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### Neutral Barcoding of Genomes Reveals the Dynamics of *Salmonella* Colonization in Cattle and their Peripheral Lymph Nodes

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#### Abstract

Feedlot cattle often contain *Salmonella*. The number of bacteria that initiate colonization of different cattle organs and the bacterial migration within these large animals are poorly understood. To investigate these questions, we constructed wild-type isogenic tagged strains (WITS) of *Salmonella* by inserting 21-base barcodes flanked by Illumina sequencing primers into a neutral genome location. We then delivered several different pools of uniquely barcoded clones orally and into multiple intradermal sites, in individual Holstein steers, and subsequently performed *Salmonella*-directed sequence tag-based analysis of microbial populations (STAMP). Using high-throughput sequencing of the barcodes of *Salmonella* grown from steer lymph nodes, organs and feces, we monitored how individual barcoded clones travel from different entry sites within animals. Data showed that gastrointestinal colonization was established by up to hundreds of *Salmonella* founder cells, whereas peripheral lymph nodes were usually colonized by very low numbers of founding bacteria, often originating from the nearest draining intradermal delivery site. Transmission of *Salmonella* from the gastrointestinal tract to the lymphatic system was frequently observed, whereas entry of intradermally delivered bacteria into the gut was rare. Bacteria undergo limited extraintestinal proliferation within or prior to arrival at peripheral lymph nodes. Overall, the application of the STAMP technique facilitated characterization of the migration routes and founder population size of *Salmonella* within feedlot cattle and their organs and lymph nodes in unprecedented detail.

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