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

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Completion Date: April 2017

Background

Salmonella enterica is a foodborne pathogen, which inhabits and colonizes a large variety of hosts and environments. Recent studies (Gragg, Loneragan, Brashears, et al. 2013; Gragg, Loneragan, Nightingale, et al. 2013) have high- lighted the presence of *Salmonella* in bovine peripheral lymph nodes, which may be present in lymph nodes in meat used to produce ground beef and therefore become a source of food contamination. In this study, whole genome sequencing (WGS) was used to characterize *Salmonella* isolates obtained from 5 animals at 5 different sites (hide, spleen, mesenteric lymph node, left sub-iliac lymph node and right sub-iliac lymph node) at slaughter to understand dispersal with the animals. Knowledge about the origin of *Salmonella* in peripheral lymph nodes will provide insight into the ecology of *Salmonella* within cohorts of cattle and offer direction for intervention opportunities.

This study was developed around 2 main objectives: (1) characterization of *Salmonella* by whole genome sequencing of fed beef cattle at slaughter, and (2) to use WGS data to infer the origin of *Salmonella* in peripheral lymph nodes of cattle.

Methodology

335 strains isolated from hide, feces and peripheral lymph nodes of cattle were sequenced using sequencing by synthesis technology. To do so, each strain was grown individually on brain-heart infusion (BHI) agar. A single colony was used to inoculate 5mL of BHI and incubated overnight at 37°C with shaking. Bacteria from 1mL of overnight culture were harvested and genomic DNA was extracted using the GenElute Bacterial Genomic DNA kit (Sigma Aldrich) following manufacturer's recommendations. All the gDNA extractions were quantified using fluorimetric quantification (Qubit, Thermofisher) and their concentration was adjusted to 0.20ng/mL. Then, libraries were prepared for each strain using 1ng of gDNA and were processed using the XT Nextera kits following manufacturer's recommendations (Illumina).

Libraries were then pooled, quantified, and sequenced on 2x75bp cartridges (Illumina) on a MiSeq sequencer. Raw sequence data were used directly to predict *Salmonella* serotype using SeqSero pipeline (Zhang et al. 2015) and SNP trees were inferred as outlined in Den Bakker et al. (Bakker et al. 2014).

Findings

The results of the study showed extreme genomic homogeneity of *Salmonella* between animals and within animals. This suggests rapid between and within animal dispersal of

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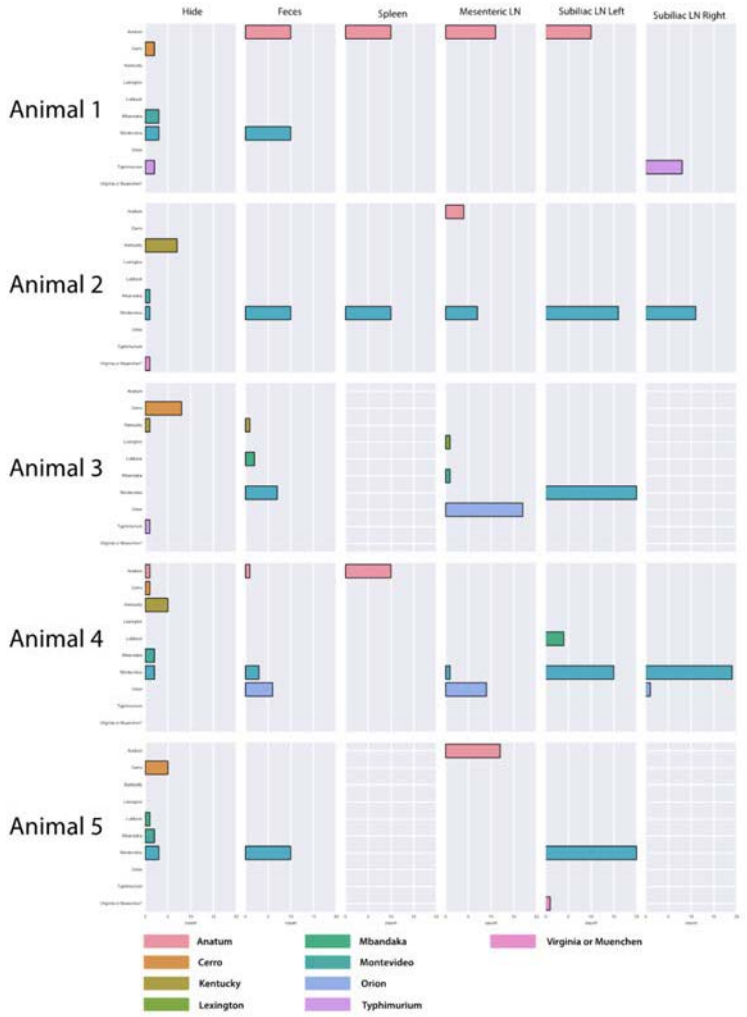
Salmonella strains but makes it difficult to infer within animal directionality of dispersal.

Implications

Knowledge of the origin of *Salmonella* in peripheral lymph nodes could help offer direction for intervention opportunities. The research presented here shows WGS cannot be used to directly infer the origin of *Salmonella* in peripheral lymph nodes.



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