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EXPLORATORY EVALUATION OF *SALMONELLA* PREVALENCE IN SYNOVIAL FLUID AND BONE MARROW FROM TRADITIONAL FEEDLOT CATTLE

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I. INTRODUCTION

Ground beef has continued to be linked to outbreaks of salmonellosis, and from 2012 to 2019, was linked to 87% of the *Salmonella* illnesses implicated from beef [1]. Previous research has identified lymphatic tissue, more specifically lymph nodes, as reservoirs in beef for *Salmonella* [2]. These areas are a food safety concern as *Salmonella* may survive postmortem antimicrobial interventions put in place during harvest and processing. As synovial fluid (SF) and bone marrow (BM) are additional components of the lymphatic system that would not be exposed to antimicrobial interventions during harvest, exposure of lean trim to these mediums may contaminate ground beef with *Salmonella*. Therefore, this study was designed to explore the prevalence of *Salmonella* in SF and BM of post-mortem traditional feedlot cattle. The objectives of this study were to evaluate the prevalence of *Salmonella* found in beef synovial fluid exposed during beef harvest and fabrication processes and the presence of *Salmonella* found in beef bone marrow.

II. MATERIALS AND METHODS

Synovial fluid (SF; *n* = 190/collection) and bone marrow (BM; *n* = 190) samples were collected from a commercial beef processing facility in Texas (USA). For collection 1, SF was collected from one stifle joint of beef carcasses and transported to the Texas A&M University Food Microbiology Laboratory (College Station, TX, USA). Upon arrival, samples were enriched in a 1:1 ratio of TSB, incubated at 37 °C for 48 hours, and centrifuged at 5000 rpm for 30 minutes. Supernatant was disposed, pellets were re-enriched with 1 mL of TSB, mixed, incubated for an additional 48 hours at 37 °C, and PCR analysis was completed using the Hygenia BAX Q7 system and for *Salmonella* Real-Time PCR Assay (Hygiena, LLC, Camarillo, CA, USA). For BM sampling, frozen, center cut femurs were transported on ice to the Texas A&M University Meat Science Laboratory (College Station, TX, USA). Prior to BM extraction, femurs were thawed, flame sterilized with a 70% ethanol solution, and the first 0.6 cm of BM from each end was aseptically removed. Using a sterile spatula, ~25 g of BM were removed from the diaphysis of the femur and placed into sterile Whirl Pack (Pleasant Prairie, Wisconsin, USA) bags. BM samples were enriched in a 3:1 ratio with TSB, massaged by hand for 60 seconds, and incubated for 48 hours at 37 °C. Samples were then prepared for qualitative confirmation and PCR analysis utilizing the Hygenia BAX Q7 system. SF and BM PCR analyses were run with positive and negative controls. To further isolate and quantify *Salmonella* from SF, an additional collection was completed. The sampling protocol was amended so that each sample (*n* = 190) consisted of SF from both stifle joints of the carcass compositely to increase sample volume to approximately 8 mL. Upon arrival to the Food Microbiology Laboratory, approximately 4 mL of SF from each sample was frozen (-65 °C) to await further isolation and quantification upon a positive PCR result, while the additional 4 mL underwent the same enrichment methodology used for PCR analysis in collection 1. Frozen sample pairs resulting in a positive PCR were thawed, pre-enriched in a 9:1 ratio of TSB, and diluted following the most probable number (MPN) guidelines under MLG Appendix 2.05 [3]. Diluted samples were incubated 42 °C for 15 to 24 hours, isolated for *Salmonella,* and positive colonies were selected following MLG 4.14 [4]. Positive colonies were stabbed into the butts of both Triple Sugar Iron (TSI; Hardy Diagnostics, Santa Maria, CA, USA) and Lysine Iron Agar (LIA; Hardy Diagnostics, Santa Maria, CA, USA) slants using the same sterile needle. Slants were incubated for 18 - 24 hours at 35°C and screened as a set presence of *Salmonella spp*. Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and MPN values from MLG Appendix 2.05 Table 2 were used to report prevalence and quantitative data by sample type and collection trip.

III. RESULTS AND DISCUSSION

Synovial fluid (SF) sample analysis resulted in prevalences of 50.53% (96/190) and 2.11% (4/190) for collections 1 and 2, respectively. Due to sufficient sampling size in collection 2, further quantification of *Salmonella* PCR positives (*n* = 4) was done following the MPN method. No plates displayed viable colonies for biochemical confirmation, determining that all four samples contained less than 0.3MPN/mL. As the Hygenia BAX Q7 system detects presence of *Salmonella* DNA, this low level of detection on plates could indicate that the bacteria present were either dead or viable non-culturable. In a bi-phasic SF study in swine, *Salmonella* prevalences of 0 and 2.63% were reported [5] whereas in a study of Holstein calves, 3.3% of SF samples were positive for *Salmonella* [6]. The

swine and calves of the studies were inoculated with *Salmonella*, as opposed to the current study where we surveyed the inherent presence post-harvest in commercial feedlot cattle. Of 190 BM samples, two tested positive (1.05%) for *Salmonella* via PCR analysis, but were not further analyzed for enumeration. As BM is part of the lymphatic and circulatory systems, it would be logical to conclude that systemic *Salmonella* infection could lead to this matrix harboring the bacterium. In *Salmonella* challenged birds, varying rates of *Salmonella* were present in BM for only a short period of time during the related infection [7]. Work in human models exploring the pathology of *Salmonella* and BM infection concluded that *Salmonella* can survive in lymphatic B cells, formed within BM [8]. Furthermore, BM may shelter *Salmonella*, as B cells are not able to leave the BM until they are fully mature [9]. Yet, a study in 1983 reported that when inoculated, beef BM had a bacteriostatic effect on *Salmonella* Anatum [10]. This suggests low BM bacterial counts in the current study may be associated with this effect. While the results of this study show that *Salmonella* can be present in the SF and BM of traditional feedlot cattle, the level at which *Salmonella* was quantified in SF was very low.

Table 1. Enrichment positives for *Salmonella* in beef synovial fluid and bone marrow samples

Sample Type	Number Examined	Number Enrichment Positive (%)[1]
Synovial Fluid		
Collection 1	190	50.5
Collection 2	190	2.1
Bone Marrow	190	1.1

[1] % Enrichment positive calculated as the ratio of enrichment positive divided by the number of samples examined (*n*)

CONCLUSION

Overall, findings of this study support that *Salmonella* can be inherently present in both SF and BM of US feedlot cattle, identifying both mediums as potential vectors of the bacteria. However, positive samples were not able to be enumerated through further serological testing. Findings support the importance of continued research focusing on *Salmonella* and its presence/pathology in the lymphatic system of feedlot cattle. Results of this study could aid in development of additional or alternative food safety control measures, thereby lessening the risk of foodborne salmonellosis.

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