Beef Cattle Salmonellosis: A Study of Oral *S. typhimurium* and Topical *S. newport* Inoculations

Ed K. Daniels, Neal E. Woollen
James S. Dickson and **E. Travis Littledike**
USDA, ARS
U.S. Meat Animal Research Center
P.O. Box 166, Clay Center, NE 68933

Introduction

Salmonellae are enteric gram-negative facultative intracellular bacteria capable of causing disease in animals and human beings. Most serotypes are not host specific.[1] Serotype typhimurium is considered a major cause of salmonellosis in cattle as well as food poisoning and enteric fever in human beings.[2,3] *Salmonella typhimurium* is not usually noted for producing a carrier state in cattle. However, a cow may have intermittently secreted *S. typhimurium* in her milk for 2 to 5 years.[4]

Cattle are frequently infected with salmonellae by fecal-oral transmission or by being fed contaminated animal protein by-products (40% are contaminated in the United States).[1] Both could propagate salmonellosis in feedlots. Two hundred feeder-calves in Tennessee tested negative for salmonellae at the point of origin and auction market. Upon feedlot entry, 1.5% were salmonella positive and at feedlot exit, 30 days later, 8% were positive[5]. It was suggested that some cattle entering the feedlot were subclinically infected and infection was spread within pens by horizontal transmission. Stress has been incriminated to induce shedding of salmonellae by asymptomatic carriers.[1] Stress factors associated with salmonellosis included: transportation, starvation, changes in ration, overcrowding, age pregnancy, parturition, exertion, anesthesia, surgery, intercurrent disease, and oral treatment with antibiotics and anthelmintics.[2]

In this study, we have attempted to correlate dosage of *S. typhimurium* inoculum with disease, persistence of infection, and environmental contamination. The persistence and spread of *S. newport* placed on the skin of cattle was also studied.

Materials and Methods

Inoculation Procedures

Three groups of 4 steers each were inoculated orally with a nalidixic acid resistant strain of *S. typhimurium*. Groups 1, 2, and 3 were inoculated orally with 4×10^7, 7×10^6, and 1×10^6 colony forming units of *S. typhimurium*, respectively. The inoculum for each steer was suspended in 1 ml of Butterfield's phosphate buffer, place in a gelatin capsule and administered with a balling gun. Ages of the steers were 19 months (group 1), 8 months (group 2), and 12 months (group 3).

Group 2 steers were also inoculated topically with a nalidixic acid resistant strain of *S. newport*. Each hind foot was placed in a plastic bag containing bovine feces inoculated with 5×10^5 salmonellae per gram. Fecal material was smeared up to the mid-metatarsus of both hind legs.

Sampling Procedures

Fecal samples were collected from the rectum and frozen at -20°C. At each sampling, rectal temperatures were recorded and observations of general appearance and clinical signs were noted.

In group 1, fecal samples were collected from 2 calves twice daily for 9 days post inoculation and then necropsied following euthanasia. The remaining 2 animals were sampled twice daily from 1 to 64 days, then once daily from 64 to 103 days, and thereafter once a day 3 days a week (Monday, Wednesday, and Friday) from 103 to 365 days post inoculation.

In group 2, fecal samples were collected once daily for 39 days post inoculation and then once a day 3 days a week to day 109. Rectal mucosa scrapings, using a wooden applicator stick, were collected from this group from day 5 to day 36 post inoculation. Microbiological samples of each foot were taken once a day (Monday through Friday) for 17 days post inoculation and then once a week for 2 additional weeks. Foot samples were collected by scraping and swabbing the hoof walls with a sterile wooden applicator stick and then a piece of sterile gauze moistened with Butterfield's phosphate buffer. On day 21 post inoculation, hair clippings from above the hoof were collected. Blood samples were taken for bacterial culture once a week for 4 weeks.

In group 3, fecal samples were collected once daily for 43 days post inoculation and then once a day 3 days a week to day 68.

Ground samples of the pens, as well as feed and

water samples, were taken once during clinical signs for groups 1 and 3, and 4 times (once a week for the first 4 weeks) for group 2.

Tissue samples were harvested from all steers at necropsy following euthanasia. Sampling included brain, spinal cord, tonsil, various muscles, heart, lung, liver, spleen, kidney, urinary bladder, gall bladder, mesenteric lymph nodes, peritoneal fluid, pericardial fluid, and blood and both tissue and contents of the rumen, omasum, abomasum, duodenum, jejunum, cecum, colon, and rectum. Two steers in group 1 were necropsied 9 days post inoculation and the remaining 2 approximately at 1 year. Group 2 steers were necropsied 125 days post inoculation and group 3 steers were necropsied 80 days post inoculation. Tissue samples were frozen at -70°C.

Microbial Analysis

Approximately 25 g of feces were added to 225 ml of lactose broth and incubated at 37°C for 20 h. From this culture, 0.25 ml were transferred to 10 ml of tetrathionate broth with 10 mg/l brilliant green and incubated at 37°C for 20 h. One ml of the tetrathionate broth culture was then diluted 1:100 in Butterfield's buffer and 1 ml of this solution was filtered through a hydrophobic grid membrane filter (HGMF)*. The filter was then placed on an EF-18 medium plate** and the plate was incubated at 43°C for 20 h. Suspect colonies were restreaked on McConkeys, and then identified by genus and species using the Sensititre Microbiology System***. *S. typhimurium* isolates were also checked for growth in trypticase soy broth containing 0.1 g nalidixic acid per liter to verify compatibility with the inoculum. Salmonella isolates were also sent to the National Veterinary Services Laboratory, Ames, Iowa, for serotyping.

In groups 2 and 3 the HGMF was not used. Instead, 10 µl of the tetrathionate broth culture was streaked directly onto an EF-18 plate. Suspect colonies were isolated and identified, as previously described.

Tissue samples were cut into pieces, approximately 1 cm^3, and macerated in a stomacher, Model 400**** for 1 min. Twenty-five gram samples were incubated in 225 ml of lactose broth at 37°C for 20 h, selectively enriched in tetrathionate broth, and suspect colonies isolated and identified, as previously described. Ground and feed samples were tested by the same protocol. Water samples (25 ml) were mixed with 225 ml lactose broth and analyzed, as previously described.

Foot swab and hair clipping samples were diluted in 10 ml of lactose broth and placed in a stomacher for 1 min. The contents were then placed in a sterile tube and incubated at 37°C for 20 h. Isolation and identification was then conducted using the techniques described above.

Blood was cultured at 37°C for 20 h by adding 10 ml of blood to 100 ml of trypticase soy broth in blood culture bottles.

Results

Group 1

Three steers showed severe clinical signs of diarrhea, elevated rectal temperatures (102 to 104°F) and ataxia by 1 day post inoculation. The marker strain of *S. typhimurium* was found in fecal samples from 2 of the clinically ill steers 1 day post inoculation. The other clinically ill steer shed the marker bacteria on day 2. Fecal shedding of salmonellae persisted for 4 days in 2 of the steers and 6 days in the third. Clinical signs in 2 of the steers increased in severity and euthanasia was necessary on day 9. Salmonellae were never isolated from the feces of the steer showing no clinical signs.

Two steers were necropsied on day 9 and the marker strain of salmonella was found in the distal jejunum of both, the proximal jejunum of 1, and the rectum of the other. *Salmonella infantis* was found in the urinary bladder, a mesenteric lymph node, and the caudal lumbar spinal cord of 1 steer. The other steer had *S. infantis* in jejunal and abomasal contents and the liver. This wild strain salmonella was never isolated from fecal samples during the experiment.

The surviving affected steer developed laminitis, but all other clinical signs gradually decreased during the year. Laminitis was not noted in any other steers (groups 1,2, or 3). At necropsy, tissues and gastrointestinal contents of the 2 surviving steers were salmonella negative.

Group 2

Mild clinical signs of ataxia, slightly elevated rectal temperatures (102 to 103°F), and diarrhea were noted in all steers. Salmonellae were found in the fecal sample of 1 steer on day 4 post inoculation and in the fecal sample of another steer 13 days post inoculation. These were the only positive fecal samples in this group.

Salmonella contamination persisted in the interdigital spaces for 8 days on 1 steer, for 3 days on another, and for 7 days on 2 steers. Attempts to isolate salmonellae by scraping the hoof wall or from clipped hair was unsuccessful. In all 4 animals, the marker

* *ISO-GRID, QA Laboratories, LTD, Toronto, Canada M9C 1C2.*
** *QA Laboratories, LTD. Toronto, Canada M9C 1C2*
*** *Radiometer America, Inc., Westlake, Ohio 44145*
**** *Tekmar, Inc., Cincinnati, Ohio 45222*

strain of salmonella was found on 1 front foot. No positive ground samples were found during the period of foot-to-foot transfer.

Group 3

No clinical signs were observed. At necropsy 3 weeks later, no gross lesions were noted and no salmonellae were isolated from tissues or gastrointestinal contents.

Conclusions

Severity of clinical signs was variable. Severity of disease appeared to be related to the infectious dosage, but individual variability was also observed.

Fecal shedding of salmonellae was not consistent. Individual variability of both onset and duration was observed in groups 1 and 2. The fact that no fecal shedding of salmonellae was observed in group 3 suggests that there is a minimal infectious dose required to induce fecal shedding of salmonella. Long-term persistence of enteric *S. typhimurium* infection with recurrent shedding was not observed. A wild strain of salmonella, *S. infantis,* was recovered from various tissue samples of clinically ill steers but never recovered from fecal samples. This microorganism was not recovered from fecal samples of clinically normal pen-mates.

Ground, feed, and water samplings were not reliable in evaluating fecal shedding of *S. typhimurium* in the cattle pens. In spite of the spread of *S. newport* infection from the interdigital spaces of hindfeet to forefeet, this microorganism was never recovered from ground samples. Even during periods of known fecal shedding, salmonellae could only be recovered from 1 sample of damp soil at the base of a watering unit.

It was demonstrated that active infection of the gastrointestinal tract can be present with no shedding of salmonellae in the feces. This observation suggests that isolation of salmonella from fecal material is a poor indicator of the salmonella infection status of beef cattle. Most of the time, during clinical signs of salmonellosis, we were unable to isolate the organism in rectal samples or rectal mucosal scrapings. It was shown that even if fecal sampling is negative, carcass tissues may be infected with salmonellae and could possibly serve as a potential source of contamination to processing facilities, employees, and consumers.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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