Dissecting *Salmonella* Serotype Patterns: A Comparative Study Between Poultry Industry Sources and Clinical Samples in the PulseNet Database

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INTRODUCTION

Salmonella serotyping is an important element of foodborne pathogen surveillance and risk-based mitigation strategies. Existing methods such as whole genome sequencing (WGS) rely on isolates and a one-sample, one-serotype approach; however, for poultry samples this introduces a risk for misidentifying clinically important serotypes co-present with less pathogenic serotypes. This study sets out to compare serotype data generated from poultry sample enrichments (Poultry Production) with clinical WGS serotype data (Isolate).

METHODS

Isolate: Dataset Preparation

Clinical isolate WGS serotype data from the PulseNet database (n = 174,053, NCBI Bioproject: PRJNA230403) was downloaded from the NCBI Pathogen Detection Isolate Browser (https://www.ncbi.nlm.nih.gov/pathogens/isolates) on 2024-01-07. The SRA submission date was treated as the primary date annotation. Any isolate without serotyping results was excluded.

Poultry Production: Dataset Preparation

Amplicon sequencing data was collected from various poultry production facilities across the U.S. (n = 83,899), between January 2020 and December 2023. Serotyping data was annotated using the Clear Safety™ *Salmonella* bioinformatics pipeline. This pipeline supports identification of the 63 most common serotypes observed in the U.S and is capable of identifying co-infections (up to 2 different serotypes) for a subset of serotypes with distinguishing genomic patterns. Serotype annotations for co-infections were treated as a separate group from single infections.

Poultry Production: Co-infection Analysis

Serotype prevalence rates were compared over the 4-year time period to understand how single serotype vs co-infection of multiple serotypes rates change over time (Figures 1,3,4,5). The frequencies of various co-present serotypes were compared to determine whether any particular serotype was more or less frequently observed. (Figure 2).

### Isolate vs. Poultry Production: Dataset Comparison

The top 10 most frequent single infection serotypes from the Poultry Production dataset were compared against their frequencies in the clinical isolate dataset (Figure 6). Serotype prevalence rates were compared over the 4-year time period to understand if any relationship existed between co-infections and single-infections. Individual serotype prevalences were compiled over annual quarters for both datasets. Prevalence

Isolate vs. Poultry Production: Dataset Comparison (cont.)

rates for Kentucky, Typhimurium, Infantis, and Enteritidis across both datasets were compared on a quarter-year basis between both datasets. To understand whether there was a correlation where higher or lower prevalence rates in one dataset correspond consistently to higher or lower rates in the other dataset for the same serotypes, a Spearman's correlation analysis was performed on these pairings (Table 1). Enteritidis frequency in the clinical isolate dataset was compared to both the the Poultry Production Enteritidis single-infection and co-infection data separately to understand if any seasonality/cyclic behavior in serotype frequencies were present (Figures 7,8).
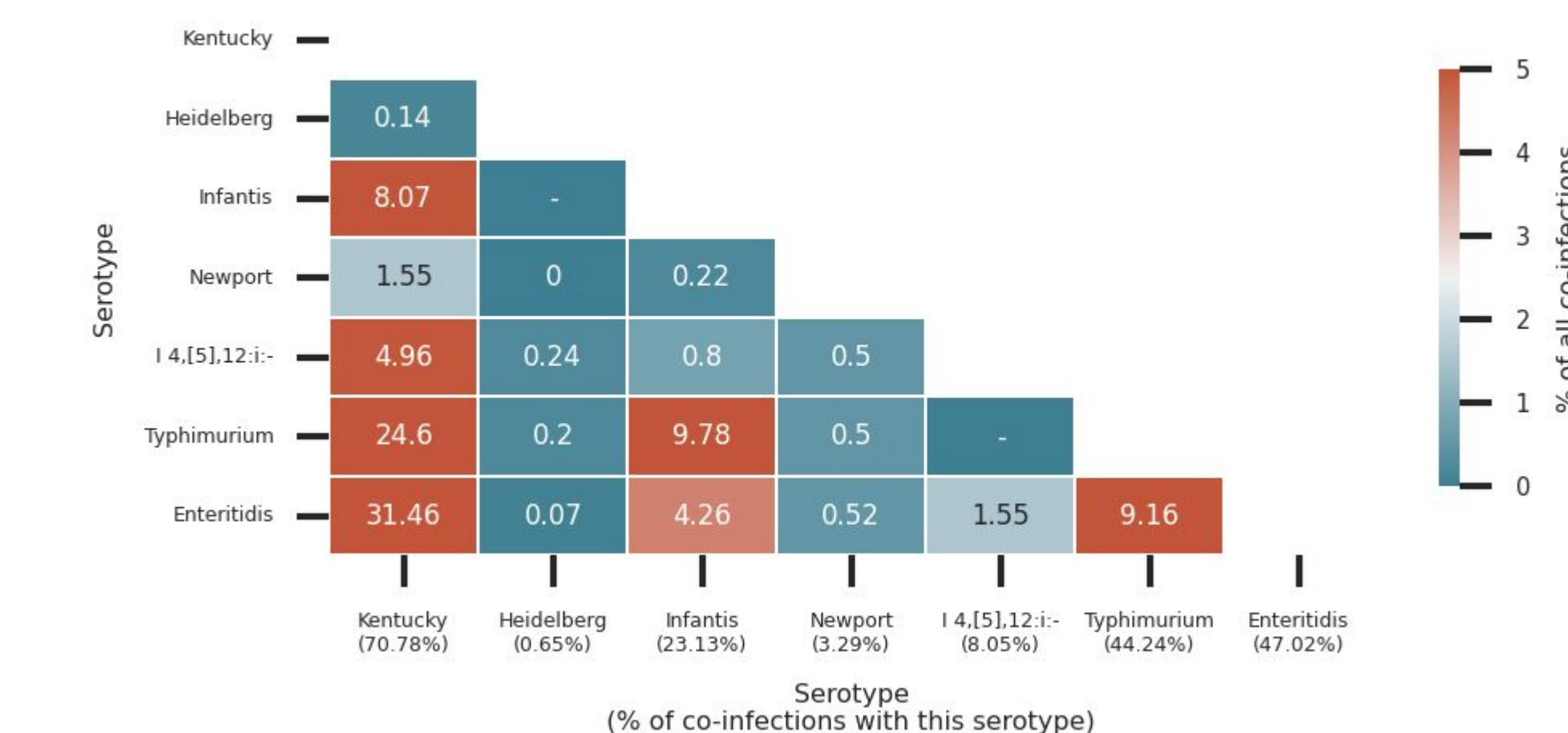
RESULTS

Figure 1: Poultry Production - Single vs. Co-infections (2020-2023)
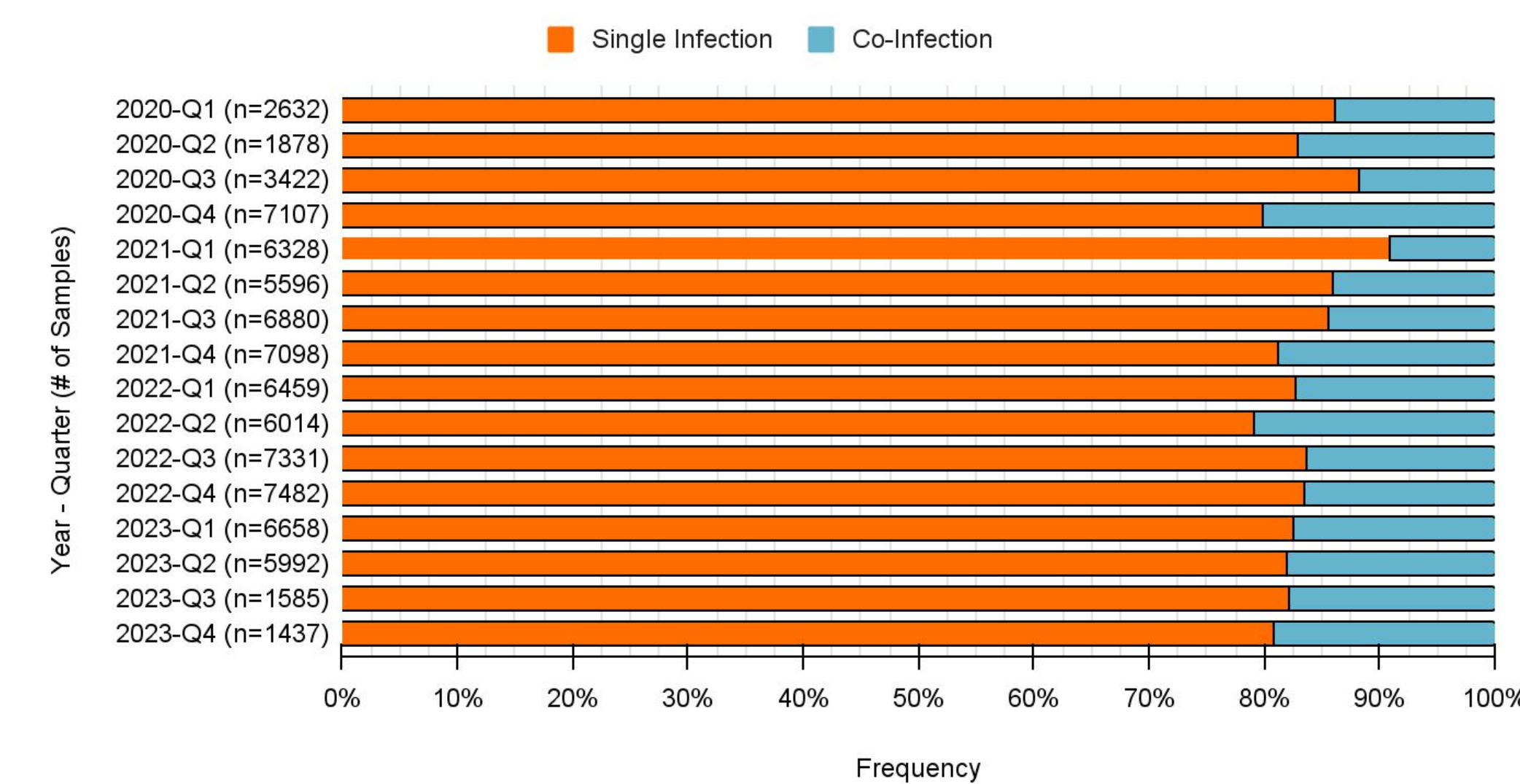
Over a 4-year period, co-infection samples were detected every quarter, with an average of 16.2% of *Salmonella enterica* positive samples with multiple serotypes present.

Figure 2: Poultry Production - Co-infection Serotype Frequency for a Subset of Serotypes

In 71% of co-infections, Kentucky was found alongside other pathogenic serotypes, emphasizing the risk of testing for only one serotype and potentially overlooking clinically relevant ones. Enteritidis and Typhimurium are involved in 44% of co-infections, illustrating the common occurrence of pathogenic serotypes in co-infection scenarios. Certain serotyping co-infection pairings are unsupported by the bioinformatics pipeline and are distinguished with a hyphen.

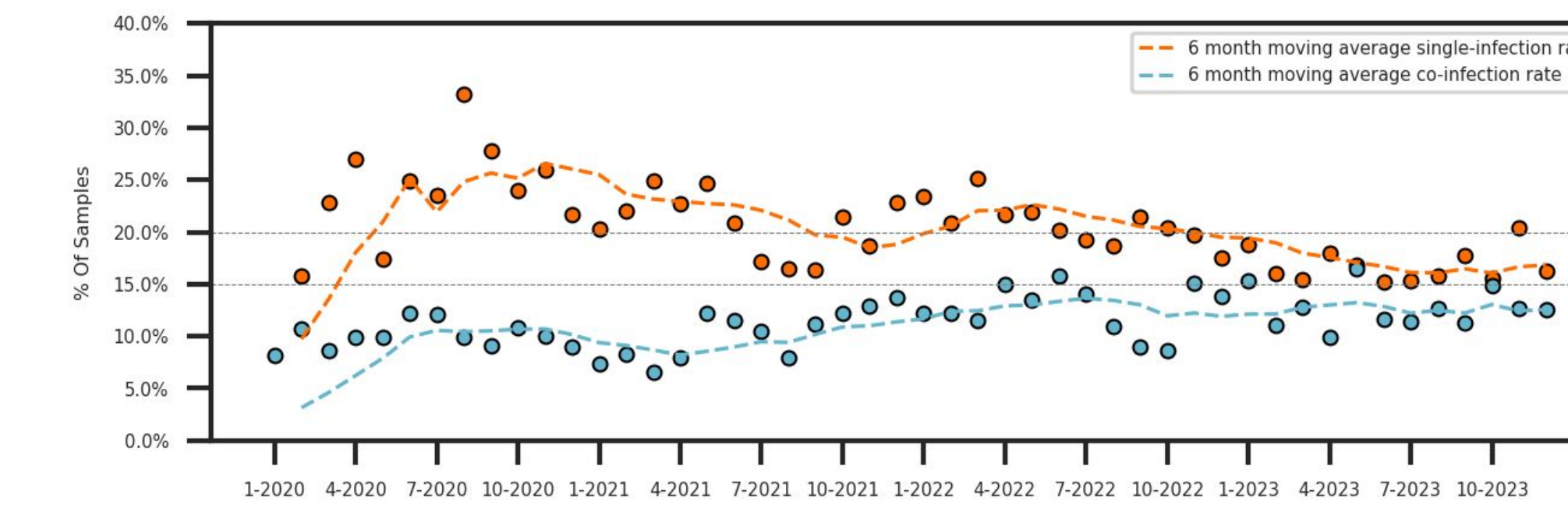**Figure 3: Poultry Production - Kentucky Single vs Co-Infection Rates**

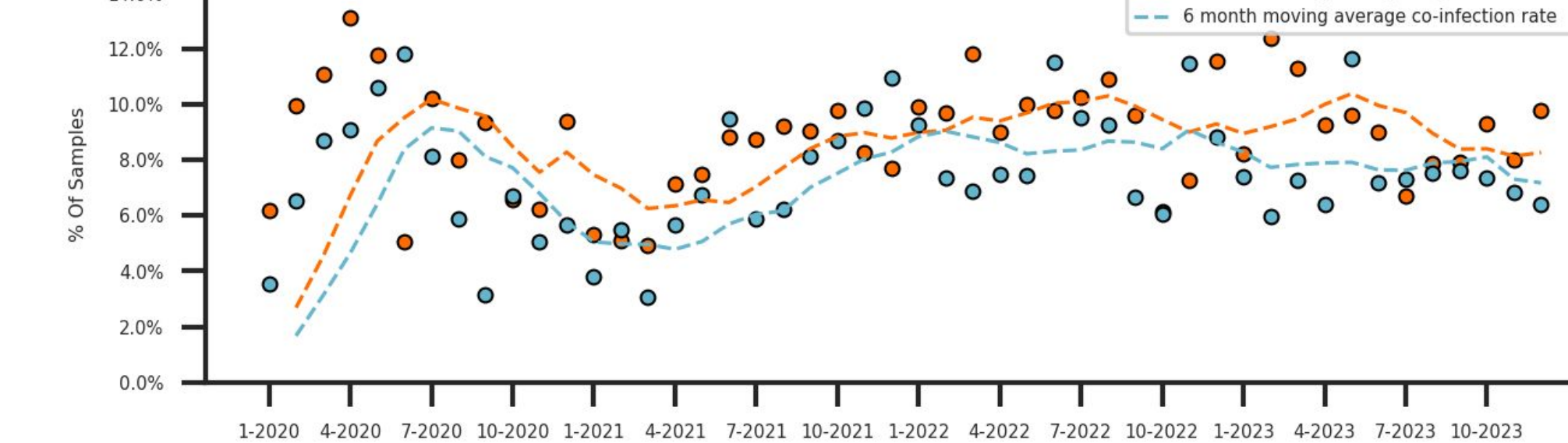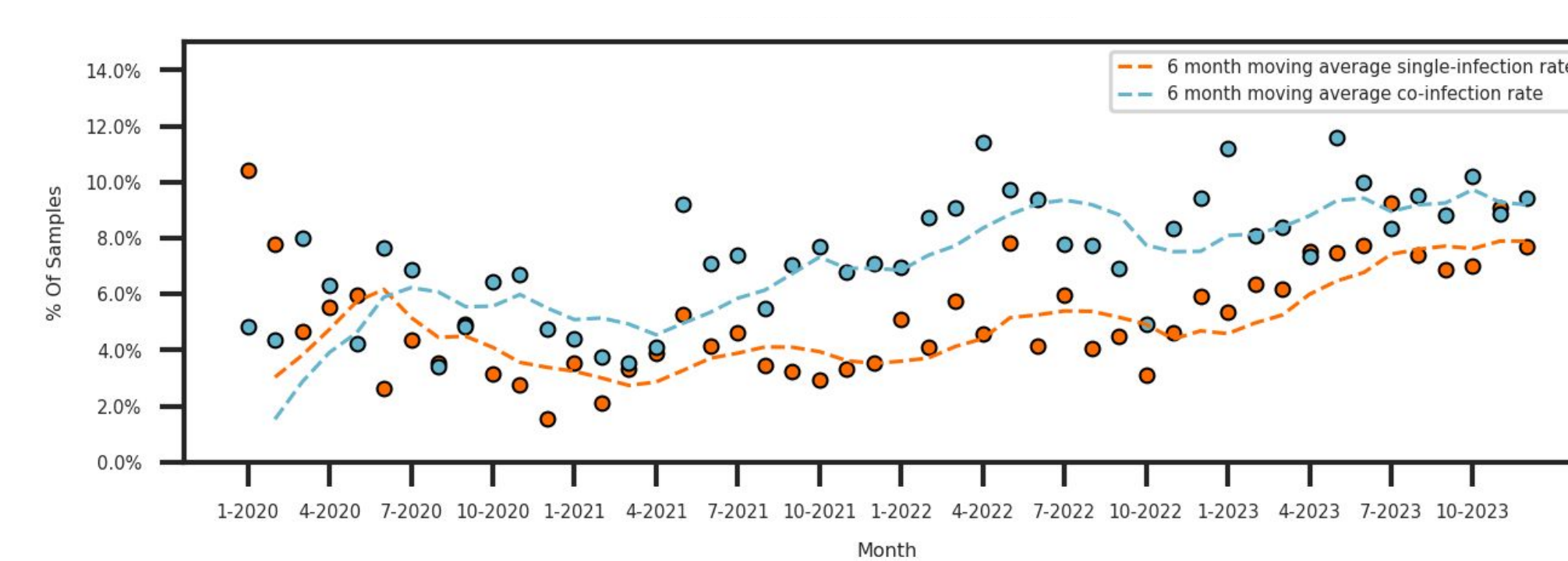**Figure 4: Poultry Production - Typhimurium Single vs Co-Infection Rates**

Figure 5: Poultry Production - Enteritidis Single vs Co-Infection Rates

Kentucky single infections appear to have a decline over time, whereas Kentucky co-infections are more stable over the four-year period. (Figure 3). Typhimurium and Enteritidis single/co-infections exhibit similar patterns of increase/decrease over time (Figure 4,5). It was more common to see Enteritidis with other serotypes than alone (Figure 5). These data suggest that co-infection rates may not necessarily follow the same behavior of a single-infection serotype. More importantly, when only testing for one serotype, it's possible to miss all clinically-relevant serotypes within a sample.

Figure 6: Comparison of the Top 10 Serotypes in Poultry Production with Clinical Isolate Frequencies
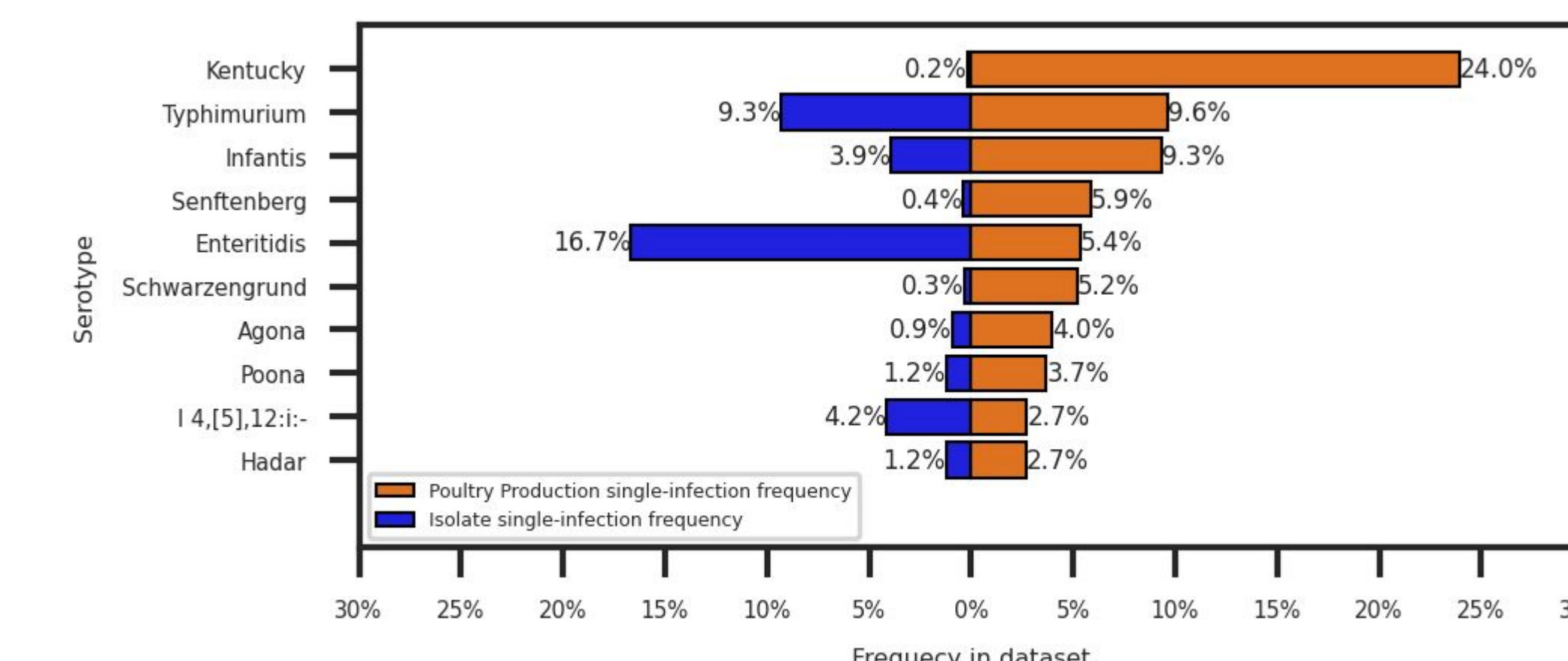
Comparing serotype prevalence between the 2 datasets reveals that Kentucky is more common in poultry but less so in clinical isolates likely due to its lower pathogenicity in humans. Typhimurium rates are similar in both datasets, while Enteritidis is more prevalent in clinical isolates. This highlights how serotype prevalence differences can provide insights into the distribution of *Salmonella* in different contexts, whether in clinical settings or in production environments linked to food safety.

Table 1: Spearman Correlation Results Comparing Single-Infection Prevalence Rates Between Isolates and Poultry Production

Salmonella enterica Serotype	P-value	Spearman's Rank correlation coefficient
Kentucky	0.117872	-0.406809
Typhimurium	0.078099	-0.452941
Infantis	0.003588	0.682353
Enteritidis	0.002798	0.695115

Figure 7: Enteritidis - 6-Month Moving Average Rates Relative to June 2020 in Isolate vs. Poultry Production Single Infections
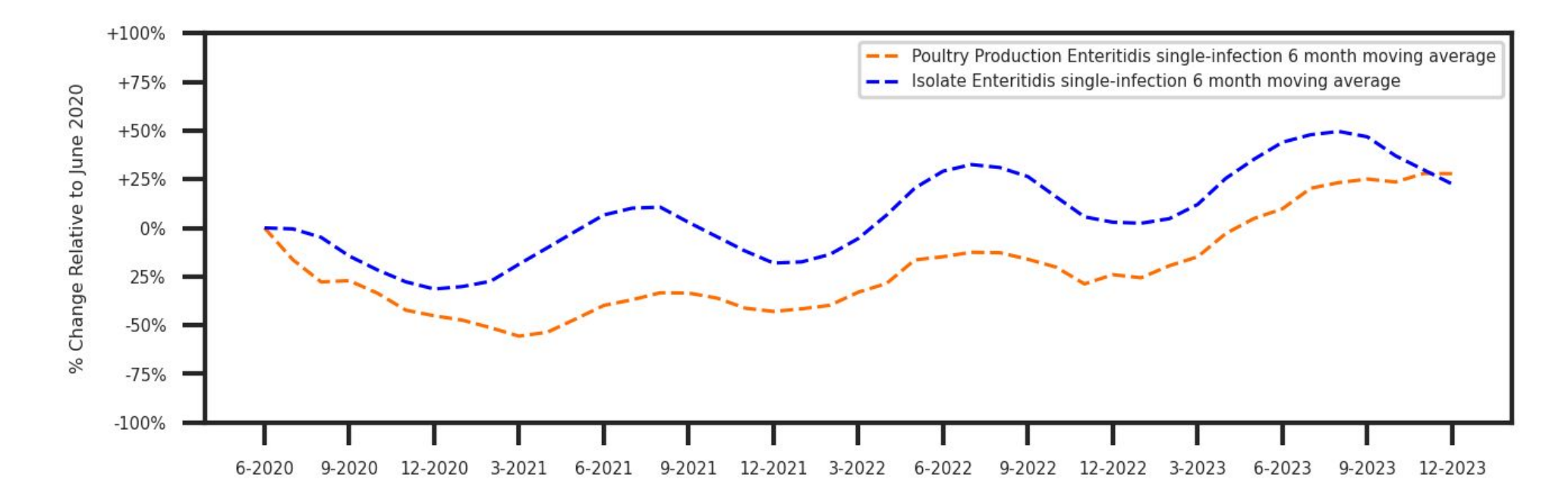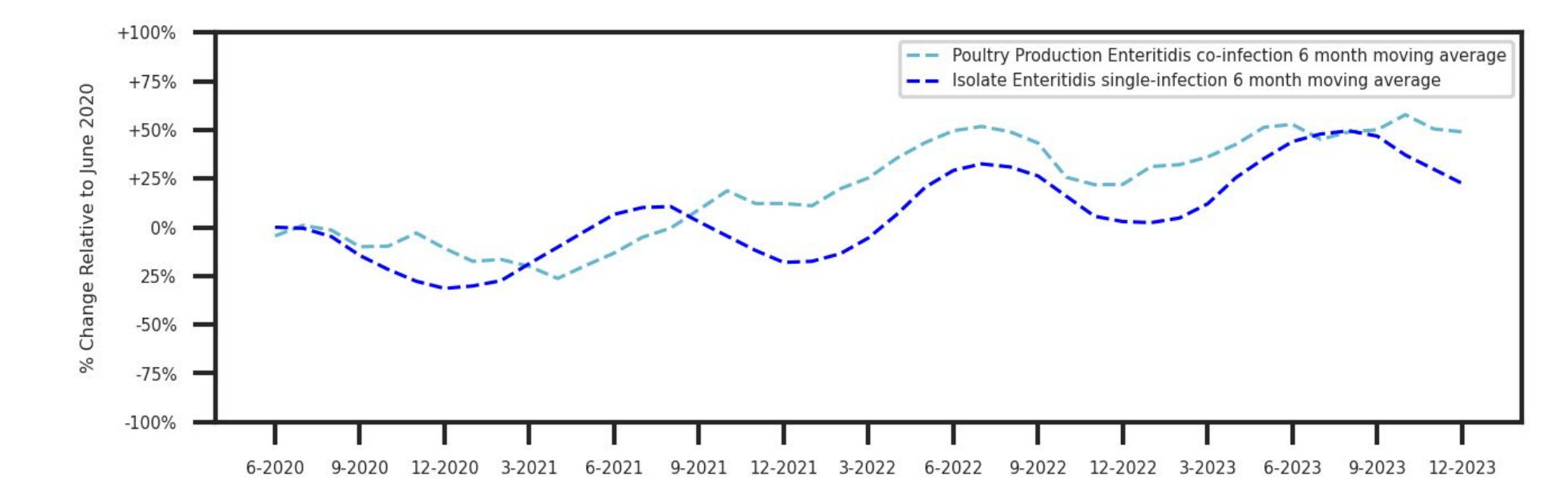
Figure 8: Enteritidis - 6-Month Moving Average Rates Relative to June 2020 in Isolate vs. Poultry Production Co-Infections

From Table 1, with a significance level of 0.05, a significant positive correlation (ρ = 0.68) was observed for Infantis and Enteritidis prevalence rates. This indicates that higher frequencies of these serotypes in one dataset corresponded to higher frequencies in the other. Kentucky showed no correlation likely due to its low representation in the clinical isolate dataset, and Typhimurium also showed no significant correlation. To further explore these similarities, a 6-month moving average for Enteritidis was calculated to assess relative changes over time, with all rates compared to the June 2020 rate. In the clinical isolate dataset, Enteritidis showed a seasonal spike during the summer months. This same seasonal increase was also present in the Poultry Production dataset, for both Enteritidis single infections and co-infections respectively. This consistency across datasets suggests an expected seasonal behavior in Enteritidis infections and highlights the importance of testing for multiple serotypes to fully understand the epidemiological picture.

CONCLUSION

- Co-infections were detected every month over the 4-year time period and on average 16% of the time, demonstrating its common occurrence in Poultry Production environments
- With 71% of co-presence results including Kentucky and a clinically relevant serotype, this highlights the potential risk/harm of testing for only one serotype for *Salmonella* surveillance and monitoring
- Results further support the USDA's recent mandate on the importance of serotyping