Managing *Salmonella* in Equine Populations

Brandy A. Burgess, DVM, MSc, PhD[a], Paul S. Morley, DVM, PhD[b,*]

KEYWORDS

• Equine • *Salmonella* • Infection control

KEY POINTS

- Veterinary practitioners have an ethical obligation to appropriately manage risks related to *Salmonella* in animal populations and their environments.
- The goal of infection control is to eliminate sources of potentially pathogenic microorganisms and to disrupt infectious disease transmission.
- Congregating animals from multiple sources increases the risk for transmission of infectious agents such as *Salmonella*.
- Practitioners should be aware of the different *Salmonella* testing methods available because this can affect test results and interpretations relative to disease control efforts.
- Managing *Salmonella* in populations can be particularly challenging because of the diversity in clinical consequences of infection and intermittent shedding.

INTRODUCTION

Congregating animals from multiple sources, as occurs at veterinary hospitals, racetracks, equestrian events, and boarding and training facilities, increases the risk for transmission of infectious agents such as *Salmonella*.[1] This article provides equine practitioners with details relevant to effectively managing *Salmonella* in these populations. It begins by focusing on the agent, *Salmonella enterica*, to develop an appreciation of its key features, including the nuances of organism detection and test interpretation. It then considers the fundamentals of veterinary infection control with the intent of developing a foundation that can be applied to both hospital and field settings. In addition, the article discusses how infection control principles and understanding of the epidemiology of *S enterica* can facilitate managing transmission risks related to this organism in hospital populations and field settings. Detailed

[a] Department of Population Health Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia-Tech, 100 Sandy Hall (MC0395), 210 Drillfield Drive, Blacksburg, VA 24061, USA; [b] Department of Clinical Sciences, James L. Voss Veterinary Teaching Hospital, Colorado State University, 1678 Campus Delivery, Fort Collins, CO 80523-1678, USA
* Corresponding author.
E-mail address: paul.morley@colostate.edu

Vet Clin Equine ■ (2014) ■–■
http://dx.doi.org/10.1016/j.cveq.2014.08.005 **vetequine.theclinics.com**
0749-0739/14/$ – see front matter

descriptions of bacteriology, pathophysiology, and treatment are beyond the scope of this article.

IMPORTANCE OF *SALMONELLA* IN EQUINE POPULATIONS

Salmonella is one of the most common causes of epidemic disease in veterinary hospitals[2] and an agent frequently associated with on-farm contamination.[3] Significant efforts are made to control its transmission among animals, especially within equine hospitals. However, these efforts are predominantly based on first principles because many prevention methods have not been critically evaluated in clinical studies. Regardless, outbreaks known to be attributable to *Salmonella* can come at a great cost, not only in terms of morbidity and case fatality to affected animals but also in terms of direct financial costs; they also present a clear risk to veterinary patients and personnel working with these animals.[4,5] Veterinary practitioners have an ethical obligation to appropriately manage risks related to *Salmonella* in animal populations and their environment. There is a recognizable standard of practice with respect to infection control and due effort must be given to control and prevention of infectious disease transmission within animal populations and facilities.[6]

When *Salmonella* spreads among patients, environmental contamination is predictably present, whether as cause or effect.[4,7–9] Although concerns about management are typically focused on clinically affected animals, subclinical infection and shedding in the absence of disease is more common than clinical infections, which can greatly exacerbate environmental contamination before the scope of the problem is recognized.[8,10] However, testing strategies for relevant veterinary samples (ie, fecal and environmental samples) for the presence of *Salmonella* is variable among laboratories and current testing methodology generally lacks in sensitivity, likely because of the intermittent nature and low level of organisms shed in animal feces. Therefore, testing strategies generally require testing of multiple samples and lengthy enrichment steps, and it can often take 3 to 5 days to obtain results. In that time, significant environmental contamination and disease transmission can occur. As a result, risk recognition and the ability to rapidly identify these patients are critical to the effective management of populations and their environments.

SALMONELLA: THE BASICS

S enterica, a member of the family Enterobacteriaceae, is a gram-negative facultative anaerobic bacterium found colonizing the small intestine, cecum, and colon of both cold-blooded and warm-blooded vertebrates. There are more than 2400 serotypes, which are distinguished by the presence of differing O-antigen (polysaccharide portion of lipopolysaccharide) and H-antigen (filamentous portion of flagella or flagellin) on the surface of the bacteria. *S enterica* subspecies *enterica*, the focus of this article, accounts for approximately 59% of all serotypes and is responsible for approximately 99% of clinical and subclinical *Salmonella* infections in warm-blooded animals.[11,12]

S enterica is considered an opportunistic pathogen that is more likely to cause clinical disease in situations of high exposure or patients that have an increased susceptibility, such as neonates and patients with severe systemic illness. Transmission occurs by the fecal-oral route and can result in enterocolitis (ie, diarrhea), bacteremia, or subclinical infection, with infection depending on the infective dose, host susceptibility, and the infecting serotype. As such, identifying subclinical fecal shedding, managing contacts among patients, and practicing effective personal and environmental hygiene are critical for protecting animals and people.

SALMONELLA TESTING AND INTERPRETATION

There are many methods available for the detection of *S enterica* in samples relevant to veterinary medicine, including enriched culture, polymerase chain reaction (PCR), and lateral flow immunoassays (LFIs), all of which require varying levels of expertise, cost, and time to detection. Practitioners should be aware of the different testing methods available, their strengths and limitations, and know which method is being used (eg, the type of enrichments used) by laboratories because this can affect test results and interpretations relative to disease control efforts.[4,13–16]

Culture of Fecal Samples

There are limitations to the detection of *Salmonella* when culturing fecal samples. In experiments, the analytical sensitivity of equine fecal culture has been found to be as few as 4 colony-forming units (cfu) per gram of feces when enriched in tetrathionate broth[17] and 100 cfu/g of feces when enriched in selenite broth.[18] However, in practice, fecal culture is an insensitive detection method, probably because of intermittent shedding of few organisms per gram of feces,[19,20] as well as the heterogeneous distribution of organisms within fecal samples.[21] Because of the need to use 1 or more enrichment steps, it can take up to 3 days to realize test results for a single fecal sample and the limited test sensitivity means that typically 3 to 5 cultures are needed per animal, interpreting tests in parallel, to achieve reasonable sensitivity for the overall diagnostic process.

The reliability of bacterial culture for *S enterica* detection can be affected by the type of sample (feces, swab, or rectal biopsy), heterogeneity of target organism in the sample, sample weight, intermittent shedding, bacterial culture method, and laboratory proficiency. In general, a fecal culture is a more sensitive detection method than a rectal swab; this is likely due to the amount of fecal material that is cultured as larger sample mass generally provides higher test sensitivity.[22] Organisms such as *Salmonella* tend to cluster within a fecal sample rather than being homogeneously distributed, therefore testing of a small aliquot (eg, swabs or <1 g) may result in false-negative test results because there is a higher probability that a single sample does not contain any *Salmonella* organisms even though the animal is actively shedding at low levels.[21] As stated, the relative sensitivity of culture increases with increasing sample weight and this can be improved upon by thoroughly mixing the sample (eg, with a paddle blender).[21,22] As an alternative, culture of a rectal mucosal sample can be performed with a reported greater sensitivity than fecal culture, although given the invasive nature of the sample it may best be reserved for those difficult-to-sample cases that have scant or liquid feces with minimal solid material.[23]

There are many different methods that can be used for aerobic culture of *Salmonella*, which use a wide variety of broth and solid culture media, as well as incubation times and temperatures.[13–16,24,25] These different methodological choices lead to differences in test accuracy and time until results are reported, which should be carefully considered by laboratories, and practitioners should have a general understanding of how methodological choices affect the ability to detect *Salmonella*. A detailed review of culture techniques for *Salmonella* is beyond the scope of this article. In general, using an enrichment step with *Salmonella* selective media (eg, tetrathionate, Rappaport-Vassiliadis) improves overall test sensitivity, allowing *Salmonella* to grow while inhibiting the growth of competing bacteria, thus enabling detection on selective plating media (eg, xylose-lysine-Tergitol 4 or Hektoen enteric agar).[24] Preenrichment of samples with low bacterial burdens (eg, environmental samples) with nonselective media (eg, buffered peptone water [BPW]) can aid in recovery of bacteria that are

damaged or stressed because of environmental conditions, but use with samples containing high bacterial burdens (ie, fecal samples) may be counterproductive because this may allow overgrowth of competing bacteria, resulting in a falsely negative test result. In general, samples should be kept refrigerated and processed as soon after collection as possible. However, the proportion of test-positive samples has been shown to not differ significantly when processed the same day, after 6 days of refrigeration (4°C), or after 14 days of freezing (−15°C),[26] suggesting that recovery is not greatly impaired, if at all, when samples are kept cool before cultures are initiated.

We have found, as have others, that the proficiency of laboratories in their ability to detect *Salmonella* in enriched cultures can vary greatly (Paul Morley, personal communication, June, 2014).[14] Some of these differences are attributable to use of suboptimal culture methods (eg, very harsh enrichment media or lower culture temperatures).[13–16,24,25] In addition, some laboratories have substantially lower recovery rates than other laboratories when using the same culture methods, even among laboratories that routinely process fecal samples for *Salmonella* culture (Paul Morley, personal communication, June, 2014).[14,27] These differences highlight the importance of asking laboratories to provide documentation of training and proficiency testing (eg, check tests) when selecting a laboratory to perform *Salmonella* cultures.[14,27]

Culture of Environmental Samples

Salmonella is an organism that is hardy in damp environments that contain organic debris, having the ability to develop biofilms and environmental reservoirs that serve as potential sources for infection. When performing environmental surveillance, the type of sample collection device and testing method should be carefully considered, because the sensitivities of each are likely to differ. For example, electrostatic wipes have been found to be an effective and more sensitive collection method than sterile sponges for detection of *Salmonella* in the hospital environment.[4,28,29] These differences can be attributed to the collection method (the device used and the size of the surface area sampled) as well as the culture method. In general, sampling a larger surface area not only provides a more representative sample but is likely to be a more sensitive method for organism detection. Organisms in the environment may be injured by harsh environmental conditions (eg, drying and ultraviolet light) and by exposure to disinfectants. As such, general practice is to perform a preenrichment step in a nutrient-rich medium (eg, BPW) before performing an enriched culture. Although this extends the lag time required to obtain results, it allows for injured or damaged organisms to repair themselves before being exposed to the harsh enrichment medium, thereby improving overall testing results.

Polymerase Chain Reaction

PCR is generally considered to be a highly sensitive and specific method of *Salmonella* detection.[30] Most PCR assays use primers that target highly conserved bacterial genes, allowing detection of many different *Salmonella* serotypes without cross-reaction with other common bacteria (eg, Enterobacteriaceae).[31] PCR assays have been reported to have an analytical sensitivity of 100 cfu/g of equine feces in testing of overnight broth cultures or 1000 cfu/g of feces in testing of nonenriched samples. They are also generally more rapid than bacterial culture, providing results in 1 to 2 days compared with 2 to 5 days for many enriched culture methods.[18,31–33] As a result, PCR may be particularly useful with samples containing low numbers of organisms and for times when it is important to obtain results quickly (eg, during epidemics). Although PCR may be useful for earlier detection of shedding and environmental contamination compared with culture, it does not necessarily detect viable organisms.

Therefore, a positive PCR result does not necessarily indicate infection risk related to environmental contamination. In addition, PCR does not replace the need for culture because it is important to have more than dichotomous (positive/negative) results to facilitate epidemiologic investigations and ongoing surveillance. It is impossible to determine the likelihood of health care–associated transmission unless additional information is available for strain differentiation, such as antimicrobial susceptibility, serogroup, serotype, or optimally the pulse field gel electrophoresis profile (ie, DNA fingerprint). As such, PCR testing should always be paired with culture of PCR-positive samples. Some laboratories have proposed using PCR for broader initial screening of all samples, which can then be followed by culture-based investigations of the samples that were PCR positive.[30]

PCR is often considered a more sensitive detection method than enriched culture for fecal and environmental samples. However, there is much debate as to the reason for this apparent higher positive detection rate, or even whether this is consistently true. In theory, PCR can detect nonviable organisms as well as degraded DNA, which may account for some of this difference. It is also clear that suboptimal laboratory methods and laboratory proficiency can likewise affect this observation. In addition, low numbers of organisms contained within samples, or poor test specificity of PCR, can also affect apparent test accuracy.[33–35] A caveat to using PCR as a method of detection for environmental samples is that disinfectants target different parts of bacterial organisms. For example, quaternary ammonium and phenolic disinfectants target cytoplasmic membranes, leaving DNA intact, whereas bleach and formaldehyde degrade DNA, which could theoretically lead to differences in PCR detection rates.[36,37]

Lateral Flow Immunoassays

Commercially available LIAs have been developed for use in food safety microbiology, and have shown promise as practical alternatives to traditional culture and PCR methods for detection of *Salmonella* in animals and their environments (**Fig. 1**).[17,38] LFIs have been shown to have an analytical sensitivity of ~4 cfu/g from enriched cultures of experimentally inoculated equine fecal samples, and can reliably detect *S enterica* in 1-g samples after only 18 hours in selective broth culture.[17] The use of these tests does not require any specialized training or equipment; just the purchase of an incubator and premade media are all that are necessary. Although there may be some differences in the ability to detect different strains (serotypes) of *Salmonella*, their low cost, ease of use, and reliability make them an appealing option for point-of-care testing in equine practice.[39] Just as with PCR, it is important to pair LFI testing with follow-up culture of samples that are LFI positive. In addition to characterization of isolates, it also allows epidemiologic investigation and assessment of transmission risks in populations. It should be highlighted that all laboratories, including those that are set up for limited testing of samples in veterinary practices, should have adequate facilities and protocols in place to ensure appropriate and safe handling of infectious materials, especially those that could lead to zoonotic infections if not properly handled.

Testing Strategy and Test Interpretation

Detecting *Salmonella* in equine practice can be challenging because horses frequently shed low numbers of organisms and do so intermittently, except in extreme situations that may or may not be accompanied by clinical disease.[19,20] Regardless of the analytical sensitivity of test methods, this causes the overall detection system (ie, sample type combined with sample processing and detection method) to have poorer

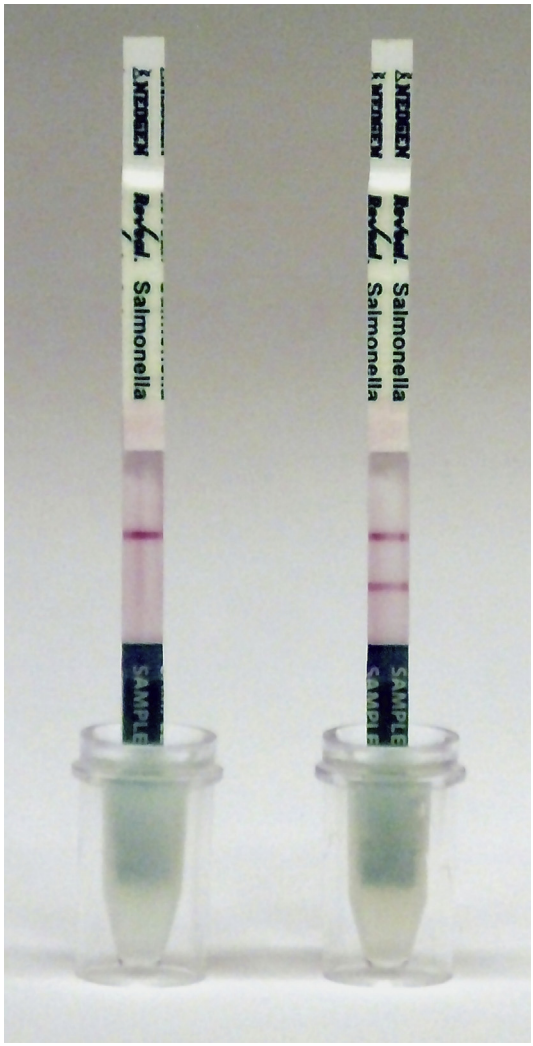

Fig. 1. Commercially available lateral flow immunoassay (Reveal 2.0, Neogen Corporation; see Ref.[38]) used for the detection of *S enterica* in equine fecal and environmental samples (see Refs.[17,39,57]). Test strip on the left indicates a negative test (only the control line is visible); the test strip on the right indicates a positive test (the test line [*lower line*] is as intense in color as the control line [the *top line*]).

epidemiologic sensitivity (ie, lower probability of detecting truly infected/shedding horses). Although testing larger sample volumes improves, up to a limit, test sensitivity,[22] it is also helpful to test multiple samples. Interpreting the results in parallel for multiple tests performed on the same patient has the benefit of greatly improving the overall sensitivity of the testing strategy.

Research suggests that a positive patient is more likely to culture positive with increased number of samples tested.[40] The generally accepted application of this idea is that a minimum of 3 to 5 negative cultures should be obtained in a short time frame (ie, sampling at intervals of 12–24 hours) to be reasonably sure that patients

have a low risk of *Salmonella* shedding.[19] Assuming independence of test results, it has been reported that the sensitivities for a series of fecal cultures using selective enrichment were 44% for a single culture, 66% for 2 cultures, 82% for 3 cultures, and 97% for 5 cultures.[41] Thus, by obtaining a series of 3 to 5 negative cultures, practitioners can be reasonably confident that a horse is truly negative.

Regardless of the detection strategy used, many different *Salmonella* serotypes can cause disease and the distribution of serotypes can change over time.[42] Thus it is important to ensure that the test being used can detect many different serotypes, especially those commonly detected in a given geographic or practice location.[16] In addition, when evaluating tests, the way they perform on the bench top may differ from samples relevant to veterinary medicine (ie, fecal and environmental samples), so methods should be appropriately validated and optimized for their intended use on relevant veterinary samples.

FUNDAMENTALS OF VETERINARY INFECTION CONTROL

Many of the practices used in veterinary infection control have not been scientifically evaluated to test their efficacy in applied circumstances. However, veterinarians can learn from infection control strategies applied in human health care. In the Study on the Efficacy of Nosocomial Infection Control (SENIC), conducted in US human health care facilities (1970–1976), the implementation of an infection control program reduced nosocomial infections by an estimated 32%. The minimum components needed for programs to achieve this impressive reduction in infection risk were simply to identify a person to oversee infection control activities, conduct some type of surveillance activity, and maintain a system for reporting.[43] Although similar data are lacking in veterinary medicine, it is realistic to presume that similar measures may be effective. In a recent epidemic of multidrug resistant *Salmonella* Newport, an ineffective infection control program was cited as an important factor in the outbreak, which resulted in patient fatalities, hospital closure, and an estimated financial cost of US$4.1 million.[4]

Infection control is achieved through all efforts used to prevent the introduction and limit the spread of contagious pathogens within a facility or population, with the goal of eliminating sources of potentially pathogenic microorganisms and of disrupting infectious disease transmission. In veterinary hospital settings, this is a challenge because clinicians are purposefully caring for patients with infectious diseases in the midst of animals whose resistance to disease may be compromised; and they are doing so in an environment in which animals from many different farms congregate.

There are several types of preventive measures that can be used to decrease infectious disease transmission risk, including optimizing environmental and personal hygiene and managing patient movement and contacts during hospitalization. Although every equine facility is distinctive with its own physical and operational features, necessitating the tailoring of infection control efforts to each facility's specific needs, all programs are based on these shared infection control principles. Detailed descriptions for program development have been provided elsewhere.[44–46] In addition, there are many available online resources that can facilitate program development.[47–49] Although the structure of specific policies enacted to promote prevention efforts are facility specific, it is important that they are designed with all patients in mind, not just those suspected of harboring an infectious disease. Consideration should be given to establishing distinct, segregated hospital areas to manage neonates, patients with severe disease (eg, colic or systemic illness vs elective surgery), inpatients versus outpatients, and different species (eg, horse vs cattle). Common

examples of this include segregating intensive care units, isolation facilities, and having separate equine and livestock hospitals.

MANAGING *SALMONELLA* RISK IN HOSPITAL POPULATIONS

Patient Management

Managing *Salmonella* in populations can be particularly challenging, in part because of the wide diversity in clinical consequences of infection, ranging from asymptomatic, intermittent shedding to acute diarrhea and fever with neutropenia to septicemia and death. In addition, horses recovering from naturally occurring acute salmonellosis can shed for extended periods of time; one-third shed for up to 30 days,[41] and can do so intermittently. Veterinarians have the challenge of caring for the patient standing before them but must consider the population of tomorrow in order to effectively control health care–associated infections (HCAIs).

Factors associated with epidemic disease

Nosocomial outbreaks of salmonellosis, representing a climactic meeting of patient and hospital factors, have repeatedly been shown to be a constant risk in all types of veterinary hospitals, resulting in significant morbidity and mortality among hospitalized patients and zoonotic infections in personnel.[4,7,8,37,50] Environmental hygiene is commonly identified as a contributing factor, including ineffective infection control policies; floor surfaces that allow contamination to accumulate; and use of porous, noncleanable surfaces in other materials such as unsealed concrete and wood.[4,7,37] In addition, contamination of common-use equipment (eg, buckets, nasogastric tubes, and rectal thermometers), and periods of high caseload with limited personnel have been found to affect the occurrence of HCAIs.[7,9,37] In the course of epidemics, horses with severe disease, such as those with colic or undergoing abdominal surgery, are frequently identified as shedding *Salmonella* and likely contribute to ongoing environmental contamination and transmission among hospitalized patients.[7,9,51,52]

Factors associated with endemic disease

During outbreaks, there is typically widespread environmental contamination and it is common for patient and environmental isolates to be phenotypically similar (ie, serotype and antimicrobial susceptibility); this phenomenon has also been identified during times of endemic disease, suggesting animals to be a likely source for this contamination.[4,8,29,53] From experience consulting with different veterinary facilities, disseminated environmental contamination is a ubiquitous feature associated with nosocomial *Salmonella* transmission,[4,7,8] although use of insensitive sampling and culture methods can impair the ability to detect this important feature (Paul Morley, personal communication, June, 2014).[4]

In the past, *Salmonella* shedding among horses was associated with a triad of clinical signs (diarrhea, fever, and leukopenia) based on early studies and observation.[54,55] A recent case-control study supports this observation, finding that horses with acute colic with clinical signs of fever (rectal temperature >39.4°C [103°F]), diarrhea, and abnormal leukocyte count ($\leq$4500 cells/μL or leukocytosis $\geq$12,500 cells/μL) were more likely to shed *Salmonella* in feces and reflux in the first 5 days of hospitalization.[56] This triad of clinical signs can occur infrequently, accounting for only 2.7% of shedding among a hospital population (the population attributable fraction).[57]

A meta-analysis of studies experimentally inoculating healthy animals (horses, cattle, sheep), found that, on average, pyrexia occurred within 1.5 days of infection (95% confidence interval [CI], 1.47, 1.55) and diarrhea occurred within 1.7 days of infection (95% CI, 1.62, 1.83).[58] The 43 studies included in this meta-analysis used inoculating

doses from 10^4 to 10^{13}; in natural infection the infective dose is expected to be at least at the low end of this range and is likely to be lower. Thus, the average times to onset reported here are probably more rapid than would be expected in natural infections. This study also reported an average time to shedding of 1.3 days (95% CI, 1.22, 1.39) after inoculation, suggesting that, by the time fever and/or diarrhea are apparent, animals are frequently shedding *Salmonella* in their feces. This finding emphasizes the utility of identifying specific factors or groups of factors associated with shedding that are easily recognizable, thus allowing prevention strategies to be implemented more rapidly, before the situation becomes an epidemic.

The duration from exposure to fecal shedding can be affected by serotype, inoculating dose, as well as the health status of the horse. Time to shedding varies by infecting serotype, ranging from approximately 3 to 5 days among naturally infected horses.[51] This is in contrast with experimental inoculation, which results in shedding within 1.3 days (95% CI, 1.22, 1.39),[58] suggesting that infecting dose plays a role because experimental inoculations are general at much higher doses than would be expected to occur naturally. Not only can time to shedding vary by serotype, it may also be affected by health status. Days from admission to shedding among horses presenting for gastrointestinal disease ranged from 1 day to 3.5 days for serotypes Saint Paul and Java, respectively,[40] likely representing an increased susceptibility in this compromised subgroup of horses, but this may have been attributable to variation in virulence among serotypes and strains.

It has been suggested that horses with severe disease are more likely to shed detectable quantities of *Salmonella* in their feces. In a recent study, horses admitted for acute colic (excluding those presenting with diarrhea) were more likely to shed *Salmonella* with surgical management versus medical management, as were those with more severe disease (eg, inflammatory and vascular compromising conditions) versus those with simple colic (eg, simple obstruction and nonstrangulating lesions) that resolved with minimal medical management.[56] Although the suggestion that horses with more severe disease are more likely to shed makes biological sense, over the years this has not been a consistent finding. Many studies have evaluated shedding risk among horses with gastrointestinal disease, and some studies have reported an increased risk associated with abdominal surgery.[40,56] However, others have not found an association between *Salmonella* shedding and abdominal surgery.[9,59,60] In addition, although it is commonly thought that antimicrobial therapy affects the probability of *Salmonella* shedding, this too has been inconsistently identified as a significant risk factor.[9,40,59–61]

Although horses with severe disease are probably more susceptible to infection than healthy horses, they are also more likely to have fecal samples tested. For this reason, extrapolating findings from studies observing limited patient populations should be done with caution. Studies to determine factors associated with endemic shedding among the general patient population have found that both patient and hospital factors may be important.[57,61–64] Patients with systemic illness, regardless of the body system affected, and those having any of the classic triad of clinical signs (fever, diarrhea, or leukopenia) have a higher likelihood of shedding, but these are not the only animals that shed *Salmonella*.[57] These indicators are specific for identifying shedding, but they are not perfectly sensitive. Patient management factors may also play a role, with transportation distance (patients within 32 km [20 miles] having a greater risk), antimicrobial therapy (specifically being treated with aminoglycosides), and duration of hospitalization affecting the probability of shedding.[57,64] Species or rearing circumstances may be important hospital factors as well, with intensively managed cattle being much more likely to shed than horses; an element that should not be overlooked

when managing horses in a multispecies hospital.[57,64] Further, reports consistently show a seasonal occurrence to shedding, generally highest in late summer and early fall, and lowest in the spring.[3,19,40,42,57] Thus, studies evaluating a limited time frame may underestimate risk factor contributions to overall patient shedding. There are also large regional differences in the shedding prevalence that have been found in similar species and rearing conditions; shedding seems to be much more likely to occur in the warmer and wetter regions of North America compared with cooler and dryer regions.[3]

Subpopulations and Salmonella Risk

Managing *Salmonella* in horse populations is challenging because horses can shed intermittently and often in the absence of clinical signs.[10] Patient shedding prevalence can vary markedly from as few as 0.5% up to 7%, with horses tested on admission typically having a lower prevalence than horses tested throughout hospitalization.[3,19,62,63] In contrast, horses admitted for elective procedures (ie, musculoskeletal disease, cryptorchidism) and as hospital companions are less likely to shed[63] and healthy horses in the general equine population have an estimated shedding prevalence of 0.8% (standard error [SE], 0.5).[3] At the Colorado State University Veterinary Teaching Hospital, from 2002 to 2010, culture-positive horses were most commonly admitted with gastrointestinal disease (60.8% [87 of 143]), followed by musculoskeletal disease (34.2% [49 of 143]), and approximately 10% were considered clinically normal by attending clinicians (10.5% [15 of 143]). Thus, differential patient management may be warranted for patient population subgroups identified as being at a high risk for *Salmonella* shedding on admission or throughout hospitalization.

Horses with gastrointestinal disease

Many facilities manage horses with gastrointestinal disease or colic separately from the general patient population because this subgroup has an increased likelihood of shedding, with prevalence ranging from 4.3% up to 13%.[40,60,63] Factors that can be associated with fecal shedding among these patients include transportation distance (travel time >1 hour), abnormal findings on nasogastric intubation, diarrhea, leukopenia ($\leq$5000 white blood cells/μL), previous antimicrobial therapy, abdominal surgery, and duration of hospitalization.[19,40,56,60]

Horses with severe disease

Horses admitted to the critical care unit are also more likely to shed *Salmonella* during hospitalization.[65] This subgroup of horses, along with critical neonates, likely represents patients with greater disease severity (as well as susceptibility to infection) compared with the general hospital population. Equine neonates present a particular challenge. Critically ill foals are typically unable to stand, require intensive management, and those with gastrointestinal disease are at higher risk for shedding *Salmonella* compared with adults.[40] For patients comprising the general inpatient population, approximately 70% of shedding risk can be attributed to systemic illness (ie, the population attributable fraction) regardless of body system affected, with more severe disease having a higher probability of shedding.[57,64]

Salmonella Surveillance Among Patients in Clinical Practice

Routine surveillance by testing fecal samples may be an effective means to identify *Salmonella* shedding among the general inpatient population but careful consideration should be given to how this might be incorporated into an infection control program (including cost and ability to manage positive patients). Targeted surveillance of

horses presenting for acute colic or diarrhea or developing diarrhea during hospitalization has been shown to be an effective method for identifying fecal shedding. However, research suggests that many horses can be shedding *Salmonella* in the absence of clinical signs.[8,36,56] A recent case-control study found that most horses presenting for acute colic were identified as shedding *Salmonella* through routine untargeted surveillance of all inpatients (64.4%, excluding isolation patients) rather than on admission (6.8%) or targeted surveillance triggered by the infection control program (28.8%).[56] Therefore, depending on the types of patients seen at a practice, routine patient surveillance may be warranted. **Box 1** shows specific components of the *Salmonella* surveillance program conducted at the James L. Voss Veterinary Teaching Hospital, Colorado State University, as part of long-term infection control efforts. Note that this is but 1 example; infection control programs must be tailored for a particular facility to ensure that they meet those facilities' specific needs and limitations.

A facility may alternatively find it more cost-effective to focus on syndromic surveillance; a method that is effective at detecting adverse events in hospitalized horses.[66] However, applying this technique to horses with *Salmonella* may be a challenge because not all patients shedding *Salmonella* develop clinical signs. As stated earlier, only 2.7% of shedding has been associated with diarrhea, fever, and leukopenia, whereas 70% of shedding has been attributed to either systemic illness or gastrointestinal disease.[64] Given this, practitioners may elect to differentially manage and conduct targeted surveillance of those patients with more severe disease or gastrointestinal disease.

In addition, environmental surveillance for *Salmonella* may also be a cost-effective means for detecting patient shedding because environmental contamination is commonly detected near where positive patients are managed.[29,61] This surveillance could be conducted as routine or periodic surveillance of high-traffic areas such as examination areas or alleyways. If contamination is detected, not only can infection control measures be heightened but more extensive patient testing could be undertaken to facilitate mitigation efforts.

Box 1
Example[a] surveillance program for *S enterica* conducted at the James L. Voss Veterinary Teaching Hospital, Colorado State University

Components	Sample for Culture	Description
Routine surveillance	Patient fecal sample[b]	All large-animal inpatients on admission and twice weekly for the duration of hospitalization
	Environmental swab[c]	Approximately 60 sites throughout the small-animal, equine, and livestock hospitals
Targeted surveillance	Patient fecal sample[b]	All patients with 2 of 3 signs (fever, leukopenia, diarrhea) or developing diarrhea during hospitalization
	Environmental swab[c]	Select locations when an increase (above baseline) in positive patients or positive environmental samples is detected
Passive surveillance	Patient fecal sample[b]	All diagnostic samples culture positive for *Salmonella* from inpatients are reported to biosecurity personnel

[a] Surveillance programs must be tailored to each facility's needs and limitations.
[b] One-gram fecal sample for enriched culture.
[c] Collected using a Swiffer (Proctor & Gamble; see Ref.[29]).

Management of the Hospital Environment

Incorporating environmental surveillance into clinical practice is a common method for managing risks associated with *Salmonella* in populations. Active surveillance of patients and the environment can complement each other to detect endemic shedding among patients and to identify outbreaks early in their course, thereby limiting the overall consequences.[8] Research shows that isolates recovered at times of endemic and epidemic disease can be phenotypically linked (serotype and antimicrobial susceptibility) to animal isolates, suggesting animals as a likely source for environmental contamination and ongoing transmission.[29,53] In addition, recovery of genetically related *Salmonella* isolates during routine patient and environmental surveillance over an extended period of time suggests environmental persistence and nosocomial transmission, despite the implementation of a rigorous infection control program.[67] As such, environmental hygiene and surveillance are critical to eliminating reservoirs for infection within the hospital environment. Again, based on experiences from consulting with a variety of veterinary practices, we have not experienced substantial nosocomial transmission of *Salmonella* without also identifying environmental contamination. Thus, sampling the environment at times of concern can be an important investigative tool for detection of HCAIs related to *Salmonella*. See **Box 1** for specific components of the *Salmonella* surveillance program conducted at the James L. Voss Veterinary Teaching Hospital, Colorado State University, as part of long-term infection control efforts. Note that this is but 1 example; infection control programs must be tailored for a particular facility to ensure that they meet those facilities' specific needs and limitations.

When incorporating environmental surveillance into practice, careful consideration should be given to locations being sampled and type of samples being collected (eg, floor-contact surface, hand-contact surface, or a composite sample of both those surfaces). For example, for mixed-species practices, samples collected in areas used to manage livestock are more likely to be culture positive, as are samples collected from floor-contact surfaces or composite samples (but hand-contact samples may be more important with respect to transmission risk).[68] In addition, sample collection and detection methods, laboratory selection, and available resources (both financial and personnel) should be taken into consideration. Methods should be appropriately validated and optimized for their intended use and practitioners should understand that different collection and testing methodologies can result in different test sensitivities.[4,14,28] In general, sampling a larger surface area provides a more representative sample and is likely to be a more sensitive method for detecting *Salmonella* in the environment.

Environments in veterinary hospitals are frequently contaminated near where positive patients are managed (eg, equine isolation, livestock hospital, calf isolation), with floor samples, floor drains, cracks, and crevices being common sites for contamination.[29,36,69,70] It is imperative to maintain nonporous, cleanable surfaces throughout the hospital environment because epidemics are commonly associated with in-stall matting and surfaces such as unsealed concrete and wood.[4,7,37] Although environmental contamination cannot be completely eliminated, the goal is to reduce contamination of the environment with potential pathogens to a level that becomes biologically irrelevant. To gain meaningful information, environmental testing should be performed regularly to establish a baseline level of environmental contamination with which future findings can be compared. In this way potential environmental reservoirs of *Salmonella* can be detected and cleaning effectiveness can be continually monitored.

MANAGING *SALMONELLA* RISK IN THE FIELD SETTING

In addition to being one of the most common causes of outbreaks of HCAI in equine hospitals,[2] *Salmonella* is also frequently detected on equine operations and farms[3] and is a recognized cause of farm outbreaks and disease in personnel.[71–73] In general, of nonhospitalized horses in the general equine population that are considered healthy, an estimated 0.8% (SE, 0.5) shed *Salmonella* in their feces.[3]

Although much of this article focuses on managing salmonella risk in the hospital setting, the same principles regarding infection control apply to other settings; namely, preventing disease introduction and transmission between facilities and among animals by breaking the cycle of transmission and practicing rigorous hygiene. On-farm infection control practices are largely owner dependent. In a survey of Colorado boarding facilities, only 50% of facility managers reported isolating new horses from resident horses and only 6.6% isolated resident horses returning to the farm after travel.[74] Among US equine operations with at least 5 resident horses, approximately 78% had nonresident horses arriving on farm.[75] Although the risk of exposure to nonresident horses increased with operation size, so too did the likelihood of implementing some biosecurity measures such as entry requirements for personnel. Advising owners and managers regarding implementation of best practices for infection control remains an excellent opportunity for veterinarians to provide service and strengthen productive veterinarian-client relationships.

It is clearly important for practitioners to maintain a minimum standard of infection control, whether in the clinic or in the field. As ambulatory practitioners move from farm to farm, it is critically important to maintain a high level of hygiene within practice vehicles, with respect to multiuse equipment, and with outer attire worn on an individual farm. There are many online resources available to help facilitate on-farm infection control program development[47–49] as well as published resources on program development[44,45] and outbreak investigation and control.[76]

SUMMARY

An international panel of infection control experts recently identified critical needs for infection control in equine populations. One of the issues specifically identified was the need to expand knowledge about the epidemiology and control of *Salmonella* infections in equine populations, along with improving methods for detection.[6] Recognizing the challenges faced by practitioners in managing this agent is the first step to improving its control. Despite the way this organism is shed, intermittently, at low levels, and often subclinically, due effort must be made to mitigate associated risks, whether in the hospital or field setting. Veterinary practitioners have an ethical responsibility to appropriately manage risks related to *S enterica* in animal populations and their environments.

REFERENCES

1. Christley RM, French NP. Small-world topology of UK racing: the potential for rapid spread of infectious agents. Equine Vet J 2003;35(6):586–9.
2. Benedict KM, Morley PS, Van Metre DC. Characteristics of biosecurity and infection control programs at veterinary teaching hospitals. J Am Vet Med Assoc 2008;233(5):767–73.
3. Traub-Dargatz JL, Barber LP, Fedorka-Cray PJ, et al. Fecal shedding of *Salmonella spp* by horses in the United States during 1998 and 1999 and detection of

Salmonella spp in grain and concentrate sources on equine operations. J Am Vet Med Assoc 2000;217(2):226–30.
4. Dallap Schaer BL, Aceto H, Rankin SC. Outbreak of salmonellosis caused by *Salmonella enterica* serovar Newport MDR-AmpC in a large animal veterinary teaching hospital. J Vet Intern Med 2010;24(5):1138–46.
5. Weaver DR, Newman LS, Lezotte DC, et al. Perceptions regarding workplace hazards at a veterinary teaching hospital. J Am Vet Med Assoc 2010;237(1):93–100.
6. Morley P, Anderson ME, Burgess BA, et al. Report of the third Havemeyer workshop on infection control in equine populations. Equine Vet J 2012;45(2):131–6.
7. Tillotson K, Savage CJ, Salman MD, et al. Outbreak of *Salmonella* Infantis infection in a large animal veterinary teaching hospital. J Am Vet Med Assoc 1997; 211(12):1554–7.
8. Steneroden KK, Van Metre DC, Jackson C, et al. Detection and control of a nosocomial outbreak caused by *Salmonella* Newport at a large animal hospital. J Vet Intern Med 2010;24(3):606–16.
9. Hird DW, Pappaioanou M, Smith BP. Case-control study of risk factors associated with isolation of *Salmonella* Saintpaul in hospitalized horses. Am J Epidemiol 1984;120(6):852–64.
10. Palmer JP, Benson CE, Whitlock RH. Subclinical salmonellosis in horses with colic. In Equine Colic Research Symposium. University of Georgia, Athens, 1982; 180–6.
11. Lan R, Reeves PR, Octavia S. Population structure, origins and evolution of major *Salmonella enterica* clones. Infect Genet Evol 2009;9(5):996–1005.
12. Brenner FW, Villar RG, Angulo FJ, et al. *Salmonella* nomenclature. J Clin Microbiol 2000;38(7):2465–7.
13. Rostagno MH, Gailey JK, Hurd HS, et al. Culture methods differ on the isolation of *Salmonella enterica* serotypes from naturally contaminated swine fecal samples. J Vet Diagn Invest 2005;17(1):80–3.
14. Voogt N, Nagelkerke NJD, van de Giessen AW. Differences between reference laboratories of the European community in their ability to detect *Salmonella* species. Eur J Clin Microbiol Infect Dis 2002;21(6):449–54.
15. Love BC, Rostagno MH. Comparison of five culture methods for *Salmonella* isolation from swine fecal samples of known infection status. J Vet Diagn Invest 2008;20(5):620–4.
16. Singer RS, Mayer AC, Hanson TE. Do microbial interactions and cultivation media decrease the accuracy of *Salmonella* surveillance systems and outbreak investigations? J Food Prot 2009;72(4):707–13.
17. Burgess BA, Noyes NR, Bolte DS, et al. Rapid *Salmonella* detection in experimentally-inoculated equine feces and veterinary hospital environmental samples using commercially available lateral flow antigen detection systems. Equine Vet J 2014.
18. Cohen ND, Neibergs HL, Wallis DE, et al. Genus-specific detection of salmonellae in equine feces by use of the polymerase chain reaction. Am J Vet Res 1994;55(8):1049–54.
19. Smith BP, Reina-Guerra M, Hardy AJ. Prevalence and epizootiology of equine salmonellosis. J Am Vet Med Assoc 1978;172(3):353–6.
20. Smith BP, Reina-Guerra M, Hardy AJ, et al. Equine salmonellosis: experimental production of four syndromes. Am J Vet Res 1979;40(8):1072–7.
21. Cannon RM, Nicholls TJ. Relationship between sample weight, homogeneity, and sensitivity of fecal culture for *Salmonella enterica*. J Vet Diagn Invest 2002;14(1):60–2.

22. Funk JA, Davies PR, Nichols MA. The effect of fecal sample weight on detection of *Salmonella enterica* in swine feces. J Vet Diagn Invest 2000;12(5):412–8.
23. Palmer JE, Witlock RH, Benson CE, et al. Comparison of rectal mucosal cultures and fecal cultures in detecting *Salmonella* infection in horses and cattle. Am J Vet Res 1985;46(3):697–8.
24. Davies PR, Turkson PK, Funk JA, et al. Comparison of methods for isolating *Salmonella* bacteria from faeces of naturally infected pigs. J Appl Microbiol 2000;89(1):169–77.
25. Waltman WD, Mallinson ET. Isolation of *Salmonella* from poultry tissue and environmental samples: a nationwide survey. Avian Dis 1995;39:45–54.
26. O'Carroll JM, Davies PR, Correa MT, et al. Effects of sample storage and delayed secondary enrichment on detection of *Salmonella spp* in swine feces. Am J Vet Res 1999;60(3):359–62.
27. Kuijpers A, Mooijman KA. EU Interlaboratory comparison study veterinary XV (2012): detection of *Salmonella* in pig faeces. RIVM Report 330604028. 2013. Available at: http://hdl.handle.net/10029/313961. Accessed September 18, 2014.
28. Ruple-Czerniak A, Bolte DS, Burgess BA, et al. Comparison of two sampling and culture systems for detection of *Salmonella enterica* in the environment of a large animal hospital. Equine Vet J 2014;46(4):499–502.
29. Burgess BA, Morley PS, Hyatt DR. Environmental surveillance for *Salmonella enterica* in a veterinary teaching hospital. J Am Vet Med Assoc 2004;225(9):1344–8.
30. Malorny B, Hoorfar J. Toward standardization of diagnostic PCR testing of fecal samples: lessons from the detection of salmonellae in pigs. J Clin Microbiol 2005;43(7):3033–7.
31. Cohen ND, Neibergs HL, McGruder ED, et al. Genus-specific detection of salmonellae using the polymerase chain reaction (PCR). J Vet Diagn Invest 1993;5(3):368–71.
32. Kurowski PB, Traub-Dargatz JL, Morley PS, et al. Detection of *Salmonella spp* in fecal specimens by use of real-time polymerase chain reaction assay. Am J Vet Res 2002;63(9):1265–8.
33. Ward MP, Alinovi CA, Couetil LL, et al. Evaluation of a PCR to detect *Salmonella* in fecal samples of horses admitted to a veterinary teaching hospital. J Vet Diagn Invest 2005;17(2):118–23.
34. Cohen ND, Martin LJ, Simpson RB, et al. Comparison of polymerase chain reaction and microbiological culture for detection of salmonellae in equine feces and environmental samples. Am J Vet Res 1996;57(6):780–6.
35. Pusterla N, Byrne BA, Hodzic E, et al. Use of quantitative real-time PCR for the detection of *Salmonella spp*. in fecal samples from horses at a veterinary teaching hospital. Vet J 2010;186(2):252–5.
36. Ewart SL, Scott HC 2nd, Robinson RL, et al. Identification of sources of *Salmonella* organisms in a veterinary teaching hospital and evaluation of the effects of disinfectants on detection of *Salmonella* organisms on surface materials. J Am Vet Med Assoc 2001;218(7):1145–51.
37. Schott HC 2nd, Ewart SL, Walker RD, et al. An outbreak of salmonellosis among horses at a veterinary teaching hospital. J Am Vet Med Assoc 2001;218(7):1152–9, 1100.
38. Bird CB, Miller RL, Miller BM. Reveal for *Salmonella* test system. J AOAC Int 1999;82(3):625–33.

39. Burgess BA, Bolte DS, Hyatt DR, et al. Rapid *Salmonella* detection in fecal and veterinary hospital environmental samples using commercially available lateral flow antigen detection systems. In American College of Veterinary Internal Medicine Forum. Seattle, June 12–15, 2013.
40. Ernst NS, Hernandez JA, MacKay RJ, et al. Risk factors associated with fecal *Salmonella* shedding among hospitalized horses with signs of gastrointestinal tract disease. J Am Vet Med Assoc 2004;225(2):275–81.
41. Palmer JE, Benson CE. *Salmonella* shedding in the equine. In International Symposium on *Salmonella*. New Orleans, July 19–20, 1984.
42. Carter JD, Hird DW, Farver TB, et al. Salmonellosis in hospitalized horses: seasonality and case fatality rates. J Am Vet Med Assoc 1986;188(2):163–7.
43. Haley RW, Culver DH, White JW, et al. The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals. Am J Epidemiol 1985;121(2):182–205.
44. Morley PS. Biosecurity of veterinary practices. Vet Clin North Am Food Anim Pract 2002;18(1):133–55, vii.
45. Traub-Dargatz JL, Dargatz DA, Morley PS, et al. An overview of infection control strategies for equine facilities, with an emphasis on veterinary hospitals. Vet Clin North Am Equine Pract 2004;20(3):507–20, v.
46. Morley PS, Weese JS. Biosecurity and infection control for large animal practices. In: Smith BP, editor. Large animal internal medicine. 5th edition. New York: Elsevier; 2015. p. 1407–31.
47. Anonymous. Equine biosecurity policies and best practices guide. 2011. Available at: http://www.albertaequestrian.com/Biosecurity. Accessed September 18, 2014.
48. Flynn K, Wilson EM, Traub-Dargatz J, et al. Biosecurity toolkit for equine events. 2012. Available at: http://www.cdfa.ca.gov/ahfss/animal_health/pdfs/Biosecurity_Toolkit_Full_Version.pdf. Accessed September 18, 2014.
49. Guelph E. Equine biosecurity risk calculator. 2011. Available at: http://www.equineguelph.ca/Tools/biosecurity_2011.php. Accessed September 18, 2014.
50. Wright J, Tengelsen LA, Smith KE, et al. Multidrug-resistant *Salmonella* Typhimurium in four animal facilities. Emerg Infect Dis 2005;11(8):1235–41.
51. House JK, Mainer-Jaime RC, Smith BP, et al. Risk factors for nosocomial *Salmonella* infection among hospitalized horses. J Am Vet Med Assoc 1999;214(10):1511–6.
52. Ekiri AB, MacKay RJ, Gaskin JM, et al. Epidemiologic analysis of nosocomial *Salmonella* infections in hospitalized horses. J Am Vet Med Assoc 2009;234(1):108–19.
53. Castor ML, Wooley RE, Shotts EB, et al. Characteristics of *Salmonella* isolated from an outbreak of equine salmonellosis in a veterinary teaching hospital. J Equine Vet Sci 1989;9(5):236–41.
54. Dorn CR, Coffman JR, Schmidt DA, et al. Neutropenia and salmonellosis in hospitalized horses. J Am Vet Med Assoc 1975;166(1):65–7.
55. Owen R, Fullerton JN, Tizard IR, et al. Studies on experimental enteric salmonellosis in ponies. Can J Comp Med 1979;43(3):247–54.
56. Dallap Schaer BL, Aceto H, Caruso MA, et al. Identification of predictors of *Salmonella* shedding in adult horses presented for acute colic. J Vet Intern Med 2012;26(5):1177–85.
57. Burgess BA. Epidemiology and prevention of *Salmonella enterica* in veterinary hospitals. Colorado State University, Libraries Fort Collins, CO, PhD Dissertation; 2014.
58. Aceto H, Miller SA, Smith G. Onset of diarrhea and pyrexia and time to detection of *Salmonella enterica* subsp *enterica* in feces in experimental studies of cattle,

horses, goats, and sheep after infection per os. J Am Vet Med Assoc 2011; 238(10):1333–9.
59. Hird DW, Casebolt DB, Carter JD, et al. Risk factors for salmonellosis in hospitalized horses. J Am Vet Med Assoc 1986;188(2):173–7.
60. Kim LM, Morley PS, Traub-Dargatz JL, et al. Factors associated with *Salmonella* shedding among equine colic patients at a veterinary teaching hospital. J Am Vet Med Assoc 2001;218(5):740–8.
61. Dunowska M, Patterson G, Traub-Dargatz JL, et al. Recent progress in controlling *Salmonella* in veterinary hospitals. In 50th Annual Convention of the American Association of Equine Practitioners. Lexington, December 4-8, 2004:350–3.
62. Traub-Dargatz JL, Salman MD, Jones RL. Epidemiologic study of salmonellae shedding in the feces of horses and potential risk factors for development of the infection in hospitalized horses. J Am Vet Med Assoc 1990;196(10):1617–22.
63. Alinovi CA, Ward MP, Couetil LL, et al. Risk factors for fecal shedding of *Salmonella* from horses in a veterinary teaching hospital. Prev Vet Med 2003;60(4): 307–17.
64. Burgess, BA, Morley PS. Factors associated with large animal inpatient shedding of *Salmonella enterica* in a veterinary teaching hospital. In 94th Annual Conference of Research Workers in Animal Diseases. Chicago, December 9-10, 2013.
65. Mainar-Jaime RC, Atashparvar N, Chirino-Trejo M. Influence of fecal shedding of *Salmonella* organisms on mortality in hospitalized horses. J Am Vet Med Assoc 1998;213(8):1162–6.
66. Ruple-Czerniak AA, Aceto HW, Bender JB, et al. Syndromic surveillance for evaluating the occurrence of healthcare-associated infections in equine hospitals. Equine Vet J 2014;46(4):435–40.
67. Dunowska M, Morley PS, Traub-Dargatz JL, et al. Comparison of *Salmonella enterica* serotype Infantis isolates from a veterinary teaching hospital. J Appl Microbiol 2007;102(6):1527–36.
68. Burgess BA, Morley PS. Hospital risk factors for environmental contamination with *Salmonella enterica*. In 93rd Annual Conference of Research Workers in Animal Diseases. Chicago, December 3-4, 2012.
69. Pandya M, Wittum T, Tadesse DA, et al. Environmental *Salmonella* surveillance in the Ohio State University Veterinary Teaching Hospital. Vector Borne Zoonotic Dis 2009;9(6):649–54.
70. Alinovi CA, Ward MP, Couetil LL, et al. Detection of *Salmonella* organisms and assessment of a protocol for removal of contamination in horse stalls at a veterinary teaching hospital. J Am Vet Med Assoc 2003;223(11):1640–4.
71. Hartmann FA, Callan RJ, McGuirk SM, et al. Control of an outbreak of salmonellosis caused by drug-resistant *Salmonella* Anatum in horses at a veterinary hospital and measures to prevent future infections. J Am Vet Med Assoc 1996; 209(3):629–31.
72. Jay-Russell MT, Madigan JE, Bengson Y, et al. *Salmonella* Oranienburg isolated from horses, wild turkeys and an edible home garden fertilized with raw horse manure. Zoonoses Public Health 2014;61(1):64–71.
73. Walker RL, Madigan JE, Hird DW, et al. An outbreak of equine neonatal salmonellosis. J Vet Diagn Invest 1991;3(3):223–7.
74. Kirby AT, Traub-Dargatz JL, Hill AE, et al. Development, application, and validation of a survey for infectious disease control practices at equine boarding facilities. J Am Vet Med Assoc 2010;237(10):1166–72.

75. Traub-Dargatz J, Kopral C, Wagner B. Relationship of biosecurity practices with the use of antibiotics for the treatment of infectious disease on U.S. equine operations. Prev Vet Med 2012;104(1–2):107–13.
76. Kane AJ, Morley PS. How to investigate a disease outbreak. In Annual Convention of the American Association of Equine Practitioners. Albuquerque, December 5-8, 1999.