Project Title: Collaborator I - Investigation into the Origin of *Salmonella* in the Peripheral Lymph Nodes of Fed Beef Cattle at Slaughter in the Southwestern United States

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Completion Date: June 2017

Background

Salmonella can often be recovered from the peripheral lymph nodes (PLNs) of cattle at harvest. When contained within the PLNs, this foodborne pathogen is protected from currently used post-harvest intervention strategies, and therefore represents a potential insidious contaminant, particularly of ground beef. Pre-harvest interventions will need to be developed and tested that will either prevent *Salmonella* uptake by PLNs and/or eliminate the bacterium from the nodes.

The objectives of the study were to 1) develop a methodology that allows the study of the dynamics of bacterial growth, dissemination and survival in feedlot animals, at a resolution beyond the total bacterial load, 2) to determine, in unprecedented detail, the population dynamics of *Salmonella* colonization, in feces and during transit of the bacteria to the cattle lymph nodes and while resident in the PLNs, 3) to monitor transmission of *Salmonella* between cattle, and 4) to determine if protective immune responses are initiated following *Salmonella* uptake by the nodes.

Methodology

A combination of an innovative bacterial transdermal delivery method and DNA-based molecular barcoding strategies was used to study how *Salmonella* travels from different entry sites within and between infected animals, and whether the bacterium can flourish within animal sites other than the gut. Differently barcoded clones of the same *Salmonella* strains from multiple serovars were simultaneously delivered orally and through the skin.

Findings

Using a combination of a transdermal inoculation technique and a molecular barcoding technology, the dynamics of *Salmonella* colonization of feedlot cattle were studied after both oral and transdermal inoculation. Using these routes of delivery, infection was readily established in cattle guts and peripheral lymph nodes, and bacterial founder population sizes for gut and lymph node infections were determined to be in the hundreds and low tens, respectively. The use of individually barcoded clones allowed these estimates to be obtained with a minimal number of animals and would facilitate straightforward surveys of the effect of pre-harvest intervention methods on protection of the animals from *Salmonella*.

The *Salmonella* transmission paths were characterized to the peripheral lymph nodes, within the gut environment and between animals after both oral and transdermal delivery of the bacteria. In most cases, bacteria drained from transdermal injection sites into the expected peripheral lymph node. However, subiliac /prefemoral nodes also often harbored bacteria that had been delivered to the rear legs. In addition, PLNs also often harbored orally delivered bacterial

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clones, while some transdermally delivered isolates were also present in the animals' gut environment.

While the total bacterial numbers in the peripheral lymph nodes remained low, evidence of bacterial replication of transdermally delivered *Salmonella* was found, after delivery, prior to arrival at the node and/or intra-node. We were unable to distinguish between these possibilities, and further experiments will be needed to establish whether the bacteria can, and do, replicate inside of the PLNs, and at what frequency.

Implications

This work accomplished several goals including verification of a technique useful for measuring the effectiveness of pre- harvest intervention strategies, elucidation of the transmission routes of bacteria inside steer, determination of the scale of the founder population sizes for bacterial colonies in different animal environments, and evidence of replication of bacteria after transdermal inoculation. Observations made during the course of our experiments suggest inter-animal bacterial transmission occurs not only via the feco-oral route, but also includes uptake via skin lesions or oral contact with animal or pen surfaces. Verification of the frequency of these novel transmission routes in further studies would be helpful to assess the risk of bacterial spread throughout cattle in the farms and feedlots

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