An outbreak of salmonellosis among horses at a veterinary teaching hospital

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During the past several decades, numerous reports of outbreaks of nosocomial salmonellosis involving large animal patients in veterinary teaching hospitals in the United States[1-7] and abroad[8-12] have been published. Most of these reports have been published within the past 15 years, but whether this indicates a true increase in the risk of nosocomial salmonellosis concurrent with the trend toward larger herd sizes and an increase in the antimicrobial resistance of *Salmonella* isolates or merely an increase in the frequency of reporting of such outbreaks is unknown.

Established risk factors for nosocomial colonization with *Salmonella* spp (ie, shedding of organisms in the feces without associated clinical abnormalities), as well as for nosocomial infection, include exposure to hospitalized horses shedding *Salmonella* spp, concurrent gastrointestinal tract disease, a change in or withholding of feed, use of common instruments (eg, nasogastric tubes, rectal thermometers), high ambient temperature, and treatment with antimicrobial agents.[2,5-7,11-16] However, morbidity and mortality rates associated with outbreaks of nosocomial salmonellosis vary widely with the serotype of *Salmonella* spp involved. As a result, control measures also vary and range from minor changes in hospital practices to hospital closure for complete disinfection. Thus, each outbreak of nosocomial salmonellosis is somewhat unique, and experiences gained with each outbreak can provide important information to equine clinicians and hospital administrators who may be faced with this problem in the future.

In the present report, we describe an outbreak of nosocomial salmonellosis involving horses hospitalized at Michigan State University's Large Animal Clinic during 1996 and measures implemented to control the problem and limit the risk of future nosocomial infections. Unique features of this outbreak included a high case fatality rate, which led to a decision to close the hospital for complete disinfection, use of a **polymerase chain reaction (PCR)**-based assay to detect *Salmonella* DNA persisting in the environment, and use of **pulsed-field gel electrophoresis (PFGE)** to characterize biotypes of *Salmonella enterica* serotype Typhimurium isolates. Subsequent experience with a monitoring program incorporating bacterial culture of fecal samples from horses considered to be at risk of salmonellosis and use of a PCR-based assay to test samples from the hospital environment is described elsewhere.[17]

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Procedures

Case selection—Medical records of all horses examined between May 2, 1996 (date of admission of the point-source foal) and Jan 29, 1997 (date of admission of the last horse documented to have nosocomial salmonellosis) for which bacterial culture of ≥ 1 fecal sample yielded *Salmonella* spp were reviewed. Data retrieved from the records included signalment, initial complaint, location in the hospital, duration of hospitalization, number of hospital days until onset of fever or diarrhea, number of fecal samples submitted for bacterial culture and results, and outcome. During the initial part of the outbreak (May and June), multiple fecal samples were collected and submitted for bacterial culture from all horses that developed fever, signs of colic, or diarrhea after hospital admission. In addition, clinicians were encouraged to submit fecal samples for bacterial culture from horses that were hospitalized for more than a couple of days or that were receiving broad-spectrum antimicrobials for another primary disease; however, a specific protocol for collection and submission of fecal samples was not in place. Cultures that yielded a *Salmonella* Typhimurium isolate with an antimicrobial susceptibility profile identical to that of the isolate recovered from the point-source foal were initially assumed to indicate nosocomial infection. Affected horses were considered to have nosocomial colonization if there was no exacerbation of the primary disease after admission to the hospital (ie, if a fever or diarrhea did not develop) or to have nosocomial infection if clinical signs of salmonellosis were identified in addition to the primary disease.

Bacterial culture of fecal samples—Fecal samples submitted for isolation of *Salmonella* spp were directly plated onto MacConkey and **brilliant green (BG)** agar plates and incubated for 24 hours at 35 to 37 C. A sample (approx 1 g) was also inoculated into 10 ml of selenite broth, and after incubation for 24 hours at 35 to 37 C, the broth was subcultured onto BG agar plates. Lactose-negative colonies on agar plates were tested for agglutination with *Salmonella* somatic antigen polyvalent antisera,[a] and colonies positive for agglutination were further confirmed to be *Salmonella* spp by use of an automated biochemical identification system.[b] *Salmonella* isolates were serotyped by means of antigen

testing, using a panel of antisera[a] directed against somatic and flagellar *Salmonella* antigens. Antimicrobial susceptibility was determined by use of the disk diffusion method.[18]

Collection and testing of environmental samples—During hospital closure and disinfection, multiple environmental samples were collected and submitted for detection of *Salmonella* spp by means of bacterial culture and PCR testing. After each area (eg, stall, aisle, room) was cleaned, disinfected, and allowed to dry, multiple sites in the area were swabbed with paired sterile swabs premoistened with sterile water. One swab from each pair was inoculated into 10 ml of selenite broth and subsequently subcultured on BG agar as described for fecal samples. The other swab was placed in a tube containing 0.3 ml of sterile water. The tip of the swab was broken off, leaving the cotton swab in the water, and the sample was tightly capped and sent to a commercial laboratory[c] for PCR testing.[19]

Pulsed-field gel electrophoresis—*Salmonella* Typhimurium isolates from 29 horses were analyzed by means of PFGE.[d] In several instances, multiple isolates from the same horse were tested. *Salmonella muenster* and *S newport* isolates from 3 additional horses were also analyzed by means of PFGE. Agarose-embedded DNA was prepared by a modification of a described method,[20] and 2 patterns were produced for each isolate by means of DNA digestion with restriction enzymes *Xba*I and *Spe*I.[e] Pulsed-field gel electrophoresis was performed, using a 1% agarose gel, with a electrophoresis cell.[f] Run time was 18 hours at 14 C and 6 V/cm, with switch times of 5 to 45 seconds (*Xba*I) or 5 to 30 seconds (*Spe*I). The bacteriophage γ DNA concatamers[g] were used as size standards. In essence, PFGE allows separation of *Salmonella* isolates on the basis of various genetic events (eg, mutations, deletions, and insertions) that result in DNA fragments of various sizes after digestion with the restriction endonucleases. Isolates that yielded PFGE patterns identical to the pattern for the isolate from the point-source foal, as well as isolates that yielded patterns differing from that isolate by only 1 or 2 genetic events, were considered related; isolates that yielded patterns differing from the pattern for the isolate from the point-source foal by ≥ 3 genetic events were considered unrelated.[21]

Results

Description of the outbreak—On May 2, 1996, a recumbent 3-day-old Arabian filly (horse 1) was referred to the Michigan State University Large Animal Clinic for evaluation and treatment of suspected neonatal septicemia and diarrhea. Later the same day, a 10-year-old Tennessee Walking Horse gelding with colic (horse 2) and a 1-day-old Standardbred filly with colic and diarrhea (horse 3) were also admitted to the hospital on an emergency basis. Although the 2 foals were hospitalized in a separate neonatal ward, all 3 horses, as well as other hospitalized patients in the main hospital, were treated by the same personnel (clinicians, technicians, and students). Horse 1 was euthanatized 24 hours after admission. Three days later, bacterial cultures of blood and fecal samples were reported to be positive for *Salmonella* Typhimurium resistant to ampicillin, gentamicin, kanamycin, tetracycline, and trimethoprim-sulfonamide and susceptible to ceftiofur and amikacin.

Horse 2 underwent abdominal surgery the day of admission and developed fever and diarrhea 3 and 5 days, respectively, after surgery. The horse was moved to an isolation stall on the day diarrhea developed, and *Salmonella* Typhimurium was recovered from feces collected on day 5. On day 8, the *Salmonella* Typhimurium isolate from horse 2 was reported to have the same antimicrobial resistance pattern as the isolate from horse 1. Horse 3 had been stabled directly across the aisle from horse 1 (the point-source foal), and *Salmonella* Typhimurium was first isolated from its feces on day 4. On day 12, the isolate was reported to have an antimicrobial resistance pattern identical to that for the isolate from the point-source foal.

Between May 2 and Jun 26, 1996, 13 additional hospitalized horses developed fever (n = 10) or diarrhea (12) between 2 and 15 days after admission and were subsequently determined to have *Salmonella* Typhimurium with an antimicrobial resistance pattern identical to the pattern for the isolate from the point-source foal in their feces. Of these, 3 were adult horses examined because of diarrhea; all 3 were placed in isolation stalls at the time of admission. The isolation facility was located in a building separate from the main hospital and consisted of 5 stalls with individual outside doors (for animal entry) and inner doors (for personnel entry) that opened into a common supply hallway. For all 3 of these horses, results of bacterial culture of initial fecal samples were negative for *Salmonella* spp, but a multiple-drug resistant *Salmonella* Typhimurium was isolated from feces collected between 6 and 8 days after admission. Diarrhea became more severe after *Salmonella* Typhimurium was isolated, prolonging hospitalization. One horse was euthanatized on hospitalization day 10.

During the same period, 3 hospitalized foals developed diarrhea at their home farms after discharge from the teaching hospital. One neonatal foal that had been hospitalized for treatment of failure of passive transfer and meconium impaction had *Salmonella* Typhimurium with an identical antimicrobial resistance pattern recovered from feces collected on hospitalization day 3, but diarrhea did not become severe until several weeks after discharge. The foal was ultimately euthanatized at approximately 2 months of age because of progressive emaciation and chronic diarrhea that was unresponsive to treatment with ceftiofur and gentamicin. The *Salmonella* Typhimurium isolate recovered from the small intestine at necropsy was resistant to ceftiofur and susceptible only to amikacin. *Salmonella* Typhimurium isolates with identical antimicrobial resistance patterns were obtained from fecal samples collected on several occasions from the other 2 foals. Both foals recovered, but fecal shedding of the multiple-drug resistant *Salmonella* Typhimurium persisted for at least 3 months in 1 foal.

During May and June of 1996, therefore, a multiple-drug resistant *Salmonella* Typhimurium was isolat-

ed from feces of 19 equine patients, including the Arabian filly that was the source of the organism (horse 1). These horses had been housed in all 3 equine wards in the main hospital, as well as in the isolation facility. During these months, 337 horses were examined at the teaching hospital (199 as outpatients and 138 as inpatients). None of the animals treated as outpatients appeared to have been affected, but feces from these animals were not submitted for bacterial culture. Further, other than the 3 foals that developed diarrhea after discharge, outbreaks of salmonellosis were not reported by any of the farms to which animals were discharged. Similarly, a history of diarrheal disease was not reported by the farm from which horse 1 had been admitted, and further testing of fecal samples from horses on that farm was not pursued.

The estimated nosocomial infection rate for hospitalized patients for this period was 13% (18/138). However, the rate may actually have been higher, because fecal samples were not submitted for all 138 hospitalized horses. In addition, the case fatality rate was high (44%), in that 8 of the 18 affected horses were euthanatized or died. For these reasons, a decision was made to close the hospital to new admissions on Jun 26, 1996.

Facility cleaning and disinfection—After the teaching hospital was closed, a strategic cleaning and disinfection plan was implemented, following recommendations made by an outside consultant (RMD) with expertise in control of enteric infectious disease. Key points of the process included establishing specific traffic patterns from least to most contaminated areas of the hospital, with cleaning and disinfection to follow these routes; disposal of all supplies and movable storage containers that could not be appropriately cleaned and disinfected; hand-scrubbing of all surfaces with a bleach solution followed by application of a phenolic disinfectant solution[h]; and bacterial culture and PCR testing of samples from all cleaned areas (ie, all stalls; barn aisles; equipment; and examination, treatment, record, and supply rooms). Cleaning crews consisted of teams of faculty, residents, technicians, and hospital staff, and each team was restricted to a specific area of the hospital on each day of cleaning.

During hospital closure and disinfection, the multiple-drug resistant *Salmonella* Typhimurium was isolated from only 3 of 241 environmental samples. All 3 samples were from sites in stalls in which horses with nosocomial salmonellosis were still housed and which had not yet been cleaned and disinfected. None of the subsequent environmental samples yielded *Salmonella* spp. In contrast, PCR testing of environmental samples yielded positive results for 28 of 237 (12%) samples collected from several areas of the hospital and the isolation facility after cleaning and disinfection. Areas for which results of bacterial culture and PCR testing of environmental samples were negative were sealed and not entered again until restocking prior to hospital reopening. When results of PCR testing of environmental samples were positive, cleaning and disinfection of the area was repeated until negative results were obtained. The isolation facility was the last area to be cleaned and disinfected. Despite 3 or more rounds of cleaning and disinfection, a few areas in the isolation facility (floors, drain bowls, and vertical drainpipes of 2 of the 5 stalls) still yielded samples with positive PCR test results. Because bacterial culture results had been consistently negative for *Salmonella* spp after cleaning and disinfection of these areas, and because it was unclear whether the positive PCR test results indicated detection of viable *Salmonella* spp, a decision was made to open the hospital for regular service on Jul 22, 1996.

Infection control policies—Simultaneous with cleaning and disinfection, infection control policies for the large animal hospital were updated by an ad hoc infection control committee. Existing policies required that all horses with diarrhea at the time of admission be placed in the isolation facility. Essential changes to the policies included adoption of stricter criteria for transferring hospitalized horses to the isolation facility (ie, horses that developed 2 of the following 3 criteria were transferred: diarrhea, fever [rectal temperature > 39.2 C], and neutropenia [< 2,000 neutrophils/µl]); decreased use of shared equipment (ie, new nasogastric tubes, funnels, and buckets were purchased and used for each patient with colic); use of disposable impermeable gowns[i] and a double layer of plastic booties[j] when working with patients in isolation stalls; use of disposable plastic booties[j] when entering the stall of any horse with gastrointestinal tract disease; further encouragement of recommended handwashing procedures between patient contacts; routine submission of 3 fecal samples for bacterial culture for *Salmonella* spp from all horses hospitalized with gastrointestinal tract disease and submission of a minimum of 5 daily fecal samples for all horses with diarrhea.

In addition, cleaning and disinfection of stalls became more stringent. Following discharge of a horse from a stall in the main hospital, the stall was initially steam cleaned, followed by hand scrubbing of all areas visibly soiled with feces, using a bleach solution (6 oz of a commercial 5.25% sodium hypochlorite solution in 1 gallon of water). All surfaces were then rinsed well with water and sprayed with a phenolic disinfectant.[k] The stall was allowed to air dry and was then considered ready for use. Stalls in the main hospital wards were already equipped with a manure disposal system that minimized retention of soiled bedding in the hospital environment. Separate underground disposal systems in each ward used a strong flow of water to carry soiled bedding to a central holding bin underneath the main hospital, and a covered drain connected to the disposal system was located in the ward aisles in front of each stall. During stall cleaning, the drain cover was removed and soiled bedding was scraped directly into the disposal system. Although this manure disposal system also essentially eliminated a rodent problem, a resident cat was removed from the hospital, even though results of bacterial culture of fecal samples were negative for *Salmonella* spp.

After the large animal clinic reopened, stalls in the main hospital from which horses were transferred to the isolation facility were considered restricted and subjected to more stringent cleaning and disinfection.

All bedding was scraped directly into the disposal system used for all stalls in the main hospital, but, rather than steam cleaning, the stall was hosed clean and the entire stall was hand scrubbed, using a bleach solution (6 oz of a commercial 5.25% sodium hypochlorite solution in 1 gallon of water). After surfaces had been sprayed with the phenolic disinfectant and allowed to dry, they were sprayed a second time; after the second disinfection with phenolic disinfectant, environmental samples were collected and submitted for bacterial culture. The stall was released for use by another patient only after results of bacterial culture of environmental samples were negative for *Salmonella* spp. Cleaning and disinfection procedures for stalls in the isolation facility were similar to those for restricted stalls, except that because of the limited number of stalls, new patients were, if necessary, placed in stalls that had been cleaned and disinfected but for which results of bacterial culture were pending.

At the end of each day, outpatient stalls (limited to use by 1 horse daily) were hosed clean, doused with bleach, and allowed to air-dry overnight. The floors of examination and treatment rooms and hospital and ward aisles were hosed daily and disinfected twice weekly with a phenolic compound,[g] using a hose-end spray applicator. Finally, each hospital ward was closed 1 or more times annually for complete cleaning and disinfection. Environmental samples were regularly collected from sites throughout the hospital and submitted for bacterial culture for *Salmonella* spp.

One member of the technical staff was designated as the infection control officer. Responsibilities included regular collection of environmental samples for bacterial culture, release of restricted stalls, and maintenance of a database for results of bacterial culture of fecal and environmental samples and the schedule for regular cleaning and disinfection of hospital areas. Finally, a summary of results of bacterial culture of fecal samples detailing the frequency at which *Salmonella* spp were isolated and information about clinical cases of salmonellosis was distributed regularly to large animal hospital faculty, residents, technicians, and hospital staff.

Salmonellosis following hospital reopening—On Jul 30, 1996, 8 days after the hospital was reopened, a mare with diarrhea was admitted to the isolation facility. After an initial favorable response to treatment, the mare's condition deteriorated and it died on hospitalization day 3. A *Salmonella* Typhimurium with an antimicrobial resistance pattern identical to that for the outbreak strain was recovered from the small intestine at necropsy. During the next 6 months, multiple-drug resistant *Salmonella* Typhimurium was recovered from 10 additional horses examined because of gastrointestinal tract problems. Of these 10 horses, 8 had been admitted directly to the isolation facility because of diarrhea, colic, or general poor condition, and 2 with colic had been admitted to the main hospital. One of the horses that was admitted to the main hospital was subsequently moved to the isolation facility after it developed a fever. The other horse admitted to the main hospital was treated for sand colic and discharged on hospitalization day 4. The day after discharge, bacterial culture of a second fecal sample collected from this horse yielded a multiple-drug resistant *Salmonella* Typhimurium.

All 9 horses admitted or transferred to the isolation facility remained in their stalls throughout the remainder of the hospitalization period, with 1 exception. A horse with chronic poor condition and intermittent diarrhea underwent exploratory celiotomy on hospitalization day 12. This horse did not have evidence of chronic salmonellosis (including negative results for bacterial culture of 4 fecal samples) and was placed in the main hospital for recovery. However, the horse developed a fever the evening after surgery and profuse diarrhea ensued 4 days later, prompting a return to its original isolation stall. The last horse from which *Salmonella* Typhimurium with an antimicrobial resistance pattern identical to that of the outbreak strain was recovered was a university-owned research horse that developed diarrhea and was admitted to the isolation facility on Jan 29, 1997. The horse remained in the isolation facility and was euthanatized when the positive culture results were reported. This horse had been healthy prior to this episode and had been housed from Jan 20 through 27 in a stall in the main hospital as part of a research study that did not involve anesthesia, surgery, or other major invasive procedures. This stall had been used previously to house a horse from which *Salmonella* Typhimurium was isolated. Bacterial culture of fecal samples from all herd mates of the research horse during the following week did not yield *Salmonella* spp.

Salmonella spp was isolated from 15 equine patients admitted during the first 6 months after the hospital reopened. *Salmonella* Typhimurium was isolated from 11 horses, and for 8 of the 11, *Salmonella* Typhimurium isolates had an antimicrobial resistance pattern identical to that for the strain that had resulted in hospital closure. Four of these 8 horses were considered to have nosocomial colonization, and the other 4 were considered to have nosocomial infection because of exacerbation of the primary problem. For the other 3 horses, the antimicrobial resistance patterns of the *Salmonella* Typhimurium isolates were different. Other *Salmonella* serotypes were isolated from the remaining 4 horses. One of these was a late-gestation mare examined because of colic that developed fever and leukopenia on hospitalization day 2 and was transferred to the isolation facility. *Salmonella newport* was isolated from feces collected on day 2, but bacterial culture of subsequent fecal samples, as well as environmental samples from the main hospital stall, yielded *Salmonella* Typhimurium with an antimicrobial resistance pattern identical to that for the strain that had resulted in hospital closure (increasing the number of nosocomially colonized horses to 5).

Characterization of *Salmonella* Typhimurium biotypes—Two methods were used to determine the similarity among *Salmonella* Typhimurium isolates and the likelihood of nosocomial infection. First, antimicrobial resistance patterns were compared to the pattern for the isolate recovered from the point-source

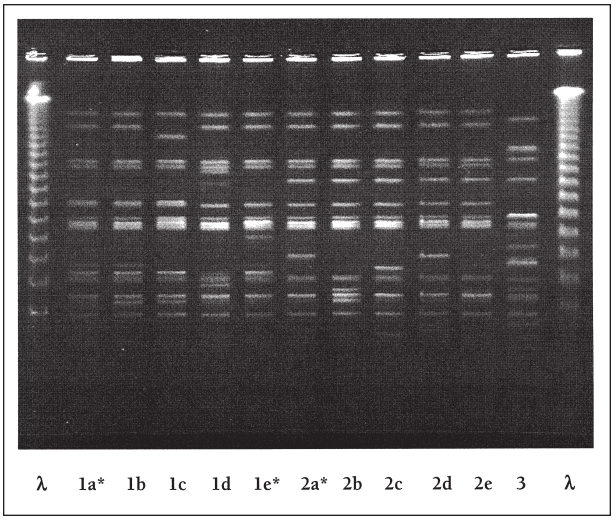

Figure 1—Pulsed-field gel electrophoresis patterns produced by DNA digestion with the restriction endonuclease *Xba*I of *Salmonella enterica* serotype Typhimurium isolates obtained from horses involved in an outbreak of salmonellosis. λ = Lambda ladder for size comparison of DNA fragments. * = Patterns for the isolate recovered from the point-source foal. Isolates with pattern 1a, 1b, 1c, 1d, 1e, 2a, 2b, 2c, 2d, or 2e were considered to be related to the strain from the point-source foal and indicative of nosocomial infection. Isolates with pattern 3 were considered to be naturally acquired.

foal. If an identical resistance pattern was detected, infection was considered to be nosocomial. Second, PFGE was performed at a later date. Three PFGE patterns were obtained for the *Salmonella* Typhimurium isolate recovered from the point-source foal. Two of these patterns were similar (1a and 1e), and the third (2a) was less closely related to the other 2 (**Fig 1**). Testing of isolates from 16 of the 18 horses suspected to have nosocomial salmonellosis prior to hospital closure revealed that 13 of these isolates had PFGE pattern 1a or a closely related pattern (1b, 1c, or 1d), and 3 isolates had PFGE pattern 2a or a closely related pattern (2b, 2c, or 2d). Isolates from 2 horses were no longer available for PFGE testing.

Testing of *Salmonella* Typhimurium isolates recovered from 11 horses after the hospital reopened revealed that 4 were PFGE pattern 1a, 2 were PFGE pattern 2a, and 2 were patterns (2b and 2d) closely related to those recovered from the point-source foal. *Salmonella* Typhimurium isolates from the remaining 3 horses all had different antimicrobial resistance patterns, and PFGE revealed that these isolates were unrelated to the isolate recovered from the point-source foal. Testing of the *Salmonella* Typhimurium isolate from the horse from which *S newport* and *Salmonella* Typhimurium were both isolated revealed PFGE pattern 2g, a pattern closely related to the pattern for the isolate from the point-source foal.

Discussion

Although nosocomial infections are a well-recognized risk of operating any hospital, the prevalence of nosocomial infection with multiple-drug resistant organisms appears to be increasing in human and veterinary hospitals.[6,22-25] An effective response to a possible outbreak of nosocomial infection requires prompt recognition of the problem, isolation of affected patients, implementation of procedures to minimize exposure of at-risk patients and monitor those patients for infection, identification of the sources and reservoirs of the infectious agent, and review and possible modification of infection control procedures.[26]

Documentation that infections are nosocomial requires characterization of the organism responsible for the outbreak. For nosocomial infections with zoonotic potential, specific infection control policies should be reviewed with at-risk hospital staff and students to minimize the risk of human infection. During the 6-month outbreak of nosocomial salmonellosis described in the present report, only 1 individual, a veterinary student, was found to have developed a zoonotic infection. The *Salmonella* Typhimurium isolate from this student had an antimicrobial resistance pattern identical to that for the isolate from the point-source foal, and although not identical, the PFGE pattern of the isolate (2e) suggested that it was likely related to the outbreak strain. The prevalence of human infection may have been underreported, because not all affected people report their disease to hospital administrators.

During the outbreak described in the present report, *Salmonella* Typhimurium with a specific antimicrobial resistance pattern was recovered from 28 horses during a 9-month period. The case fatality rate was 46% (13/28). The point-source foal and the 18 horses with nosocomial salmonellosis prior to hospital closure were examined at the teaching hospital because of primary gastrointestinal tract disease, had been admitted for abdominal surgery, or were foals. The 9 horses affected after the hospital reopened all were examined because of primary gastrointestinal tract disease. These findings were consistent with previous reports of increased susceptibility to *Salmonella* infection among horses with gastrointestinal problems, horses undergoing surgical procedures, and neonates.[2,7,8,13-16,27] For horses discharged alive, a 30-day isolation period was recommended, followed by submission of 5 serial fecal samples for bacterial culture prior to reintroduction into the herd. We are unaware of subsequent salmonellosis problems in affected horses or of outbreaks of salmonellosis on farms to which these horses returned. Further, in the 3 years after this outbreak, we have not isolated this multiple-drug resistant *Salmonella* Typhimurium from any environmental samples or large animal patients subsequently admitted to the teaching hospital.[17]

Although the mode of transmission could not be documented for all horses involved in the outbreak described in the present report, several factors played an important role. Two of the initially affected horses were admitted on the same evening as the point-source filly, and it seems likely that hospital personnel and shared equipment contributed to the spread of the organism. Although more stringent guidelines for admission of horses (including foals) with diarrhea, hand washing between patients, removal of soiled protective clothing, and minimizing use of shared equipment between horses have been emphasized in updated infection control policies, this type of nosocomial

infection remains a risk in the operation of any large animal hospital. This is especially true of hospitals with large caseloads and of those located in more temperate climates.[2,7] Persistence of the organism in the environment, despite more stringent stall and ward cleaning, was another likely factor involved in nosocomial infection of several horses in the present report. In retrospect, results of PCR tests suggested that a reservoir persisted in the isolation facility during hospital closure, and 14 of 28 samples for which results of PCR tests were positive were from the isolation facility. Samples from 2 isolation stalls in particular consistently yielded positive PCR test results despite 3 cycles of cleaning and disinfection. Although a positive PCR test result could be indicative of live or dead *Salmonella* organisms, a reservoir of viable organisms became apparent, because the next 3 horses to develop nosocomial salmonellosis had all been initially placed in 1 of these 2 stalls. Results of repeated PCR testing suggested that *Salmonella* organisms had survived in vertical drainpipes. Subsequently, drain plugs were installed at the top of these vertical pipes and leaving the drain bowl filled with a concentrated disinfectant solution was added to the cleaning protocol for all vacated isolation stalls.

Persistence of the organism in the environment despite increased cleaning and disinfection efforts was also supported by analysis of housing records for 2 horses with nosocomial salmonellosis. One of these horses was examined because of poor general condition and chronic intermittent diarrhea; the horse was initially housed in an isolation stall but was moved to a stall in the main hospital after undergoing abdominal surgery. The horse became febrile after surgery and developed profuse diarrhea and was transferred back to the isolation facility. The stall in the main hospital in which this horse was housed was cleaned and disinfected, and results of bacterial culture and PCR testing of environmental samples were negative. However, a healthy research horse housed in the stall for a week subsequently developed nosocomial salmonellosis approximately 4.5 months later. Similarly, 2 horses examined because of colic were initially housed in the same stall in the main hospital approximately 4.5 months apart. Although other horses had occupied this stall in the interim, recovery of *Salmonella* Typhimurium with PFGE patterns 2b and 2g (both likely related to 2a) suggested a common source of infection in the stall. Although none of the horses with nosocomial salmonellosis prior to hospital closure had been housed in this stall, the stall is located at the end of an aisle and could have been contaminated by material hosed from the front of other stalls or from an adjacent stall that had housed horses with nosocomial salmonellosis. Interestingly, the only environmental samples positive for *Salmonella* spp in the 3 years since the hospital reopened were obtained from this stall after the stall had been cleaned. This suggests that a reservoir in the stall was the likely source of colonization for these 2 horses.

The control of this outbreak was likely the result of several factors. First, although not 100% effective, hospital closure and disinfection helped to slow the outbreak. Second, institution of stricter criteria for isolation of hospitalized patients that have been shedding *Salmonella* spp likely helped contain the organism and prevented wider spread through the hospital. Third, other modifications to infection control policies that were instituted likely helped control the spread of the organism. Although some of these changes may have had a minor impact on controlling spread of infectious agents, they were instrumental in heightening infection control efforts and awareness on a daily basis. For example, it became a new policy that any individual entering the stall of a horse hospitalized in the main hospital because of gastrointestinal tract problems had to wear disposable plastic booties. Booties were hung on the stall doors, and despite the inconvenience associated with their use, their presence on the stall door was instrumental in changing the culture of hospital personnel. Finally, the decrease in new nosocomial infections paralleled decreases in caseload and ambient temperature during the winter months.

Documentation that infection with *Salmonella* Typhimurium was nosocomial was initially based on clinical data (ie, development of fever or diarrhea during hospitalization) and recovery of an isolate with an antimicrobial resistance pattern identical to that for the outbreak strain. In addition, in some horses from which *Salmonella* Typhimurium was subsequently isolated, results of bacterial culture of initial fecal samples were negative, again suggesting that salmonellosis was nosocomial.

Two horses in the present report were shedding *Salmonella* Typhimurium resistant to multiple antimicrobials (ampicillin and tetracycline); however, the antimicrobial resistance pattern was different from the pattern for the outbreak strain, suggesting that these horses had naturally acquired, not nosocomial, salmonellosis. Both of these horses were examined because of diarrhea, and *Salmonella* Typhimurium was isolated from the initial fecal samples collected after admission to the hospital.

Although finding an antimicrobial resistance pattern identical to that of the outbreak strain was useful in documenting that salmonellosis was nosocomial in this outbreak and others,[4,5,28] further phenotypic and genotypic analyses of *Salmonella* isolates are often required to support or refute the contention that infection is nosocomial, because antimicrobial resistance can be rapidly exchanged via plasmid transfer.[29] Other techniques that have been used include bacteriophage typing and plasmid profile analysis.[3,4,9,28,30,31] More recently, emphasis has been placed on characterizing bacterial isolates by analysis of chromosomal DNA for phylogenetic classification and comparison to source strains recovered in outbreaks of disease.[32,33] Pulsed-field gel electrophoresis is a relatively simple procedure, in comparison to bacteriophage typing, that separates variably sized fragments of chromosomal DNA after digestion with 1 or more restriction endonucleases.[19,20] Because PFGE provides information about a bacterial strain's genotype rather than phenotype, comparison to source or outbreak strains is more reliable. This technique has been used in a few previous outbreaks of salmonellosis thought to be nosocomial[28,31,33] and is being investigated for use in *Streptococcus equi* outbreaks.[34]

In the outbreak described in the present report, PFGE of *Salmonella* Typhimurium isolates yielded results supportive of nosocomial infection for most affected horses. Three PFGE patterns (1a, 1e, and 2a) were identified for the *Salmonella* Typhimurium isolate recovered from the point-source foal. Possibly, the foal was infected with multiple strains of the pathogen with these distinct PFGE patterns. Alternatively, it is possible that overwhelming septicemia was accompanied by rapid proliferation of *Salmonella* Typhimurium and spontaneous genetic events in situ, leading to development of multiple PFGE patterns. Unfortunately, detection of multiple PFGE patterns for the isolate from the point-source foal complicated the epidemiologic use of PFGE during this outbreak. As a consequence, we conservatively interpreted that all isolates with similar PFGE patterns were likely related, suggesting that infection was nosocomial when supported by clinical data (development of fever or diarrhea after admission). In contrast, results of PFGE supported the assumption that *Salmonella* Typhimurium isolates from 3 horses were naturally acquired.

To our knowledge, this is the first report to document changes in PFGE patterns during the natural course of an outbreak of salmonellosis in horses. Pattern changes were evident between initial isolates and subsequent isolates recovered from 5 horses. All changes could be explained by 1 or 2 genetic events. Detection of these changes provided support for the suggestion that salmonellosis was nosocomial in horses affected with isolates that had closely related PFGE patterns.

At the time of the outbreak, it was unclear as to whether 7 horses examined because of colitis should be interpreted as having nosocomial infections when *Salmonella* Typhimurium isolates with identical or related PFGE patterns were recovered from feces. Exacerbation of clinical disease coupled with negative results for bacterial culture of initial fecal samples supported the contention that salmonellosis was nosocomial in 4 patients. The other 3 did not have an exacerbation of disease, but results of bacterial culture of initial fecal samples were negative for these horses. As in a previous report,[5] these horses with colitis raised the question of whether a multiple-drug resistant *Salmonella* Typhimurium had become endemic in the equine population in the region. However, because *Salmonella* Typhimurium isolates with these PFGE patterns have not been recovered from any other large animal patients examined at the veterinary teaching hospital during the past 3 years, we believe that these horses with colitis had nosocomial infection.

The fact that the organism that caused this outbreak was able to persist in the environment despite hospital closure for extensive cleaning and disinfection was disconcerting. However, a recent survey[35] of French broiler chicken houses found that *Salmonella* spp could be found in 38% of the facilities after depopulation and complete cleaning and disinfection. Terminal disinfection by fogging with formaldehyde- or glutaraldehyde-based disinfectants was associated with the lowest rate of persistence, but even this practice was not 100% effective. We considered fogging the teaching hospital's isolation facility with a formaldehyde-based disinfectant, but that procedure was not pursued because of human and environmental health concerns.

[a]Bacto-Salmonella antisera, DIFCO Laboratories, Detroit, Mich.
[b]Gram-negative identification+ card, bioMérieux Vitek Inc, Hazelwood, Mo.
[c]Diagnostic and Biologic Technologies, San Antonio, Tex.
[d]PFGE, State of Michigan Department of Community Health, Lansing, Mich.
[e]Boehringer-Mannheim, Indianapolis, Ind.
[f]CHEF DRII, Bio-Rad Laboratories, Hercules, Calif.
[g]DNA concatamers, Bio-Rad Laboratories, Hercules, Calif.
[h]Tek-Trol, Bio-Tek Industries Inc, Atlanta, Ga.
[i]Tyvek ProctectiveWear, Isolyser Co Inc, Norcross, Ga.
[j]AgTek MaxiBoot, Kane Enterprises, Sioux Falls, SD.
[k]Wex-cide-128, Wexford Labs Inc, Kirkwood, Mo.

References

1. Dorn CR, Coffman JR, Schmidt-DA, et al. Neutropenia and salmonellosis in hospitalized horses. *J Am Vet Med Assoc* 1975;166:65–67.
2. Carter JD, Hird DW, Farver TB, et al. Salmonellosis in hospitalized horses: seasonality and case fatality rates. *J Am Vet Med Assoc* 1986;188:163–167.
3. Rumschlag HS, Boyce JR. Plasmid profile analysis of salmonellae in a large-animal hospital. *Vet Microbiol* 1987;13:301–311.
4. 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;9:236–241.
5. 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;209:629–631.
6. 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;211:1554–1557.
7. House JK, Mainer-Jaime R, Smith BP, et al. Risk factors for nosocomial *Salmonella* infection among hospitalized horses. *J Am Vet Med Assoc* 1999;214:1511–1516.
8. Baker JR. An outbreak of salmonellosis involving veterinary hospital patients. *Vet Rec* 1969;85:8–10.
9. Bauerfeind R, Wieler LH, Weiss R, et al. Comparative plasmid profile analysis of *Salmonella typhimurium* var. *copenhagen* strains from a *Salmonella* outbreak in hospitalized horses. *Berl Munch Tierarztl Wochenschr* 1992;105:38–42.
10. van Duijkeren E, Sloet van Oldruitenborgh-Oosterbaan MM, Houwers DJ, et al. Equine salmonellosis in a Dutch veterinary teaching hospital. *Vet Rec* 1994;135:248–250.
11. Larsen J, Dolvik NI, Teige J. Acute post-treatment enterocolitis in 13 horses treated in a Norwegian surgical ward. *Acta Vet Scand* 1996;37:203–211.
12. Ravary B, Fecteau G, Higgins R, et al. Prevalence of infections caused by *Salmonella* spp in cattle and horses at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine of the University of Montreal. *Can Vet J* 1998;39:566–572.
13. Hird DW, Pappaioanou M, Smith BP. Case-control study of risk factors associated with isolation of *Salmonella saint paul* in hospitalized horses. *Am J Epidemiol* 1984;120:852–864.
14. Palmer JE, Benson CE, Whitlock RH. *Salmonella* shed by horses with colic. *J Am Vet Med Assoc* 1985;187:256–257.
15. Traub-Dargatz JL, Salman MD, Jones RL. Epidemiologic study of salmonellae shedding in feces of horses and potential risk factors for development of the infection in hospitalized horses. *J Am Vet Med Assoc* 1990;196:1617–1622.
16. Pare J, Carpenter TE, Thurmond MC. Analysis of spatial and temporal clustering of horses with *Salmonella krefeld* in an intensive care unit of a veterinary hospital. *J Am Vet Med Assoc* 1996;209:626–628.
17. Ewart SL, Schott HC, Robison R, 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;218:1145–1151.

18. Quinn PJ, Carter ME, Markey BK, et al. *Clinical veterinary microbiology*. London: Mosby-Year Book Europe Ltd, 1994;95–102.

19. Cohen ND, Wallis DE, Neibergs HL, et al. Detection of *Salmonella enteritides* in equine feces using the polymerase chain reaction and genus-specific oligonucleotide primers. *J Vet Diagn Invest* 1995;7:219–222.

20. Smith CL, Klco SR, Cantor CR. Pulsed-field gel electrophoresis and the technology of large DNA molecules. In Davies K, ed. *Genome analysis: a practical approach*. Oxford: IRL Press, 1988;41–73.

21. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;33:2233–2239.

22. McFarland LV, Mulligan ME, Kwok RYY, et al. Nosocomial acquisition of *Clostridium difficile* infection. *N Engl J Med* 1989;320: 204–210.

23. Murray BE. Vancomycin-resistant enterococcal infections. *N Engl J Med* 2000;342:710–721.

24. Donaldson MT, Palmer JE. Prevalence of *Clostridium perfringens* enterotoxin and *Clostridium difficile* toxin A in feces of horses with diarrhea and colic. *J Am Vet Med Assoc* 1999;215:358–361.

25. Seguin JG, Walker RD, Caron JP, et al. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. *J Clin Microbiol* 1999;37:1459–1463.

26. Dwyer RM. Review of equine *Salmonella* serotypes causing outbreaks of disease: disinfection and management protocols for use in disease outbreaks, in *Proceedings*. 9th Int Cong Anim Hyg 1997;770–772.

27. Walker RL, Madigan JE, Hird DW, et al. An outbreak of equine neonatal salmonellosis. *J Vet Diagn Invest* 1991;3:223–227.

28. Walker RL, de Peralta TL, Villanueva MR, et al. Genotypic and phenotypic analysis of *Salmonella* strains associated with an outbreak of equine neonatal salmonellosis. *Vet Microbiol* 1995;43: 143–150.

29. Ikeda JS, Hirsh DC. Common plasmid encoding resistance to ampicillin, chloramphenicol, gentamicin, and trimethoprim-sulfadiazine in two serotypes of *Salmonella* isolated during an outbreak of equine salmonellosis. *Am J Vet Res* 1985;46:769–773.

30. Sato G, Nakaoka Y, Marufuji, S, et al. A plasmid profile study of *Salmonella typhimurium* strains isolated from horses in Japan. In: Powell DG, ed. *Equine infectious diseases V*. Lexington, Ky: University Press of Kentucky, 1988;223–230.

31. Hartmann FA, West SEH. Utilization of both phenotypic and molecular analyses to investigate an outbreak of multidrug-resistant *Salmonella anatum* in horses. *Can J Vet Res* 1997;61:173–181.

32. Liu SL, Schryvers AB, Sanderson KE, et al. Bacterial phylogenetic clusters revealed by genetic structure. *J Bacteriol* 1999;181: 6747–6755.

33. Hansen LM, Jang SS, Hirsh DC. Use of random fragments of chromosomal DNA to highlight restriction site heterogeneity for fingerprinting isolates of *Salmonella typhimurium* from hospitalized animals. *Am J Vet Res* 1993;54:1648–1652.

34. Lindmark H, Jonsson P, Engvall EO, et al. Pulsed-field gel electrophoresis and distribution of the genes *zag* and *fnz* in isolates of *Streptococcus equi*. *Res Vet Sci* 1999;66:93–99.

35. Rose N, Beaudeau F, Drouin P, et al. Risk factors for *Salmonella* persistence after cleaning and disinfection in French broiler-chicken houses. *Prev Vet Med* 2000;44:9–20.