.shapeb,")  ## Prior for Sp of Test B
  Se_", TestC, " ~ dbeta(",Se.TestC.shapea,",
",Se.TestC.shapeb,")  ## Prior for Se of Test C
  Sp_", TestC, " ~ dbeta(",Sp.TestC.shapea,",
",Sp.TestC.shapeb,")  ## Prior for Sp of Test C
  Se_", TestD, " ~ dbeta(",Se.TestD.shapea,",
",Se.TestD.shapeb,")  ## Prior for Se of Test D
  Sp_", TestD, " ~ dbeta(",Sp.TestD.shapea,",
",Sp.TestD.shapeb,")  ## Prior for Sp of Test D

#=== CONDITIONAL DEPENDENCE STRUCTURE ===#

  covp ~ dunif(minp,maxp)
  covn ~ dunif(minn,maxn)
  minp <- (1-Se_", TestA, ")*(Se_", TestB, "-1)
  minn <- (Sp_", TestA, "-1)*(1-Sp_", TestB, ")
  maxp <- min(Se_", TestA, ",Se_", TestB, ") - Se_", TestA,
"*Se_", TestB, "
```

```
  maxn <- min(Sp_", TestA, ",Sp_", TestB, ") - Sp_", TestA,
"*Sp_", TestB, "

}")

# write as a text (.txt) file ---------------------------------
------------
write.table(rapidtest_4test_3pop_dep,
            file="rapidtest_4test_3pop_dep.txt",
            quote=FALSE,
            sep="",
            row.names=FALSE,
            col.names=FALSE)

# Initialize values for Prev parameters, and the Ses and Sps of
the 3 tests for the 3 chains ---------------------------------
-----------
inits <- list(list(Prev1=0.05,
                   Prev2=0.15,
                   Prev3=0.30,
                   Se_TETXLT4=0.85,
                   Sp_TETXLT4=0.95,
                   Se_SELHE=0.75,
                   Sp_SELHE=0.95,
                   Se_PCR=0.90,
                   Sp_PCR=0.95,
                   Se_LFI=0.70,
                   Sp_LFI=0.60),


              list(Prev1=0.15,
                   Prev2=0.25,
                   Prev3=0.50,
                   Se_TETXLT4=0.95,
                   Sp_TETXLT4=0.99,
                   Se_SELHE=0.85,
                   Sp_SELHE=0.99,
                   Se_PCR=0.95,
                   Sp_PCR=0.99,
                   Se_LFI=0.85,
                   Sp_LFI=0.70),


              list(Prev1=0.01,
                   Prev2=0.10,
                   Prev3=0.10,
                   Se_TETXLT4=0.75,
                   Sp_TETXLT4=0.75,
                   Se_SELHE=0.65,
                   Sp_SELHE=0.75,
```

```
                    Se_PCR=0.80,
                    Sp_PCR=0.80,
                    Se_LFI=0.45,
                    Sp_LFI=0.45)

)

# Run the Bayesian model ---------------------------------------
-------
set.seed(123)
bug.out.2 <- jags(data=datalist,
                  model.file="rapidtest_4test_3pop_dep.txt",
                  parameters.to.save=c("Prev1", "Prev2",
"Prev3", "Se_TETXLT4", "Sp_TETXLT4", "Se_SELHE", "Sp_SELHE",
"Se_PCR", "Sp_PCR", "Se_LFI", "Sp_LFI", "covp", "covn"),
                  n.chains=3,
                  inits=inits,
                  n.iter=101000,
                  n.burnin=1000,
                  n.thin=1,
                  DIC=FALSE)
```

APPENDIX 3C: DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF SALMONELLA DETECTION METHODS IN HORSES – EXPERT OPINION SURVEY

Diagnostic Sensitivity and Specificity of Salmonella Detection Methods in Horses
Expert Opinion Survey

Part 1: Respondent Information

The following questions will collect general identifying information and details regarding your educational background and expertise.

1. First name

2. Last name

3. Degree(s) held (select all that apply).

- [ ] Doctor of Veterinary Medicine (DVM) or equivalent
- [ ] Master of Science (MS) or equivalent
- [ ] Master of Public Health (MPH)
- [ ] Doctor of Philosophy (PhD)
- [ ] Other (please specify)

4. Board certification(s) held (select all that apply)

- [ ] Internal medicine (American College of Veterinary Internal Medicine [ACVIM], European College of Veterinary Internal Medicine [ECVIM], European College of Equine Internal Medicine [ECEIM])
- [ ] Microbiology (American College of Veterinary Microbiologists [ACVM]), European College of Veterinary Microbiology [EVCM])
- [ ] Preventive Medicine (American College of Veterinary Preventive Medicine [ACVPM])
- [ ] Pathology (American College of Veterinary Pathologists [ACVP], European College of Veterinary Pathologists [ECVP])
- [ ] None
- [ ] Other (please specify)

5. What do you consider to be your **primary** area of expertise?

- ◯ Epidemiology
- ◯ Microbiology
- ◯ Internal Medicine
- ◯ Clinical Pathology
- ◯ Anatomic Pathology
- ◯ Other (please specify)

Diagnostic Sensitivity and Specificity of Salmonella Detection Methods in Horses
Expert Opinion Survey

Test 1: Tetrathionate-enriched culture

Please answer the questions in this section regarding the following diagnostic test scenario:

A 3-gram equine fecal sample was selectively enriched in 30 ml tetrathionate broth, incubated overnight at 43°C, streaked for isolation onto XLT4 agar, and incubated overnight at 43°C for the detection of *Salmonella enterica*.

6. In your opinion, what is the **most likely** value of the **sensitivity** of this test for the detection of *Salmonella*?

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

7. Please provide the lower and upper limits of a 95% confidence interval for the **sensitivity** of this test (i.e., "I am 95% confident that the sensitivity of this test lies between ___ and ___%")

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

Lower limit

Upper limit

8. In your opinion, what is the **most likely** value of the **specificity** of this test for the detection of *Salmonella*?

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

9. Please provide the lower and upper limits of a 95% confidence interval for the **specificity** of this test (i.e., "I am 95% confident that the specificity of this test lies between ___ and ___%")

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

Lower limit

Upper limit

Diagnostic Sensitivity and Specificity of Salmonella Detection Methods in Horses
Expert Opinion Survey

Test 2: Selenite-enriched culture

Please answer the questions in this section regarding the following diagnostic test scenario:

A 3-gram equine fecal sample was selectively enriched in 30 ml selenite broth, incubated overnight at 35°C, streaked for isolation onto Hektoen enteric (HE) agar, and incubated overnight at 35°C for the detection of *Salmonella enterica*.

10. In your opinion, what is the **most likely** value of the **sensitivity** of this test for the detection of *Salmonella*?

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

11. Please provide the lower and upper limits of a 95% confidence interval for the **sensitivity** of this test (i.e., "I am 95% confident that the sensitivity of this test lies between ___ and ___%")

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

Lower limit

Upper limit

12. In your opinion, what is the **most likely** value of the **specificity** of this test for the detection of *Salmonella*?

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

13. Please provide the lower and upper limits of a 95% confidence interval for the **specificity** of this test (i.e., "I am 95% confident that the specificity of this test lies between ___ and ___%")

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

Lower limit

Upper limit

Diagnostic Sensitivity and Specificity of Salmonella Detection Methods in Horses
Expert Opinion Survey

Test 3: qPCR

Please answer the questions in this section regarding the following diagnostic test scenario:

A 3-gram equine fecal sample was selectively enriched in 30 ml tetrathionate broth and incubated overnight at 43°C. The enrichment broth was subjected to qPCR for the detection of *Salmonella enterica*.

14. In your opinion, what is the **most likely** value of the **sensitivity** of this test for the detection of *Salmonella*?

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

15. Please provide the lower and upper limits of a 95% confidence interval for the **sensitivity** of this test (i.e., "I am 95% confident that the sensitivity of this test lies between ___ and ___%")

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

Lower limit

Upper limit

16. In your opinion, what is the **most likely** value of the **specificity** of this test for the detection of *Salmonella*?

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

17. Please provide the lower and upper limits of a 95% confidence interval for the **specificity** of this test (i.e., "I am 95% confident that the specificity of this test lies between ___ and ___%")

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

Lower limit

Upper limit

Diagnostic Sensitivity and Specificity of Salmonella Detection Methods in Horses
Expert Opinion Survey

Test 4: Rapid test (lateral flow immunoassay)

Please answer the questions in this section regarding the following diagnostic test scenario:

A 3-gram equine fecal sample was selectively enriched in 30 ml tetrathionate broth and incubated overnight at 43°C. The enrichment broth was tested for *Salmonella enterica* using a rapid test. This test is a lateral flow immunoassay designed for food safety applications.

18. In your opinion, what is the **most likely** value of the **sensitivity** of this test for the detection of *Salmonella*?

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

19. Please provide the lower and upper limits of a 95% confidence interval for the **sensitivity** of this test (i.e., "I am 95% confident that the sensitivity of this test lies between ___ and ___%")

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

Lower limit

Upper limit

20. In your opinion, what is the **most likely** value of the **specificity** of this test for the detection of *Salmonella*?

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

21. Please provide the lower and upper limits of a 95% confidence interval for the **specificity** of this test (i.e., "I am 95% confident that the specificity of this test lies between ___ and ___%")

Enter as a number with one decimal place (e.g., 50.0 or 75.5). Do not include the % symbol.

Lower limit

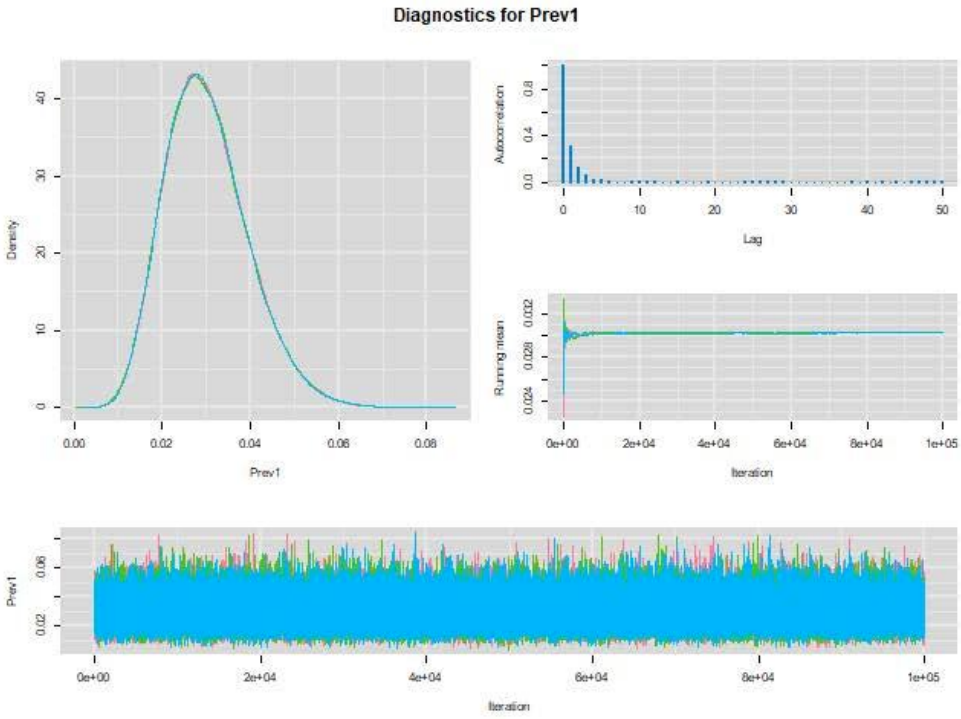
Upper limit

APPENDIX 3D: BAYESIAN LATENT CLASS MODEL DIAGNOSTIC PLOTS

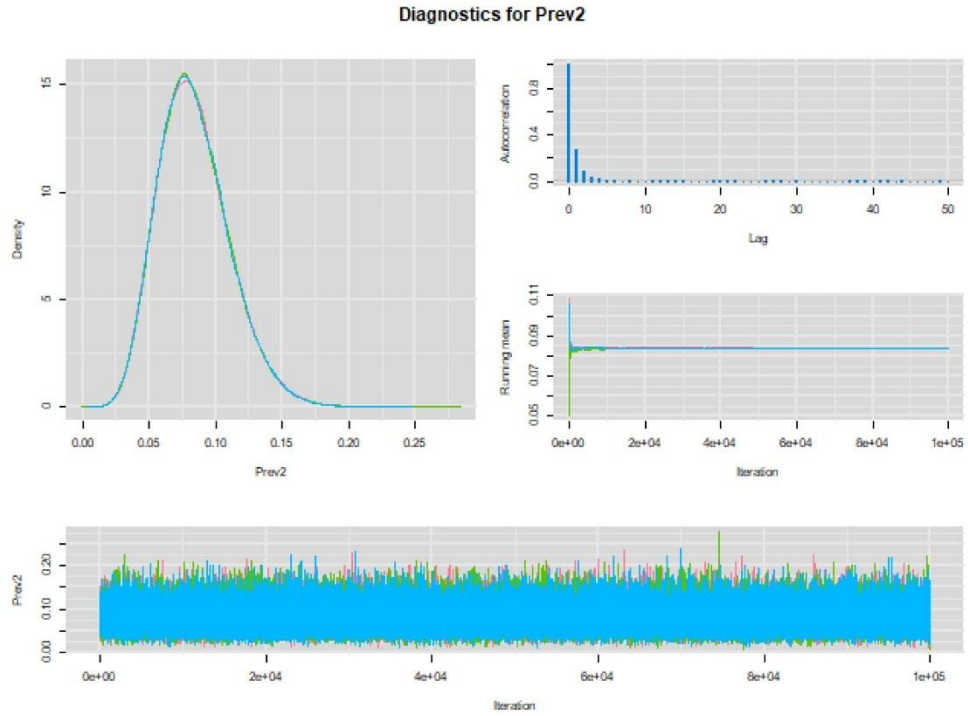
Diagnostic plots - 3 Populations, 4 Tests, Dependent

Table of Contents

Plots for Prev1



Plots for Prev2



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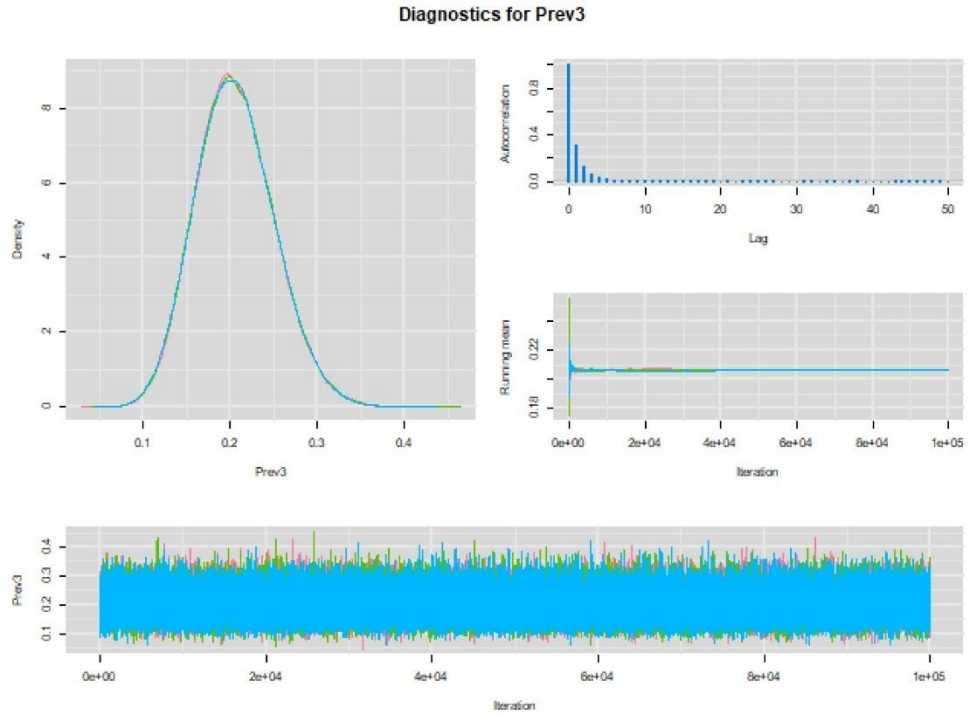
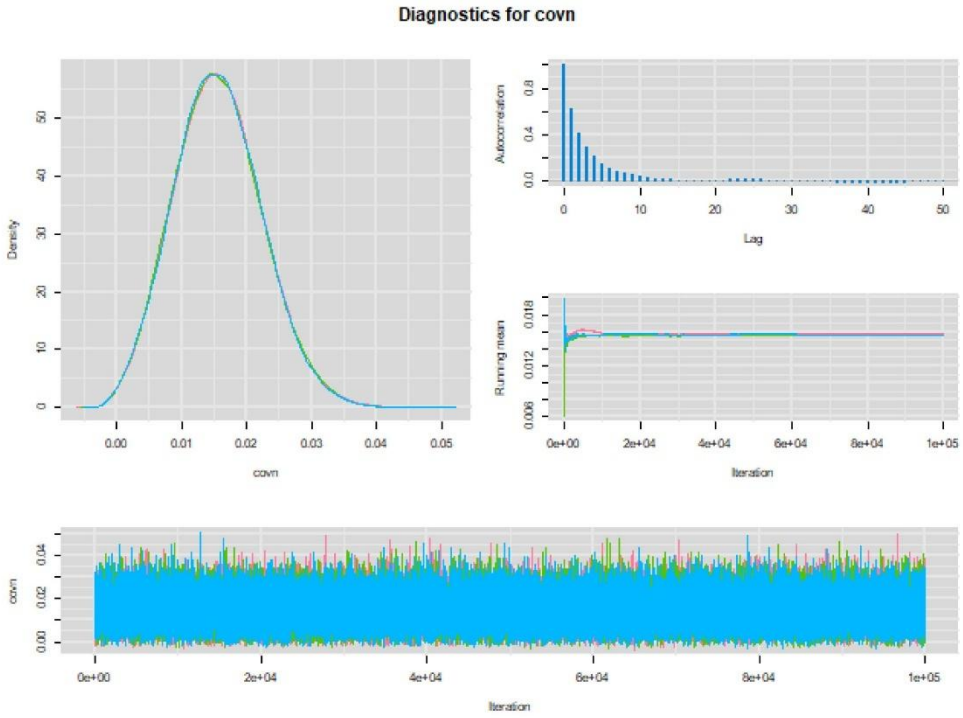
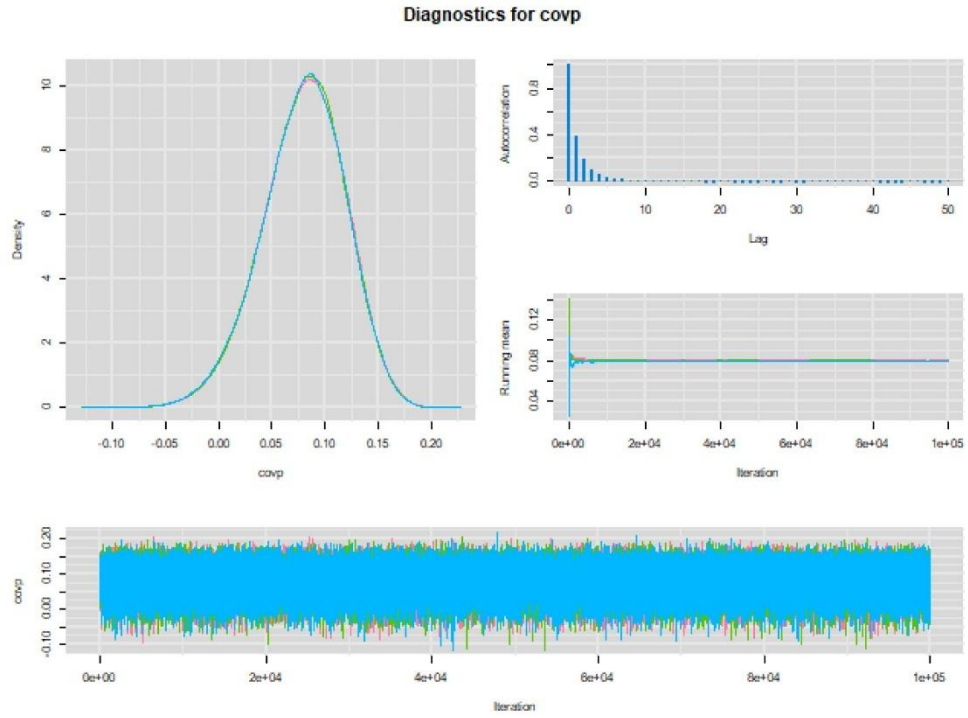


Plots for covn



Plots for covp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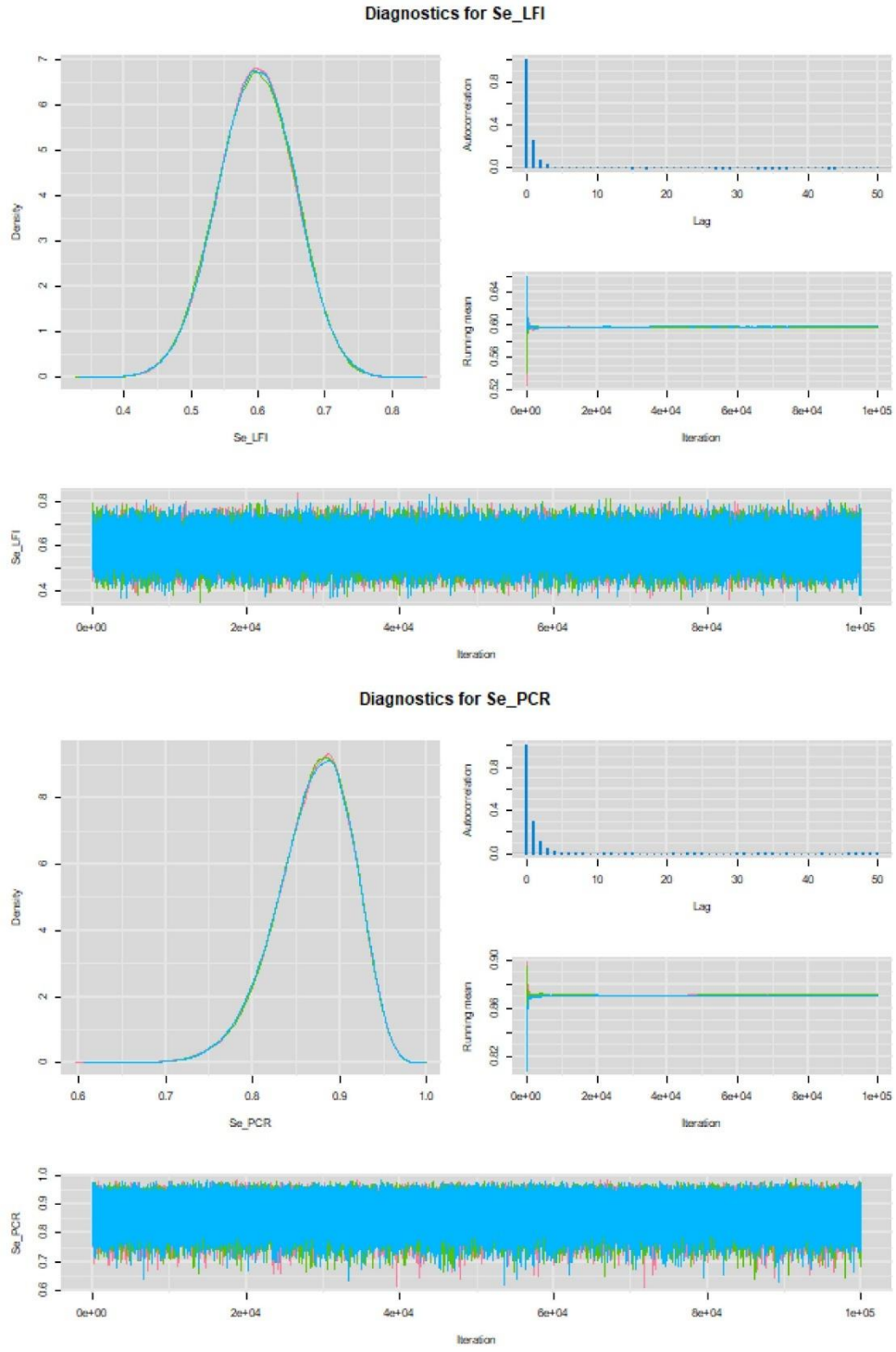
Density
covp
Autocorrelation
Lag
Running mean
Iteration
covp
Iteration

Plots for Se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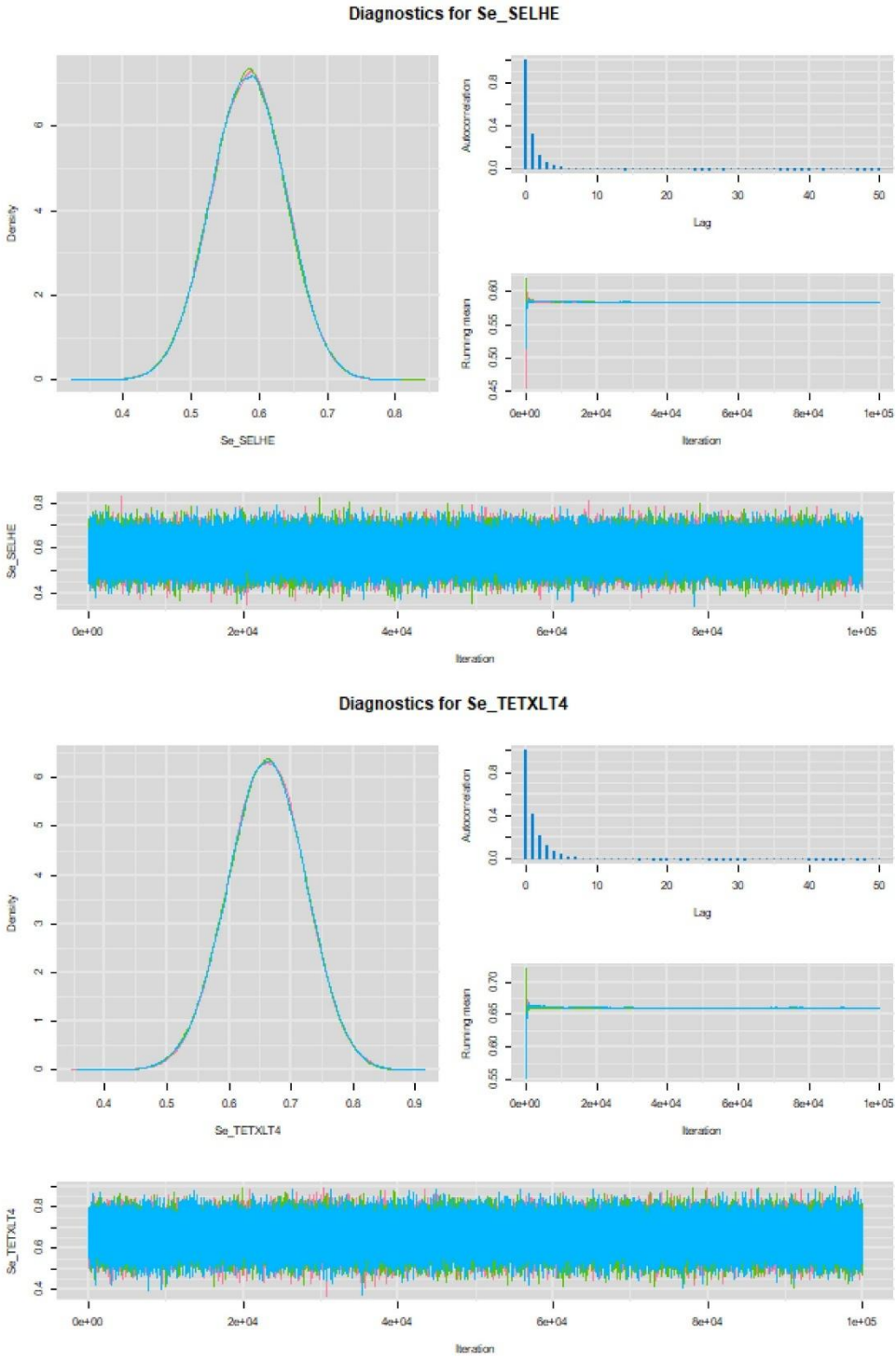
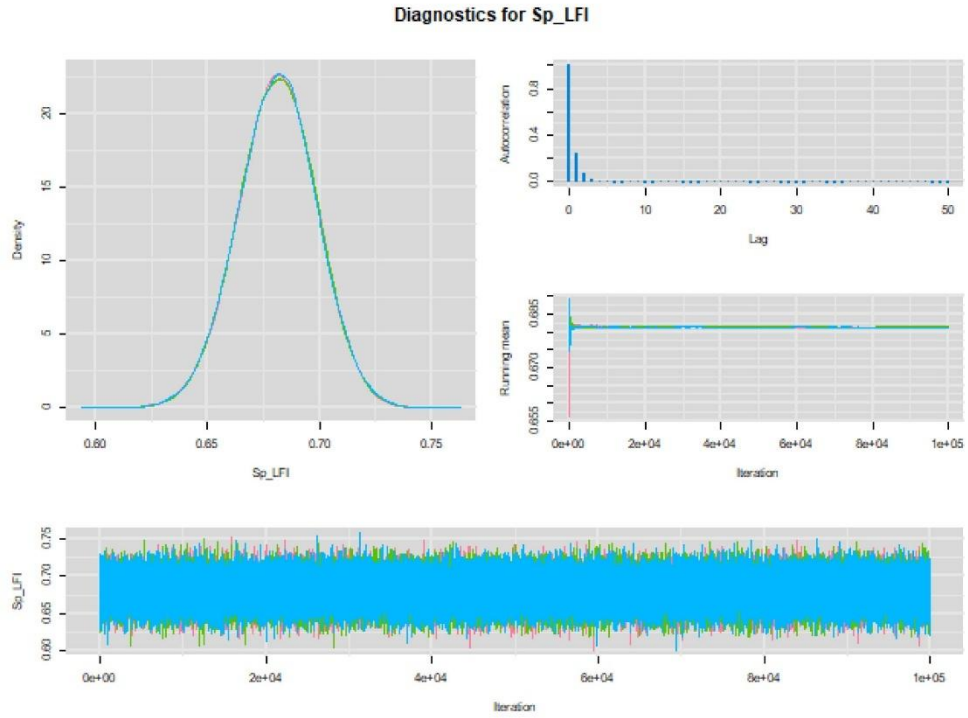
Density
Se_LFI
Autocorrelation
Lag
Running mean
Iteration
Se_LFI
Iteration
Diagnostics for Se_PCR
Density
Se_PCR
Autocorrelation
Lag
Running mean
Iteration
Se_PCR
Iteration


Diagnostics for Se_SELHE
Density
Se_SELHE
Autocorrelation
Lag
Running mean
Iteration
Se_SELHE
Iteration
Diagnostics for Se_TETXLT4
Density
Se_TETXLT4
Autocorrelation
Lag
Running mean
Iteration
Se_TETXLT4
Iteration

Plots for Sp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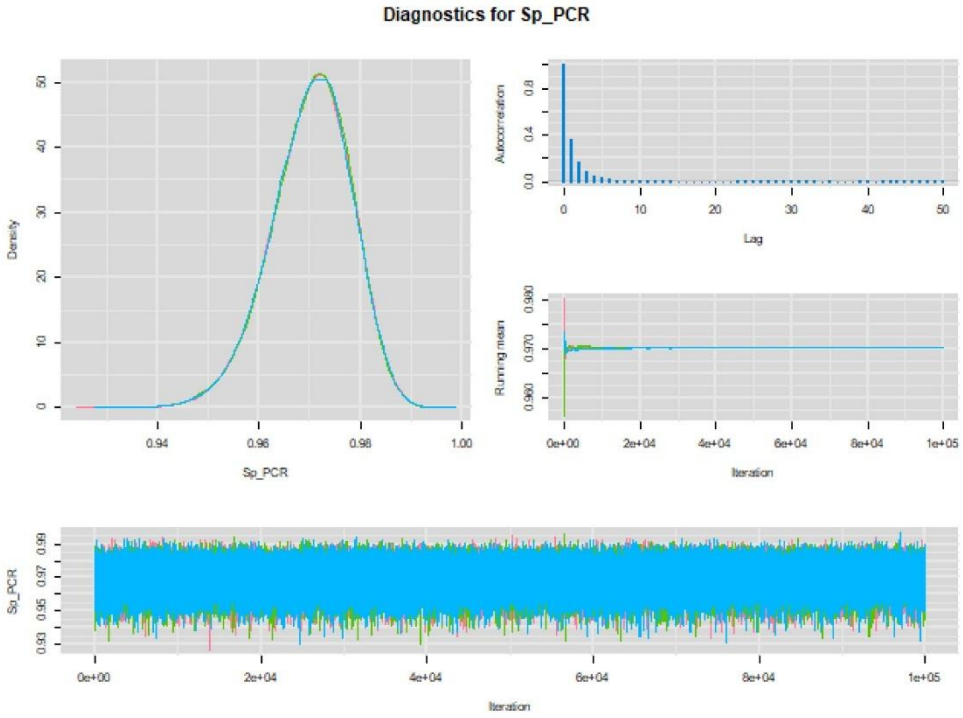
Density
Sp_LFI
Autocorrelation
Lag
Running mean
Iteration
Sp_LFI
Iteration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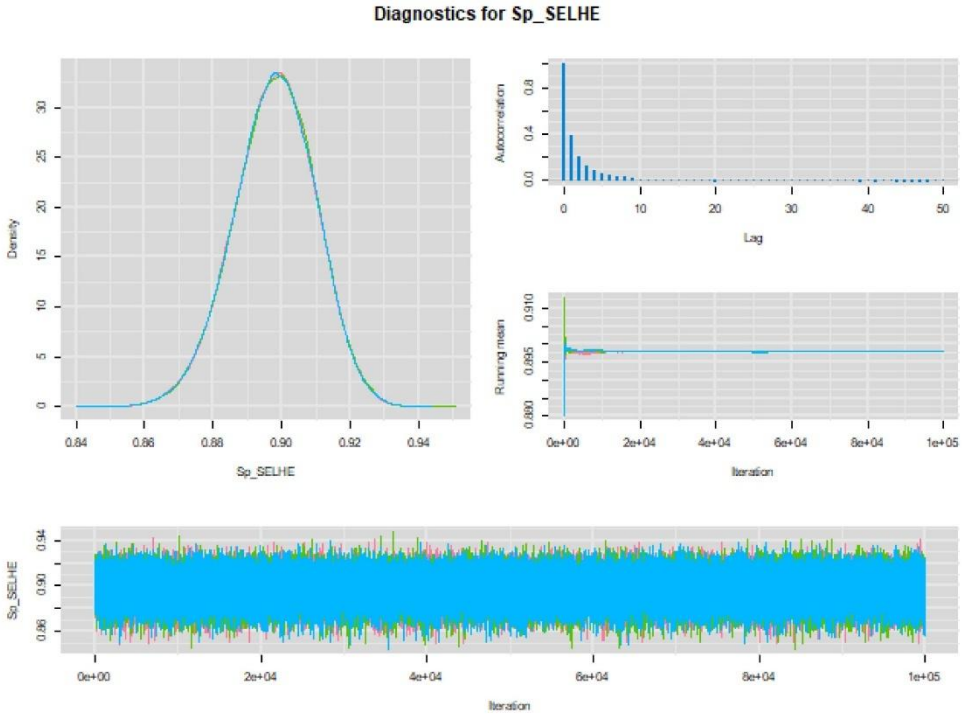
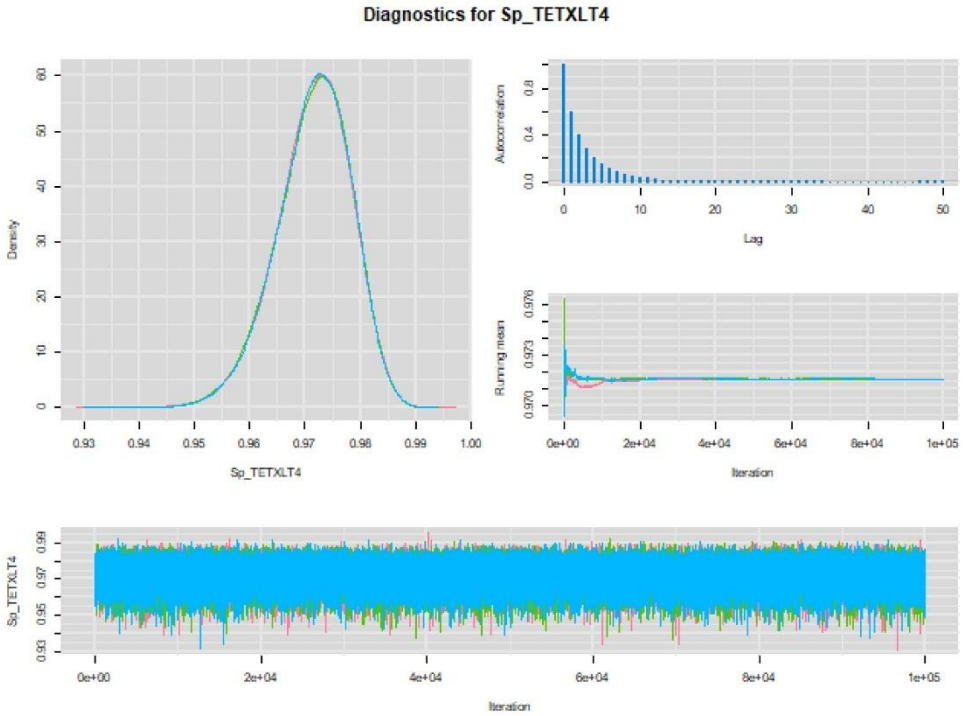
Density
Sp_PCR
Autocorrelation
Lag
Running mean
Iteration
Sp_PCR
Iteration


Diagnostics for Sp_SELHE
Density
Sp_SELHE
Autocorrelation
Lag
Running mean
Iteration
Sp_SELHE
Iteration


Diagnostics for Sp_TETXLT4
Density
Sp_TETXLT4
Autocorrelation
Lag
Running mean
Iteration
Sp_TETXLT4
Iteration

APPENDIX 4A: ENROLLMENT SURVEY – DURATION OF FECAL *SALMONELLA* SHEDDING IN HORSES

How long will my horse shed *Salmonella*
-- Participant Enrollment Form --

Owner and Horse Demographics

1. Enrolling institution (i.e., study site)

- ◯ Colorado State University
- ◯ Hagyard Equine Medical Institute
- ◯ Louisiana State University
- ◯ University of Georgia
- ◯ University of Pennsylvania
- ◯ Chaparral Veterinary Medical Center
- ◯ Other (please specify)

2. Referring Veterinarian Name

3. Referring Veterinarian Email Address

4. Owner Name

5. Mailing/shipping address (Street, City, State, Zip Code) [Note: We are using FedEx for all shipping]

6. Owner email address

7. Owner phone number

8. Horse Name

9. Horse age (months and years)

10. Horse sex

- ◯ Mare
- ◯ Gelding
- ◯ Stallion

11. Horse breed

12. Admit date (MM/DD/YY)

13. Discharge Date (MM/DD/YY)

14. What is this horses use or occupation?

- ☐ Breeding (e.g., broodmare, stallion, nurse mare)
- ☐ Eventer
- ☐ Pleasure
- ☐ Race Horse
- ☐ Western (e.g., reining, cutting, roping)
- ☐ Working on farm or ranch
- ☐ None
- ☐ Other (please specify)

15. Does this horse have a history of culture-confirmed *Salmonella* shedding prior to this instance?

- ◯ Yes
- ◯ No
- ◯ Unknown

16. If yes, how long ago (years and months)?

17. Does this horse come from a farm with a history of salmonellosis (e.g., positive stablemates or previous outbreak)?

- ◯ Yes
- ◯ No
- ◯ Unknown

18. If yes, how long ago (years and months)?

19. What types of feed/grain does this horse routinely receive? (check all that apply)

- ☐ Pasture
- ☐ Grass hay
- ☐ Alfalfa hay
- ☐ Sweet feed
- ☐ Oats
- ☐ Commercial horse feed (e.g., Purina Equine Senior)
- ☐ Other (please specify)

How long will my horse shed *Salmonella*
-- Participant Enrollment Form --

Medical Management During Hospitalization

20. Presenting complaint

21. Systemic illness summary (past 48 hours)

- ◯ Healthy or minimal illness (e.g., minor lameness, arthroscopy, COPD, mare with admitted foals, reproductive problems, etc.)
- ◯ Minor or moderate systemic illness (e.g., lacerations, recovering fractures, animals RTG recovering from more serious illness such as colic, FUO, mild respiratory infections, etc.)
- ◯ Major systemic illness (e.g., severe fractures, renal failure, liver failure, colic, severe strangles, pleuritis, pneumonia, colitis or enteritis, peritonitis, etc.)

22. Diarrhea of soft fecal consistency in past 48 hours

- ◯ Yes
- ◯ No
- ◯ Unknown

23. Febrile in past 48 hours (rectal temperature >101.5F)

- ◯ Yes
- ◯ No
- ◯ Unknown

24. Leukopenia in past 48 hours (WBC < 5000/ul)

- ◯ Yes
- ◯ No
- ◯ Unknown

25. Anesthesia or surgery in past 48 hours

- ◯ Yes
- ◯ No

26. Antimicrobials in past 48 hours (check all that apply)

- [ ] None
- [ ] Oral
- [ ] Parenteral
- [ ] Topical or Ophthalmic

27. Antimicrobial class(es) administered during hospital visit (check all that apply)

- [ ] None
- [ ] Aminoglycoside
- [ ] Beta-lactam
- [ ] Cephalosporin (any generation)
- [ ] Florfenicol
- [ ] Fluoroquinolone
- [ ] Macrolide
- [ ] Sulfas
- [ ] Tetracycline
- [ ] Other (please specify)

28. Gastroprotectant class(es) administered during hospitalization (check all that apply)

- [ ] Antidiarrheal
- [ ] H2-Blocker
- [ ] Proton pump inhibitor
- [ ] Mucosal protectant
- [ ] Other (please specify)

29. Significant reduction in dietary intake during past 48 hours (e.g., inappetance, withholding feed)

- ◯ Yes
- ◯ No
- ◯ Unknown

30. Body system affected (check all that apply)

- [ ] Normal
- [ ] Musculoskeletal
- [ ] GI
- [ ] Respiratory
- [ ] Renal
- [ ] Hepatic
- [ ] Reproductive
- [ ] Other (please specify)

31. Highest 'level of care' during hospitalization (what level out of number possible OR n/a)

APPENDIX 4B: FECAL SAMPLE SUBMISSION FORM

College of Veterinary Medicine
UNIVERSITY OF GEORGIA

Fecal Sample Submission Form

If you have any questions or concerns, please do not hesitate to contact your local Co-Investigator:

University of Georgia
Brandy A. Burgess, DVM, MSc, PhD, DACVIM, DACVPM
Brandy.Burgess@uga.edu

Clare Ryan, DVM, PhD, DACVIM
cryan@uga.edu

Colorado State University
Paul S. Morley, DVM, PhD, DACVIM
Paul.Morley@colostate.edu

University of Pennsylvania
Helen Aceto, PhD, VMD
helenwa@vet.upenn.edu

Louisiana State University
Ann Chapman, DVM, MS, DACVIM
achapm2@lsu.edu

Hagyard Equine Medical Institute
Nathan M. Slovis, DVM, DACVIM, CHT
nmslovis@yahoo.com

General Information

1. Owner name: _____
2. Sample Date: sample 1_____; sample 2_____; sample 3_____
3. Sample number: _____ of 8 weekly fecal samples
4. Referring hospital: _____
5. Horse's name: _____
6. Number of horses currently at the facility: _____

Horse Health Information

- **Since being discharged from the hospital, has THIS HORSE had...**
  a. Any episodes of colic? Yes No
     If yes, how many episodes? _____
  b. Any soft or loose feces? Yes No
     If yes, how many times? _____

- **Since this horse was discharged, have ANY STABLEMATES had...**
  a. Any episodes of colic? Yes No
     If yes, how many horses? _____
  b. Any soft or loose feces? Yes No
     If yes, how many horses? _____

- **Since this horse was discharged, have ANY STABLEMATES been...**
  a. Hospitalized? Yes No
     If yes, how many horses? _____
  b. Culture-positive for *Salmonella*? Yes No
     If yes, how many horses? _____

APPENDIX 4C: FECAL SAMPLE COLLECTION GUIDE

College of Veterinary Medicine
UNIVERSITY OF GEORGIA

How to:
Collect a Fecal Sample

If you have any questions or concerns, please do not hesitate to contact your local Co-Investigator:

University of Georgia
Brandy A. Burgess, DVM, MSc, PhD, DACVIM, DACVPM
Brandy.Burgess@uga.edu

Clare Ryan, DVM, PhD, DACVIM
cryan@uga.edu

Colorado State University
Paul S. Morley, DVM, PhD, DACVIM
Paul.Morley@colostate.edu

University of Pennsylvania
Helen Aceto, PhD, VMD
helenwa@vet.upenn.edu

Louisiana State University
Ann Chapman, DVM, MS, DACVIM
achapm2@lsu.edu

Hagyard Equine Medical Institute
Nathan M. Slovis, DVM, DACVIM, CHT
nmslovis@yahoo.com

Instructions

Make sure to put icepacks in freezer 24-hours before shipping

1. Put on disposable gloves (there should a 3 pairs in your sample kit)
2. Collect THREE 1-gram samples at 12-24 hour intervals from freshly voided feces (1-gram is approximately 10mls) – store in fridge until shipping
3. Place ALL samples in same sample container provided
4. Disinfect sample container using a household disinfectant (e.g., Lysol)
5. Ensure that the container is properly sealed and fill in label information on the container (i.e., owner name, sample date, horse name, sample number X of 8, referring hospital)
6. Place the container in the provided biohazard bag
7. Remove your gloves and wash hands thoroughly
8. Store sample in refrigerator until shipping to UGA
9. Complete a Fecal Sample Submission Form (included in sample kit)
10. Pack sample, submission form, import permit, and ice packs into Styrofoam shipper
11. Apply FedEx return label (found in the sample kit) and drop by your nearest FedEx location for 'Standard Overnight' shipping
12. If the return label is not in the kit – contact Dr. Brandy Burgess (brandy.burgess@uga.edu) to receive a shipping label via email

v6

APPENDIX 4D: INFORMATION SHEET – MANAGING *SALMONELLA*

Colorado State University
Veterinary Teaching Hospital

Managing Salmonella

What you should know about *Salmonella*:

Salmonella spreads through contaminated feces. As horses do not have the same qualms humans do about laying in dirt and manure, any part of their body and anything in their environment is potentially contaminated. Flies and other insects may also spread the bacteria from surface to surface. Fortunately, if appropriate precautions are taken, horses with *Salmonella* are not likely to infect other horses or humans.

Created by:

Kimberly J. Pattison BA, MPH

Brandy A. Burgess DVM, MSc, PhD, DACVIM, DACVPM

Paul S. Morley DVM, PhD, DACVIM

Salmonella -- The Basics

Salmonella is a common problem among horses that can result in diarrhea and other gastrointestinal symptoms. While most people think of *Salmonella* as a bacteria found in food, it also spreads through contact with the feces of infected individuals and anything contaminated with their feces (water, food, hands, surfaces, etc). Stablemates of infected horses and their human caretakers have a heightened risk for *Salmonella* infection.

Sometimes, horses with *Salmonella* do not have any symptoms. They can be healthy, but still contagious. A horse is contagious when it is "shedding" *Salmonella* bacteria in its feces. Infected, but healthy, horses have *Salmonella* contained in their intestines and only shed occasionally. Stress increases the likelihood that a horse will shed *Salmonella*. Sources of stress might include transportation, changing diets, competition, moving to a new property, disease such as colic, and hospitalization or anesthesia. Horses exhibiting symptoms of a *Salmonella* infection should always be assumed to be shedding until their veterinarian has determined they are cleared of the bacteria.

Along with gastrointestinal symptoms, horses with Salmonella tend to develop laminitis (founder). If your horse has *Salmonella*, monitor it closely for signs of hoof pain so it can be treated promptly. Cushioning its stall with deep bedding may help alleviate the problem. Dehydration is also a concern. Make sure ill horses have continuous access to water and a clean environment.

Humans who work with horses have a higher risk for contracting *Salmonella*. They may also carry the bacteria home on their clothes and unwashed hands to others in their household. *Salmonella* infections in young children and immune compromised people tend to be especially severe. If you are caring for a horse with *Salmonella*, it's important to take precautions to protect yourself and those around you.

How Do I Know if My Horse is Contagious?

If your horse has *Salmonella*, your veterinarian may want to run tests to determine when it is no longer shedding the bacteria. Generally, veterinarians believe 3 to 5 negative tests in a row are enough to say a horse is probably finished shedding *Salmonella*. This is not a guarantee that *Salmonella* is entirely out of your horses system and it may begin shedding again later. Research from the CSU Veterinary Teaching Hospital shows that horses can be well protected from infections like *Salmonella* if you take appropriate precautions, such as those outlined on the opposite side of this handout.

Even if your horse is not actively shedding *Salmonella* bacteria, it is always wise to continue using basic infection prevention practices to keep your horses healthy. Your veterinarian can give you more information about the risk *Salmonella* poses to humans and other animals.

* *Immune compromised people should always take greater precautions, as they are susceptible to infections that the general population is not.*

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