Potential for Clearing *Salmonella* spp. from Beef Cattle for Wholesome Meat Production

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Abstract

Beef products were reported in 1989 to be 1.5% contaminated with salmonellae. They were also reported to have been the cause of 19% of human salmonellosis. Our research goals are directed towards minimizing the amount of salmonellae that enter the abattoir in and on the live steer or heifer. In this discussion, brief overviews of research projects will be presented directed at further characterization of bovine salmonellosis and optimization of diagnosis and preventive therapeutics.

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.

Introduction

Beef products were reported in 1989, by the U.S. Animal Health Association, to be 1.5% contaminated with salmonellae. They also reported that contaminated beef products caused 19% of the cases of human salmonellosis. Managing this zoonosis has multidisciplinary potential extending from management of live cattle to the preparation of properly cooked beef products. Our focus has been on reducing the number of salmonella infected and/or skin contaminated cattle that enter the abattoir for slaughter.

Known sources of infectious salmonellae are carrier animals, contaminated feces, contaminated feed, birds, and rodents.[1] Identifying sources of infection and mechanisms of persistence are integral to controlling salmonella infection in the feedlot. Although salmonellae are thought to persist in cattle primarily through constant fecal-oral transmission, local persistence can also be due to the persistent infection of rodents with environmental and feed contamination from fecal shed salmonellae.[2] There is also new evidence that viable but non-culturable salmonellae can persist in soil.[3] These factors make the identification of risk factors and subsequent control oriented management more challenging.

Identification and control of infectious agents capable of causing epidemic or endemic problems are dependent on the reliability of available diagnostic techniques. Salmonellae are considered a diagnostic challenge. The success of standard microbial culture is dependent on proper enrichment and the use of selective media. Immunologic diagnostic techniques and gene probes are becoming available, but not yet widely used. Identification is also complicated by the fact that a number of cattle can be infected without clinical evidence of disease.

Preventing salmonella infection has been described as extremely difficult and probably a futile effort with respect to nonadapted serotypes.[1] The host adapted bovine serotype is *Salmonella dublin*. Since the salmonellae of greatest zoonotic concern are nonadapted serotypes, clearing beef cattle of salmonellae with respect to wholesome meat production is a formidable challenge. This paper will briefly summarize both completed and ongoing research efforts conducted in our laboratories. For detailed information the reader is encouraged to consult the publications cited for each study.

Brief Methods and Results of Completed Studies

Studies on Bovine Salmonellosis and Detectable Fecal Shedding

Twelve feedlot steers were divided into 3 inoculation groups of 4 steers each and the groups were inoculated with 40,000,000, 7,000,000, or 1,000,000 colony forming units (cfu) of *S. typhimurium* orally. The *S. typhimurium* used in this study was a nalidixic-acid-resistant mutant which allowed us to differentiate between the inoculum and a wild strain of the same species.[4]

In the 40,000,000 cfu inoculation group, 3 steers showed severe clinical signs of diarrhea, elevated rectal temperatures, and ataxia by 1-day post-inoculation. Severity of clinical illness progressed in 2 of the steers to a point, 9-days post-inoculation, that euthanasia and

necropsy were deemed necessary. The third steer showed signs of recovery at this time and was allowed to continue in the experiment without treatment. He developed chronic laminitis, but recovered from all other signs of infection. Fecal shedding of salmonellae was not detected in samples from any steer after 6-days post-inoculation. *S. infantis* was isolated from tissue samples and upper gastrointestinal contents collected from the 2 steers necropsied on day 9. *S. infantis* was never isolated from fecal samples. The 2 surviving steers remained healthy with no evidence of recurrent fecal salmonella shedding and gained weight normally for the remaining year of observation. At 1-year post-inoculation, the steers were necropsied and no salmonellae were cultured from either tissue or intestinal content samples.[4]

In the 7,000,000 cfu inoculation group, clinical signs similar to but milder than those described for the previous group were observed in all steers. Inoculum salmonellae were cultured from 1 fecal sample on day 4 and from 1 fecal sample from another steer on day 13. All steers recovered from clinical disease. Following euthanasia, at 125-days post-inoculation, all steers were necropsied. No salmonellae were cultured from either tissue or intestinal content samples collected at necropsy.[4]

In the 1,000,000 cfu inoculation group, no clinical signs were observed and no salmonellae were cultured from fecal samples. Following euthanasia, at 80-days post-inoculation, all steers were necropsied. No salmonellae were cultured from either tissue or intestinal content samples collected at necropsy.[4]

Study of Persistence and Transfer Following Salmonella Contamination of the Bovine Hindlimb

Steers in the 7,000,000 cfu inoculation group described above were used in this study. At the time of oral inoculation, we also immersed both hindlimbs to the mid-metatarsus in salmonella contaminated bovine feces. The inoculum for this study was fresh bovine feces mixed with 500,000 cfu of nalidixic-acid-resistant *S. newport* per gram of feces.[4]

Culturable levels of salmonellae persisted in the interdigital spaces for a maximum of 8 days. Salmonellae could not be cultured from either the hoof wall or distal limb skin scrapings. Inoculum salmonellae were cultured from the interdigital space of at least 1 forelimb from each steer during the period of organism viability. During the time period of limb to limb transfer, salmonellae could not be cultured from ground samples.[4]

Diagnostic Potential of Frozen and Chilled Samples

Fresh bovine feces were inoculated with 1,000,000 cfu of *S. typhimurium* per gram of feces. The inoculated feces were then divided into 10 gram aliquots and equal numbers were frozen at either -20 or -70°C for periods of 1, 2, 7, 14, or 28 days. Samples were then thawed and viability counts were conducted on each of these days.[5]

There was an approximate 85% reduction at both storage temperatures after 1 day of storage. For all sample-storage times greater than 1 day, viability in samples stored at -20°C was significantly less than those stored at -70°C. Following the initial loss in viability, there was no significant change in viability with prolonged storage at -70°C. The samples stored at -20°C showed a continual loss of viability with prolonged storage.[5]

Fresh ground beef of 2 different leanness values, 80 and 95%, were inoculated with 1,000,000 cfu of *S. typhimurium* per gram of ground beef. The 2 types of inoculated ground beef were then divided into 10 gram aliquots and equal numbers were placed in storage at either 4 or -20°C for periods of 1, 3, 7, 14, or 28 days. Samples were then brought to room temperature and viability counts were conducted on each of these days (unpublished data).

With the slight exception of 2 values at 3 days, all samples maintained at least 50% viability through 7 days of storage. Contaminant bacteria became a problem in both tissue groups held at 4°C, but the problem manifested itself sooner, 14 days versus 28 days, in the samples containing more fat. However, salmonellae in the fattier samples appeared to be better protected from freezing damage. Only 3 time-temperature groups in the 80% lean beef group showed significantly reduced viability in comparison to 7 in the 95% lean beef group. For diagnostic purposes, where prolonged storage is necessary, the 28-day values showed that freezing samples will yield results far superior to refrigeration (unpublished data).

Salmonella Diagnostic Techniques

Bovine feces inoculated with S. typhimurium were used to evaluate several protocols recommended for culturing salmonellae. Buffered peptone water (BPW) and tetrathionate broth (TTB) were compared as pre-enrichment media variables. Rappaport's media enrichment was compared to no enrichment. Final culture media comparisons were conducted using brilliant green agar with .008% sulfadiazine (BGS), brilliant green agar-sulpha-mandelate (BGM), brilliant green agar-novobiocin (BGN), EF-18, and XLT-4 (unpublished data).

Tetrathionate broth was found to be superior to BPW as a pre-enrichment media. Enrichment with Rappaport's media significantly improved salmonella recovery over techniques where it was not used. Brilliant green agar-novobiocin and BGS were the poorest performing final culture media, with EF-18 and XLT-4 showing modest superiority over BGM (unpublished data).

Brief Methods and Preliminary Results of Ongoing Studies

Salmonella Diagnostic Techniques

A structural protein unique to salmonellae has been identified and used for the production of a monospecific polyclonal antibody and a monospecific monoclonal antibody. The antibodies have been used in enzyme linked immunosorbent assay testing of various bacteria.

No cross-reactivity has been found with bacteria from the genera *Citrobacter, Escherichia, Serratia, Klebsiella, Enterobacter, Shigella, Pseudomonas, Listeria, and Acinetobacter.* Reactivity has been demonstrated with all salmonellae tested so far. They include: *S. typhimurium, S. newport, S. dublin, S. enteritidis, and S. choleraesuis.*

Salmonella Vaccine Trial

In this study, we divided 50 sheep into 5 treatment groups, all were inoculated orally with 20,000,000 cfu of *S. typhimurium*, with half of each group being necropsied at 1-week post-inoculation and half at 2-weeks post-inoculation. The treatment groups included: non-vaccinated controls, adjuvant- vaccinated controls, heat-killed *S. typhimurium* vaccinated animals, phenol attenuated- live *S. typhimurium* vaccinated animals, and *S. typhimurium*-purified structural-protein subunit vaccinated animals. Data has been collected from all groups, except the subunit vaccine group. The protein subunit used in this vaccine was the same as that used to produce the diagnostic antibodies described above. Preliminary results in mice show that it stimulates antibody production reactive with several species of salmonellae.

Twenty million cfu of *S. typhimurium* produced marked enteritis as well as septicemia in non-vaccinated sheep. There was no protection from either enteritis or septicemia noted in either the adjuvant- or the killed-vaccine group. Culture of salmonellae in the attenuated live-vaccine group was confined to the intestine and mesenteric lymph nodes, with reduced incidence from the 1 week to the 2 week necropsy groups. Gross pathological lesions in this group were mild to not perceptible.

Discussion

The potential for clearing beef cattle of salmonellae for wholesome meat production will be dependent on the ability to accurately diagnose salmonellae with increased sensitivity. Most of the current diagnostic techniques rely on critical levels of viable organisms to be present in samples that are submitted for testing. Sample type and handling are well recognized variables in diagnostic success. We have re-emphasized with our studies the importance of fresh sample testing and also the need for diagnostic tests with increased sensitivity. Although we have shown that prolonged sample storage adversely affects salmonella viability, we have also demonstrated that options are available to minimize the deleterious affects. In the realm of meat-quality assurance, diagnostic techniques will be required to detect organisms in numbers below the threshold of disease. We feel that diagnostic antibodies directed against highly antigenic subunits is a step in that direction. Further characterization of these subunits may eventually lead to tests of even greater sensitivity.

Salmonella disease management is quite effectively handled through maintenance of a clean environment. Once again, in the realm of meat-quality assurance we need to look beyond the management of disease and concentrate on the control of infection. The oral-inoculation studies demonstrated that the feedlot-age steer is capable of consuming 1,000,000 infectious salmonellae and show no clinical signs or detectable shedding of organisms in the feces. Our findings of natural *S. infantis* infection in the experimentally infected steers points out that apparently normal individuals can harbor even nonadapted infectious salmonellae. The oral-inoculation studies also pointout the individual variability that can exist between similarly infected steers. With all the existing possible sources of infection, we need to develop safe efficacious methods to control the colonization of salmonellae in the beef-producing animal when sanitation fails to prevent their exposure. We are hopeful that subunit vaccines will offer that protection. The protein subunit we are currently studying shows promise. If it offers good protection, experiments directed at methods of delivery capable of stimulating mucosal and cell-mediated immunity will be conducted.

Management of hide contamination will be difficult. We have shown that salmonellae can be transferred from foot-to-foot and probably animal-to-animal even when the bacteria is not culturable from the environment. Our current approach to this problem is to reduce the level of fecal shed salmonellae in a herd through enhanced control of intestinal colonization in the individual animal.

References

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