Integrative Metagenomic and Metatranscriptomic Profiling of *Salmonella* and Microbial Communities in Fecal and Environmental Holding Pond Samples from Cattle Feedlots

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INTRODUCTION

- High incidence of ***Salmonella***-related foodborne illnesses
  - Continuous shedding into the environment by affected animals through **fecal waste**
- Use of **AMD** in livestock operations
- Runoff management in feedlots
  - **Holding ponds**
- **Aquatic agricultural environments**
  - Composition and function of *Salmonella* populations, overall **microbial communities, AMR and virulence gene pools, functional profiling**.
- Use of **metatranscriptomics**
  - Genetic potential vs metabolically active members

OBJECTIVES

- Characterize the **diversity and composition of *Salmonella*** populations in holding ponds and feces from nearby cattle pens
- Conduct **taxonomic, antimicrobial resistance (AMR), virulence factor, and functional profiling** on both fecal and pond samples.
- Compare the **genetic potential** of microbial populations (passive metagenome) to their **metabolically active** members (active metatranscriptome).

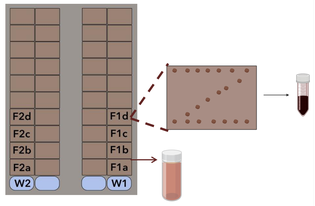
METHODS

Sample Collection

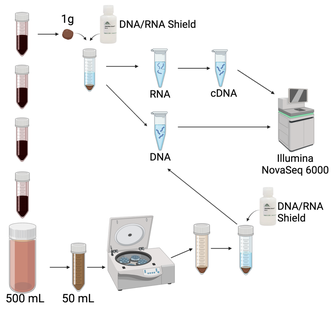
- Sampled 5 feedlots in the Texas Panhandle
- Holding pond water (n = 13)
- Fecal samples from 4 nearby pens (n = 48)

Sample Processing

- Samples were divided into 2 aliquots:
  - *Salmonella* testing
  - Shotgun metagenomic sequencing
- Salmonella detection:
  - Culture + PCR
  - Serovar identification (CRISPR-SeroSeq)



- RNA and DNA were co-isolated using the RNeasy PowerSoil Total RNA Kit and DNA Elution Kit (Qiagen)
  - RNA was reverse transcribed into cDNA (qScript cDNA Synthesis Kit, Quantabio)
- Shotgun metagenomic (n = 60) and metatranscriptomic (n = 60) sequencing libraries were prepared using the Illumina DNA Prep Kit
- Illumina NovaSeq 6000 platform



Bioinformatics

- Kraken2: Taxonomic classification
- AMR++/MEGARes: AMR gene pools
- HUMAnN3: Functional profiling
- VFDB BLAST-based alignment: Virulence factor genes

Statistical Analysis

- All data was analyzed in R version 4.5.0
- The R packages phyloseq, microbiome, vegan, metagMisc, ANCOM-BC, and MaAsLin3 were used to analyze, visualize, and compare microbial diversity and composition

RESULTS

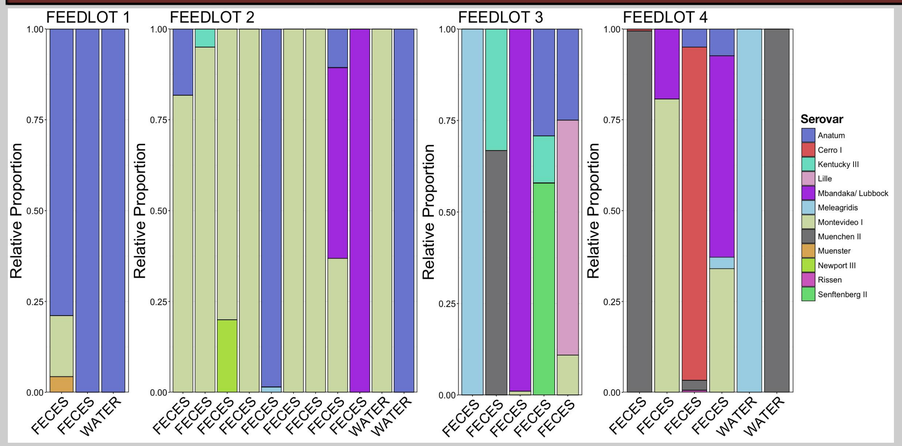


Figure 1. Overall, 12 *Salmonella* serovars were detected, and most of the samples (88%) contained a serovar of concern to animal health. While most of the culture-positive fecal samples contained more than 1 *Salmonella* serovar (70%), only 1 serovar was detected in all the culture-positive lagoon samples.

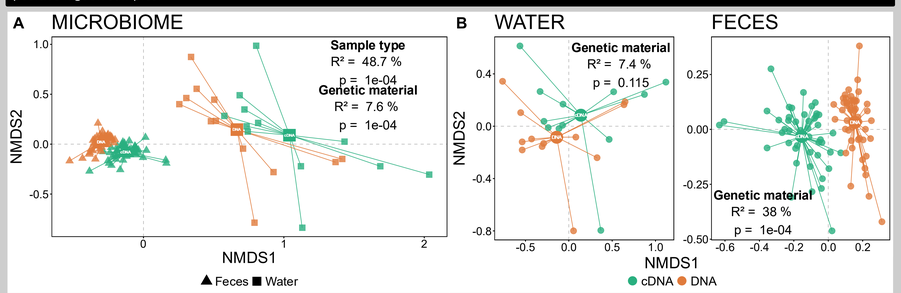


Figure 2. A) Both **sample type** (pond vs fecal samples) and **genetic material** (DNA vs RNA/cDNA) influenced the overall **microbial community composition**. **B)** Fecal samples showed distinct clustering based on genetic material. While a similar trend was observed in pond samples, this clustering was not statistically significant. Ordination is based on Bray-Curtis distances.

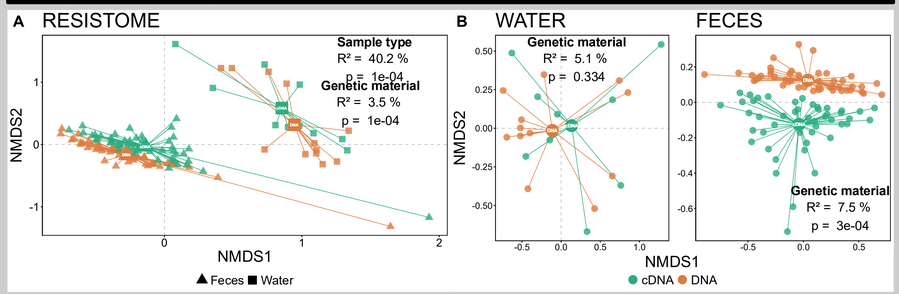


Figure 3. A) Both **sample type** (pond vs fecal samples) and **genetic material** (DNA vs RNA/cDNA) influenced the overall **AMR gene pool** structure. **B)** Fecal samples showed distinct clustering based on genetic material. While a similar trend was observed in pond samples, this clustering was not statistically significant. Ordination is based on Bray-Curtis distances.

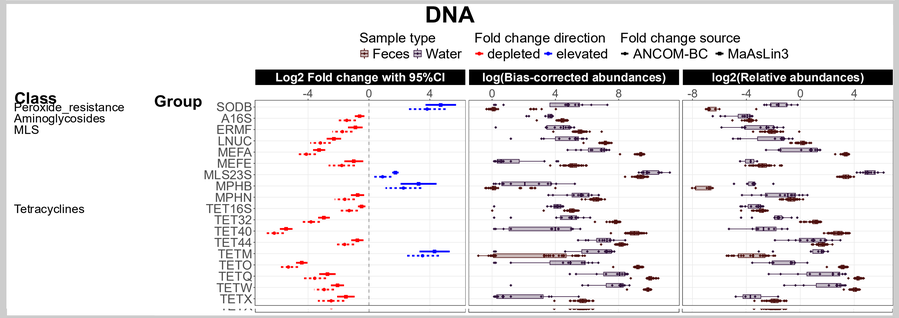


Figure 4. For the passive communities (DNA), several **AMR gene groups** showed significant **differences in abundance** between fecal and pond samples as calculated by both ANCOM-BC and MaAsLin3.

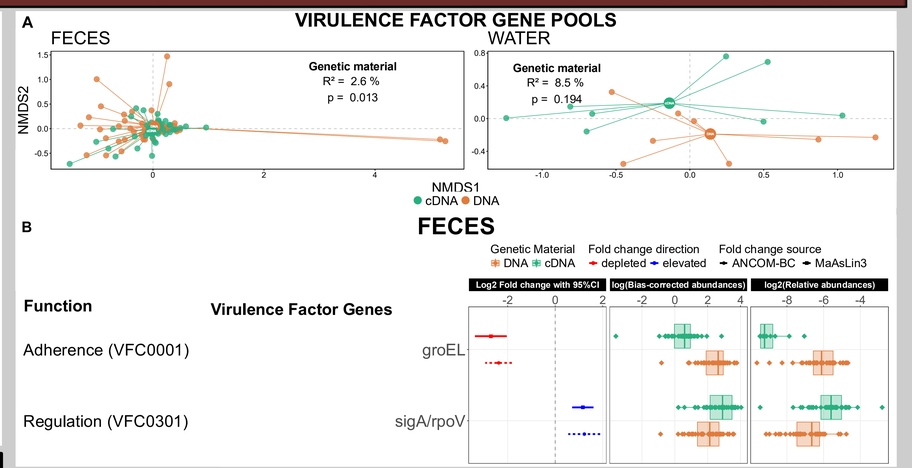


Figure 5. A) Only **genetic material** (DNA vs RNA/cDNA) influenced the overall **virulence factor gene pool** structure. Ordination is based on Bray-Curtis distances. **B)** Virulence factors related to adherence and regulation of expression were differentially abundant between DNA (passive) and RNA/cDNA (active) in fecal samples.

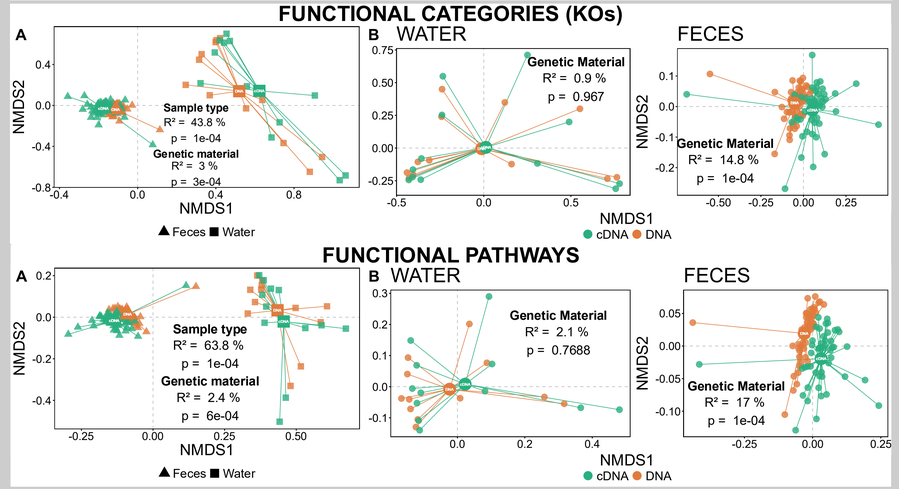


Figure 6. A) Sample type (pond vs fecal samples) influenced the overall **functional profiles**. **B)** However, genetic material (DNA vs RNA/cDNA) only significantly influenced the functional profile of fecal samples. Ordination is based on Bray-Curtis distances.

CONCLUSIONS

- *Salmonella* can **persist** in feedlot holding ponds.
- The serovar-level diversity of *Salmonella* in holding ponds did not reflect that observed in fecal samples.
- Fecal and aquatic communities share little similarity in community composition, AMR/virulence gene pools, and functional profiles
- Large differences between active and passive fecal communities (microbiome, resistome, virulence factors, functional profiles).

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