RESEARCH REPORT
PORK SAFETY

Title: An analysis of primary research and meta-analysis to investigate temporal changes in serotypes of Salmonella in US-based pigs and pork products - **NPB #15-070**

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Industry Summary:

The goal of our project was to determine if there are important changes in the prevalence of Salmonella serotypes in swine. This is important because if the serotypes remain unchanged on 20 years, then we know that there are no changes required in our currently effective Salmonella control approaches. However, new serotypes are emerging or patterns are shifting, this might signal a change in the ecology of Salmonella and we would need to determine if the controls we are remain effectives. The pork industry does currently do not have evidence that Salmonella control programs need to be serotypes specific, however it is important to be proactive and understand which pathogens are circulating in swine populations and found of pork products.

We used 4 longitudinal datasets which looked at Salmonella from 1996 to 2014. We observed decreasing proportions of S. enterica serovar Typhimurium, serovar Derby, and serovar Heidelberg and increasing proportions of S. enterica serovar 4,[5],12:i:-, serovar Infantis, and serovar Johannesburg in swine. We also observed positive correlations for the yearly changes in S. enterica serovar 4,[5],12:i:-, serovar Anatum, and serovar Johannesburg between swine and human data; in S. enterica Worthington between avian and human data; and in S. enterica serovar 4,[5],12:i:- between bovine and human data were observed
These data suggested that some serovars are emerging while others are decreasing. In particular, serovar 4,[5],12:i:- is found commonly in swine submitted to diagnostic laboratories in the past 5 years. As this organism has been associated with large outbreaks of disease, this is an important finding. These data also suggested that veterinary diagnostic laboratory data are potentially timely for detecting changes in serotypes and that serovar 4,[5],12:i:- is common in swine submitted to VDLs.

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Keywords:

diagnostic laboratory, NARMS, Salmonella, surveillance, swine

Scientific Abstract:

As Salmonella enterica is an important pathogen of food animals, surveillance programs for S. enterica serovars have existed for many years in the United States. Surveillance programs serve many purposes, one of which is to evaluate alterations in the prevalence of serovars that may signal changes in the ecology of the target organism. The primary aim of this study was to evaluate changes in the proportion of S. enterica serovars isolated from swine over a 20-year observation period using four longitudinal datasets from different food animal species. The secondary aim was to evaluate correlations between changes in S. enterica serovars frequently recovered from food animals and changes in S. enterica serovars associated with disease in humans. We found decreasing proportions of S. enterica serovar Typhimurium, serovar Derby, and serovar Heidelberg and increasing proportions of S. enterica serovar 4,[5],12:i:-, serovar Infantis, and serovar Johannesburg in swine over time. We also found positive correlations for the yearly changes

in S. enterica serovar 4,[5],12:i:-, serovar Anatum, and serovar Johannesburg between swine and human data; in S. enterica Worthington between avian and human data; and in S. enterica serovar 4,[5],12:i:- between bovine and human data. We found negative correlations for the yearly changes in S. enterica serovar 4,[5],12:i:- and serovar Johannesburg between avian and human data.

Introduction:

As *Salmonella enterica* is an important pathogen of animal-based proteins, surveillance programs for *S. enterica* serovars have existed for many years in the United States. Periodic review of data from these surveillance programs can help identify changes in the prevalence of certain serovars, which may indicate emerging issues [Stärk and Häsler (2015); Iwamoto et al. (2017)]. Currently, the pork industry has an effective *S. enterica* control program based on an understanding of the epidemiology of Salmonellosis and the ecology of *S. enterica* from years of prior basic and field-based research [Denagamage et al. (2007); O'Connor et al. (2008);Denagamage et al. (2010);Wilhelm et al. (2012)]. This swine-based *S. enterica* control program relies on a pathogen reduction approach at the abattoir [Totton et al. (2016)] based on the rationale that this approach is the most effective and cost-efficient [O'Connor et al. (2012); Alban and Stärk (2005)]. However, observations of changes in *S. enterica* serovars could be a result of different ecologies, for which currently employed control measures might be less effective. Therefore, to realize the value of surveillance programs, it is critical to periodically evaluate trends in the prevalence of *S. enterica* serovars over time to determine whether certain patterns indicate a need for modification or action. Data from long-running surveillance programs provide this opportunity. The primary aim of this study was to evaluate changes in *S. enterica* serovars in swine over a 20-year period in the United States. The secondary aim was to correlate changes in proportions of *S. enterica* serovars between food-producing species (bovine, avian, and swine) and humans. To achieve these aims, we used four longitudinal datasets to detect changes in the proportion of *S. enterica* serovars commonly isolated from swine from specimens submitted from diagnostic laboratories (two datasets) or collected at slaughter (one dataset) or retail (one dataset).

Objectives:

From your research proposal. A time series analysis of longitudinal datasets that measure Salmonella serovars on carcasses at slaughter (1997-2012), retail product (1997-2012) and diagnostic laboratory submissions (years available include 2003 to 2014)

Materials & Methods:

2.1 Study design and data sources

We used observational data from four sources: the Iowa State University (ISU) Veterinary Diagnostic Laboratory (VDL), National Antimicrobial Resistance Monitoring System (NARMS), Centers for Disease Control and Prevention (CDC) Laboratory-based Enteric Disease Surveillance (LEDS) program, and United States Department of Agriculture (USDA) National Veterinary Services Laboratory (NVSL).

2.2 CDC LEDS dataset

The Division of Foodborne, Waterborne, and Environmental Diseases in the National Center for Emerging and Zoonotic Infectious Diseases maintains national human Salmonella surveillance data through the CDC LEDS program. We directly requested and obtained the most recently available and complete data from the CDC. Details of the LEDS program and data collection approach are described elsewhere (https://www.cdc.gov/nationalsurveillance/salmonella-surveillance.html).

2.3 NARMS datasets

The NARMS program for monitoring S. enterica data is accomplished by three different agencies: the CDC collects human specimens (NARMS-H), the USDA collects animal specimens at slaughter (NARMS-S), and the Food and Drug Administration (FDA) collects animal specimens at retail (NARMS-R). This project, used NARMS data for animals only, as we used CDC LEDS data for humans. For the NARMS-S dataset, isolates of Salmonella, Campylobacter, Enterococcus, and Escherichia coli are obtained from food-producing animal specimens at federally inspected slaughter and processing plants throughout the United States. Details of data collection are available at https://www.cdc.gov/narms/. For the NARMS-R dataset, participating sites collect specimens of chicken, ground turkey, ground beef, and pork chops for culturing. Isolates of Salmonella, Campylobacter, Enterococcus, and E. coli are sent to the FDA for serotyping, antimicrobial susceptibility testing, and genetic analysis. Retail meat surveillance is conducted by the FDA in collaboration with FoodNet sites and state departments of public health, which have changed over time. When the retail program was launched in 2002, participating states included Connecticut, Georgia, Maryland, Minnesota, and Tennessee, with Oregon joining the program later that year. New York, California, Colorado, and New Mexico joined in 2003 and 2004; Pennsylvania joined in 2008; and Missouri, Louisiana, and Washington joined in 2013. We obtained NARMS datasets from a publicly available source (Error! Hyperlink reference not valid.). The laboratory methods used by NARMS are described in the inter-agency Manual of Laboratory Methods (available at Manual of Laboratory Methods).

2.4 ISU VDL dataset

The ISU VDL obtains 40% of swine specimens, 68% of avian specimens, and 76% of bovine specimens from Iowa, with the remaining specimens obtained from other states. Specimens were tested for Salmonella spp based on the supervising pathologistâ€™s or submitting veterinarianâ€™s request. The majority of isolates would be from pigs with enteric diseases, but may also include isolates from surveillance testing. Isolates from research cases were not included in the query.

2.5 NVSL dataset

The NVSL dataset contains information on Salmonella isolates submitted to the Diagnostic Bacteriology Laboratory for serotyping or genotyping. These isolates originate from states across the United States and are primarily submitted by state and private veterinary

diagnostic laboratories. In these cases, the state in which the submitting laboratory is located may not be the same state from which the isolates originated, and NVSL does not always have the originating state information. As the USDA does not require submissions to the NVSL, these data represent voluntary submissions, often from laboratories lacking in-house typing capabilities. For the data analyzed in this study, the purpose of submission included clinical cases, environmental surveillance, outbreak investigations, or unknown purposes. Any isolates clearly associated with research projects or likely duplicates in other datasets were removed from analysis i.e. isolates from ISU VDL, USDA Food Safety Inspection Service, USDA Agricultural Research Service, National Animal Health Monitoring System and others identified as research during submission. Serotyping performed at the NVSL was based on previously described methods [Ewing (1986)]. Serovar designation was based on antigenic formulae for somatic (O) and flagellar (H) antigens [Grimont and Weill (2007)].

2.6 Management of datasets

1. Swine-associated data:

• ISU VDL dataset. Data describing 11681 isolates were included in the original dataset. After removing several non-Salmonella isolates accidentally included in the provided dataset, the dataset contained information on 11631 isolates collected from 2003 to 2015. We also included data describing 132 isolates of S. enterica serovar Cholerasuis identified using a novel in-house approach.

• NVSL-S dataset. Data describing 9785 isolates were included in the original dataset. After removing non-Salmonella isolates, the dataset contained information on 9785 Salmonella isolates collected from 2006 to 2015.

• NARMS-S dataset. Data describing 4795 isolates were included in the original dataset. After removing the non-Salmonella isolates, the dataset contained data that related to 4795 Salmonella isolates collected from 1997 to 2011.

• NARMS-R dataset. Data describing 202 isolates collected from 2002 to 2015. As this dataset was filtered before it was downloaded, no non-Salmonella isolates were removed post-hoc.

2. Non-swine data:

• ISU VDL avian dataset. This dataset included isolates from chickens and turkeys, which were combined to form a single avian category. Data describing 2843 isolates were included in the original dataset. After removing non-Salmonella isolates, the dataset contained information on 2765 Salmonella isolates collected from 2003 to 2015.

• ISU VDL bovine dataset. This dataset included isolates from beef and dairy animals, which were combined to form a single bovine category. Data describing 1994 isolates were included in the original dataset. After removing the non-Salmonella isolates, the dataset contained information on 1986 Salmonella isolates collected from 2003 to 2015.

• NVSL avian dataset. Data describing 51001 isolates were included in the original dataset. After removing the non-Salmonella isolates, the dataset contained information on 50999 Salmonella isolates collected from 2006 to 2015.

• NVSL bovine dataset. Data describing 23160 isolates were included in the original data set. After removing the non-Salmonella isolates, the dataset contained information on 23120 Salmonella isolates collected from 2006 to 2015.

• NARMS-S avian dataset: This dataset included isolates from chickens and turkeys, which were combined to form a single avian category. The dataset contained information on 21065 isolates. After removing the non-Salmonella isolates, there were 21065 isolates collected from 1997 to 2013.

• NARMS-S bovine dataset. Data describing 9461 isolates were included in the original data set. After removing the non-Salmonella isolates, the dataset contained information on 9461 Salmonella isolates collected from 1997 to 2013.

• NARMS-R avian dataset. This dataset included isolates from chickens and turkeys, which were combined to form a single avian category. The dataset contained information on 4138 isolates collected from 2002 to 2015.

• NARMS-R bovine dataset. Data describing 169 Salmonella isolates were available from 2002 to 2015.

3. CDC LEDS dataset. Data describing 755086 isolates were included in the original dataset. After removing the non-Salmonella isolates, the dataset contained information on 751095 Salmonella isolates collected from 1997 to 2016.

2.7 Mapping S. enterica serovars across data sets

For each dataset, all unique serovars were identified. Serovar names that appeared to be typographic errors were identified and verified by consulting with co-authors with expertise in microbiology. For example, â€œserovar Infanisâ€ was assumed to be â€œserovar Infantis,â€ â€œserovar 4,5,12:1:-â€ was assumed to be â€œserovar 4,5,12:i:-,â€ and â€œphade DT12â€ was assumed to be â€œphage DT12.â€ After correcting such errors, co-authors with expertise in microbiology created a map that linked like serovars appropriately prior to analysis. In particular, S. enterica serovar 4,5,12:i:- and S. enterica serovar 4,12:i:- were mapped to a single group labeled S. enterica serovar 4,[5],12:i:-. S. enterica serovar 4,[5],12 with any letter other than i in the flagella section of the antigenic formula, such as S. enterica serovar 4,12:d:âˆ’, were mapped to a single group labeled S. enterica serovar Group B. S. enterica serovar Typhimurium (antigenic formula 4,[5],12:i:1,2) and S. enterica serovar Typhimurium var. Copenhagen or S. enterica serovar Typhimurium var. 5- were mapped to a single group labeled S. enterica serovar Typhimurium (antigenic formula 4,[5],12:i:1,2) because the NARMS-S dataset does not differentially report the 5- variant. For this S. enterica serovar Typhimurium group, all phage types were combined (i.e., DT12, DT104, DT104a, and DT104b).

2.8 Statistical analysis

All analyses were implemented using open software R [R Core Team (2016)]. Our focus was on estimation rather than hypothesis testing because the data were observational; therefore, the sample size was a matter of convenience rather than reflective of an a priori desired power to test a specific hypothesis. For the first aim, we first examined changes in common S. enterica serovars over time by defining the 10 most frequently isolated serovars based on the proportion of S. enterica serovars in the ISU VDL swine dataset. We next determined the proportion of isolates of each serovar out of the total serovar count each year for the other datasets. We performed simple linear regression with the yearly proportion change regressed on year to obtain an estimate of the change in proportion of the

given serovar over years within each dataset (i.e., the slope of the regression line). We also calculated 95% confidence intervals (CIs) for the slope estimates. For the second aim, we computed pairwise correlations between species in the relative changes in serovar proportions for certain years using Spearman's rank-order correlation coefficient. Given the high number of all possible pairwise correlations, we considered only the 10 most common S. enterica serovars in the ISU VDL swine dataset (i.e., the same serovars of interest in the first aim). We also limited the correlations to animal versus human isolates (e.g., we did not assess correlations between swine and bovine isolates). We calculated correlations and corresponding 95% CIs for the following datasets:

1. CDC LEDS dataset with ISU VDL swine, avian, and bovine datasets,
2. CDC LEDS dataset with NARMS-S swine, avian, and bovine datasets,
3. CDC LEDS dataset with NARMS-R swine, avian, and bovine datasets,
4. CDC LEDS dataset with NVSL-S swine, avian, and bovine datasets.

We calculated three types of correlations:

1. Correlations between concurrent years (e.g., correlations for the 2006-2007 proportion change in S. enterica serovar Typhimurium between the ISU VDL and CDC LEDS datasets). More specifically, for a given serovar, X(t) denotes the yearly proportion at year t in the CDC LEDS dataset, and Y(t) denotes the yearly proportion at year t in the other dataset; thus, Spearman's rank-order correlations were performed between $X(t+1)-X(t)$ and $Y(t+1)-Y(t)$, with t starting from the overlapped year.
2. Correlations across a 1-year lag with the animal data preceding the human data (e.g., correlations for the 2006-2007 proportion change in S. enterica serovar Typhimurium between the ISU VDL and CDC LEDS datasets). More specifically, for a given serovar, X(t) denotes the yearly proportion at year t in the CDC LEDS dataset, and Y(t) denotes the yearly percentage at year t in the other dataset; thus, Spearman's rank-order correlations were performed between $X(t+2)-X(t+1)$ and $Y(t+1)-Y(t)$.
3. Correlations across a 2-year lag with the human data preceding the animal data (e.g., correlations for the 2006-2007 proportion change in S. enterica serovar Typhimurium between the CDC LEDS and NARMS-S datasets). More specifically, for a given serovar, X(t) denotes the yearly proportion at year t in the CDC LEDS dataset, and Y(t) represents the yearly proportion at year t in the other dataset; thus, Spearman's rank-order correlations were performed between $X(t+1)-X(t)$ and $Y(t+3)-Y(t+2)$.

The rationale for assessing these time lags was our working hypothesis that if changes in S. enterica proportions in one species lead to changes in another species, then correlations might be observed across years. We used a 1-year lag from animals to humans because we assumed that if S. enterica serovars transfer from animals to humans, they are likely to more rapidly transfer through the food supply. We used a 2-year lag from humans to animals because we assumed that transfer from humans to animals is likely to be less rapid, as no ubiquitous vehicle exists for rapid transfer in this direction. Correlations were computed for the 10 S. enterica serovars most frequently reported in the ISU VDL swine dataset. Spearman's rank-order correlations were computed for each pairwise comparison due to the skewness of the data for some serovars. During the analysis, we computed correlations only across years when both datasets had recorded specimens.

3 Results

3.1 Changes in common swine *S. enterica* serovars over time

The frequency of all *Salmonella* serovars with more than 10 isolates over time are provided in the supplementary materials (Fig.S1, Fig.S2, Fig.S3, Fig.S4 and Fig.S5).

The most frequently isolated *S. enterica* serovars in the ISU VDL swine dataset as well as the slope estimates of the yearly changes in serovar proportions and 95% CIs are provided in Table 1. The frequency of isolation and slope estimates for the same serovars in the other datasets (i.e., NARMS-S, NARMS-R, NVSL, and CDC LEDS) are provided in Table 2. It is important to note the large differences in numbers of isolates used in the analysis. For example, the NARMS-R swine dataset (n=202) contained only 39 *S. enterica* serovar Typhimurium isolates from 2002 to 2015, whereas the CDC LEDS dataset (n=751095) contained 125403 such isolates during the same period. As a consequence, the precision of estimation varies enormously across datasets. Therefore, the point estimate, precision around the point estimate (i.e., 95% CI), and the number of isolates contributing to the calculation should be considered when interpreting the results.

Based on the negative upper and lower boundaries of the 95% CIs, the proportions of *S. enterica* serovar Typhimurium, serovar Derby, and serovar Heidelberg decreased over time (Table 1, Fig.1, Fig.2, and Fig.3). The proportions of *S. enterica* serovar Typhimurium also appeared to decrease over time in the NARMS-R, NARMS-S, NVSL, and CDC LEDS datasets. However, based on the 95% CIs, we can only conclude that the proportions of *S. enterica* serovar Typhimurium decreased in the CDC LEDS and NVSL datasets. For the NARMS-S and NARMS-R datasets, the 95% CIs were bounded by positive and negative estimates, which might be due to the small number of isolates in the NARMS-R dataset (n= 39) but not the NARMS-S dataset (n= 655). The patterns of temporal changes in the proportions of other serovars in the ISU VDL swine dataset were less consistent. Decreases in the proportion of *S. enterica* serovar Derby over time were observed in the ISU VDL, NARMS-S, and CDC LEDS datasets. The proportion of *S. enterica* serovar Derby appeared to increase over time in the NARMS-R dataset (Fig.2), although this dataset contained only 27 isolates. The proportions of *S. enterica* serovar Heidelberg appeared to show consistent decreases in all datasets (Fig.3).

Over time, the proportions of *S. enterica* serovar 4,[5],12:i:-, serovar Infantis, and serovar Johannesburg increased in the ISU VDL swine dataset. These data are provided in Table 1 and plotted in Fig.4, Fig.5, and Fig.6. It is interesting to note that the proportion of *S. enterica* serovar 4,[5],12:i:- increased by around 2 % (95% CIs [1.02, 2.98])each year, which is higher than that of other serovars. An increase in the proportion of *S. enterica* serovar 4,[5],12:i:- was also observed in the NVSL-S ((2.56 % [1.33, 3.78])), CDC LEDS (0.23 % [0.18, 0.29]), and NARMS-S (0.11 % [0.07, 0.16]) datasets but not in NARMS-R dataset (0.41 % [-0.03, 0.84]). However, considering the 95% CIs, the CDC LED, NVSL-S, and NARMS-S datasets provided the strongest evidence of an increasing

proportion of *S. enterica* serovar 4,[5],12:i:âˆ’. For the NARMS-R dataset, the 95% CI was bounded by positive and negative estimates, again likely due to the small number of isolates (n= 4). Following the same approach to interpreting the results, increases in the proportions of *S. enterica* serovar Infantis appeared to be consistent across datasets (Fig.5), whereas changes in the proportion of *S. enterica* serovar Johannesburg were inconsistent across datasets (Fig.6).
Plots of temporal changes in the proportions of the remaining top 10 ISU VDL swine isolates in the other datasets are provided in the supplementary materials (*S. enterica* subsp. *enterica* serovar Agona: Fig.S6, *S. enterica* subsp. *enterica* serovar Anatum: Fig.S7, and *S. enterica* subsp. *enterica* serovar Senftenberg: Fig.S8).

3.2 Between-species correlations for changes in common *S. enterica* serovars over time

Our second aim was to assess correlations for changes in proportions of serovars between food animals species and human. We first correlated ISU VDL species-level data with human CDC LEDS data within concurrent time periods. We observed consistent positive correlations for yearly changes in *S. enterica* serovar 4,[5],12:i:-, serovar Anatum, and serovar Johannesburg between ISU VDL swine and CDC LEDS datasets. For other serovars, however, the correlations involved positive and negative estimates bounding the 95% CIs. There were positive correlations for the yearly changes in *S. enterica* serovar Worthington between ISU VDL avian and CDC LEDS datasets and for the yearly changes in *S. enterica* serovar 4,[5],12:i:- between ISU VDL bovine and CDC LEDS datasets. There were negative correlations for the yearly changes in *S. enterica* serovar 4,[5],12:i:- and serovar Johannesburg between the ISU VDL avian and CDC LEDS datasets. These data are presented in Fig.7. There were no consistent correlations within concurrent time periods between the NARMS-S species-level and CDC LEDS datasets or NARMS-R species-level and CDC LEDS datasets. These data are presented in Fig. 8 and Fig.9 respectively. For example, we observed positive correlations only between swine *S. enterica* serovar Anatum and bovine *S. enterica* serovar 4,[5],12:i:-. For *S. enterica* serovar Worthington, there was a negative correlation between NARMS-R avian and CDC LEDS datasets but a positive correlation between ISU VDL avian and CDC LEDS datasets. Again, however, the avian NARMS-R dataset contained only 10 *S. enterica* serovar Worthington isolates (three from chicken and seven from turkey), which limits the confidence of our estimates. There were no consistent 1-year-lag correlations between ISU VDL and CDC LEDS datasets, NARMS-S and CDC LEDS datasets, or NARMS-R and CDC LEDS datasets. These data are presented in Supplementary Fig.S9, Fig.S10, and Fig.S11, respectively. Similarly, there were no consistent 2-year-lag correlations between ISU VDL and CDC LEDS datasets, NARMS-R and CDC LEDS datasets, or NARMS-R and CDC LEDS datasets (see Fig.S12, Fig.S13, and Fig.S14).

4 Discussion

Our results show changes in the proportion of *Salmonella* serovars over time. Our main findings are that there was an increase in *S. enterica* serovar 4,[5],12:i:- in veterinary diagnostic submissions (ISU VDL and NVSL) over time and that this increase mirrored that observed in human data (CDC LEDS). Other veterinary diagnostic laboratories have also reported an increase in the isolation of *S. enterica* serovar 4,[5],12:i:- [Hong et al. (2016)]. Interestingly, the prevalence of *S. enterica* serovar 4,[5],12:i:- was very low in the NARMS-S swine dataset, and it is unclear why this might be the case. One possible explanation is that the population of animals examined at diagnostic laboratories is different from that arriving at slaughter, as might be expected. If *S. enterica* serovar 4,[5],12:i:- is associated with clinical disease in pigs, this might explain the large difference in prevalence among datasets. However, we are unaware of published studies showing that *S. enterica* serovar 4,[5],12:i:- is associated with clinical disease. An alternative explanation is that the prevalence of *S. enterica* serovar 4,[5],12:i:- is increasing in both animals examined at diagnostic laboratories and those going to slaughter, although the efficacy of in-plant pathogen-reducing treatments reduces overall *Salmonella* prevalence to a level that is so low that detection is difficult [Totton et al. (2016); O'Connor et al. (2012); Alban and Stärk (2005)]. If this latter explanation holds true, then this suggests that the NARMS-S program does not sensitively estimate the prevalence of *Salmonella* on farms. As most people come into contact with pork rather than pigs, it is normally assumed that NARMS-S and NARMS-R data are of greater public health relevance than ISU VDL data; however, this may not be the case. It is also possible that the differences observed reflect differences in samples types. This would have interesting implications, because if difference samples have difference serotypes within animals, then which sample represents the risk an animals poses to human health has an impact on the utility of the surveillance programs. Of course even more explanations are possible, however our data does not answer which of these scenarios is correct. The large increase in *S. enterica* serovar 4,[5],12:i:- warrants investigation into the impact of the ecology on on-farm *Salmonella*. Our correlation analysis provides additional insights into the patterns of temporal changes in *Salmonella* serovars. Although correlations do not denote causation, it is interesting that increases in *S. enterica* serovar 4,[5],12:i:-, an emerging food-borne pathogen, in humans were correlated in increases in swine and bovine specimens but not in avian specimens. Interestingly, others have observed an increase in *S. enterica* serovar 4,[5],12:i:- in pigs but not beef [Hong et al. (2016)]. However, the magnitude of the observed correlation was quite high (i.e., 0.55), suggesting a meaningful association rather than a weak association that was significant merely due to a large sample size. We also observed positive correlations for changes in the proportion of *S. enterica* serovar 4,[5],12:i:âˆ’ between human and ISU VDL data and between human and NARMS-S bovine data. It should be noted that these associations do not point toward the origin of *S. enterica* serovar 4,[5],12:i:-. We detected no changes in the proportion *S. enterica* serovar 4,[5],12:i:- in meat products before or after changes in humans. We acknowledge that even if these correlations were found, they would simply serve a hypothesis-generating function. In conclusion, we propose that data from surveillance programs should be periodically evaluated to identify emerging patterns that suggest action. For our first aim of analyzing changes in *Salmonella* serovars that have predominated in swine, we found consistent evidence of changes in the predominant serovar in swine over time. We propose that our observed increase in *S. enterica* serovar 4,[5],12:i:- is likely due to an increased overall prevalence in swine, although it may be useful to determine whether pathogen-reducing treatments used at the abattoir are effective against this serovar. For our second aim of evaluating correlations for temporal changes in the prevalence of *Salmonella* serovars between animal surveillance data and human data, we found that increases in *S. enterica* serovar 4,[5],12:i:- were correlated between humans and swine diagnostic submissions and between bovine diagnostic

submissions and NARMS-S data. These findings underscore our suggestion that investigation of the control of *S. enterica* serovar 4,[5],12:i:- at the abattoir is warranted.

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6 Tables and Figures

Table 1: Most frequent serotypes in ISU VDL data set

Serotype Name	**Frequency**	**Slope Est (CIs)**
Typhimurium	3681	-1.64[-2.24, -1.03]
Derby	1477	-0.40[-0.63, -0.17]
4,[5],12:i:-	932	2.00[1.02, 2.98]
Agona	775	0.13[-0.17, 0.42]
Heidelberg	583	-0.50[-0.65, -0.35]
Infantis	548	0.15[0.00, 0.30]
Anatum	352	-0.06[-0.25, 0.12]
Johannesburg	335	0.25[0.13, 0.37]
Senftenberg	274	0.07[-0.07, 0.21]
Worthington	242	0.06[-0.02, 0.15]

Table 2: Most frequent serotypes in VDL swine data set in other data set

Serotype Name	Freq / NARMS-S Est(CIs)	Freq / NARMS-R Est(CIs)	Freq / NVSL Est(CIs)	Freq / CDC-LEDS Est(CIs)
Typhimurium	655 / -0.43[-1.00, 0.13]	39 / -0.48[-2.19, 1.22]	2711 / -1.55[-2.56, -0.55]	125403 / -0.88[-0.96, -0.80]
Derby	1156 / -0.81[-1.54, -0.09]	27 / 1.83[0.23, 3.44]	1122 / 0.12[-0.34, 0.59]	2518 / -0.02[-0.02, -0.02]
4,[5],12:i:-	9 / 0.11[0.07, 0.16]	4 / 0.41[-0.03, 0.84]	595 / 2.56[1.33, 3.78]	18968 / 0.23[0.18, 0.29]
Agona	144 / 0.11[-0.04, 0.26]	4 / -0.32[-0.84, 0.20]	856 / 0.43[-0.08, 0.94]	8922 / -0.07[-0.10, -0.04]
Heidelberg	174 / -0.26[-0.45, -0.07]	15 / -1.59[-3.66, 0.49]	554 / -0.46[-0.70, -0.22]	29346 / -0.23[-0.28, -0.19]
Infantis	327 / 0.39[0.15, 0.62]	22 / 1.26[-0.38, 2.89]	386 / 0.12[-0.18, 0.43]	14175 / 0.05[0.01, 0.08]
Anatum	354 / 0.23[-0.35, 0.82]	4 / -0.19[-1.08, 0.71]	175 / -0.14[-0.23, -0.05]	4414 / 0.00[-0.01, 0.01]
Johannesburg	330 / 0.37[-0.05, 0.80]	9 / 0.21[-1.78, 2.21]	99 / -0.06[-0.19, 0.07]	681 / 0.00[0.00, 0.00]
Senftenberg	35 / -0.02[-0.10, 0.06]	4 / -0.11[-0.69, 0.46]	272 / -0.03[-0.20, 0.14]	2704 / -0.01[-0.02, 0.00]
Worthington	58 / -0.01[-0.05, 0.04]	1 / 0.04[-0.10, 0.18]	270 / 0.01[-0.15, 0.16]	591 / 0.00[0.00, 0.00]

Figure 1: Observed prevalence of *S. enterica* serovar Typhimurium over years in all swine datasets. The β coefficients and 95% CIs for the covariate year are reported.

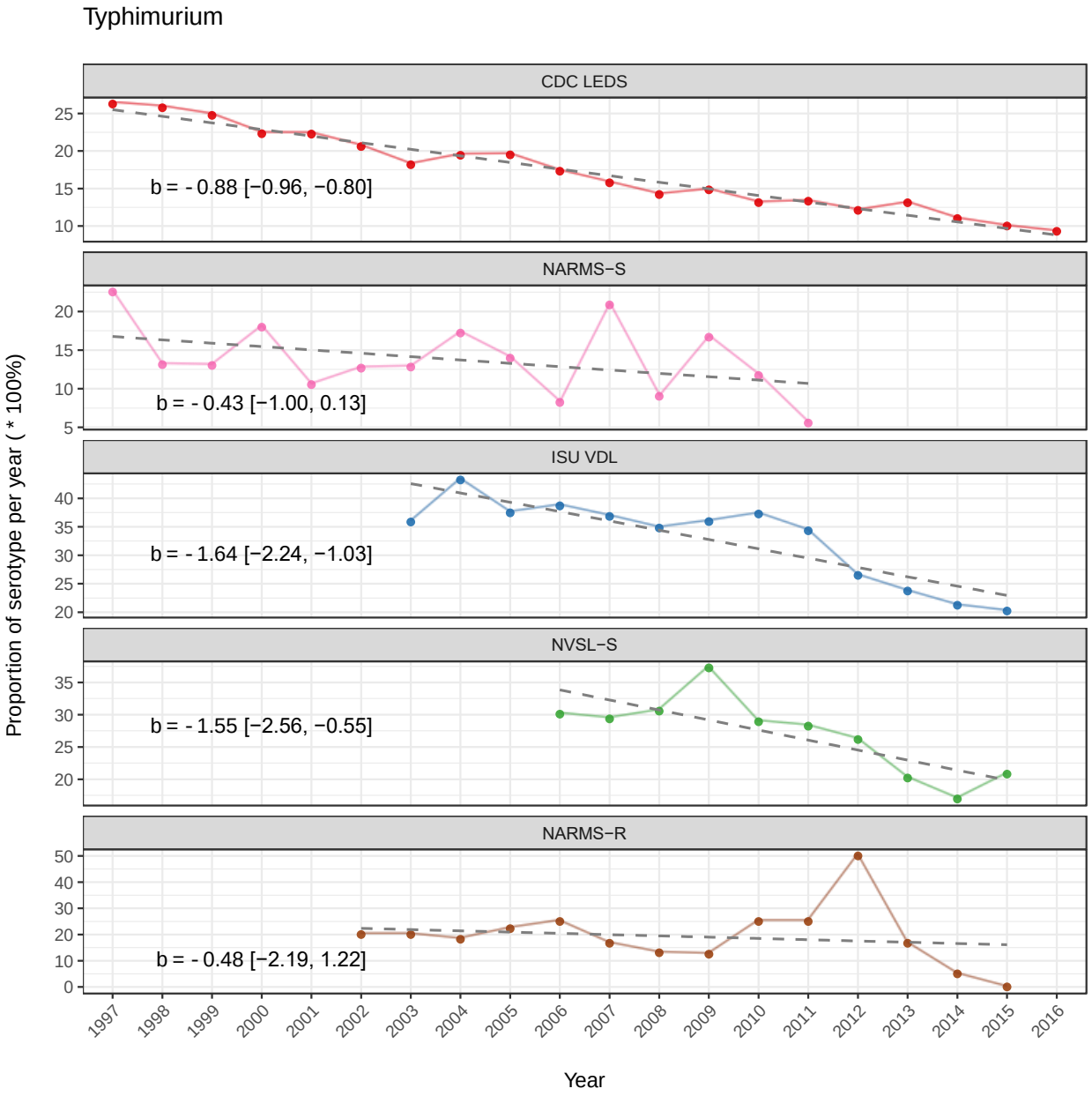


Figure 2: Observed prevalence of *Salmonella enterica* subsp. Derby over years in all swine data sets. The β coefficient and 95% confidence interval for the covariate year are reported.

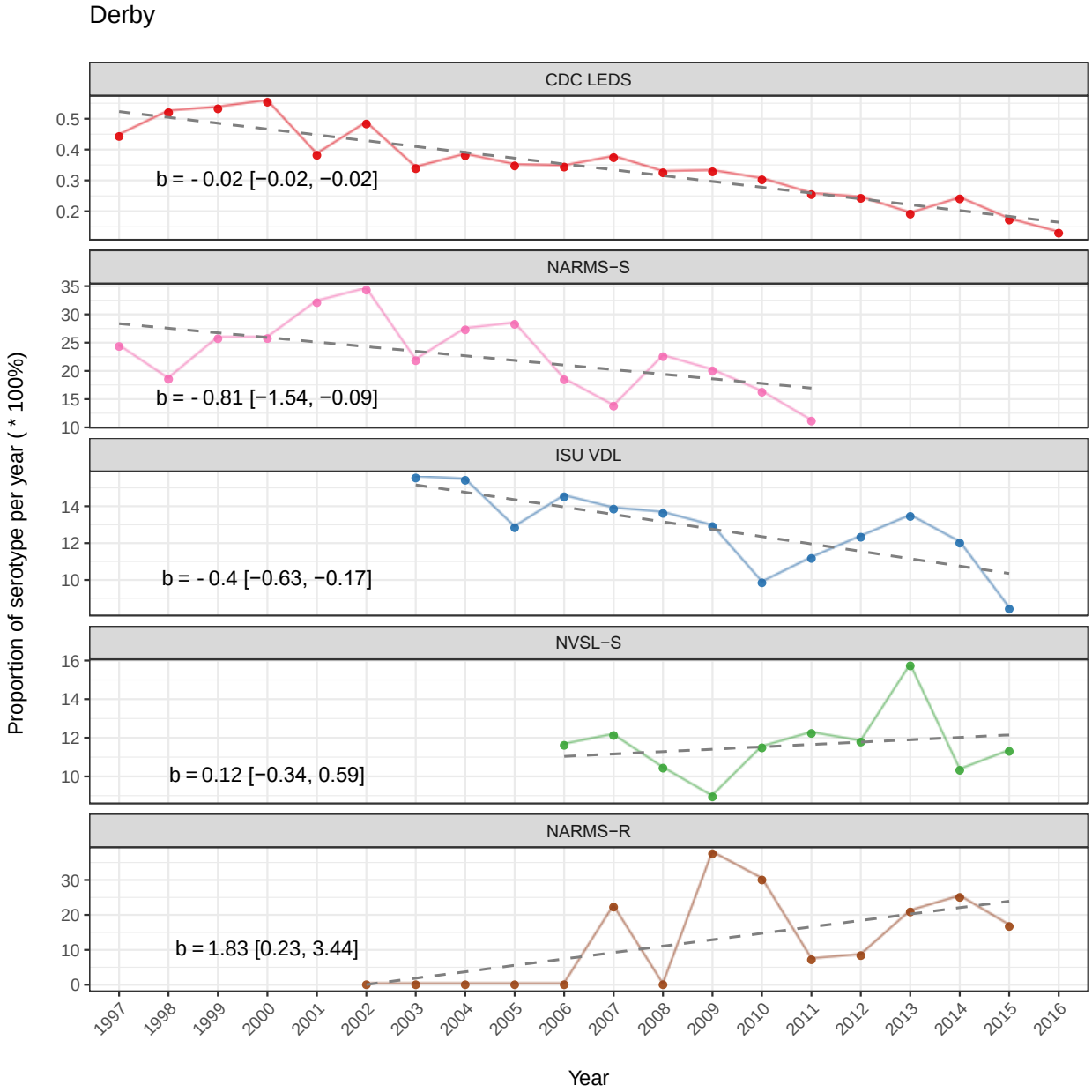


Figure 3: Observed prevalence of *Salmonella enterica* subsp. Heidelberg over years in all swine data sets. The β coefficient and 95% confidence interval for the covariate year are reported.

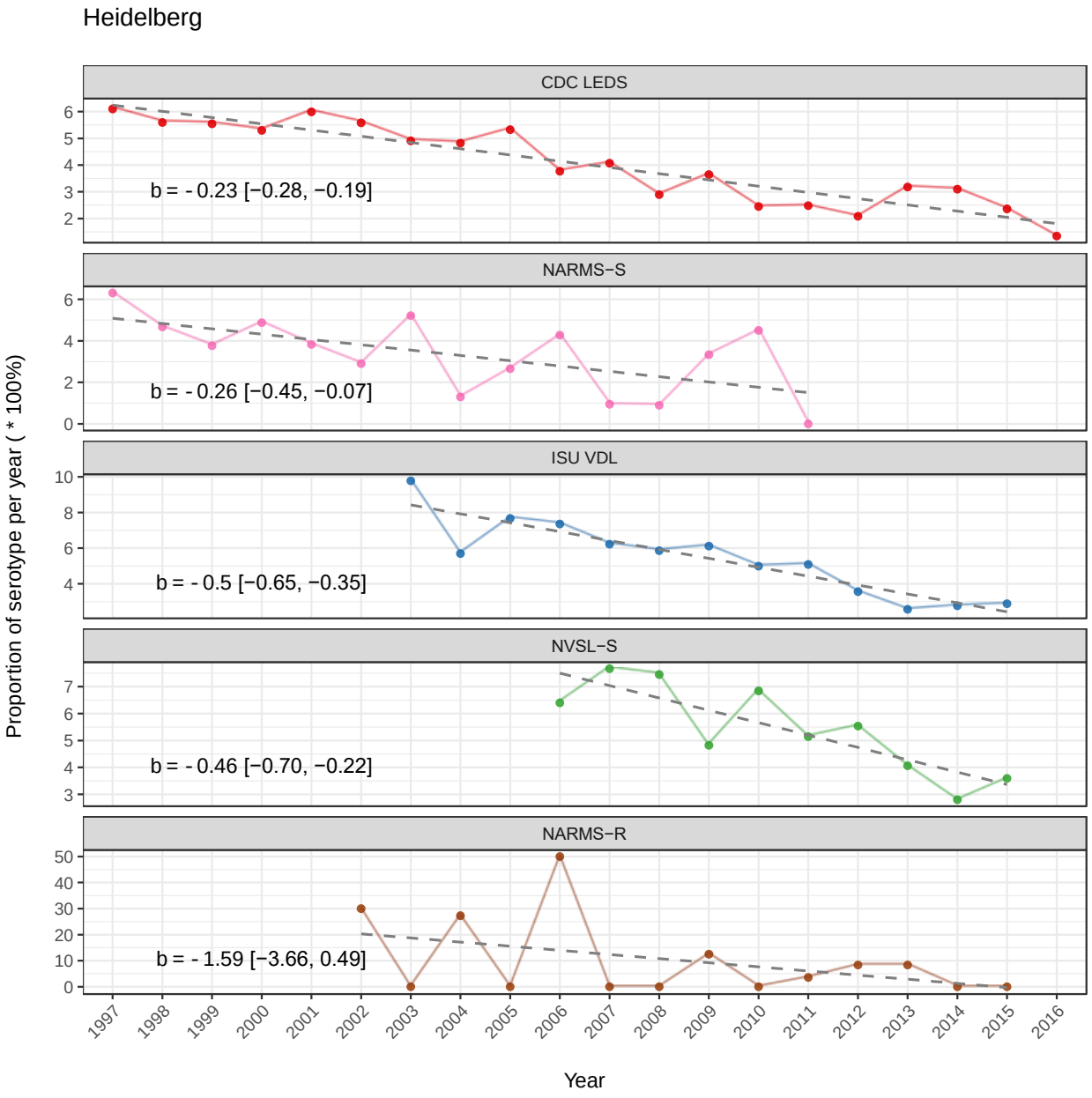


Figure 4: Observed prevalence of *Salmonella enterica* serovar 4,[5],12:i:- over years in all swine data set. The β coefficient and 95% confidence interval for the covariate year are reported.

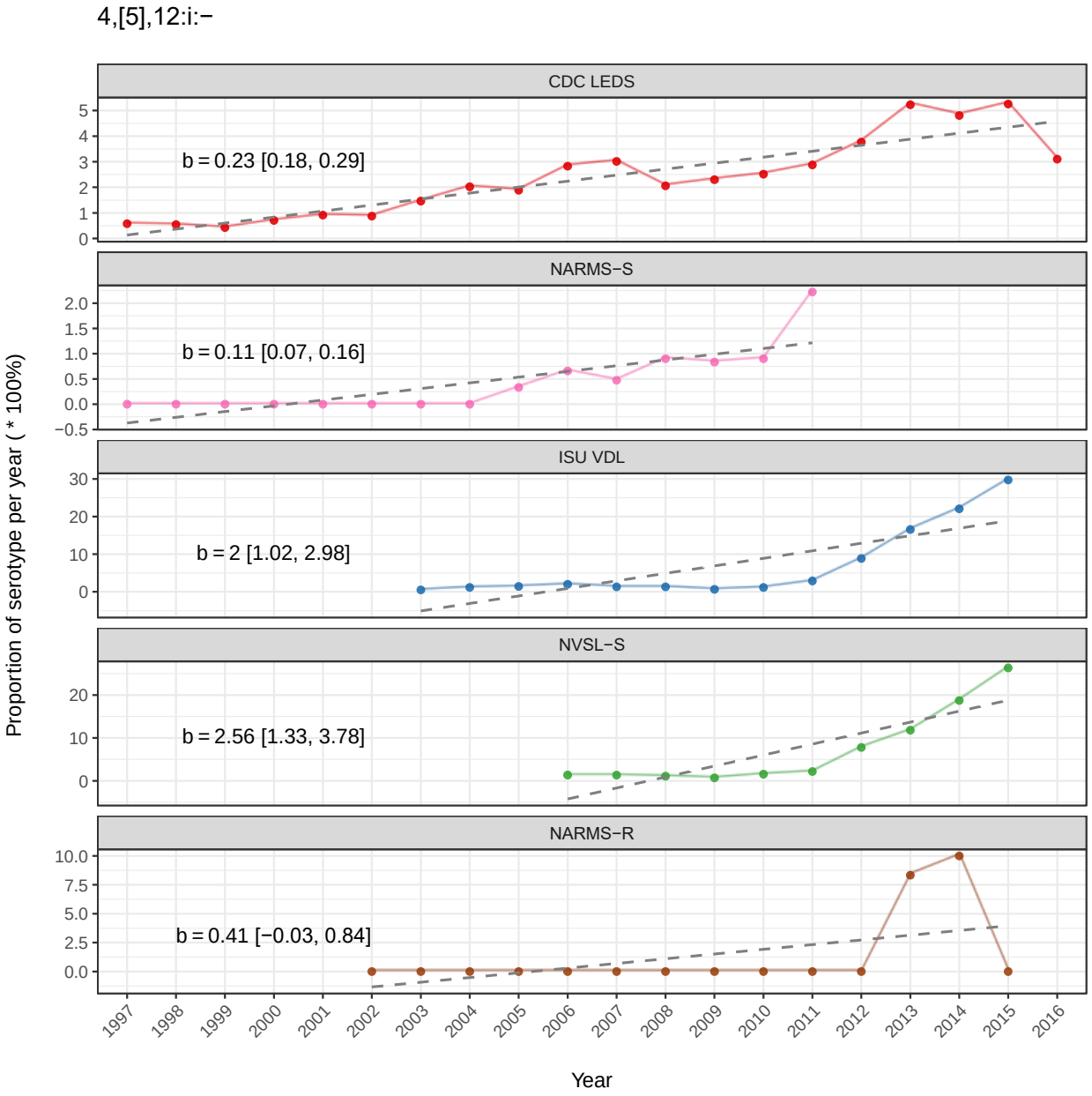


Figure 5: Observed prevalence of *Salmonella enterica* serovar Infantis over years in all swine data set. The β coefficient and 95% confidence interval for the covariate year are reported.

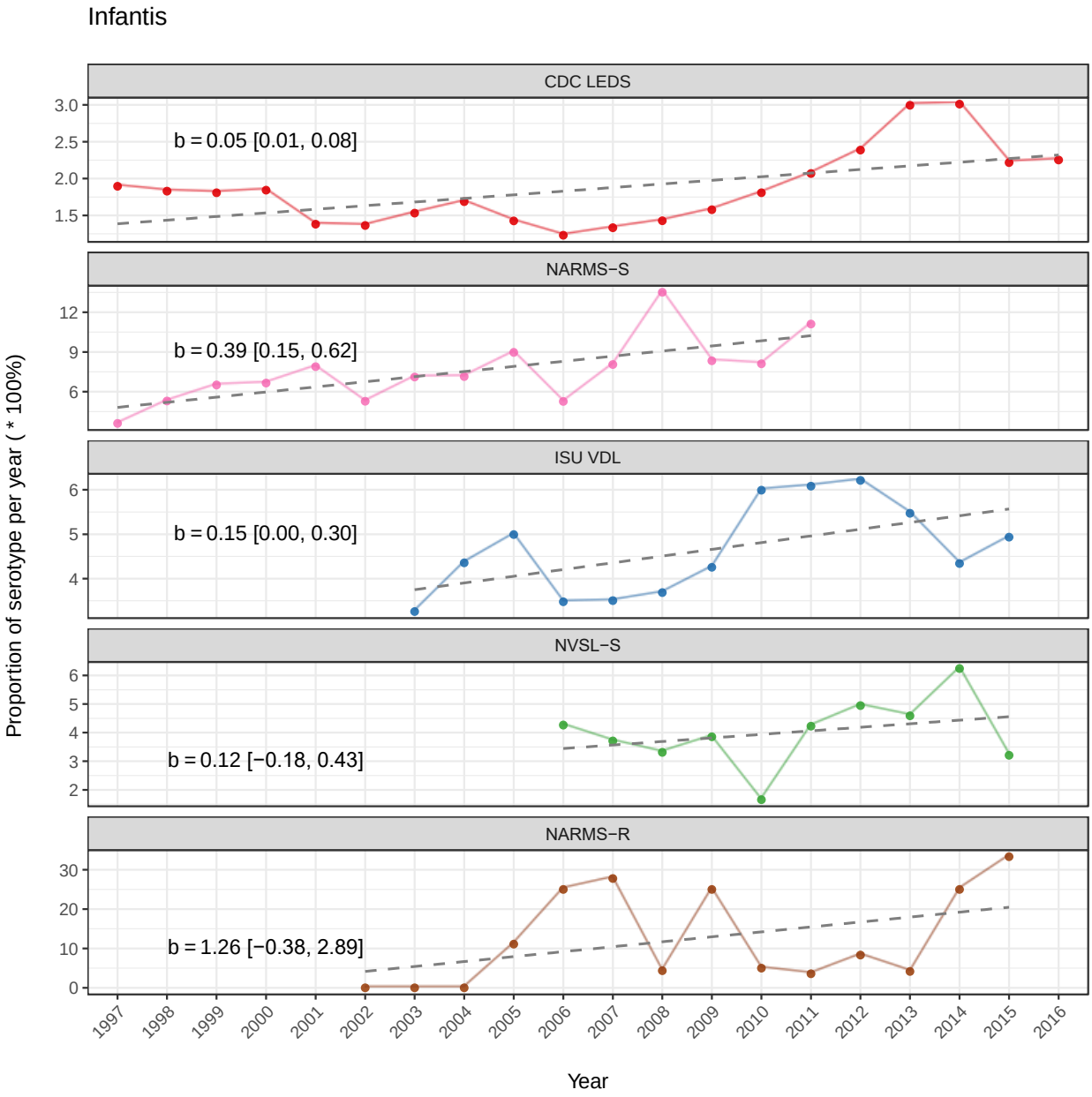


Figure 6: Observed prevalence of *Salmonella enterica* serovar Johannesburg over years in all swine data sets. The β coefficient and 95% confidence interval for the covariate year are reported.

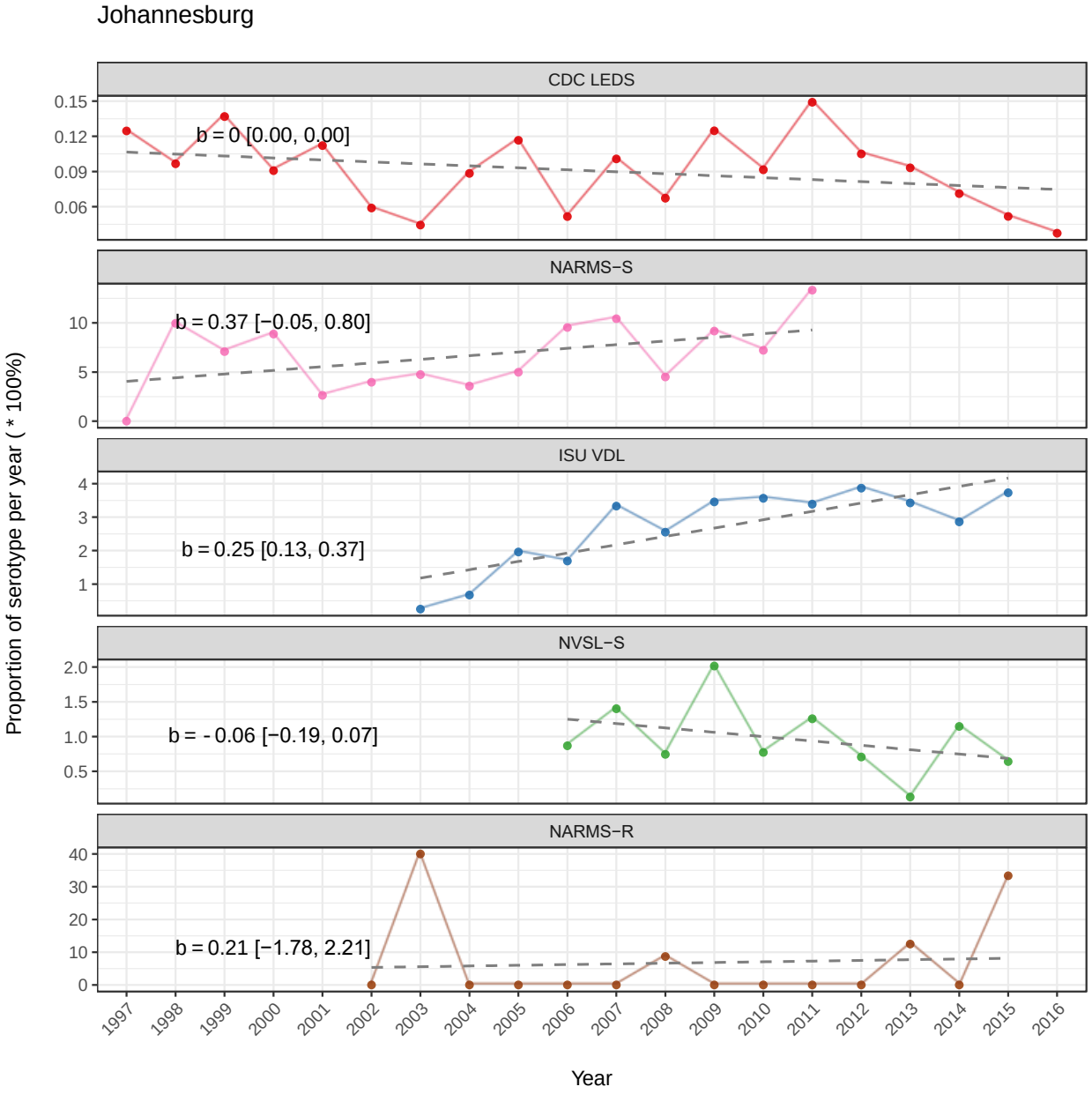


Figure 7: Spearman's rank-order correlation coefficients and 95% CIs for associations between proportion changes in the CDC LEDS dataset and those in ISU VDL swine, avian, and bovine datasets during concurrent years.

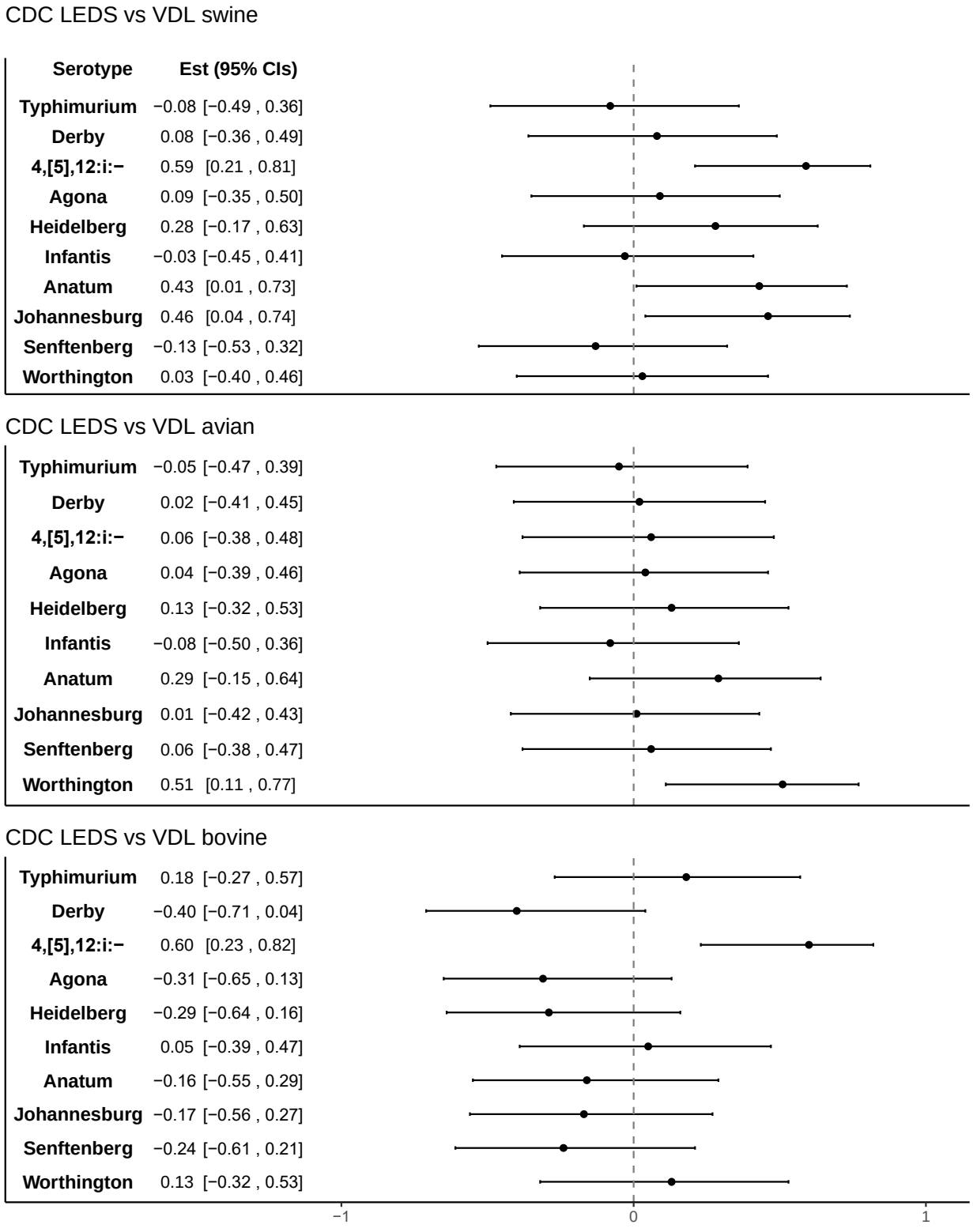


Figure 8: Spearman's rank-order correlation coefficients and 95% CIs for associations between proportion changes in the CDC LEDS dataset and those in NARMS-S swine, avian, and bovine datasets during concurrent years.

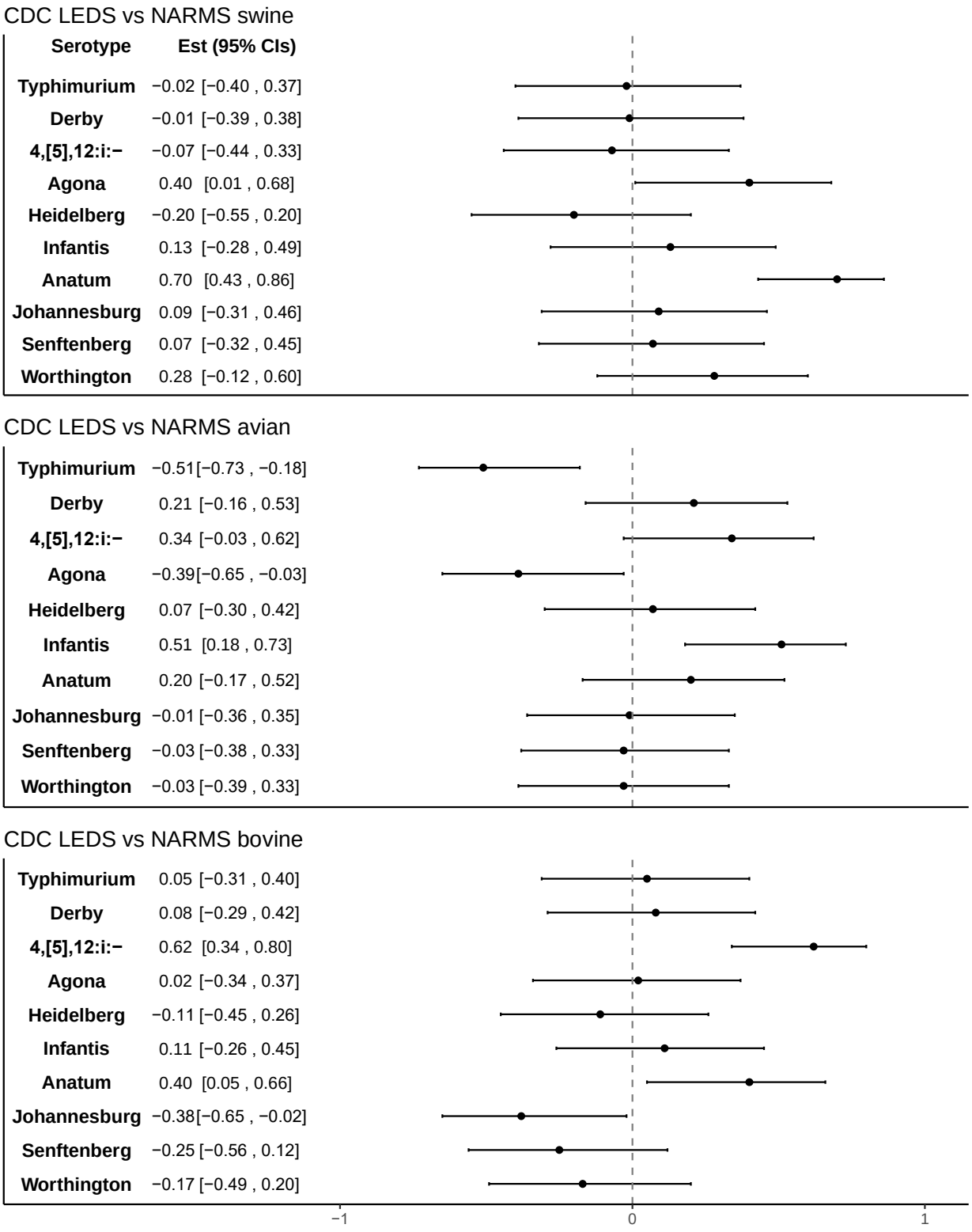
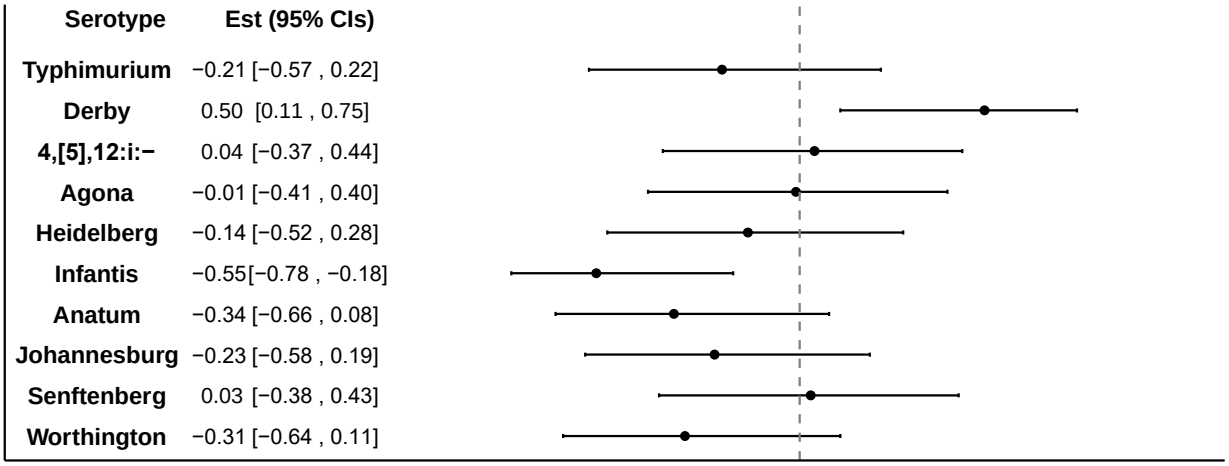
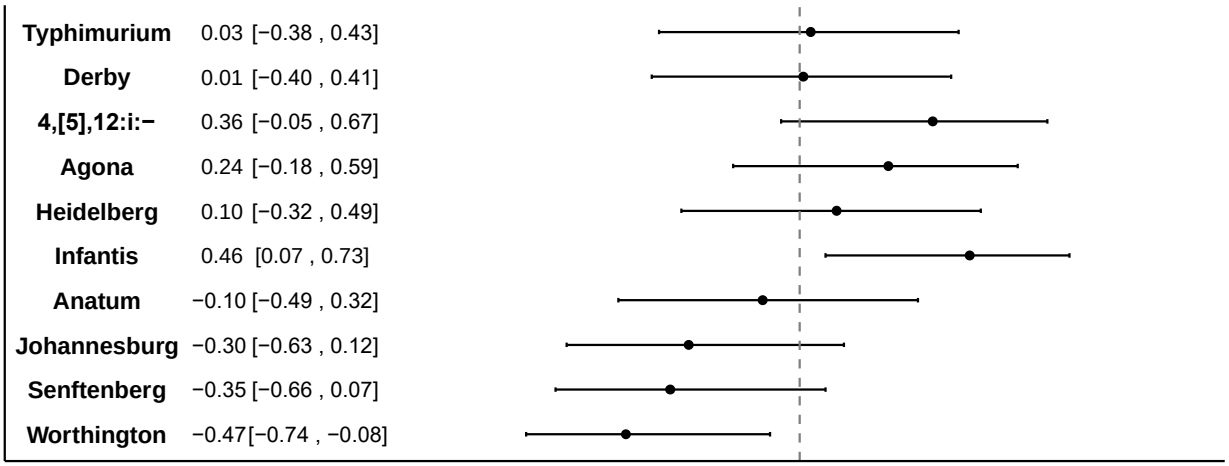


Figure 9: Spearman's rank-order correlation coefficients and 95% CIs for associations between proportion changes in the CDC LEDS dataset and those in NARMS-R swine, avian, and bovine datasets during concurrent years.

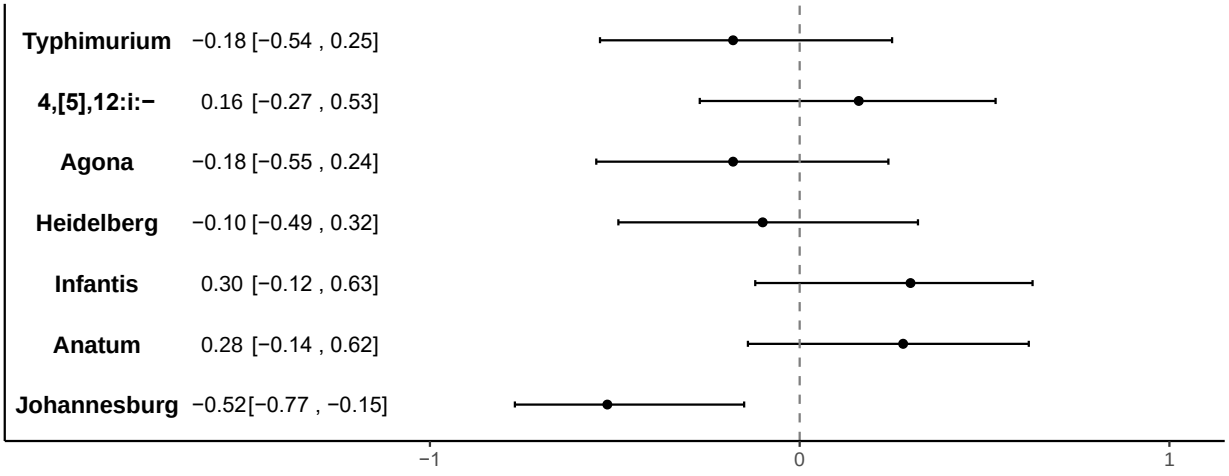
CDC LEDS vs Retail swine



CDC LEDS vs Retail avian



CDC LEDS vs Retail bovine



7 Supplementary

Figure 1: The frequency of all *S. enterica* serovars with more than 10 isolations from 2003 to 2015 in ISU VDL swine dataset.

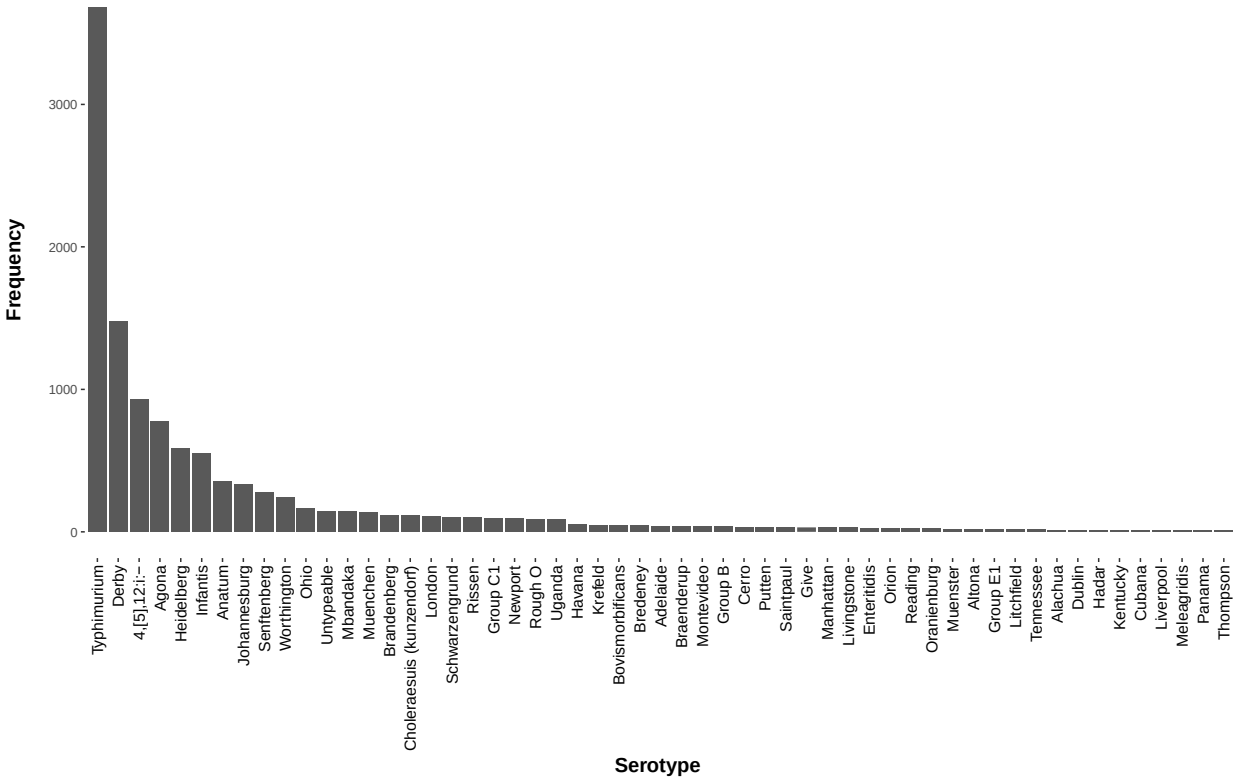


Figure 2: The frequency of all *S. enterica* serovars with more than 10 isolations from 2006 to 2015 in NVSL-S swine dataset.

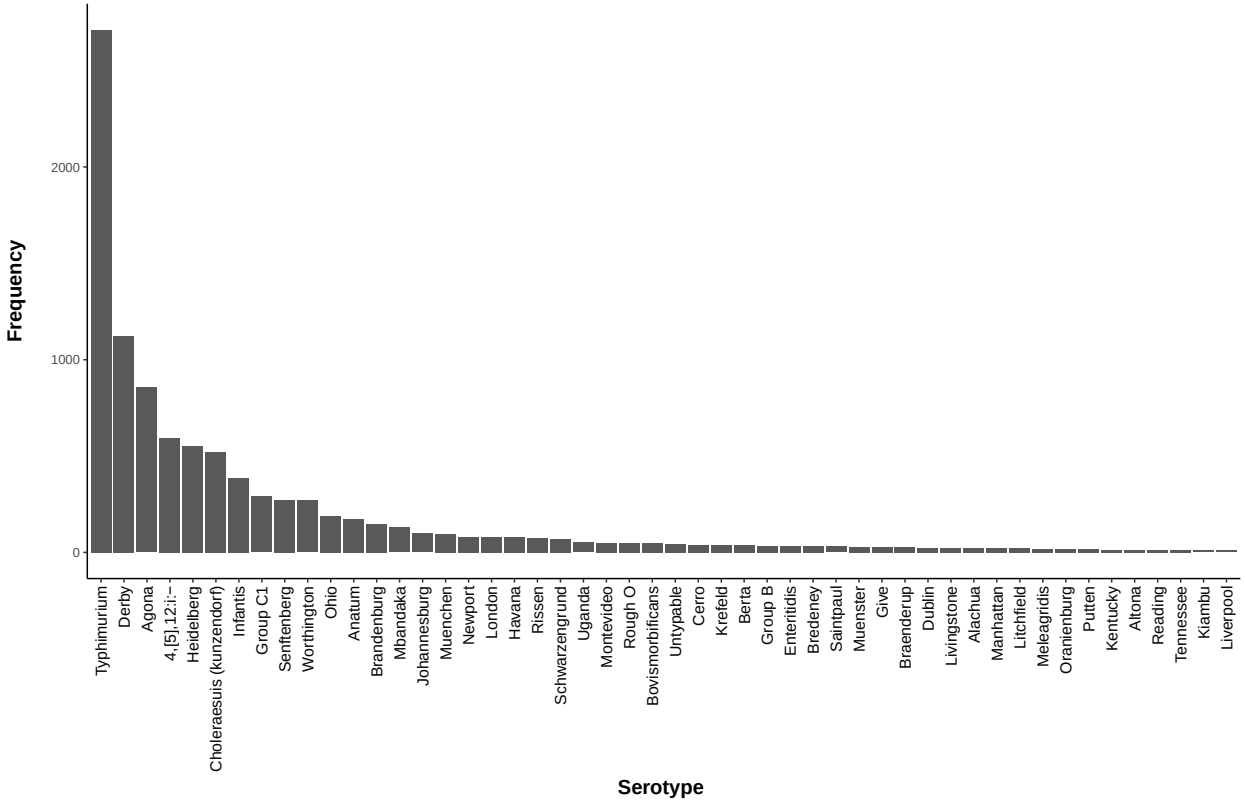


Figure 3: The frequency of all *S. enterica* serovars with more than 10 isolations from 1997 to 2011 in NARMS-S dataset.

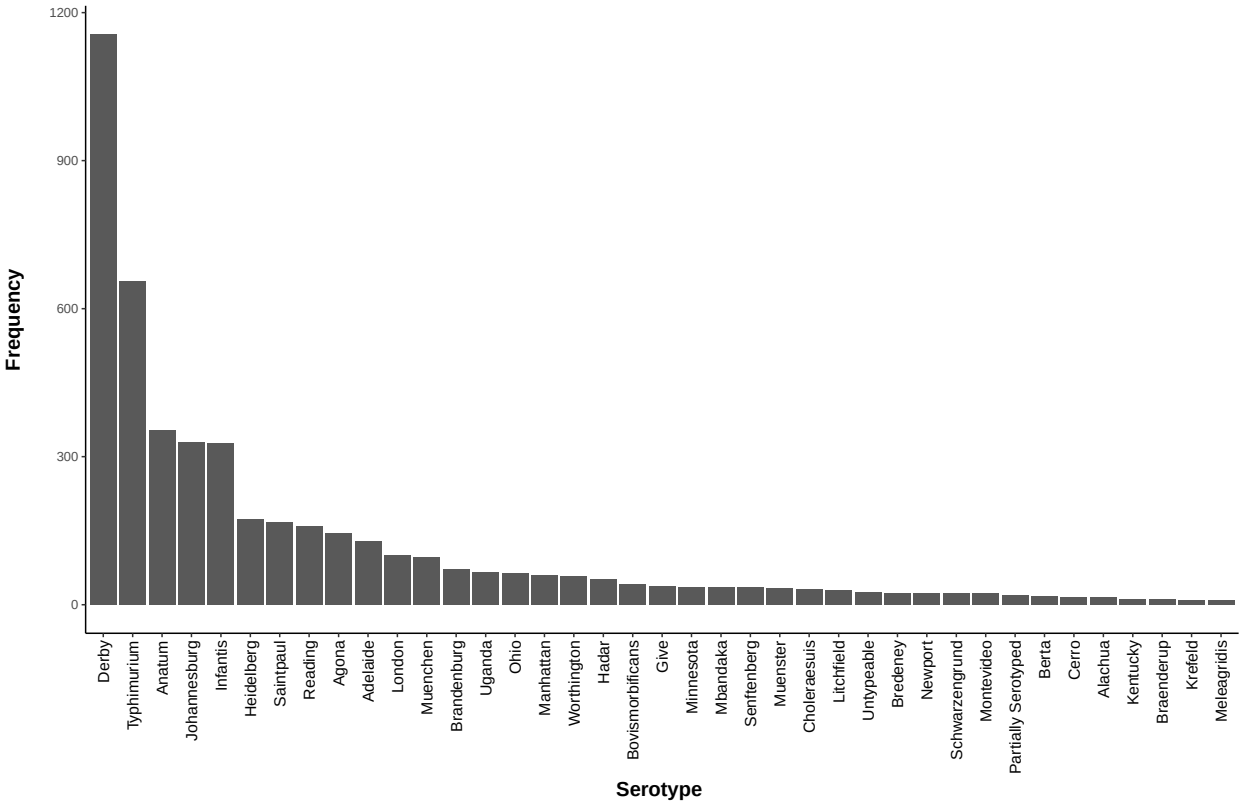


Figure 4: The frequency of all *S. enterica* serovars with more than 200 isolations from 1997 to 2016 in CDC LEDS dataset.

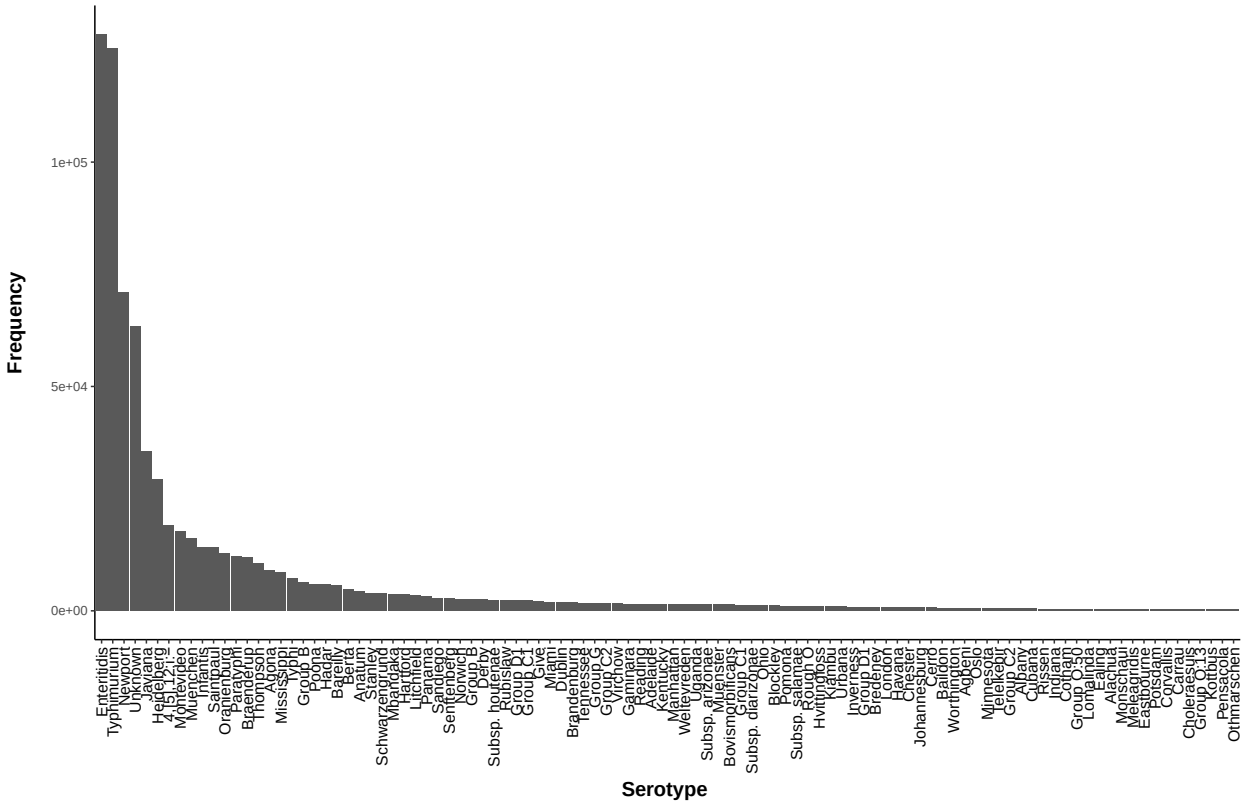


Figure 5: The frequency of all *S. enterica* serovars from 2002 to 2015 in NARMS-R swine dataset.

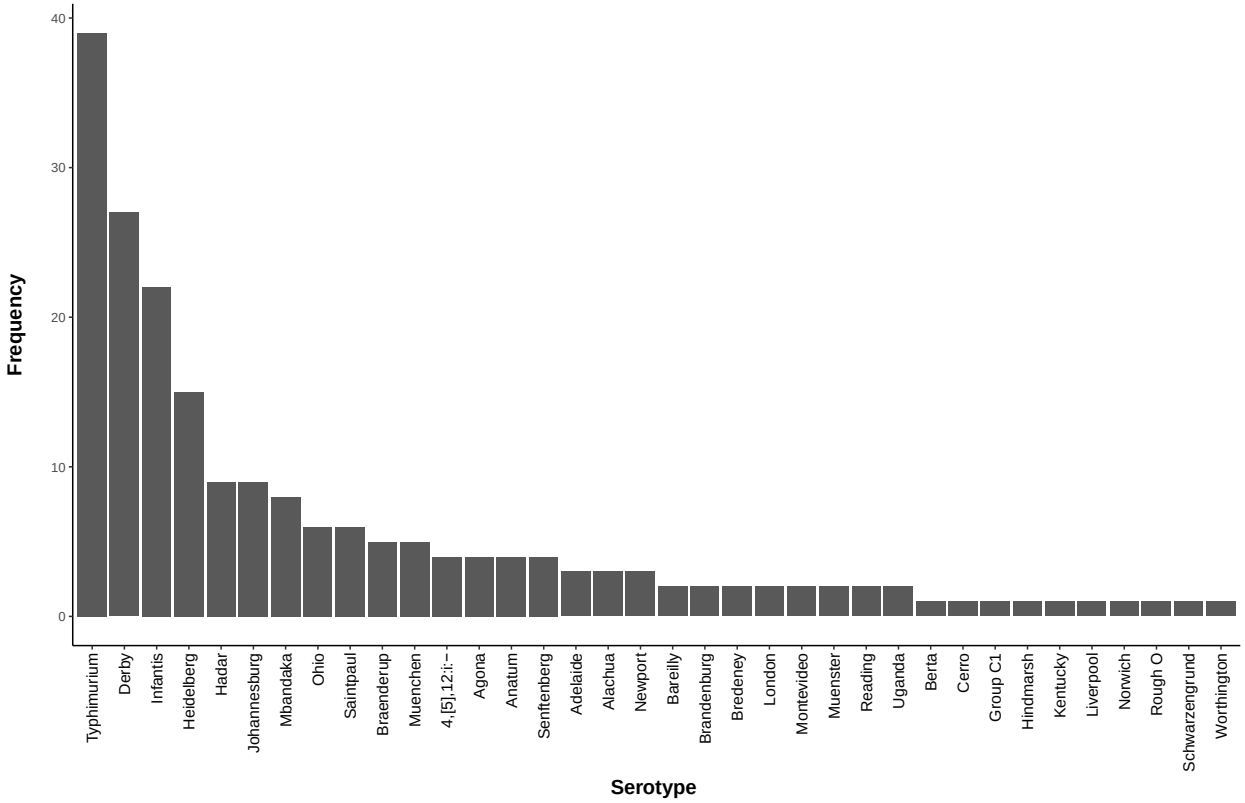


Figure 6: Observed percentage of *Salmonella enterica* serovar Agona over years. The β coefficient and 95% confidence interval for the covariate year are reported.

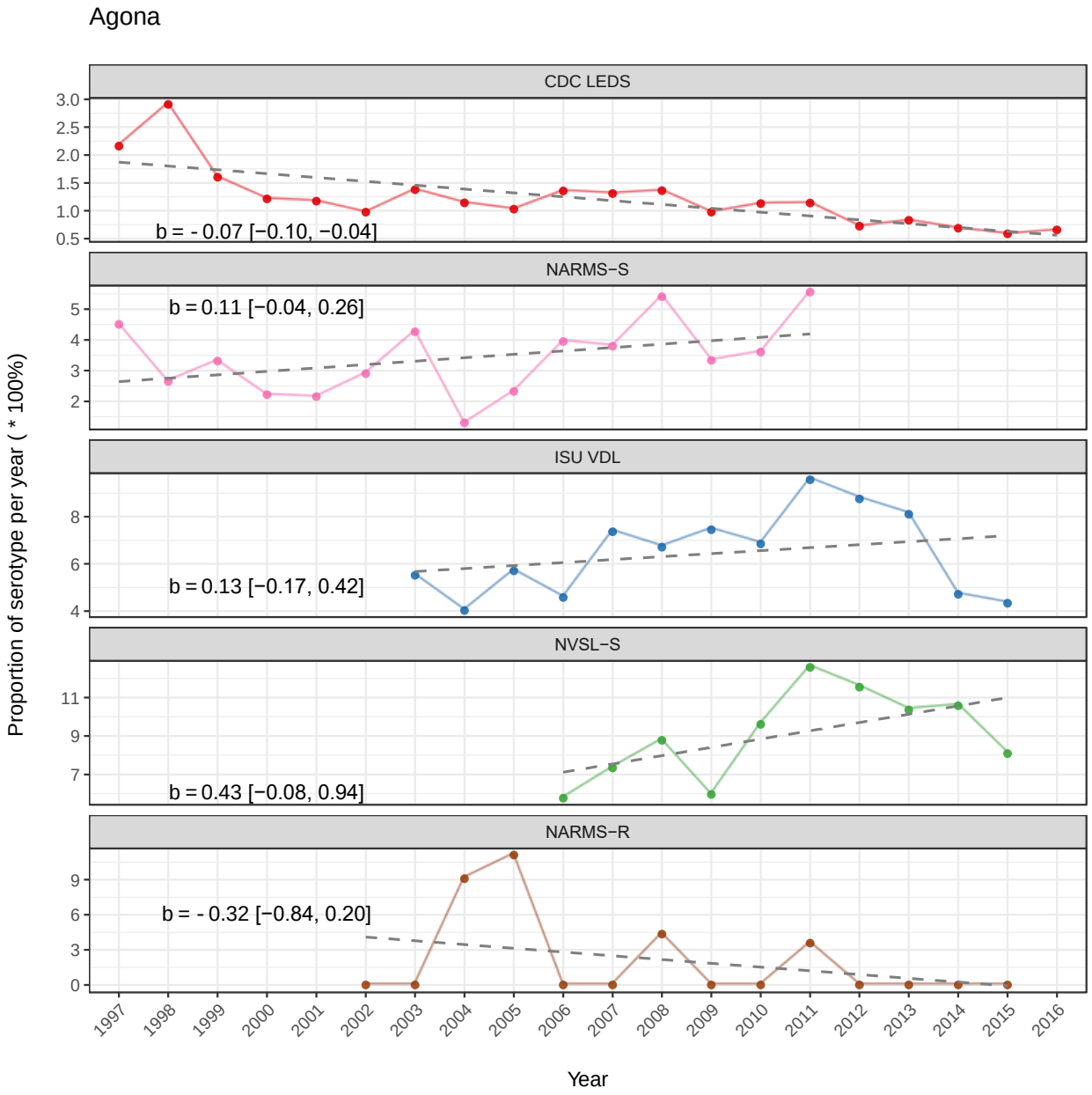


Figure 7: Observed percentage of *Salmonella enterica* serovar Anatum over years. The β coefficient and 95% confidence interval for the covariate year are reported.

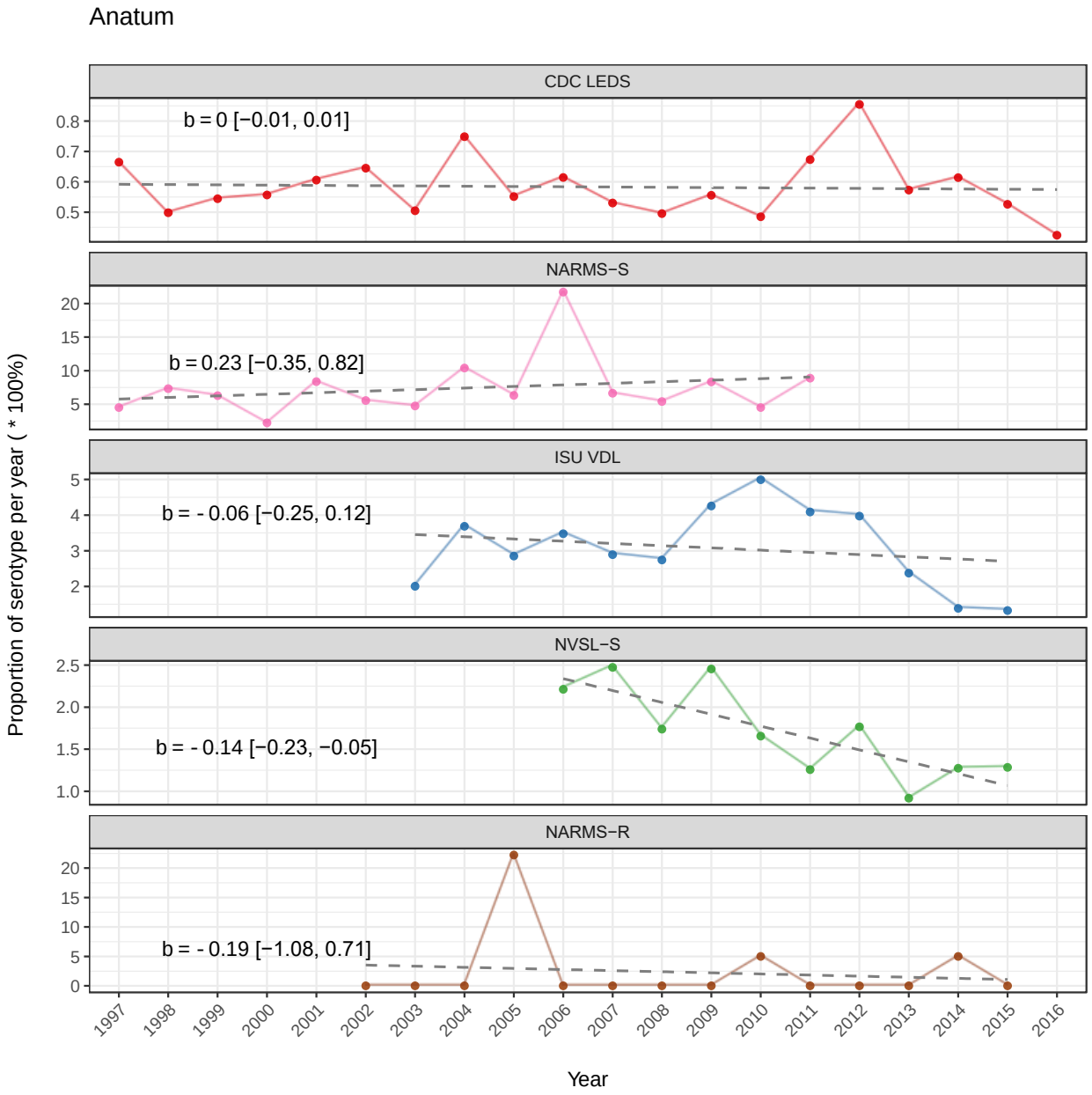


Figure 8: Observed percentage of *Salmonella enterica* serovar Senftenberg over years. The β coefficient and 95% confidence interval for the covariate year are reported.

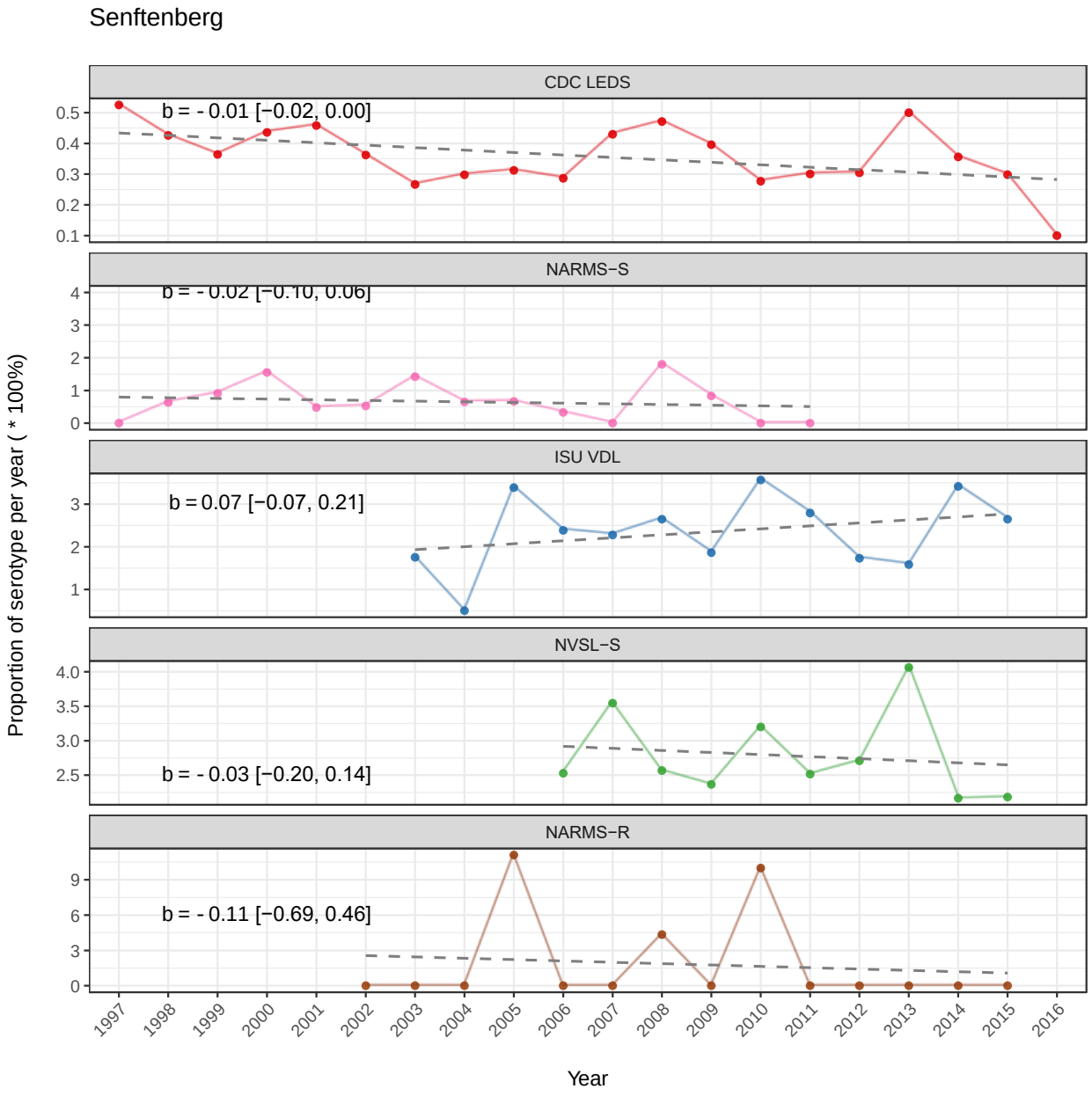


Figure 9: 1-year lag Spearman's rank-order correlation coefficients and 95% confidence intervals between ISU VDL species-level data and CDC LEDS data.

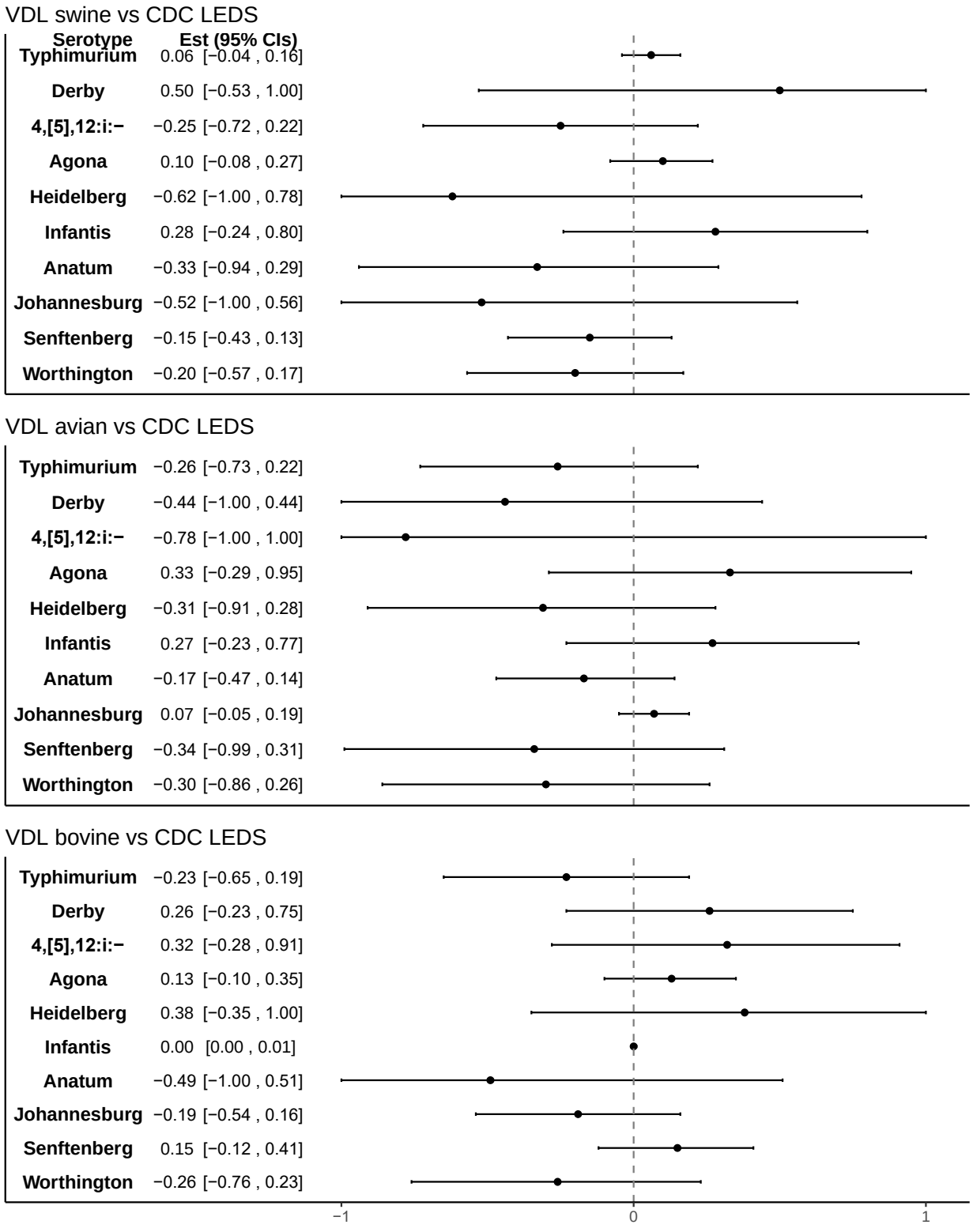


Figure 10: 1-year lag Spearman's rank-order correlation coefficient and 95% confidence intervals between NARMS-S species-level data and CDC LEDS data.
shadecolorrgb0.969, 0.969, 0.969

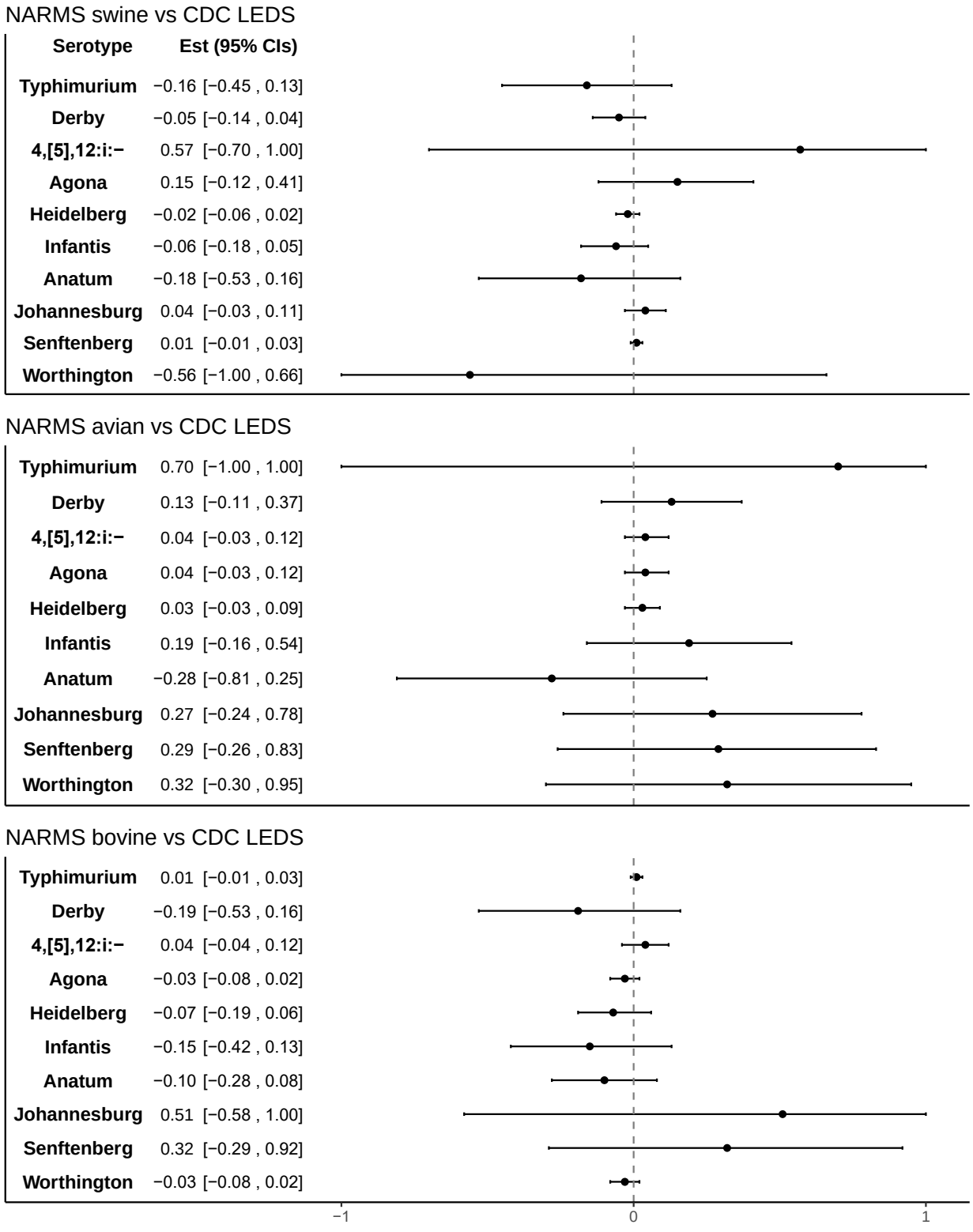


Figure 11: 1-year lag Spearman's rank-order correlation coefficient and 95% confidence intervals between NARMS-R species-level data and CDC LEDS data.

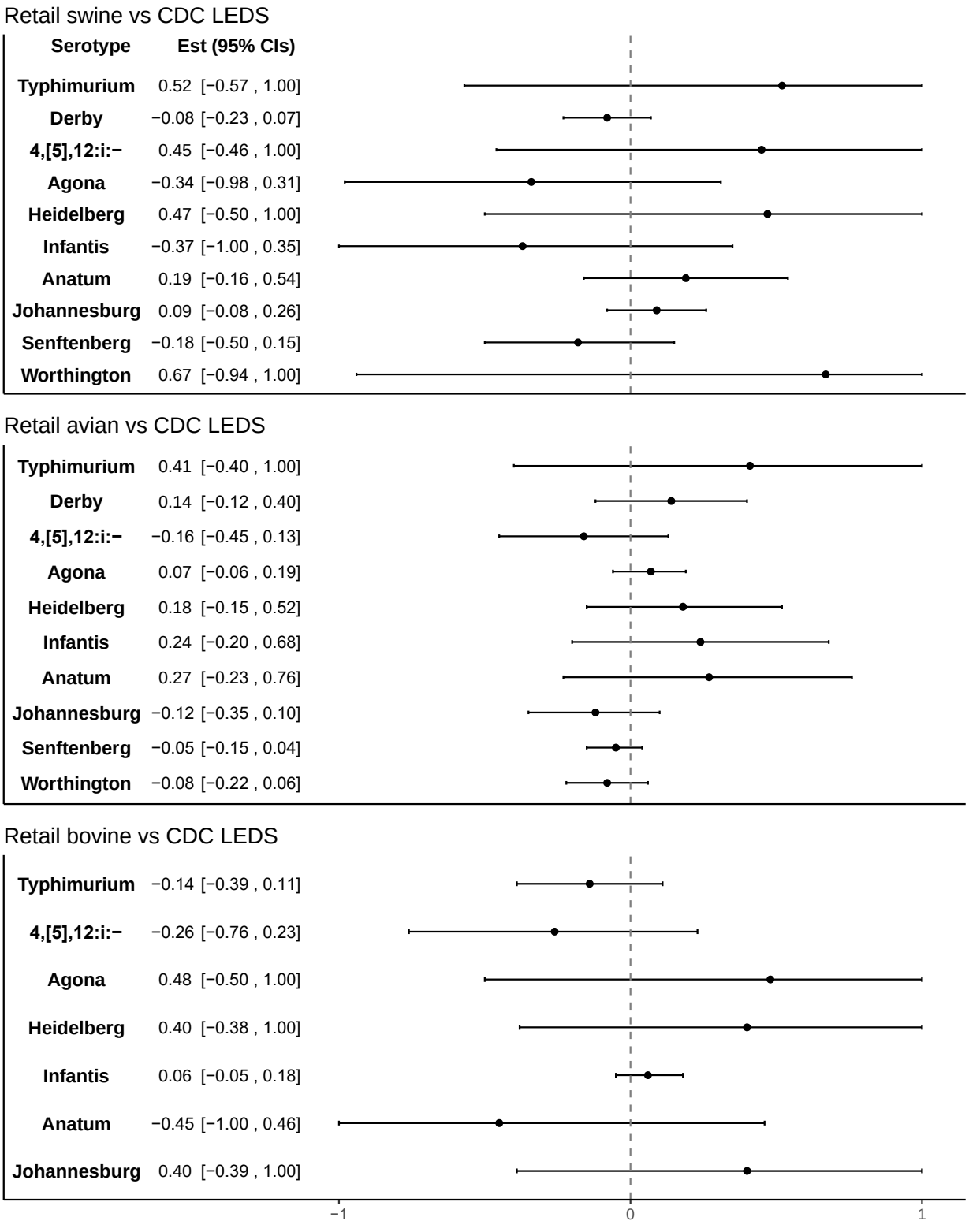


Figure 12: 2-year lag Spearman's rank-order correlation coefficient and 95% confidence intervals between VDL species-level data and CDC LEDS data.

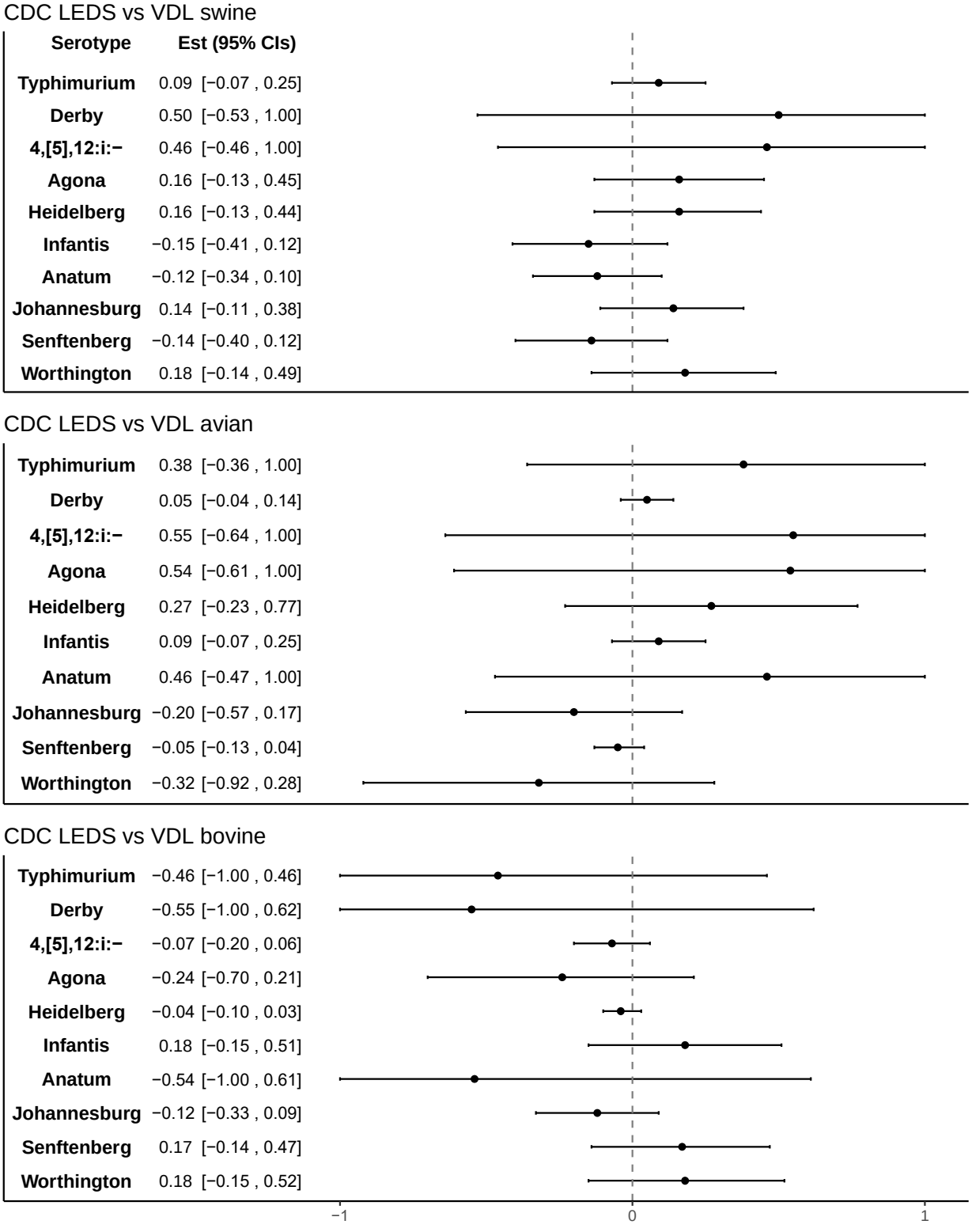


Figure 13: 2-year lag Spearman's rank-order correlation coefficient and 95% confidence intervals between NARMS-S species-level data and CDC LEDS data.

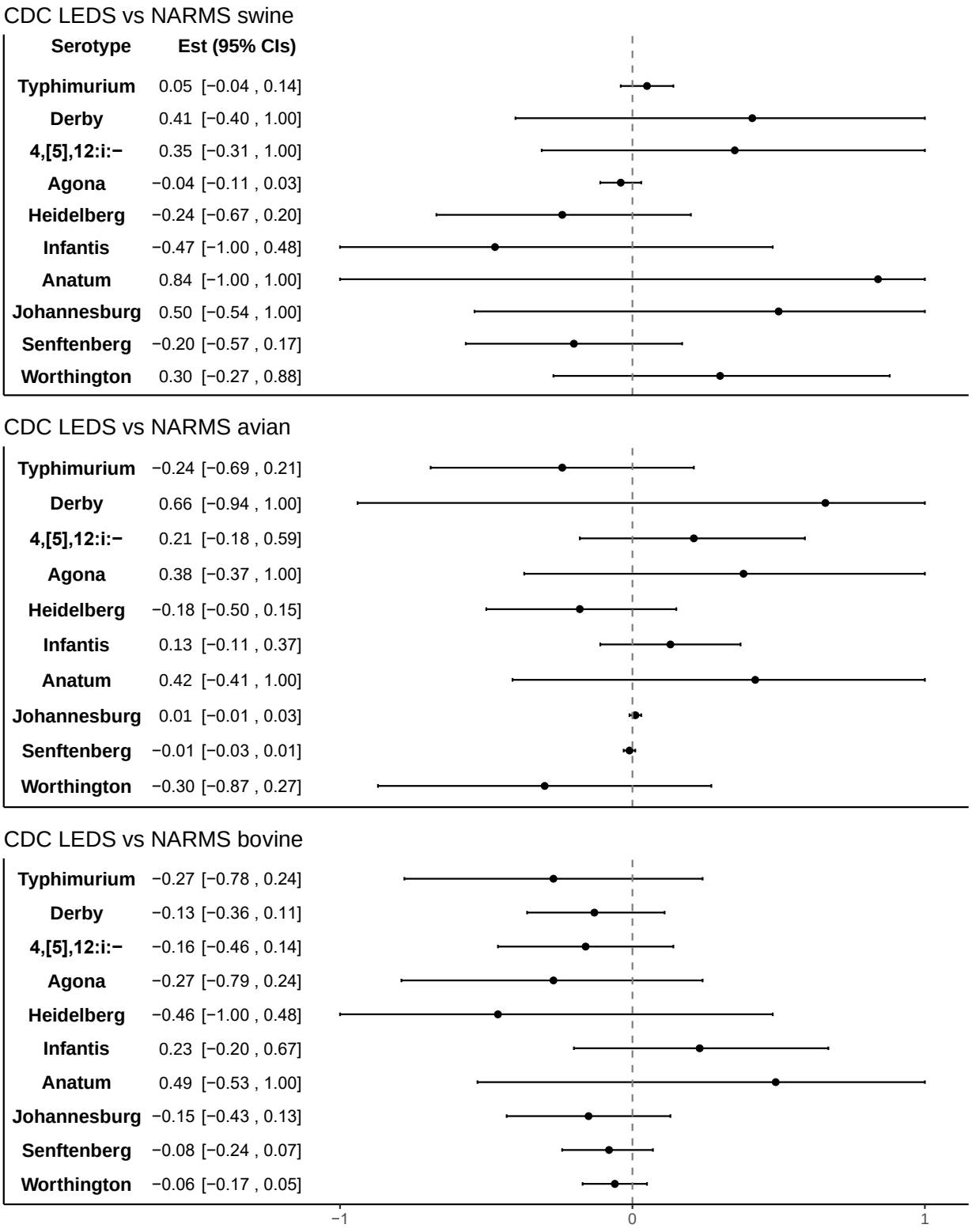
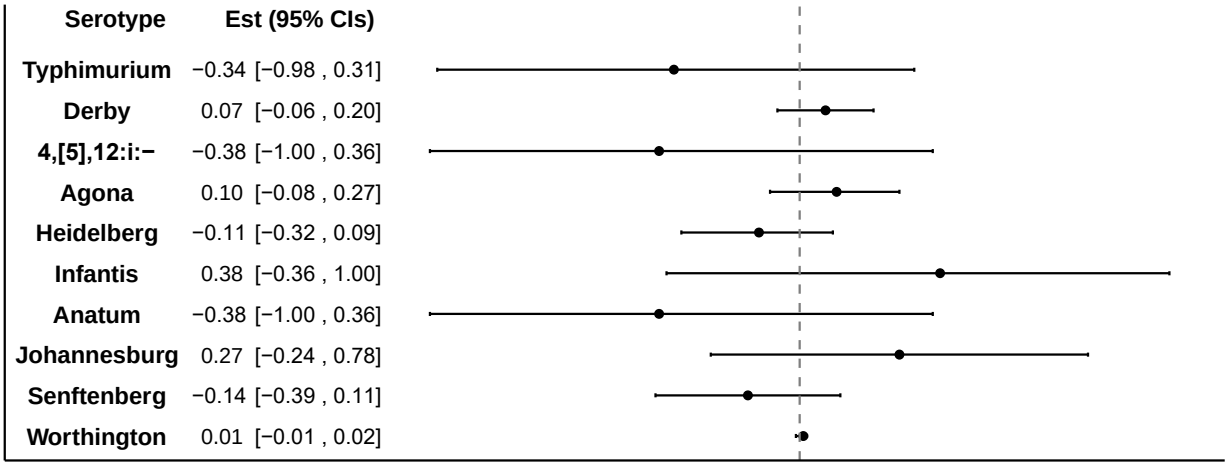
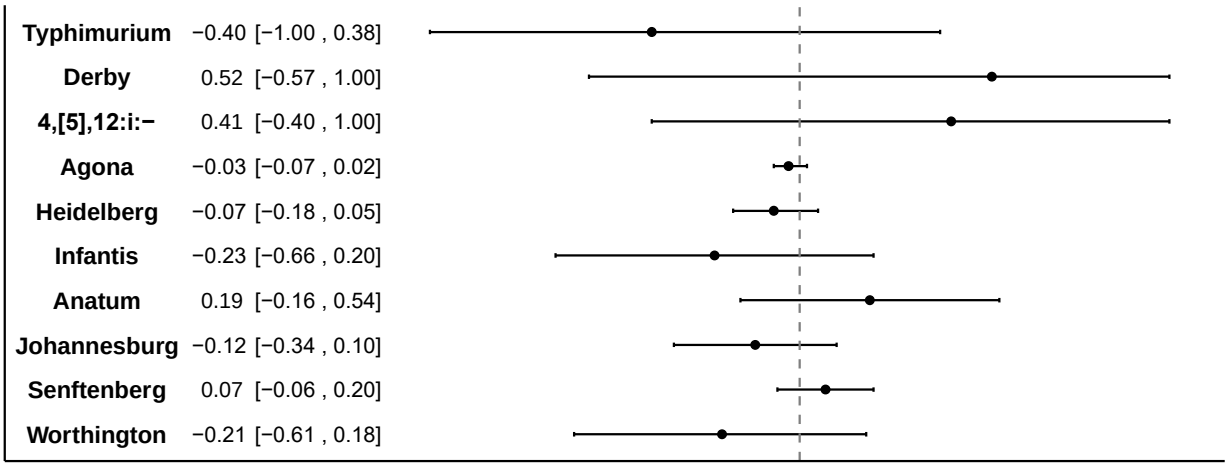


Figure 14: 2-year lag Spearman's rank-order correlation coefficient and 95% confidence intervals between NARMS-R species-level data and CDC LEDS data.

CDC LEDS vs Retail swine



CDC LEDS vs Retail avian



CDC LEDS vs Retail bovine