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Changes in the prevalence of Salmonella serovars associated swine production and correlations of avian, bovine and swine-associated serovars with human-associated serovars in the United States (1997–2015)

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Summary

As *Salmonella enterica* is an important pathogen of food animals, surveillance programmes for *S. enterica* serovars have existed for many years in the United States. Surveillance programmes 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 near 20-year observation period (1997–2015) using four longitudinal data sets 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.

KEYWORDS
avian, bovine, *Salmonella*, serovars, surveillance, swine

1 | INTRODUCTION

As *Salmonella enterica* is an important pathogen of food-producing animals, surveillance programmes for *S. enterica* serovars have

existed for many years in the United States. Periodic review of data from these surveillance programmes can help identify changes in the prevalence of certain serovars, which may indicate emerging issues (Iwamoto et al., 2017; Stärk & Häsler,

2015). Currently, the pork industry has an effective *S. enterica* control programme 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, O'Connor, Sargeant, & McKean, 2010; Denagamage, O'Connor, Sargeant, Rajić, & McKean, 2007; O'Connor, Denagamage, Sargeant, Rajić, & McKean, 2008; Wilhelm et al., 2012). This swine-based *S. enterica* control programme relies on a pathogen reduction approach at the abattoir (Totton, Glanville, Dzikamunhenga, Dickson, & O'Connor, 2016) based on the rationale that this approach is the most effective and cost-efficient (Alban & Stärk, 2005; O'Connor, Wang, Denagamage, & McKean, 2012). 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 programmes, 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 programmes 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 data sets to detect changes in the proportion of *S. enterica* serovars commonly isolated from swine from specimens submitted from diagnostic laboratories (two data sets) or collected at slaughter (one data set) or retail (one data set).

2 | MATERIALS AND 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) programme and United States Department of Agriculture (USDA) National Veterinary Services Laboratory (NVSL).

2.2 | Centers for Disease Control and Prevention Laboratory-based Enteric Disease Surveillance data set

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 programme. We directly requested and obtained the most recently available and complete data from the CDC. Details of the LEDS programme and data collection approach are described elsewhere (https://www.cdc.gov/national surveillance/

Impacts

- The 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 suggest that the populations are not static and regular evaluation is warranted.
- We detected 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).
- An impact of these findings might be that veterinary diagnostic submission could be evaluated as more sensitive methods of detecting emerging Salmonella serotypes.

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salmonella-surveillance.html). It is important to note that these LEDS data arise as a result of passive surveillance and serotyping completeness varies by reporting laboratory and over time.

2.3 | National Antimicrobial Resistance Monitoring System data sets

The NARMS programme 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 data set, 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 data set, 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 Food Net sites and state departments of public health, which have changed over time. When the retail programme was launched in 2002, participating states included Connecticut, Georgia, Maryland, Minnesota and Tennessee, with Oregon joining the programme 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 data sets from a publicly available source (https://wwwn.cdc.gov/narmsnow/). The laboratory methods used by NARMS are described in the inter-agency Manual of Laboratory Methods (available at Manual of Laboratory Methods).

2.4 | Iowa State University Veterinary Diagnostic Laboratory data set

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 pathologists' or submitting veterinarian'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 | United States Department of Agriculture (USDA) National Veterinary Services Laboratory (NVSL). data set

The NVSL data set 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 analysed 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 data sets were removed from analysis, that is, 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 & Weill, 2007).

2.6 | Management of data sets

2.6.1 | Swine-associated data

1. ISU VDL data set. Data describing 11681 isolates were included in the original data set. After removing several non-Salmonella isolates accidentally included in the provided data set, the data set contained information on 11631 isolates collected from 2003 to 2015. We also included data describing 132 isolates of *S. enterica* serovar Choleraesuis identified using a novel in-house approach.
2. NVSL-S data set. Data describing 9785 isolates were included in the original data set. After removing non-Salmonella isolates, the data set contained information on 9785 Salmonella isolates collected from 2006 to 2015.
3. NARMS-S data set. Data describing 4795 isolates were included in the original data set. After removing the non-Salmonella isolates, the data set contained data that related to 4795 Salmonella isolates collected from 1997 to 2011.
4. NARMS-R data set. Data describing 202 isolates collected from 2002 to 2015. As this data set was filtered before it was downloaded, no non-Salmonella isolates were removed post hoc.

2.6.2 | Non-swine data

1. ISU VDL avian data set. This data set included isolates from chickens and turkeys, which were combined to form a single avian category. Data describing 2843 isolates were included in the original data set. After removing non-Salmonella isolates, the data set contained information on 2765 Salmonella isolates collected from 2003 to 2015.
2. ISU VDL bovine data set. This data set 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 data set. After removing the non-Salmonella isolates, the data set contained information on 1986 Salmonella isolates collected from 2003 to 2015.
3. NVSL avian data set. Data describing 51001 isolates were included in the original data set. After removing the non-Salmonella isolates, the data set contained information on 50999 Salmonella isolates collected from 2006 to 2015.
4. NVSL bovine data set. Data describing 23160 isolates were included in the original data set. After removing the non-Salmonella isolates, the data set contained information on 23120 Salmonella isolates collected from 2006 to 2015.
5. NARMS-S avian data set: This data set included isolates from chickens and turkeys, which were combined to form a single avian category. The data set contained information on 21065 isolates. After removing the non-Salmonella isolates, there were 21065 isolates collected from 1997 to 2013.
6. NARMS-S bovine data set. Data describing 9461 isolates were included in the original data set. After removing the non-Salmonella isolates, the data set contained information on 9461 Salmonella isolates collected from 1997 to 2013.
7. NARMS-R avian data set. This data set included isolates from chickens and turkeys, which were combined to form a single avian category. The data set contained information on 4138 isolates collected from 2002 to 2015.
8. NARMS-R bovine data set. Data describing 169 Salmonella isolates were available from 2002 to 2015.
9. CDC LEDS data set. Data describing 755086 isolates were included in the original data set. After removing the non-Salmonella isolates, the data set contained information on 751095 Salmonella isolates collected from 1997 to 2016.

2.7 | Mapping *S. enterica* serovars across data sets

For each data set, all unique serovars were identified. Serovar names that appeared to be typographic errors were identified

TABLE 1 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]

TABLE 2 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]

and verified by consulting with coauthors with expertise in microbiology. For example, "serovar Infantis" was assumed to be "serovar Infantis," "serovar 4,5,12:1:-" was assumed to be "serovar 4,5,12:i:-," and "phade DT12" was assumed to be "phage DT12." After correcting such errors, coauthors 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 labelled *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:-, was mapped to a single group labelled *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 labelled *S. enterica* serovar Typhimurium (antigenic formula 4,[5],12:i:1,2) because the NARMS-S data set 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 2017). 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 data set. We next determined the proportion of isolates of each serovar out of the total serovar count each year for the other data sets. 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 data set (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
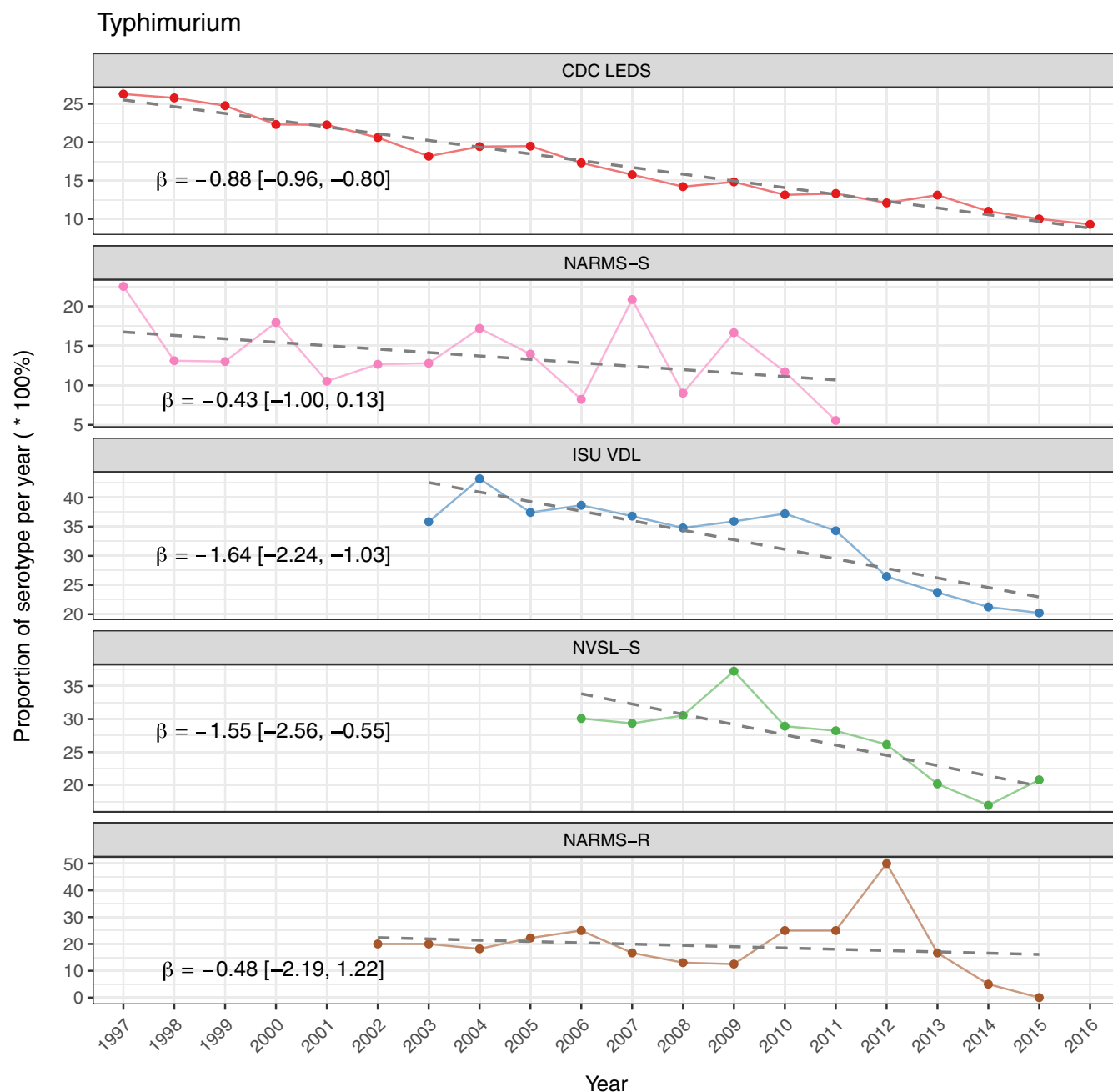
Typhimurium

FIGURE 1 Observed prevalence of *Salmonella enterica* serovar Typhimurium over years in all swine data sets. The coefficients and 95% CIs for the covariate year are reported [Colour figure can be viewed at wileyonlinelibrary.com]

S. enterica serovars in the ISU VDL swine data set (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 data sets:

1. CDC LEDS data set with ISU VDL swine, avian and bovine data sets,
2. CDC LEDS data set with NARMS-S swine, avian and bovine data sets,

3. CDC LEDS data set with NARMS-R swine, avian and bovine data sets,
4. CDC LEDS data set with NVSL-S swine, avian and bovine data sets.

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 data sets). More specifically, for a given serovar, $X(t)$ denotes the yearly
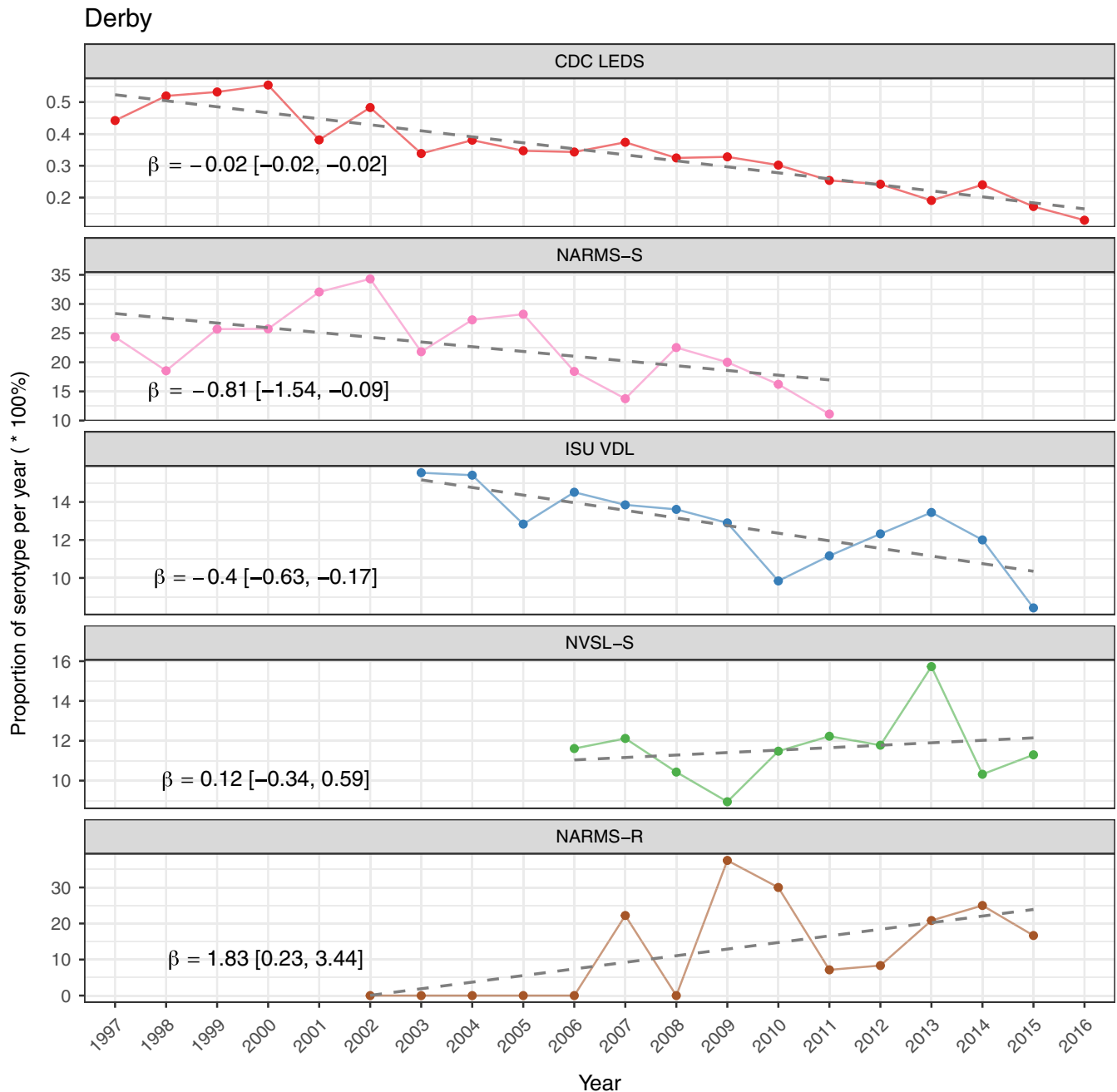
Derby

FIGURE 2 Observed prevalence of *Salmonella enterica* subsp. Derby over years in all swine data sets. The coefficient and 95% confidence interval for the covariate year are reported [Colour figure can be viewed at wileyonlinelibrary.com]

proportion at year *t* in the CDC LEDS data set, and *Y*(*t*) denotes the yearly proportion at year *t* in the other data set; 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 data sets). More specifically, for a given serovar, *X*(*t*) denotes the yearly proportion at year *t* in the CDC LEDS data set, and *Y*(*t*) denotes the yearly percentage at year *t* in the other

data set; 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 data sets). More specifically, for a given serovar, *X*(*t*), denotes the yearly proportion at year *t* in the CDC LEDS data set, and *Y*(*t*) represents the yearly proportion at year *t* in the other data set; thus, Spearman's rank-order correlations were performed between *X*(*t* + 1) - *X*(*t*) and *Y*(*t* + 3) - *Y*(*t* + 2).
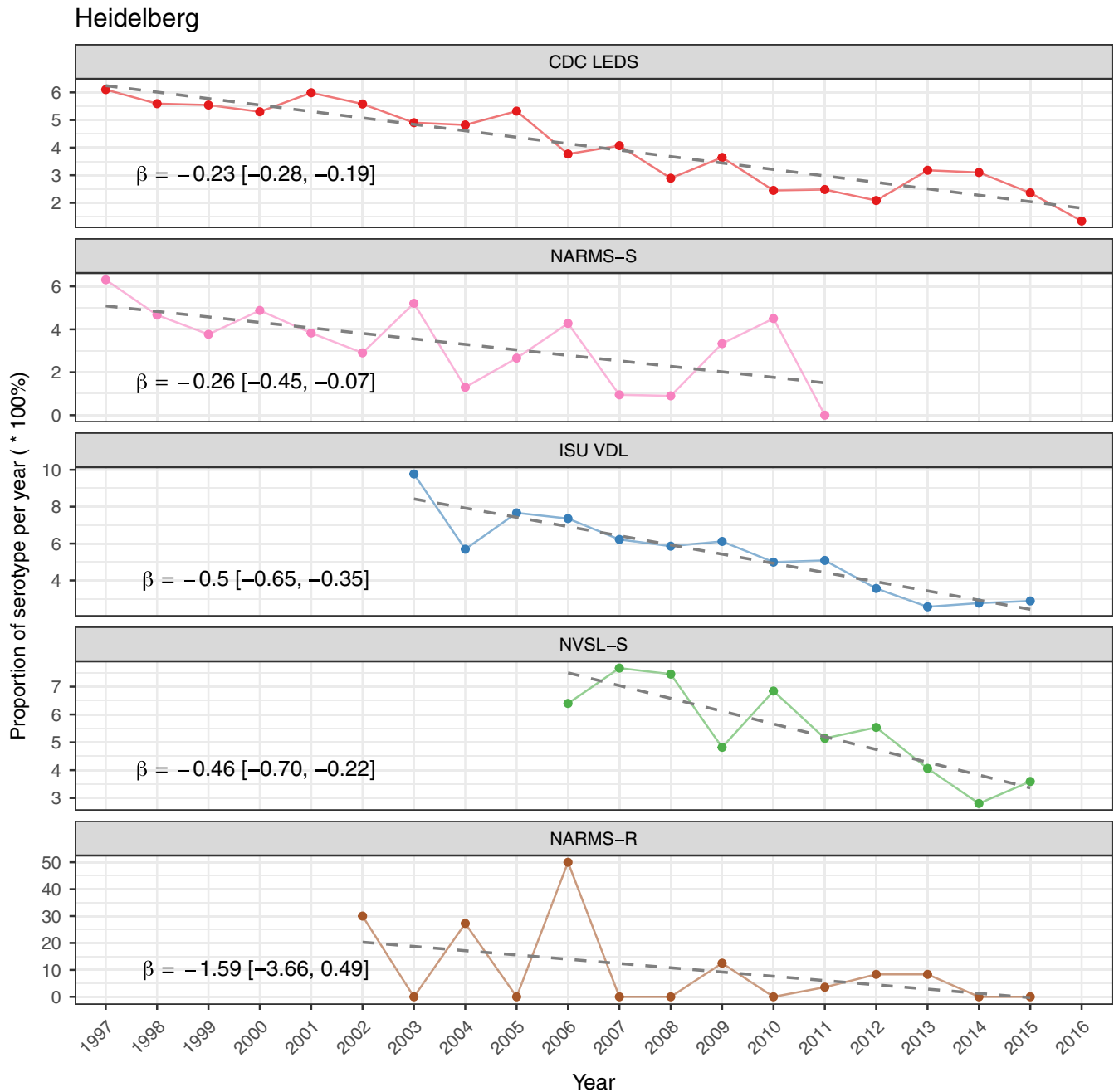
Heidelberg

FIGURE 3 Observed prevalence of *Salmonella enterica* subsp. Heidelberg over years in all swine data sets. The coefficient and 95% confidence interval for the covariate year are reported [Colour figure can be viewed at wileyonlinelibrary.com]

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 data set. 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 data sets 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 is provided in the supplementary materials (see Figure S1, Figure S2, Figure S3, Figure S4 and Figure S5).
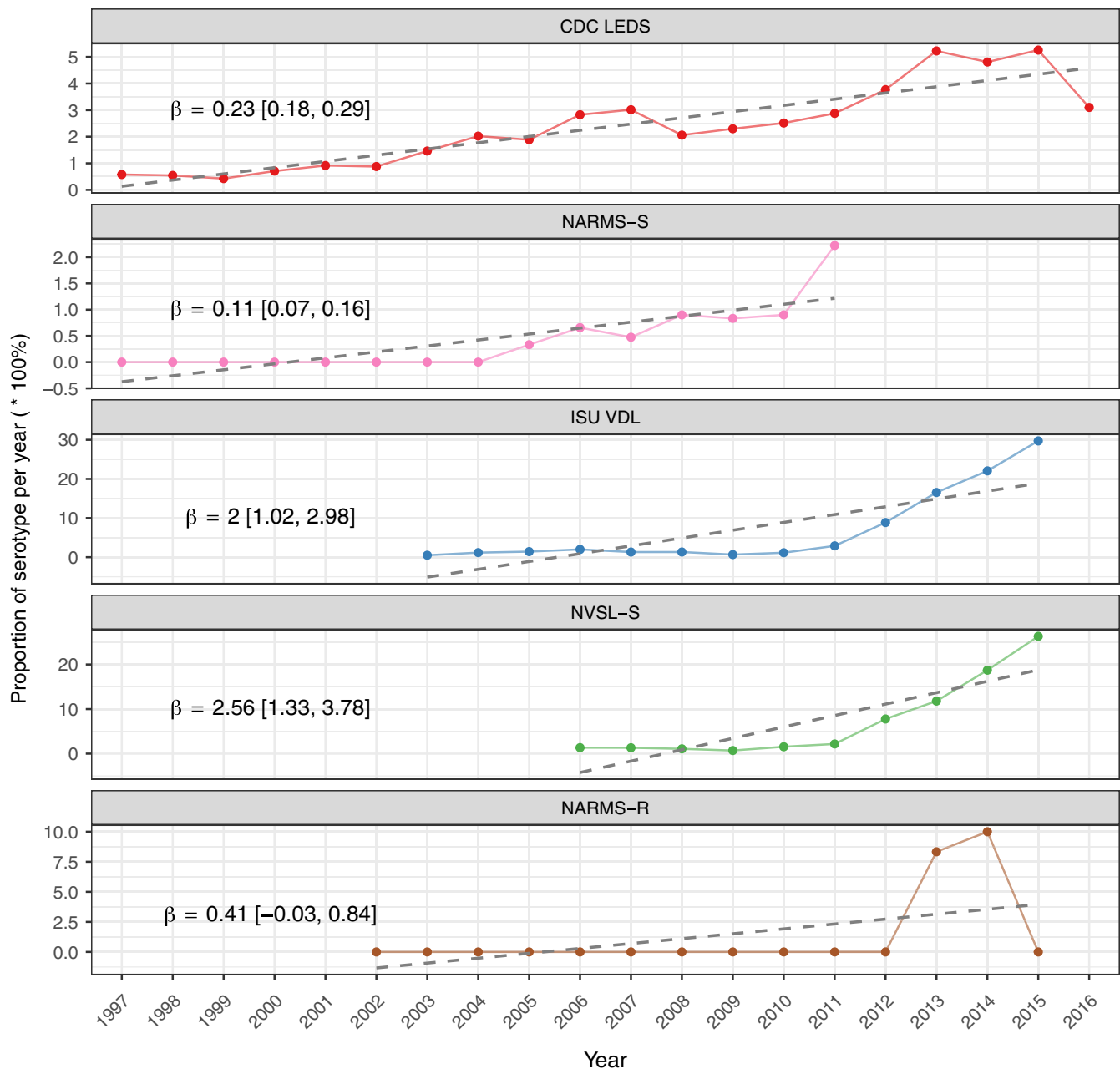
4,[5],12:i:−

FIGURE 4 Observed prevalence of *Salmonella enterica* serovar 4,[5],12:i:- over years in all swine data set. The coefficient and 95% confidence interval for the covariate year are reported [Colour figure can be viewed at wileyonlinelibrary.com]

The most frequently isolated *S. enterica* serovars in the ISU VDL swine data set 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 data sets (i.e. NARMS-S, NARMS-R, NVSL and CDC LEDS) are *provided in* Table 1. It is important to note the large differences in numbers of isolates used in the analysis. For example, the NARMS-R swine data set (*n* = 202) contained only 39 *S. enterica* serovar Typhimurium isolates from 2002 to 2015, whereas the CDC LEDS data set (*n* = 751095) contained 125403 such isolates during

the same period. As a consequence, the precision of estimation varies enormously across data sets. 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 (see Table 2, Figure 1, Figure 2, and Figure 3). The proportions of *S. enterica* serovar Typhimurium also appeared to decrease over time in the
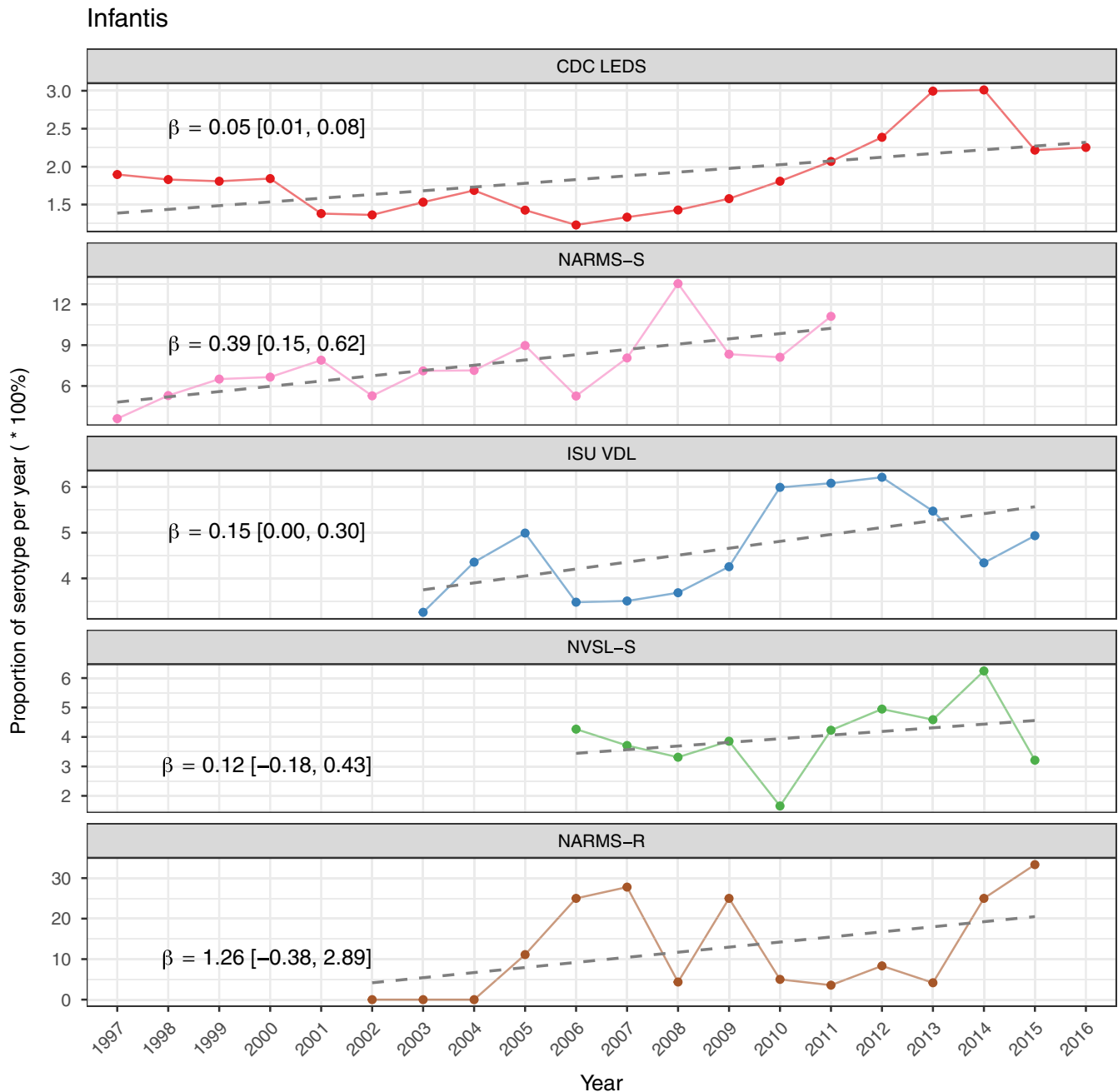
Infantis

FIGURE 5 Observed prevalence of *Salmonella enterica* serovar Infantis over years in all swine data set. The coefficient and 95% confidence interval for the covariate year are reported [Colour figure can be viewed at wileyonlinelibrary.com]

NARMS-R, NARMS-S, NVSL and CDC LEDS data sets. 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 data sets. For the NARMS-S and NARMS-R data sets, the 95% CIs were bounded by positive and negative estimates, which might be due to the small number of isolates in the NARMS-R data set (*n* = 39) but not the NARMS-S data set (*n* = 655). The patterns of temporal changes in the proportions of other serovars in the ISU VDL swine data set 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 data sets. The proportion of *S. enterica* serovar Derby appeared to increase over time in the NARMS-R data set (see Figure 2) although this data set contained only 27 isolates. The proportions of *S. enterica* serovar Heidelberg appeared to show consistent decreases in all data sets (see Figure 3).

Over time, the proportions of *S. enterica* serovar 4,[5],12:i:-, serovar Infantis and serovar Johannesburg increased in the ISU VDL swine data set. These data are provided in Table 2 and plotted in Figure 4, Figure 5 and Figure 6. It is interesting
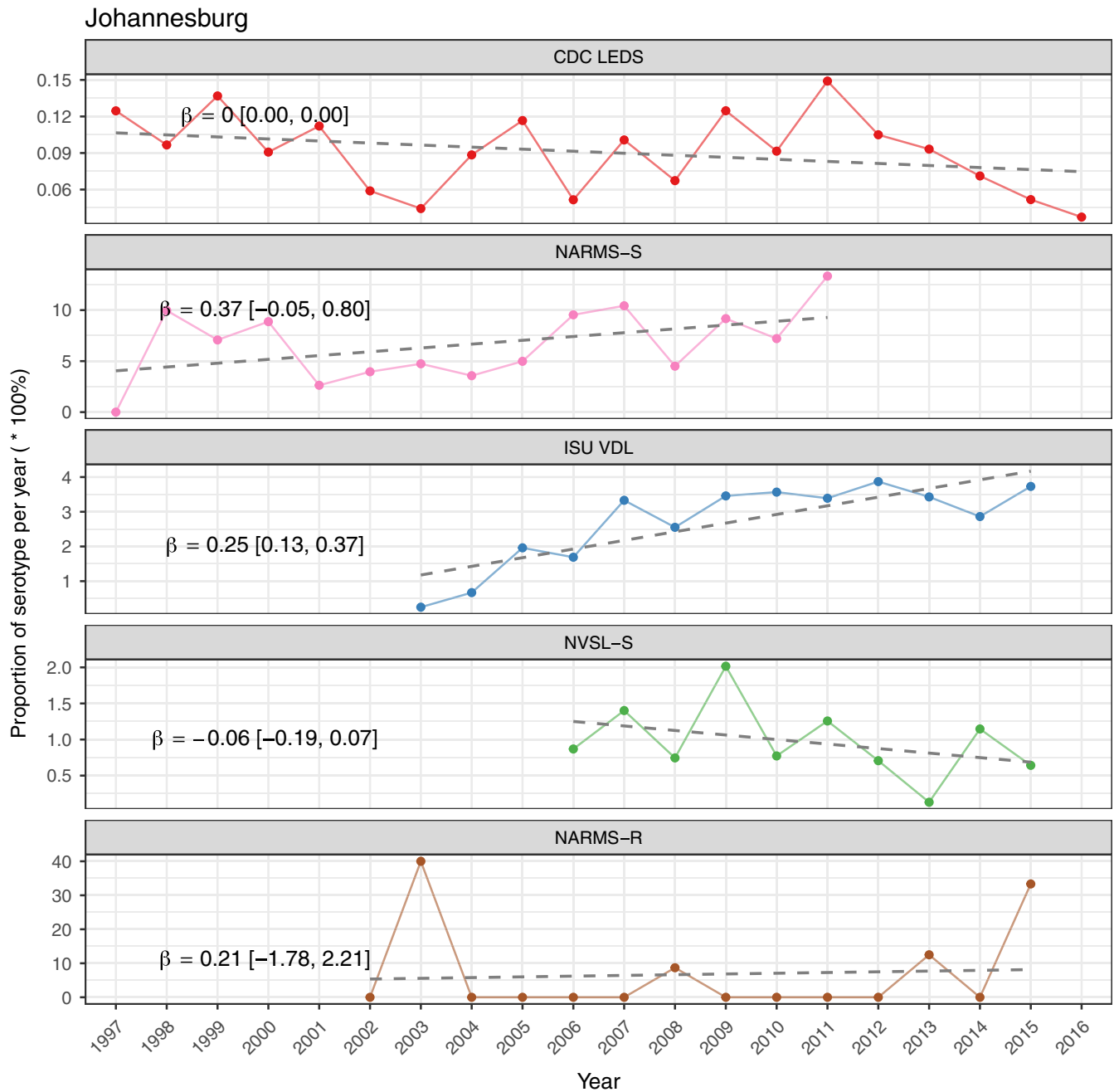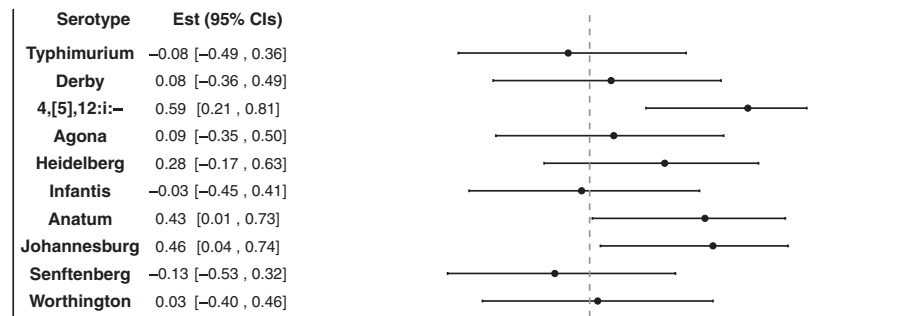
Johannesburg

FIGURE 6 Observed prevalence of *Salmonella enterica* serovar Johannesburg over years in all swine data sets. The coefficient and 95% confidence interval for the covariate year are reported [Colour figure can be viewed at wileyonlinelibrary.com]

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]) data sets but not in NARMS-R data set (0.41% [−0.03, 0.84]). However, considering the 95% CIs, the CDC LED, NVSL-S and NARMS-S data sets provided the strongest evidence of an increasing proportion of *S. enterica* serovar 4,[5],12:i:-. For the NARMS-R data set, the 95% CI was bounded
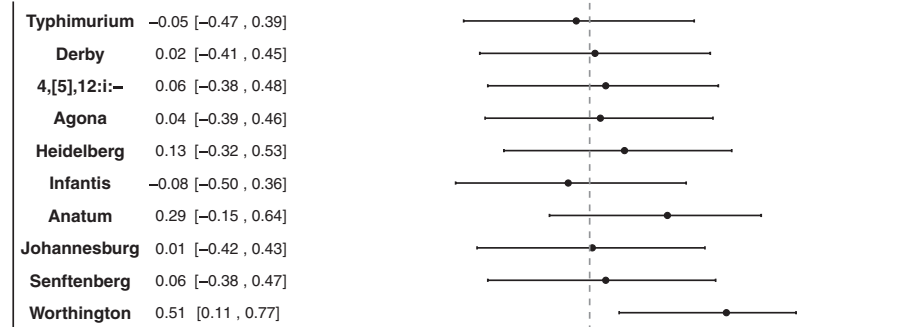
by positive and negative estimates, again likely due to the small number of isolates (*n* = 4). Following the same approach to interpreting the results, increases in the proportions of *S. enterica* serovar Infantis appeared to be consistent across data sets (see Figure 5), whereas changes in the proportion of *S. enterica* serovar Johannesburg were inconsistent across data sets (see Figure 6).

Plots of temporal changes in the proportions of the remaining top 10 ISU VDL swine isolates in the other data sets are provided in the supplementary materials (*S. enterica* subsp. *enterica* serovar

CDC LEDS vs VDL swine

Serotype	Est (95% CIs)
Typhimurium	−0.08 [−0.49 , 0.36]
Derby	0.08 [−0.36 , 0.49]
4,[5],12:i:−	0.59 [0.21 , 0.81]
Agona	0.09 [−0.35 , 0.50]
Heidelberg	0.28 [−0.17 , 0.63]
Infantis	−0.03 [−0.45 , 0.41]
Anatum	0.43 [0.01 , 0.73]
Johannesburg	0.46 [0.04 , 0.74]
Senftenberg	−0.13 [−0.53 , 0.32]
Worthington	0.03 [−0.40 , 0.46]

CDC LEDS vs VDL avian

Typhimurium	−0.05 [−0.47 , 0.39]
Derby	0.02 [−0.41 , 0.45]
4,[5],12:i:−	0.06 [−0.38 , 0.48]
Agona	0.04 [−0.39 , 0.46]
Heidelberg	0.13 [−0.32 , 0.53]
Infantis	−0.08 [−0.50 , 0.36]
Anatum	0.29 [−0.15 , 0.64]
Johannesburg	0.01 [−0.42 , 0.43]
Senftenberg	0.06 [−0.38 , 0.47]
Worthington	0.51 [0.11 , 0.77]

CDC LEDS vs VDL bovine

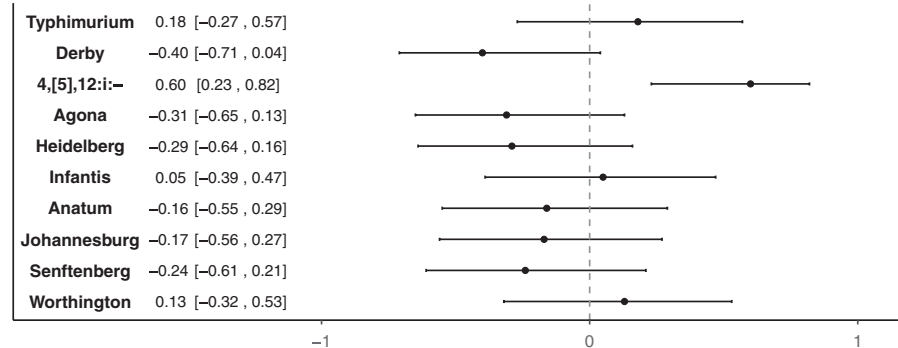|	
Typhimurium	0.18 [−0.27 , 0.57]
Derby	−0.40 [−0.71 , 0.04]
4,[5],12:i:−	0.60 [0.23 , 0.82]
Agona	−0.31 [−0.65 , 0.13]
Heidelberg	−0.29 [−0.64 , 0.16]
Infantis	0.05 [−0.39 , 0.47]
Anatum	−0.16 [−0.55 , 0.29]
Johannesburg	−0.17 [−0.56 , 0.27]
Senftenberg	−0.24 [−0.61 , 0.21]
Worthington	0.13 [−0.32 , 0.53]

FIGURE 7 Spearman's rank-order correlation coefficients and 95% CIs for associations between proportion changes in the CDC LEDS data set and those in ISU VDL swine, avian and bovine data sets during concurrent years

Agona: Figure S6, *S. enterica* subsp. *enterica* serovar Anatum: Figure S7 and *S. enterica* subsp. *enterica* serovar Senftenberg: Figure 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 data sets. 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 data sets and for the yearly changes in *S. enterica* serovar 4,[5],12:i:- between ISU

VDL bovine and CDC LEDS data sets. 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 data sets. These data are presented in Figure 7. There were no consistent correlations within concurrent time periods between the NARMS-S species-level and CDC LEDS data sets or NARMS-R species-level and CDC LEDS data sets. These data are presented in Figure 8 and Figure 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 data sets but a positive correlation between ISU VDL avian and CDC LEDS data sets. Again, however, the avian NARMS-R data set 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 data sets,
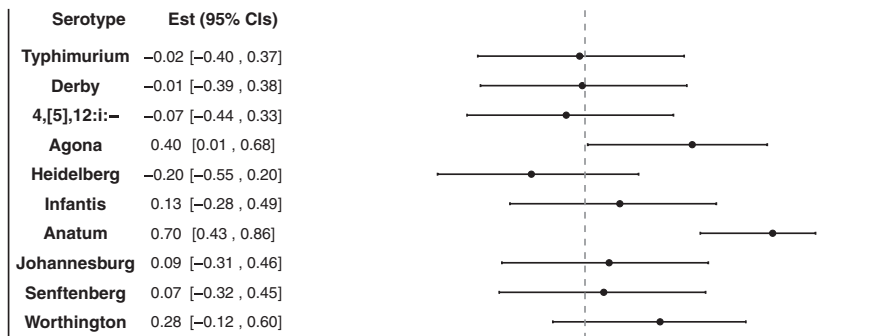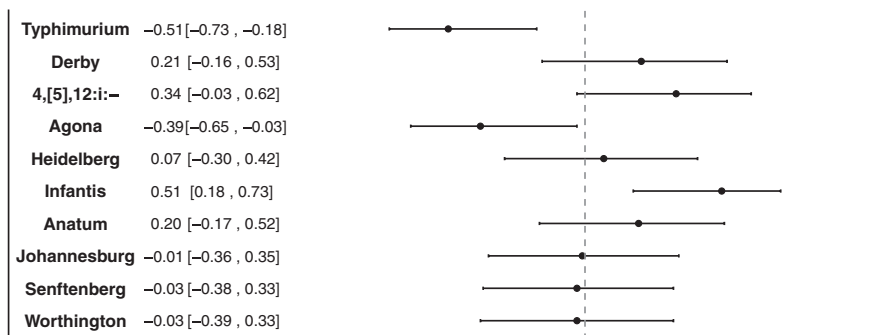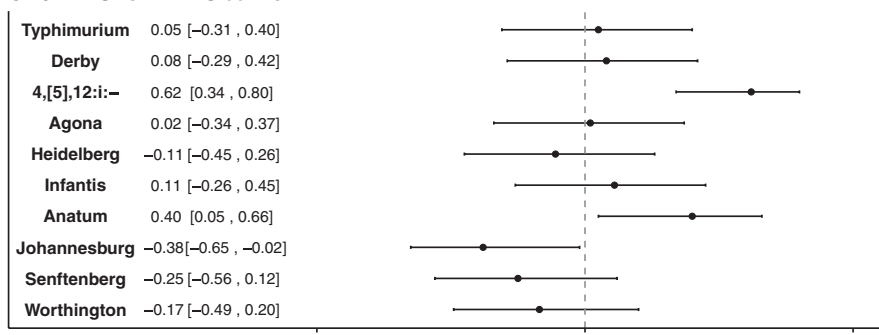
FIGURE 8 Spearman's rank-order correlation coefficients and 95% CIs for associations between proportion changes in the CDC LEDS data set and those in NARMS-S swine, avian and bovine data sets during concurrent years

NARMS-S and CDC LEDS data sets or NARMS-R and CDC LEDS data sets. These data are presented in Supplementary Figure S9, Figure S10 and Figure S11, respectively. Similarly, there were no consistent 2-year lag correlations between ISU VDL and CDC LEDS data sets, NARMS-R and CDC LEDS data sets or NARMS-R and CDC LEDS data sets (see Figure S12, Figure S13 and Figure 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

data set, 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 data sets. 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 too low for detection (Alban & Stärk, 2005; O'Connor et al., 2012; Totton et al., 2016). 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
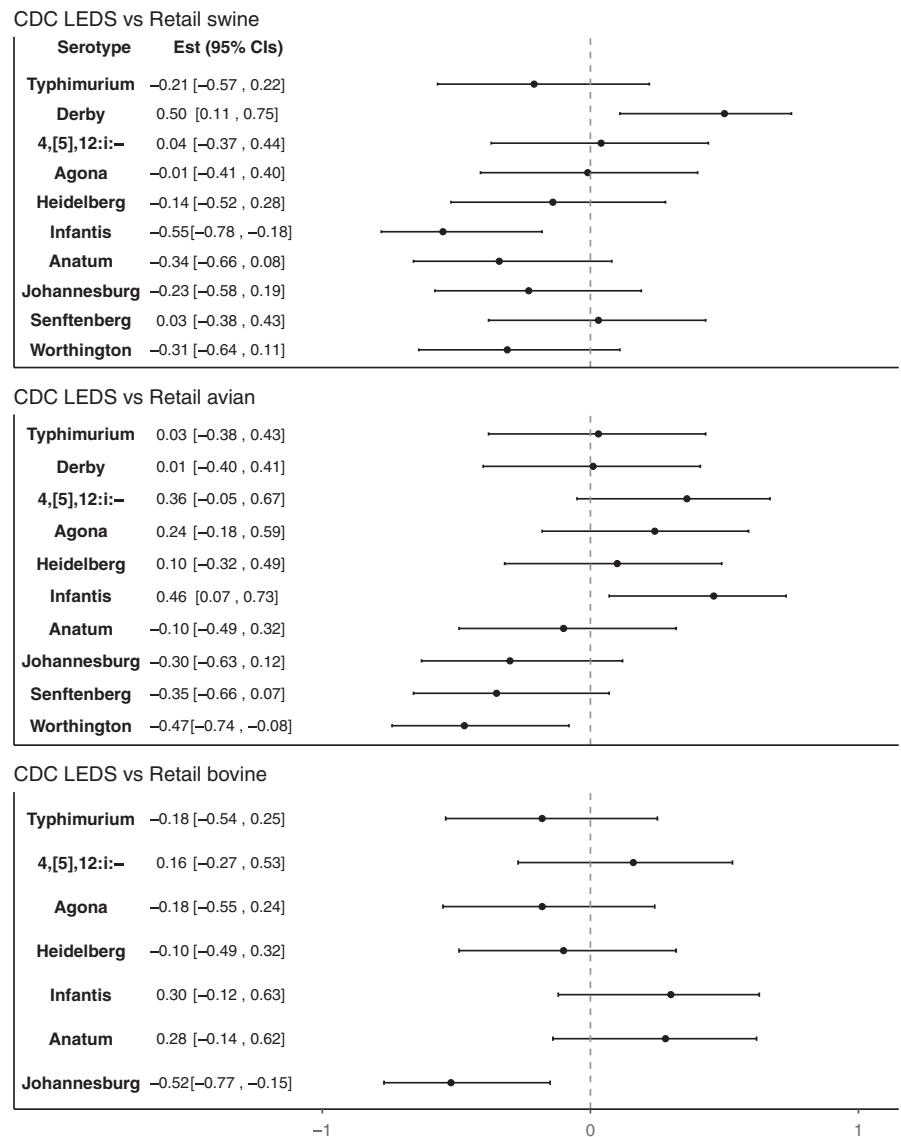
FIGURE 9 Spearman's rank-order correlation coefficients and 95% CIs for associations between proportion changes in the CDC LEDS data set and those in NARMS-R swine, avian and bovine data sets during concurrent years

reflect differences in samples types. Of course, even more explanations are possible; however, our data do 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:- between human and ISU VDL data and between human and NARMS-S bovine data. It should be noted that these associations do not point towards

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. The interpretation of changes in serotypes is, of course, contingent on the concept that the decision to fully serotype an isolate is not differential over the years or in data sets. We have no evidence to suggest that particular serotypes are preferentially fully serotyped and such an approach would fundamentally undermine the value of surveillance programmes. Further, the interpretation of the changes in serotypes is based on the principle that any misclassification of serotypes, such as misclassifying serotype 4,[5],12:i:- as Typhimurium, is random and not related to years.

In conclusion, we propose that data from surveillance programmes should be periodically evaluated to identify emerging patterns that suggest action. For our first aim of analysing 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 the 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 observations simply serve as hypothesis-generating observations about the possible links between changes observed in animal-based surveillance programmes and human disease.

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