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Project Title: Phenotypic and Genomic Characterization of the Newly Discovered Serotype Lubbock of *Salmonella enterica*

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Objective: To probe genomic and phenotypic characteristics of *Salmonella* Lubbock and ancestors, *S. Montevideo* and *S. Mbandaka*, in order to elucidate the role of different routes of infection of bovine lymph nodes.

Experimental Design & Analysis:

Whole genome sequencing of 30 *S. Montevideo*, 20 *S. Mbandaka* and 32 *S. Lubbock* isolates recovered from bovine lymph nodes or feces, was performed. Sequences were assembled using SPAdes, and annotated using RAST and Prokka pipelines. Representative *Salmonella* isolates were tested for their ability to survive and multiply in bovine macrophages, and their toxicity to bovine macrophages and *Caenorhabditis elegans* nematodes.

Key Results:

The genome sequence analysis revealed that the newly described *S. Lubbock* serotype emerged due to homologous recombination in the *fliC* operon, encoding the flagellar protein of the first flagellar phase, between a *S. Montevideo* donor and into a *S. Mbandaka* recipient. Two *S. Lubbock* lineages were found among the 32 investigated *S. Lubbock* isolates, each characterized by a different recombination event in the *fliC* operon.

Macrophage assays demonstrated that *S. Lubbock* and *Mbandaka* show similar phenotypes with regard to uptake, intracellular survival, growth and cytotoxicity, while *S. Montevideo* differs from these two serotypes. For *S. Montevideo* numbers of intracellular bacteria remained

stable during a 72h-test period, while numbers of intracellular bacteria of *S. Mbandaka* and Lubbock within macrophages increased 24h post-infection. Furthermore, *S. Mbandaka* and *S. Lubbock* are less deleterious to bovine macrophages than *S. Montevideo*. Phenotypic similarities between *S. Mbandaka* and Lubbock are not surprising as these two serotypes are genomically very similar, with the exception of the *fliC* operon.

A difference in toxicity in *C. elegans* nematodes was observed for *S. Mbandaka* and Lubbock. Both *S. Montevideo* and Lubbock display similar 50% killing values after 150 hours (6 days and 6 hours) and 146 hours (6 days and 2 hours), while, *S. Mbandaka* shows a 50% killing value after 186 hours (7 days and 18 hours). Both *S. Montevideo* and *S. Lubbock* are the two most common *Salmonella* serotypes isolated from bovine lymph nodes, while *S. Mbandaka* is rarely encountered. The similarly high deleterious effect of *S. Montevideo* and *S. Lubbock* in comparison to *S. Mbandaka* suggests that nematodes such as *C. elegans* might not represent a vector for the colonization of bovine lymph nodes by *Salmonella*.

How can this information be applied in the industry?

Increasing the understanding of the routes in the colonization of food animals provides new insights in the biology of *Salmonella*. This knowledge can be used to alter critical points in the management of feedlots and processing facilities to minimize the *Salmonella* burden in cattle and impact public health.