Review Article

Review of the epidemiology and infection control aspects of nosocomial Salmonella infections in hospitalised horses

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Summary

Outbreaks of nosocomial Salmonella infections in hospitalised horses can occur when surveillance and infection control protocols are not in place, or not well structured and enforced. The aim of this article is to present a review of published studies that have contributed to the literature of the epidemiology and infection control aspects of nosocomial Salmonella infections in hospitalised horses. The review highlights important elements that must be taken into consideration during the formulation, implementation and evaluation of a hospital surveillance and infection control programme designed to reduce the risk of an outbreak of nosocomial Salmonella infection in hospitalised horses.

Introduction

Over the past 30 years, outbreaks of nosocomial Salmonella infections in hospitalised horses have been reported, with varying consequences. In 1981–82, the University of California-Davis, Veterinary Medicine Teaching Hospital experienced an outbreak of nosocomial salmonellosis due to Salmonella enterica serotype Saint-paul (S. Saint-paul) (Hird et al. 1984). The outbreak extended for 10 months, resulting in severe disruption of hospital routine and temporary closure of the hospital for 3 months to facilitate remodelling and disinfection. In 1995, an outbreak of nosocomial salmonellosis due to S. Infantis at Colorado State University resulted in temporary closure of the hospital for 3 months and an estimated $500,000 in lost revenues and facility renovation (Tillotson et al. 1997; Dunowska et al. 2007). In 1996, an outbreak of equine salmonellosis due to S. Typhimurium occurred at the Michigan State University (Schott et al. 2001). Unique features of this outbreak included a high case fatality, zoonotic infection and closure of the hospital for one month for complete disinfection. In 2000, an outbreak due to a multi-drug resistant (MDR) strain of S. Typhimurium occurred at Purdue University (Ward et al. 2005a), and the hospital was closed for 3 months. In this outbreak, estimated losses included equine mortality, $250,000–300,000 for cleaning and disinfection, reduced caseload and impaired student education. In 2004, an outbreak of equine salmonellosis due to S. Newport occurred at the University of Pennsylvania veterinary teaching hospital (Jennifer 2004; Rankin et al. 2005). The hospital was closed for 3 months and students had to go out-of-state for large animal clinical training. Estimated losses during this outbreak included equine mortality and over $4 million for hospital renovations, cleaning and disinfection procedures, as well as a reduced caseload for 10 months following re-opening. The most recent reported outbreak of salmonellosis was associated with S. Newport at Colorado State University veterinary teaching hospital in 2006 (Steneroden et al. 2010). In this outbreak, a total of 8 animals (4 alpacas, 2 horses, one goat, one cow) were found to be shedding the outbreak strain; all but one shed Salmonella in the absence of clinical signs or before the onset of disease. Unlike previous outbreaks reported above, in this case the hospital was not closed. The threat of closure was avoided primarily because of aggressive surveillance and mitigation strategies (Steneroden et al. 2010).

The purpose of this article is to review published studies that have contributed to the literature of the epidemiology and infection control aspects of nosocomial Salmonella infections in hospitalised horses. A search for published articles on nosocomial Salmonella infections in hospitalised horses was performed using the databases PubMed and CAB. In general, most of the published articles on nosocomial outbreaks of Salmonella infections in hospitalised horses originated from veterinary hospitals located in North America (e.g. Hird et al. 1984, 1986; Castor et al. 1989; Hartmann et al. 1996; Tillotson et al. 1997; Schott et al. 2001; Rankin et al. 2005; Ward et al. 2005a; Ekiri et al. 2010).

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This review article is structured in 3 parts: 1) surveillance and infection control; 2) epidemiological research; and 3) discussion (Fig 1). The first part includes 6 elements: case definition, environmental contamination, sampling, diagnostics, risk management, and guidelines for surveillance and infection control. The second part is limited to a review of published studies that have examined exposure factors associated with nosocomial Salmonella infections in hospitalised horses. Finally, the third part is a discussion section relevant to infection control issues and areas of research that can help improve existing hospital surveillance and infection control programmes.

**Surveillance and infection control**

Control and prevention of outbreaks of nosocomial Salmonella infections in hospitalised horses require careful formulation, implementation and evaluation of a hospital surveillance and infection control programme. For an early detection system, the monitoring of nosocomial Salmonella infections in hospitalised horses is fundamental. From a hospital risk management point of view, a working case definition of nosocomial infection must be formulated, even when Salmonella serotyping data are not readily available.

**Case definition**

In general, salmonellosis is suspected to be of nosocomial origin when an infection is identified after animals have been hospitalised for 72 h or longer and when the serotype and antimicrobial susceptibility pattern of Salmonella isolates from the primary and nosocomial cases are the same. However, in published epidemiological studies, the criteria used to define nosocomial Salmonella infections in horses have varied. For example, in one study, an outbreak was considered nosocomial in origin because a relatively large number of cases shedding the same serotype were detected within a short period of time and the serotype involved had seldom previously been isolated from hospitalised animals at that hospital (Hird et al. 1984). In another study, infection with S. Krefeld or S. Typhimurium was considered nosocomial when the mean time from admission to shedding was ≥4 days (House et al. 1999).

In a recent study (Ekiri et al. 2009), horses were classified as nosocomial Salmonella cases using the following criteria: Nosocomial cases were horses that tested negative for Salmonella in samples obtained at the time of admission and tested positive in samples collected 48 h after admission, and the primary case or source of nosocomial infection was an index case that had positive results for Salmonella in a sample collected at the time of admission, and shared the same serotype and antibiogram as the isolate from the nosocomial case, and there was an overlap between admission and discharge dates of the primary and nosocomial cases. Another source of nosocomial infection was environmental contamination. Horses that had negative results for Salmonella in samples obtained at the time of admission but positive results thereafter and shared the same serotype and antibiogram as a Salmonella-positive environmental sample collected during the period of hospitalisation were also classified as nosocomial cases. When environmental contamination was attributed as the source of infection, the nosocomial case was never exposed to the primary case associated with the environmental contamination (i.e. there was no overlap between admission and discharge dates of the primary and nosocomial cases because the primary case had already been discharged from the hospital or subjected to euthanasia before the nosocomial case was admitted).

In addition to phenotyping methods such as serotyping and antimicrobial susceptibility testing, genotyping...
methods such as pulsed field gel electrophoresis (PFGE), plasmid profile analysis and phage typing have been used to determine if Salmonella isolates are genetically related, providing further evidence of nosocomial infection (Castor et al. 1989; Amavisit et al. 2001; Schott et al. 2001; Ward et al. 2005a). In a study that followed an outbreak of equine salmonellosis, presence of plasmids, examination of antibiotic profiles and production of bacteriocins and haemagglutination of erythrocytes were used to indicate that equine and environmental isolates had a common origin (Castor et al. 1989). In another study, PFGE was used in addition to serotyping and antimicrobial susceptibility testing, to characterise the outbreak strain and isolates were considered nosocomial if the PFGE pattern was similar to that of the outbreak strain (Schott et al. 2001). Another outbreak investigation used PFGE and phage typing to identify the outbreak strain, in addition to serotyping and antimicrobial susceptibility testing (Ward et al. 2005a). Finally, in a study that examined the epidemiological relationship between Salmonella cases by comparing isolates from a veterinary hospital over a period of 6 years, PFGE, presence of the insertion element IS200, plasmid profiles and antimicrobial resistance patterns were used to show that isolates from the veterinary hospital originated from a common source (Amavisit et al. 2001).

From a hospital risk management point of view, a working case definition must be formulated even when Salmonella serotyping data are not readily available. At the University of Florida: Large Animal Hospital (UF LAH), the serotyping of Salmonella isolates is performed at the US Department of Agriculture: National Veterinary Services Laboratory in Ames, Iowa, and it may take 4 weeks or longer to obtain the serotyping results. Thus, it becomes imperative to have a working case definition that excludes serotyping data but permits early detection of nosocomial Salmonella infections, so that enhanced infection control measures can be implemented to reduce the risk of disease transmission. At the UF LAH, the following 2 working case definitions are used based on 2 potential sources of nosocomial infection: another in-patient (index case) or environmental contamination (Fig 2).

First, if the source of nosocomial infection is another patient, then a horse will be considered a suspect for nosocomial Salmonella infection or colonisation when: 1) it tests positive on a faecal sample that is collected \( \geq 48 \) h after admission; 2) the serogroup of the isolate from the suspect nosocomial case is the same as that of the isolate from the suspect primary case; 3) the antimicrobial susceptibility pattern of the isolate from the suspect nosocomial case is the same as that of the isolate from the suspect primary case. In addition, the following 2 parameters are evaluated: 4) if there is a temporal overlap of admission and discharge dates of the suspected nosocomial and primary cases; and 5) there is a spatial overlap of housing location for the suspected nosocomial and primary cases (e.g. if both the suspected nosocomial and primary cases are housed in the same barn). The first 4 conditions above are required, but the last may or may not affect the decision to classify a horse as a nosocomial case. Second, if environmental contamination is considered the source of infection, then a horse will be considered a suspect for nosocomial Salmonella infection when: i) it tests positive on a faecal sample that is collected \( \geq 48 \) h after admission; ii) the serogroup of the isolate from the suspect nosocomial case is the same as that of the environmental isolate; iii) the antimicrobial susceptibility pattern of the isolate from the suspect nosocomial case is the same as that of the environmental isolate; and iv) there is no time overlap between admission and discharge dates of the suspect nosocomial and primary cases; however, the Salmonella isolate recovered from the suspect case shares the same serogroup and antimicrobial susceptibility pattern as a Salmonella-positive environmental sample collected during the period of hospitalisation. These 2 working definitions of nosocomial Salmonella infection have served well at the UF LAH over the last 10 years to identify nosocomial Salmonella infections and to justify the need to implement enhanced infection control measures to reduce the risk of disease transmission in hospitalised horses. A retrospective analysis using Salmonella serotyping results obtained \( \geq 4 \) weeks after each suspect

![Fig 2: A diagram showing 2 potential sources of nosocomial infection: an index case or environmental contamination.](image-url)
The environment can be an important source and reservoir for exposure and transmission of Salmonella in veterinary hospitals. In several reports of nosocomial outbreaks of salmonellosis, environmental contamination was implicated in the spread of salmonellae among patients and in the persistence of salmonellae in the hospital environment (Castor et al. 1989; Tillotson et al. 1997; Amavisit et al. 2001; Schott et al. 2001; Ward et al. 2005a; Dunowska et al. 2007). In one study, persistence of S. Typhimurium in the environment was identified as the source of nosocomial infection for several horses (Schott et al. 2001). In that study, S. Typhimurium was isolated from hospital personnel, shared equipment and stalls. A hospitalised foal was identified as the point source of infection. To confirm if the environment was a source of nosocomial infection, serotype information, antimicrobial susceptibility and PFGE patterns were used to compare the environmental isolates and the isolate recovered from the point-source foal. In another study, environmental contamination was suggested to be the source of infection for other horses during an outbreak of salmonellosis due to S. Typhimurium in a university hospital (Ward et al. 2005a). The point source of environmental contamination was a horse that presented with colic. S. Typhimurium was isolated from stall drains, surgery pads, forklift tires, and the ambulatory garage floor. Similarities of isolates based on serotyping, antibiogram, phage typing and PFGE patterns were used to indicate that a common source strain of S. Typhimurium was responsible for environmental contamination. In a different study, environmental contamination contributed to the wide spread nature of infection during an outbreak of salmonellosis due to S. Infantis at a veterinary hospital (Ward et al. 2005a). The point source of environmental contamination was a horse that presented with diarrhoea and horses with fever and leucopenia. At Colorado State University, faecal samples are obtained from all bovine inpatients for Salmonella culture, as well as from all equine colic patients at arrival and every other day after that (Morley 2002). At Michigan State University, faecal samples are collected on the day of admission and at various times thereafter from all horses with evidence of gastrointestinal tract abnormalities for diagnosis of Salmonella using bacteriological culture (Ewart et al. 2001). Additionally, at the attending clinician’s discretion, faecal samples are collected from horses without clinical signs of gastrointestinal tract disease that are considered to be at risk for shedding Salmonella such as neonatal foals with systemic disease, mare and foal pairs when only one of the pair has diarrhoea and horses treated with antimicrobials for long periods. At Purdue University, faecal samples are collected from horses that present with diarrhoea, or that develop diarrhoea with leucopenia or fever after admission to test for Salmonella using bacteriological culture (Ward et al. 2005a). Several university veterinary hospitals have environmental sampling as part of their surveillance and infection control programmes. At Colorado State University, stalls in which Salmonella-positive animals are housed are cultured before being made available for use with other patients (Dunowska et al. 2007). At Michigan State University, stalls used for horses with diarrhoea or that shed Salmonella in their faeces are sampled and tested using bacteriological culture (Ewart et al. 2001). Environmental samples are also collected from other hospital areas that are considered at risk for Salmonella
contamination such as surgery rooms, anaesthesia induction and recovery rooms.

**Diagnostics**

Most veterinary hospitals with a surveillance and infection control programme use bacteriological culture for detection of *Salmonella* spp. colonisation or infection in horses, as well as environmental contamination of hospital facilities. While bacteriological culture is the most common diagnostic procedure used for identification of horses infected with *Salmonella*, culture procedures are not standardised among veterinary microbiology laboratories (Hyatt and Weese 2004). For example, in a recent study (Ekiri et al. 2009), 1–2 g of fresh faeces were placed in 10 ml of selenite cystine broth (ratio, 1:10 to 1:8), and the broth was incubated at 37°C overnight for selective enrichment of *Salmonella*. This laboratory procedure differs from that reported in another study (House et al. 1999) in which investigators placed 5 g of fresh faeces in 100 ml of selenite broth (ratio, 1:20) and incubated the broth at 37°C overnight. These differences can affect the epidemiological sensitivity (i.e. false negatives) for diagnosis of *Salmonella*. For example, in one study, weight of the faecal sample obtained from pigs had an effect on detection of *Salmonella*: sensitivity increased from 32 to 63% when weight of the faecal sample increased from 1 to 10 g [diluted 1:9 [wt : wt] with buffer peptone water solution] (Funk et al. 2000). It is difficult to know how those results in swine can be extrapolated to horses. It is particularly difficult to assess how much misclassification of infected or noninfected horses may have occurred in previous epidemiological studies. The study in swine used only one sample per pig. In the equine study that used 1–2 g of fresh faeces (Ekiri et al. 2009), the median number of samples collected was 3 for control horses and 4 for nosocomial case horses. Assuming the concentration of *Salmonella* in the first faecal sample collected in that study population was similar to that in swine (Funk et al. 2000), it is possible that the surveillance system may have missed *Salmonella* shedders at admission because of low sensitivity.

Several PCR protocols have been standardised and evaluated for potential use in hospital surveillance programmes for early detection of *Salmonella* spp. in hospitalised horses. The advantage of using PCR protocols to monitor hospitalised horses shedding *Salmonella* in faeces is that test results can be obtained more quickly compared to bacterial culture (24 h vs. 3–5 days), thus allowing an earlier implementation of preventative measures such as isolation and barrier nursing, and therefore minimising the risk of a potential outbreak of nosocomial infection (Cohen et al. 1994; Ward et al. 2005b). Several studies (Table 1) have reported different estimates of sensitivity and specificity of real-time PCR protocols for detection of *Salmonella* spp. in faecal samples of horses.

These studies (Gentry-Weeks et al. 2002; Kurowski et al. 2002; Bohaychuk et al. 2007; Pusterla et al. 2009) concluded that PCR is a rapid, sensitive and specific assay for detection of *Salmonella* spp. that can be an alternative to conventional culture methods for surveillance, and can reduce the risk of nosocomial infections through the provision of highly accurate and rapid pathogen detection.

Other studies, however, have identified some limitations on the proposed use of PCR as a surveillance tool for *Salmonella* in hospital infection control programmes. An early study (Cohen et al. 1996) compared the diagnostic performance of PCR and microbiological culture for detection of *Salmonella* spp. in equine faeces. The PCR protocol used primers for the highly conserved segment of the histidine transport operon gene of *S. Typhimurium*. Overall, more faecal samples were classified as positive using PCR, compared to culture. Among equine outpatients without clinical signs of salmonellosis, 26/152 (17%) tested positive by PCR and 0/152 (0%) tested positive by culture. In addition, among hospitalised horses, 71/110 (65%) tested positive by PCR and 11/110 (10%) tested positive by culture. This study revealed that PCR can yield many false-positive results (especially among equine outpatients without clinical signs of salmonellosis). In a hospital setting, the consequences of false-positive results are significant because equine in-patients with a positive result must be placed in isolation stalls, increasing the cost of hospital fees.

Another study (Ward et al. 2005b) compared the diagnostic performance of PCR and culture for detection of *Salmonella* spp. in equine faeces. The PCR protocol used the same primers described by Cohen et al. (1996). Results from this study were very informative because the analysis was focused on 116 horses without clinical signs of gastrointestinal tract disease from which ≥5 faecal samples were collected daily and cultured for *Salmonella* spp.; the median number of samples collected per horse

<table>
<thead>
<tr>
<th>Primer probe</th>
<th>Sensitivity % (no. samples tested)</th>
<th>Specificity % (no. samples tested)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>spaQ gene</td>
<td>100.0 (80)</td>
<td>97.3 (150)</td>
<td>Kurowski et al. 2002</td>
</tr>
<tr>
<td>invE-A gene</td>
<td>80.0 (45)</td>
<td>98.6 (729)</td>
<td>Gentry-Weeks et al. 2002</td>
</tr>
<tr>
<td>hisJ gene</td>
<td>93.0 (15)</td>
<td>85.5 (291)</td>
<td>Gentry-Weeks et al. 2002</td>
</tr>
<tr>
<td>invA gene</td>
<td>100.0 (28)</td>
<td>98.3 (345)</td>
<td>Bohaychuk et al. 2007</td>
</tr>
<tr>
<td>invA gene</td>
<td>100.0 (6)</td>
<td>98.2 (893)</td>
<td>Pusterla et al. 2009</td>
</tr>
</tbody>
</table>

**TABLE 1: Reported sensitivity and specificity estimates of real-time PCR protocols for detection of *Salmonella* spp. in equine faecal samples**
was 8. A total of 87 (75%) horses had one or more samples classified as positive by PCR versus 11 (9.5%) by culture. In this study, if culture were used as the gold standard, the specificity of the PCR protocol used would be very low. Among the 105 horses that were culture-negative on ≥5 samples, only 29 (28%) were PCR negative for all samples tested. The authors concluded that the reasons why some faecal samples from which Salmonella spp. cannot be cultured are PCR positive need to be determined before PCR can be incorporated into Salmonella surveillance programmes for hospitalised horses.

Use of real-time PCR protocols has the potential to improve the diagnostic performance of conventional PCR protocols used for the detection of Salmonella spp. in equine faeces. The use of a sequence-specific fluorogenic probe in addition to conventional PCR primers allows for greater specificity for detecting a target sequence (Heid et al. 1996). In addition, the fluorogenic nature of the probe allows for rapid detection, because a fluorescent signal can be detected as the reaction progresses, negating the need for post PCR assay sample processing. Another advantage of real-time PCR over conventional PCR is that it can minimise post amplification cross-contamination or reduce the risk of ‘carry over’ contamination because closed tube systems are used (Bohachuk et al. 2007; Pusterla et al. 2009).

Enrichment of faecal specimen prior to PCR amplification can improve sensitivity of a PCR assay for diagnosis of Salmonella spp. in equine faeces. In one study in which enrichment was used prior to PCR amplification, PCR detected 10⁰ colony forming units (CFU) of S. Enteritidis/g of faeces (Cohen et al. 1995), and in another study, performed by the same author, where enrichment was not used, PCR detected 10⁰–10⁴ CFU of Salmonella spp./g of faeces (Cohen et al. 1994). In another study where real-time PCR was used to detect Salmonella spp. in 911 faecal and enriched broth samples, 3 of the 911 (0.3%) faecal samples tested positive while 22 (2.4%) enriched broth samples tested positive (Pusterla et al. 2009).

**Risk management**

Several studies have demonstrated that isolation, cleaning and disinfection, and traffic control can be effective in the control of Salmonella outbreaks in hospitals (Hartmann et al. 1996; Tillotson et al. 1997; Schott et al. 2001; Smith et al. 2004). The most commonly used protocol has been isolation of horses that pose a risk to other horses; these include horses with clinical signs of salmonellosis (diarrhoea, fever and leucopenia), and horses that are suspected or confirmed to be Salmonella-positive. Most veterinary hospitals maintain isolation units for this purpose, and horses are considered infectious and contagious until proven otherwise. A number of methods are employed to prevent and control microbial contamination during isolation, including the use of barrier nursing precautions such as examination gloves, protective coveralls or gowns, disposable boots when handling infected horses and foot baths or foot mats. Foot baths and foot mats have been shown to be effective in reducing bacterial contamination in veterinary hospital environments when used properly (Dunowska et al. 2006; Stockton et al. 2006).

In addition to barrier nursing precautions, promoting personal hygiene habits among hospital personnel can raise awareness of the importance of such behaviours in controlling nosocomial disease (Smith et al. 2004). These behaviours include washing hands or using alcohol-based hand wipers or lotions between patients, cleaning boots, avoiding walking on animal’s hay, wearing clean clothing, wearing gloves when handling contaminated wounds or soiled bandages, cleaning up manure promptly and not bringing personal pets into barn areas. One of the most important aspects of personal hygiene is washing one’s hands before and after coming into contact with any patient. Automatic or treadle-operated sinks and soap dispensers or alcohol based hand disinfectant solutions should be available at key locations in the hospital (such as isolation facilities).

Effective cleaning and disinfection of contaminated environments is one of the most important measures in preventing and controlling the spread of Salmonella in veterinary hospitals. Thorough cleaning of areas with faecal contamination such as stalls, water buckets or automatic watering apparatuses and drains is recommended (Murray 1996; Smith et al. 2004). The use of bleach in the environment after initial cleaning procedures is effective for additional elimination of environmental bacteria, and it has been shown to be the most effective product in eliminating detectable Salmonella organisms from hospital surfaces (Ewart et al. 2001). Several guidelines for the use of different disinfectants and disinfection techniques for materials, stalls and horse facilities have been published (Dwyer 1995, 1999; Ewart et al. 2001; Smith et al. 2004).

Traffic control measures have been used to prevent and control the spread of Salmonella organisms. Elements of traffic control include the designation of individuals to deal with infected/isolated animals only, restricting the number of hospital personnel or attendants entering isolation stalls, the cleaning of healthy horse stalls before cleaning stalls of sick animals, and control of excessive and unnecessary movement between and through barns (Tillotson et al. 1997). Traffic should always flow from cleaner to less clean areas. Other management practices recommended for controlling microbial contamination include: use of separate equipment (thermometers, nasogastric tubes, switches) and cleaning tools (grooming tools, manure carts, forks, brooms and shovels) for suspicious or confirmed Salmonella-positive animals and their stalls (Ernst et al. 2004). Animals should not be moved from one stall to another without due consideration for infection control protocols (Smith et al. 2004).
Guidelines for surveillance and infection control

Guidelines for surveillance and infection control programmes that are tailored to the needs and limitations of veterinary hospitals have been published (Morley 2002, 2004; Smith 2004; Smith et al. 2004; Traub-Dargatz et al. 2004; Ekiri et al. 2009; Steneroden et al. 2010). The following is a summary of surveillance and infection control procedures at the UF LAH. The objective is to reduce the risk of an outbreak of nosocomial Salmonella infections in horses and food animals.

The UF LAH has an infection control committee and an infection control officer (ICO). The committee consists of 10 members including clinicians, a microbiologist, an epidemiologist, hospital personnel (veterinary technicians) and the ICO. The committee meets quarterly and its responsibilities include formulation, implementation and evaluation of hospital surveillance and infection control protocols and policy. The responsibilities of the ICO include: overseeing sample collection from hospitalised horses and the hospital environment; microbiological procedures; collection, analysis and reporting of epidemiological data; and the implementation of all infection control protocols. In addition, the ICO periodically conducts training and orientations on surveillance and infection control practices to new hospital personnel, faculty, residents, interns and DVM students. The ICO is supervised by a senior hospital epidemiologist, who also serves as chair of the infection control committee.

All equine patients that present with or later develop signs of gastrointestinal (GI) disease are targeted for early detection of shedding of Salmonella spp. in faeces at the time of admission and during hospitalisation. A faecal sample (or swab) is collected from the rectum of each horse within 12 h after admission and submitted for bacteriological culture; thereafter, additional samples are collected from the stall floor (or rectum if possible) each morning prior to stall cleaning, every Monday and Thursday during hospitalisation. In the past, horses were sampled and tested at admission and every 48 h (i.e. Monday, Wednesday and Friday) during hospitalisation but because of funding limitations, a maximum of 2 faecal samples per animal per week are now collected. Faecal samples are submitted to the microbiology laboratory (located within the hospital complex) for bacteriological culture of Salmonella spp. On average a negative result is reported within 2–3 days after the sample is submitted. In contrast, a positive result is reported within 4–5 days due to additional required testing procedures (e.g. subculturing, serogrouping and antimicrobial susceptibility testing).

In addition to sampling hospitalised horses, routine environmental sampling is carried out monthly or bimonthly to evaluate cleaning and disinfection procedures, or more frequently during periods of high Salmonella activity in the hospital. During each event of routine environmental sampling, 25 of 100 hospital sites are targeted for sampling, and bacteriological culture is used to recover Salmonella from the environmental samples. The selection of sites is not random; instead, sites considered to be at high risk of contamination are sampled. This sample size is considered sufficient to detect (with 95% confidence) one or more sites with evidence of Salmonella contamination if the prevalence of contamination among the 100 sites is 10% or higher, which can be expected during an outbreak situation (Steneroden et al. 2010). Furthermore, stalls used by horses that were Salmonella-positive are cultured, and the stall must test negative before other patients are placed in such stalls (unless there is no other stall available, then that stall is used after proper cleaning and disinfection procedures have been completed). Also, a new round of environmental samples is collected (e.g. n = 25) whenever there is evidence that a nosocomial Salmonella infection has occurred in the hospital, or when a Salmonella-positive environmental sample is reported following routine environmental sampling; the objective is to assess cleaning and disinfection procedures and the magnitude of potential residual environmental contamination of hospital facilities.

Horses known to be shedding Salmonella spp. or that exhibit signs of diarrhoea or fever and leucopenia are housed in an isolation unit. Horses that present with signs of GI disease such as colic and do not exhibit signs of diarrhoea, or fever and leucopenia, and are not known to have tested positive for Salmonella spp. are housed in one barn assigned to patients with gastrointestinal disease or the intensive care unit. Horses that present for other complaints such as orthopaedic, reproductive or ophthalmological problems are housed in another barn. Horses that exhibit signs of diarrhoea or fever and leucopenia are sometimes housed in the GI barn under ward or stall isolation (i.e. isolated in the individual stall) if the isolation unit is full. To notify hospital personnel that a stall in the colic barn is under ward isolation, a rope barricade and isolation sign is placed around the stall entrance. Isolation procedures include the use of gloves, plastic boots, gowns and footbaths by all hospital personnel when attending to patients housed under isolation. Gloves, plastic boots, gowns and footbaths are placed outside the patient stalls and can be readily accessed. Foot mats containing a quaternary ammonium compound are placed at all points of entry and exit in all barns, the isolation unit and different railways within the LAH. Hand hygiene is strongly encouraged including washing of hands and using alcohol-based hand disinfectants. Sinks and soap dispensers, and alcohol-based hand disinfectant solutions are available at key locations within the hospital.

Every time there is evidence of nosocomial Salmonella infection or potential nosocomial Salmonella infection, enhanced infection control measures are instituted immediately for all hospitalised patients. The enhanced infection control measures include mandatory use of gloves, plastic boots and footbaths by all hospital personnel when attending to every patient in the hospital.
In addition, there is restricted movement of patients, for example, patients are not allowed out of stalls to graze or exercise. Rigorous cleaning and disinfection of the hospital (stalls, equipment) is conducted and thereafter, environmental sampling is conducted immediately to assess the magnitude of disease transmission and potential contamination of hospital facilities and equipment. Enhanced infection control measures are suspended after laboratory and epidemiological data reveal no further evidence of nosocomial *Salmonella* infection or hospital environment contamination.

Communication is an integral part of a hospital surveillance and infection control programme. At the UF LAH, information on the hospital infection control status is relayed quickly and clearly to all stakeholders including clinicians, clients or animal owners, referral veterinarians (rDVM), hospital personnel, DVM students, the infection control committee, hospital board and media. *Figure 3* shows communication channels in place at the UF LAH, where the ICO plays a pivotal role in communication. First, every time an in-patient tests positive for *Salmonella* spp., the laboratory immediately notifies both the attending clinician and the ICO. The attending clinician then informs the client or animal owner and the referral veterinarian about the infection status of the in-patient. Clients are educated on the potential risk to people and other animals on the farm, and how to manage that patient after hospital discharge. At the time of discharge, clients are given a *Salmonella* fact sheet by the attending clinician. The fact sheet includes a brief description of the surveillance programme at the hospital, symptoms of clinical salmonellosis, and measures that should be implemented to reduce the risk of infection to people and other animals at the farm. If *Salmonella*-positive laboratory results are reported after the patient has been discharged, the clinician relays the information to clients by telephone. In addition, all new hospital clients are given a brochure that describes the hospital surveillance and infection control programme. The aim is to inform clients about the surveillance and infection control procedures that are implemented at the hospital to optimise patient care.

Second, the ICO makes an assessment to determine if the source of infection is an in-patient with a community-acquired infection or a potential nosocomial infection. The ICO establishes communication with the hospital epidemiologist to confirm the source of infection and to define infection control measures that are considered most appropriate. If the patient is classified as a primary case, then the ICO communicates and coordinates with hospital personnel to ensure that appropriate infection control measures are implemented for that patient such as isolation and barrier nursing precautions. However, if the patient is classified as a potential nosocomial case, then enhanced hospital infection control measures are instituted as previously described. Before implementing enhanced measures, the ICO has to seek approval from at least 2 members of the infection control committee. Next, the ICO immediately notifies (by email) all clinicians, veterinary technicians, DVM students and the barn crew about the new infection control status in the hospital and the enhanced measures that must be observed.

Third, the ICO is responsible for scheduling hospital infection control committee meetings every 3 months (or more frequently). The main objectives of these meetings are to provide committee members with a summary report of hospital surveillance and infection control activities for the past 3 months and to review and update existing standard operating procedures, as well as the performance of key surveillance parameters (i.e. **Clinical Case Definitions**).

*Fig 3: Surveillance and infection control communication channels in place at the University of Florida: Large Animal Hospital.*
frequency of nosocomial cases, compliance in sample collection from horses at admission, rapid reporting of laboratory results, efficacy of cleaning and disinfection). Draft meeting minutes are prepared and submitted to all committee members by the infection control committee chair within 3 days for review and approval. Ten days after the meeting, the minutes are submitted to the hospital board by the committee chair for approval. Finally, when necessary, communication with the media is performed with the assistance of the UF College of Veterinary Medicine: Office of Public Relations.

Epidemiological research

Identification of risk factors associated with nosocomial Salmonella infection in hospitalised horses is important, so that effective preventative and control measures can be instituted to reduce the risk of disease transmission and potential outbreaks. Initial studies conducted in the 1980s and 1990s in California provided an initial epidemiological framework for investigation of risk factors associated with nosocomial Salmonella infection in hospitalised horses. An initial study investigated an outbreak of nosocomial salmonellosis due to S. Saint-paul in a veterinary hospital (Hird et al. 1984). In that study, a presenting complaint of colic, nasogastric intubation and treatment with antibiotics were associated with isolation of S. Saint-paul. In a second study conducted at the same hospital, a presenting complaint of colic, nasogastric intubation and treatment with antibiotics were again associated with isolation of Salmonella (Hird et al. 1986). Finally, in a third study, diagnosis of large colon impaction, withholding feed, number of days fed bran mash, treatment with potassium penicillin G and increase in mean daily ambient temperature were identified as risk factors for nosocomial Salmonella infection (House et al. 1999).

A recent epidemiological study identified abdominal surgery as a risk factor for nosocomial Salmonella infection in horses (Ekiri et al. 2009). There are several explanations for the observed association between abdominal surgery and nosocomial Salmonella infection in horses. In horses that undergo abdominal surgery, the large intestine may be evacuated and lavaged, feed may be withheld or changed, antimicrobial drugs are usually administered and various degrees of ileus may develop (Linerode and Goode 1970; Argenzio 1975; Smith et al. 1978; Owen et al. 1983; Clarke et al. 1990; House et al. 1999). These events can cause stress in surgical patients and alter the gastrointestinal physiology and microflora. In man and mice, abdominal surgery has been associated with severe alterations of host-defence mechanisms (Qui-shi and Guizhen, 1986; Ziegler-Heitbrock and Ulevitch 1993; Zellweger et al. 1995; Kawasaki et al. 2001, 2007). These alterations suppress the innate immune system during the perioperative period and cause substantial impairment of cell-mediated immunity. In view of the effects of abdominal surgery on the immune system in man and mice, it is possible that surgical stress similarly suppresses the innate and adaptive immune systems of equine surgical patients. As a result, abdominal surgery may increase their susceptibility to nosocomial Salmonella infections. Studies are necessary to confirm if abdominal surgery is a predisposing factor for nosocomial Salmonella infection in hospitalised horses.

While a high caseload has been suspected as a predisposing factor for nosocomial Salmonella infection in hospitalised horses, previous epidemiological studies (Hird et al. 1984, 1986; House et al. 1999; Ekiri et al. 2009) failed to identify high caseload as a risk factor. A study in Florida (Ekiri et al. 2009) explained that the lack of an association between a high caseload and nosocomial Salmonella infections could be attributed to the fact that the number of horses shedding Salmonella at the time of admission during periods of high or low caseload was not different. Another explanation was that the hospital surveillance and infection control programme was well established and the degree of personnel compliance was acceptable. It is possible that high caseload may have an effect on risk of nosocomial infection in hospitalised horses when the number of shedders in the hospital is high and infection control standards are suboptimal.

Discussion

In previous outbreaks where hospitals had to close, the lack of a case definition for nosocomial Salmonella infections, day-to-day monitoring of nosocomial cases, routine environmental sampling, infection control protocols and guidelines for hospital closure due to an increased number of nosocomial Salmonella infections, contributed to the onset of outbreaks of Salmonella infections in hospitalised horses. A question that is dreaded and yet warrants discussion by directors of hospital infection control programmes and hospital administrators is when to temporarily close a hospital due to an increased number of nosocomial infections in horses. Currently, there are no published reports with standardised criteria or guidelines to decide when a hospital should be temporarily closed to stop a nosocomial outbreak of Salmonella infections in hospitalised horses. A review of previous outbreak reports (Table 2) revealed several parameters that can be taken into consideration.

The consequences of previous nosocomial outbreaks of Salmonella infections in horses clearly justify the need to formulate standards for hospital surveillance and infection control measures, including specific guidelines for when to close a hospital operation to prevent unnecessary morbidity and mortality in hospitalised horses. Three key parameters that can be used by hospital administrators when making a decision to temporarily close a veterinary hospital include: 1) weekly number of nosocomial Salmonella cases with or without clinical disease; 2) case fatalities; and 3) zoonotic disease.
Although time to obtain *Salmonella* test results by bacteriological culture is a limitation, bacteriological culture is a good diagnostic tool to use in hospitals where compliance with surveillance and infection control protocols is good. Clinicians often argue that bacteriological test results have limited value for patient care because laboratory results are often reported days after the in-patient has been discharged and other patients may have been exposed by the time laboratory results are reported. While this argument is valid, hospital surveillance and infection control protocols are designed to manage the infection control status of a hospital rather than that of individual in-patients.

The proposed use of PCR protocols as a surveillance tool for early detection of *Salmonella* faecal shedding in horses is a good idea, provided the frequency of false positives is acceptably low and does not significantly affect the hospital cost. The study by Ward et al. (2005b) justifies the need to investigate further why horses without clinical signs of salmonellosis that are classified as negative (i.e. after 5 consecutive faecal samples using bacteriological culture) test positive by PCR.

In an effort to standardise hospital surveillance and infection control programmes and to institute a measure of accountability, the reporting of nosocomial *Salmonella* infections in hospitalised horses (and other species) should be considered as one parameter of excellence in hospital veterinary care. Reporting of nosocomial *Salmonella* infections may encourage improvement in the quality of patient care, and the overall efficiency of a hospital surveillance and infection control programme.

In light of the recent epidemiological finding (Ekiri et al. 2009) that abdominal surgery was identified as a risk factor for nosocomial *Salmonella* infection in hospitalised horses, there is need to further investigate this finding to confirm if surgical stress suppresses the innate and adaptive immune systems of equine surgical patients, thereby increasing their susceptibility to nosocomial *Salmonella* infections. Results from additional studies will help determine if abdominal surgery is a predisposing factor for nosocomial *Salmonella* infections in hospitalised horses, and justify the need to enhance infection control measures (e.g. isolation and use of gloves, gowns, plastic boots and footbaths) to protect equine inpatients that undergo abdominal surgery.

**References**


