

Geospatial and temporal changes in Wisconsin bovine *Salmonella enterica* isolates between 2006 and 2014

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Abstract

Bovine salmonellosis carries a high economic burden for cattle operations and presents a public health risk due to zoonosis. The objective of this study was to determine the frequency and concurrent location of *Salmonella enterica* subspecies *enterica* serovars isolated in Wisconsin from bovine diagnostic samples submitted to the Wisconsin Veterinary Diagnostic Laboratory from 2006 to 2014, by using geographic information system and statistical analysis tools. *Salmonella* serovars and zip codes of animal location were retrospectively collected. Accessions were limited to bovine fecal and tissue samples that yielded a typeable *Salmonella* serovar either by culture or polymerase chain reaction. A total of 4,829 accessions were included in the study that yielded definitive *Salmonella* serovars. The 5 most frequently isolated serovars were Dublin (23%), Cerro (15%), Newport (14%), Kentucky (9%), and Montevideo (9%). After normalization, the region with the highest incidence was the east-central region for all serovars combined. Overall incidence decreased during the 9 years except for *Salmonella* ser. Cerro, which increased. Incidence of *Salmonella* serovars in densely populated dairy regions in Wisconsin was variable. Continued study and monitoring of the spatial disease ecology for salmonellosis in Wisconsin is warranted to improve animal health and milk production, and minimize the risk of zoonosis.

Key words: bovine, *Salmonella*, Wisconsin, geographic information system (GIS)

Résumé

La salmonellose bovine constitue un lourd fardeau économique pour les élevages bovins. Cette zoonose pose aussi un risque de santé public. L'objectif de cette étude était de déterminer la fréquence et la localisation de sérotypes de *Salmonella enterica* spp *enterica* isolés au Wisconsin à partir d'échantillons de diagnostic bovin soumis au laboratoire de

diagnostic vétérinaire du Wisconsin entre 2006 et 2014 en utilisant un système d'information géographique et des outils d'analyse statistique.

Les sérotypes de *Salmonella* et les codes postaux ont été obtenus rétrospectivement. Les accès ont été limités aux échantillons fécaux ou de tissus bovins qui produisaient des sérotypes typables de *Salmonella* soit par culture ou soit par test d'amplification en chaîne par polymérase. Un total de 4829 accès produisant des sérotypes définitifs de *Salmonella* ont été inclus dans l'étude. Les cinq sérotypes les plus fréquemment isolés étaient Dublin (23%), Cerro (15%), Newport (14%), Kentucky (9%) et Montevideo (9%). Après normalisation, la région du centre-est montrait la plus forte incidence pour tous les sérotypes combinés. L'incidence en général a chuté au cours des neuf années d'étude sauf pour *Salmonella* ser. Cerro qui a augmenté. L'incidence des sérotypes de *Salmonella* dans les régions de production laitière densément peuplées était variable. Il est donc conseillé de poursuivre les travaux sur la salmonellose au Wisconsin et de continuer la surveillance de l'écologie spatiale de la maladie afin d'améliorer la santé animale et la production laitière et de minimiser le risque de zoonose.

Introduction

Bovine salmonellosis results in a high economic burden for dairy and beef producers. Disease costs for producers include treatment of clinical disease and loss of production with milk or rate of daily gain.²⁰ Because cattle, and the food products they produce, are frequently identified as vehicles and reservoirs for human infections with *Salmonella enterica* subspecies *enterica*, it is important to understand the pathogenesis, transmission, and ecology of this pathogen.^{7,18,30} Currently, 2,659 serotypes, or serovars, of *Salmonella enterica* subspecies *enterica* have been recognized by the World Health Organization (WHO) Collaborating Centre for Reference and Research,¹⁶ but the virulence, zoonotic potential, pathogenesis or epidemiology are not characterized for the vast majority of the serovars.

Of particular interest to the cattle industry, *Salmonella* ser. Dublin is a bovine-specific *Salmonella* serovar that has become endemic in some dairy and beef herds.¹³ Although infections with *Salmonella* ser. Dublin are typically associated with dysentery, the host-adapted strain is also immunosuppressive and associated with sepsis, interstitial pneumonia and abortion. *Salmonella* ser. Dublin infections in calves typically occur between 6 and 12 weeks of age from fecal-oral contamination.¹³ There is also evidence supporting vertical transmission and potential shedding of *Salmonella* bacteria into colostrum.^{15,26} Abortion induced by *Salmonella* ser. Dublin not only results in loss of production, but also represents a risk to herd health because the carrier can actively shed mainly in feces for up to 4 weeks after parturition or abortion.¹³

Prevention of shedding of *Salmonella* in the environment is complicated by persistently infected carrier animals that do not show any clinical signs of the disease. These carrier cows can shed 10^8 to 10^9 colony forming units per gram (CFU/g) of feces sporadically and unpredictably.¹⁹ *Salmonella* can persist in the water, soil, dust, and moist areas out of direct sunlight, and on or within feedstuffs for 4 to 5 years.¹⁹ *Salmonella* ser. Dublin can survive in feces adhered to stall surfaces for 10 months to 6 years.¹³ Additionally, transmission can occur between farms via contaminated farm equipment and workers, or through contaminated water sources.^{7,13,19} Due to environmental persistence, *Salmonella* transmission occurs readily on farms between animals, and zoonotic transmission occurs through the fecal-oral route or consumption of contaminated bovine food products.^{19,27}

Descriptive geo-spatial information (GIS) for incidence of bovine salmonellosis can aid the study of *Salmonella* disease ecology to better understand the potential for transmission and spread of disease in a region. High incidence in an area could be a result of close distribution of farms, frequent movement of infected cows through the production system and environmental contamination, particularly with runoff into waterways. Other localized risk factors include local feed supplies and presence of reservoir wildlife in the area.¹² To aid comprehension of Wisconsin's bovine salmonellosis, the objective of this study was to investigate the geospatial and temporal distribution of bovine salmonellosis from 2006 to 2014 in Wisconsin from diagnostic samples submitted to the Wisconsin Veterinary Diagnostic Laboratory (WVDL).

Materials and Methods

Data were retrospectively collected from the laboratory information management system (LIMS)^a at the WVDL from 2006 to 2014. Bovine diagnostic samples that yielded a *Salmonella enterica* subspecies *enterica* isolate were used in this study. Sample types included tissues such as liver, intestine, lung, kidney, spleen, stomach, fetus, and lymph nodes or fecal material submitted to the diagnostic laboratory for bacterial culture and molecular biology analysis. Milk

samples were not queried, as *Salmonella* isolates from milk are more often the result of poor hygiene during milking and subsequent processing.²⁶ Each case was assigned a unique identifier called an accession number and each accession was traceable. Information from the isolates used in this study included *Salmonella enterica* serovar type, geographical location of the premises where the bovine was housed and sampled in zip code scale, and day of test finalization in days, month, and year.

Salmonella were cultured as previously described²⁹ and following standard operating procedures (SOPs) approved by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). In brief, samples were enriched in selenite F (Sel) and Rappaport-Vassilliadis R10 (Rapp) broth for 18 to 24 hours at 96.8°F (36°C). Enrichment broth was subcultured onto brilliant green agar with novobiocin, eosin methylene blue, and xylose-lysine tergitol 4 (XLT-4) and reincubated for an additional 18 to 24 hours at 96.8°F (36°C) if found to be negative for *Salmonella* after the initial incubation. *Salmonella*-suspect colonies were identified by black or red colonies with or without black centers on XLT-4 or pink and opaque with a smooth appearance and a red color surrounding the colony on BGN.^{1,29} Colonies were confirmed by matrix-associated laser desorption/ionization time-of-flight (MALDI TOF) mass spectroscopy (MS).^b

Following confirmation of *Salmonella enterica*, 3 or more colonies from more than 1 agar, if possible, were serotyped using the Kauffman-White serotyping scheme,^{3,14} as described in a SOP. First, *Salmonella* colonies were serogrouped using polyvalent *Salmonella* 'O' antisera and then further serotyped using *Salmonella* 'H' antisera including Spicer Edwards and single-factor antisera. For an accession with multiple *Salmonella* isolates, all isolates were serogrouped but only 1 isolate per serogroup was serotyped per accession for economic reasons. In some instances, a definitive *Salmonella* serovar was not identified and those results were reported as serogroup only. These cases were not included in this study. The WVDL personnel performing these assays are proficiency tested for culture and serotyping by the National Veterinary Services Laboratories (NVSL).

Unfortunately, *Salmonella* ser. Dublin is difficult to isolate from fecal samples both due to low shedding during infection and its fastidious nature. Because the overall culture rate ranges from 0 to 25%,²⁴ a *Salmonella* ser. Dublin real-time PCR was implemented at the WVDL in 2014. In brief, samples were pre-enriched in a 1:10 dilution of buffered peptone water (BPW) for 18 to 24 hours at 96.8°F (36°C). BPW was extracted and real-time PCR performed as described previously.²⁸ Results were reported as positive (cycle threshold of <40) or negative (cycle threshold >40).

Cases were defined as definite bovine *Salmonella* serovars found in the state of Wisconsin from January 1, 2006 to December 31, 2014 submitted and identified at the WVDL. *Salmonella* ser. Bardo was reclassified as *Salmonella* ser. Newport as recommended by the Centers for Disease Control and

Prevention.^{2,4} Cases with multiple animals or multiple tissues from a single farm with identical positive diagnosis for the same *Salmonella* serovar were counted as a single positive accession. Accessions with more than 1 *Salmonella* serovar identified were recorded as separate cases.

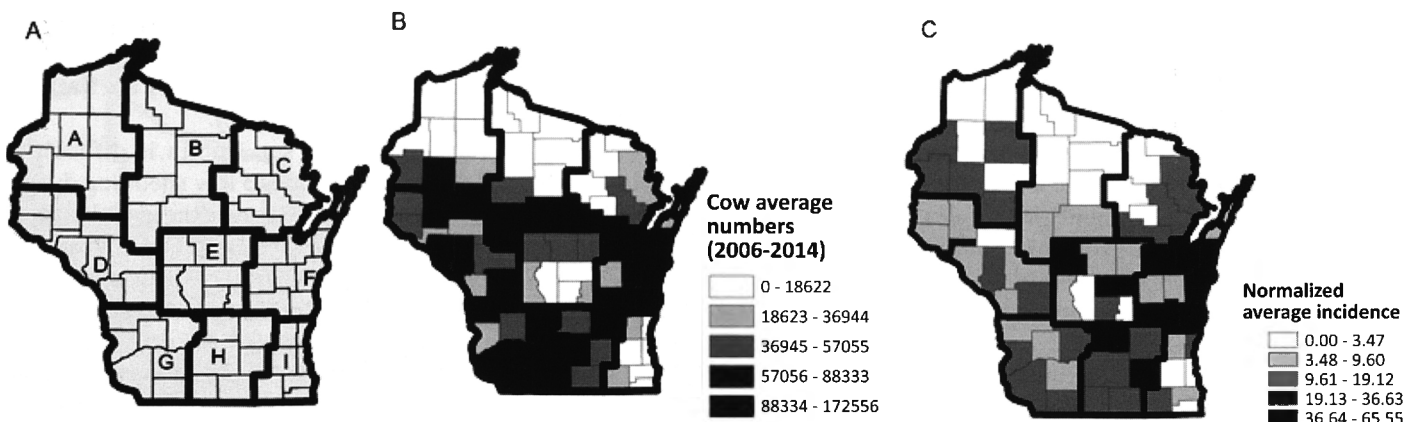
Results with valid data were grouped by serovar type and year of finalized test dates. The finalized dates of serovar test were used to group accessions by year instead of test initiation dates arbitrarily, because tests have variable times to finalization.

Zip codes used in the geographic analyses were from the 2015 March zip codes available from the United States Postal Service. During the periods between 2006 and 2014, zip codes were changed in 2006, 2007, 2008, 2009, 2011, 2013, and 2015. Hence, adjustments to align with 2015 zip codes were made in locations with zip code changes in corresponding years. Zip codes from *Salmonella*-positive accessions were used to assign the Wisconsin County of origin, which was then assigned to 1 of the 9 regions that the Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) uses for comparative study of spatial and temporal distribution (Figure 1A). Letters were assigned to designate the regions. Region A is the North-west region, which contains Douglas, Bayfield, Burnett, Washburn, Sawyer, Polk, Barron, Rusk, and Chippewa Counties. Region B is North-central region, which contains Ashland, Iron, Vilas, Price, Oneida, Taylor, Lincoln, Clark, and Marathon Counties. Region C is North-east region, which contains Forest, Florence, Marinette, Langlade, Oconto, Menominee, and Shawano Counties. Region D is West-central region, which contains Saint Croix, Peirce, Dunn, Pepin, Eau Claire, Buffalo, Trempealeau, Jackson, La Crosse, and Monroe Counties.

Region E is Central region, which contains Wood, Portage, Waupaca, Juneau, Adams, Waushara, Marquette, and Green Lake Counties. Region F is East-central region, which contains Outagamie, Brown, Door, Kewaunee, Winnebago, Calumet, Manitowoc, Fond du Lac, and Sheboygan Counties. Region G is South-west region, which contains Vernon, Richland, Sauk, Crawford, Grant, Iowa, and Lafayette Counties. Region H is South-central region, which contains Columbia, Dodge, Dane, Jefferson, Green, and Rock Counties. Lastly, region I is South-east region, which contains Washington, Ozaukee, Waukesha, Milwaukee, Walworth, Racine, and Kenosha Counties.

Cow population information was only available at the county level and not by zip code. Cow numbers in each county are shown in Figure 1B with natural break (jenks) categorization to show where and how many cows were housed in the state. Natural break jenks is a method of manual data classification that seeks the best arrangement of values into different classes by pursuing to minimize each class's average deviation from the class mean, while maximizing each class's deviation from the means of the other groups in order to achieve the reduction of the variance within classes and maximize the variance between.¹¹ With this method, representation of events on maps with clusters in limited area and no events in other areas do not get skewed and missed substantially; instead, detailed information on clusters becomes more available. *Salmonella*-positive accession numbers for each county were normalized per 100,000 dairy cows from the dairy production data obtained from the National Agricultural Statistics Service.³⁵ Figure 1C shows distribution of normalized average bovine *Salmonella enterica* subspecies *enterica* serovar incidences identified at WVDL during the 9 years in the state. The figure was produced by

Figure 1. Regional distribution of cattle and *Salmonella* incidences in Wisconsin.



The State of Wisconsin in 9 distinct regions designated by the Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP). Region A is the North-west, B North-central, C North-east, D West-central, E Central, F East-central, G South-west, H South-central, and I South-east region (A). The average dairy cow populations at the county level in Wisconsin from 2006-2014 from the National Agricultural Statistics Service (NASS) at the United States Department of Agriculture (USDA) (B). The average bovine *Salmonella enterica* subspecies *enterica* serovars incidences in Wisconsin from 2006 to 2014 with normalization for 100,000 cows over the same period, with the incidence categories of 0.00 - 3.47, 3.48 - 9.60, 9.61 - 19.12, 19.13 - 36.63, and 36.64 - 65.55(C).

using the natural break (jenks) classification with categories from lighter color to darker; 0.00 to 3.47, 3.48 to 9.60, 9.61 to 19.12, 19.13 to 36.63, and 36.64 to 65.55 per 100,000 cows.

For spatial distribution, 3-year averages (from 2006 to 2008, from 2009 to 2011, and from 2012 to 2014) of accession numbers were calculated with a spreadsheet application^c to identify temporal trends and minimizing yearly fluctuations. During analysis, we found that for some years and for some serovars there were only a few incidences or no incidences, which would have made interpretation difficult by year. As a way to avoid this, we chose to combine data into 3-year averages. This allows readers to see short-term trends and maximizes readability. Figures A 2-6, Figures B 2-6, and Figures C 2-6 demonstrate time lines of 2006-2008, 2009-2011, and 2012-2014, respectively. Shape files for counties and attributes necessary for GIS software^d were obtained from the Wisconsin Department of Natural Resources online resources.³⁸ County level choropleth maps were produced for all *Salmonella* serovar incidences combined and the top 5 serovar incidences in regional boundaries. For maps with all *Salmonella* serovar incidences combined, 5 different categories of incidence were assigned: 0.00 to 2.00, 2.01 to 10.00, 10.01 to 25.00, 25.01 to 70.00, and 70.01 to 137.93 per 100,000 cows (Figures 2A, 2B, and 2C) based on natural break categorization. Five categories were used on maps to represent 5 different color values of gray. For maps of individual serovars, maximum incidence of 43.16 cases per 100,000 cows was set as the upper limit and categorized to divisions of 0.00 to 1.00, 1.01 to 5.00, 5.01 to 10.00, 10.01 to 20.00, and 20.01 to 43.16 per 100,000 cows (Figures 3-6, A-C). Smaller intervals, compared to intervals for Figure 2 A-C, were applied due to small accession numbers in many counties. Shades of gray were used to represent the frequen-

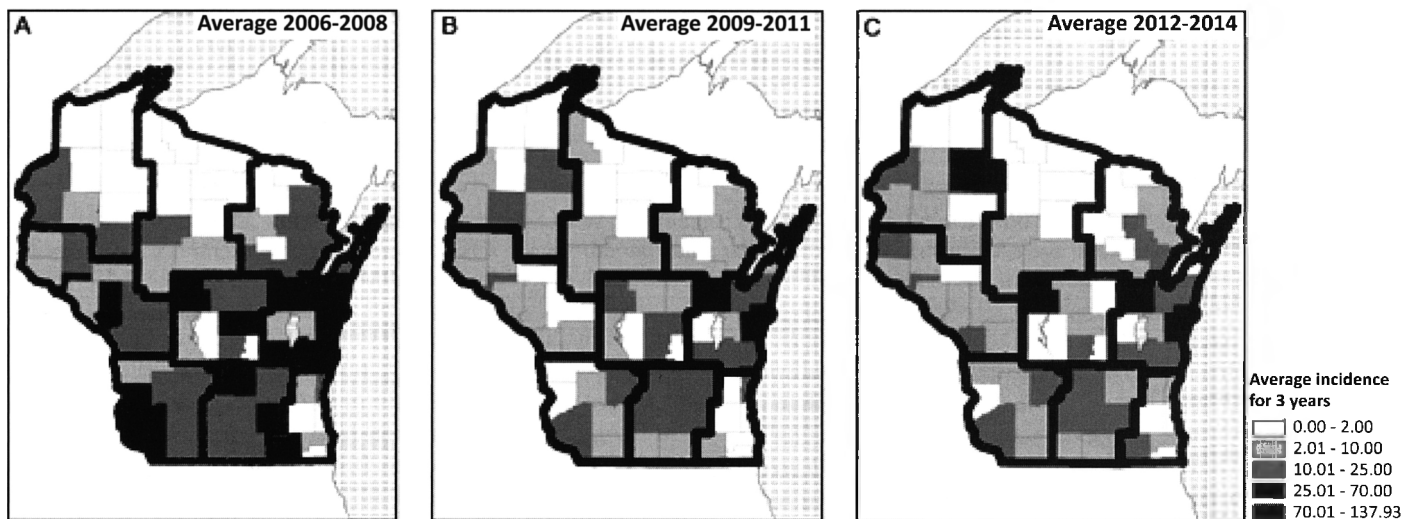
cies where the higher the number, the darker the shade of gray. Out of total 9 regions shown in Figure 1A, only 3 regional graphs were shown in Figures 2-6 D, E, F, because they had the highest incidence numbers of each *Salmonella* serovar investigated over the 9-year time period making them the most statistically significant. Each point on the graphs represents the yearly average, with the 95% confidence intervals shown as bars extending from the midpoint. The 95% confidence interval was calculated as: $(n \pm 1.96 \times) / N \times 100,000$, where n is the number of positive isolates in a year, and N is the number of cows for that year in the region. The 95% confidence interval was calculated to demonstrate confidence around the estimates and detect any differences compared to the 9-year regional and state averages. Additionally, for that purpose, the regional (dotted line) and state (solid line) averages were placed on each graph for comparison purpose (Figures 2-6, D-F).

For temporal distribution, Poisson regression graphs for the 9 regions were prepared with commercially available statistical software.^e Poisson regression was used for this study because *Salmonella* incidences were relatively rare events in the whole study population of cattle in the state over the 9 years, and the incidences were assumed to follow Poisson distribution.³² Year of analysis was the independent variable, whereas number of cows in each top 3 regions in the state and incidence number of each *Salmonella* serovar were the covariates.

Results and Discussion

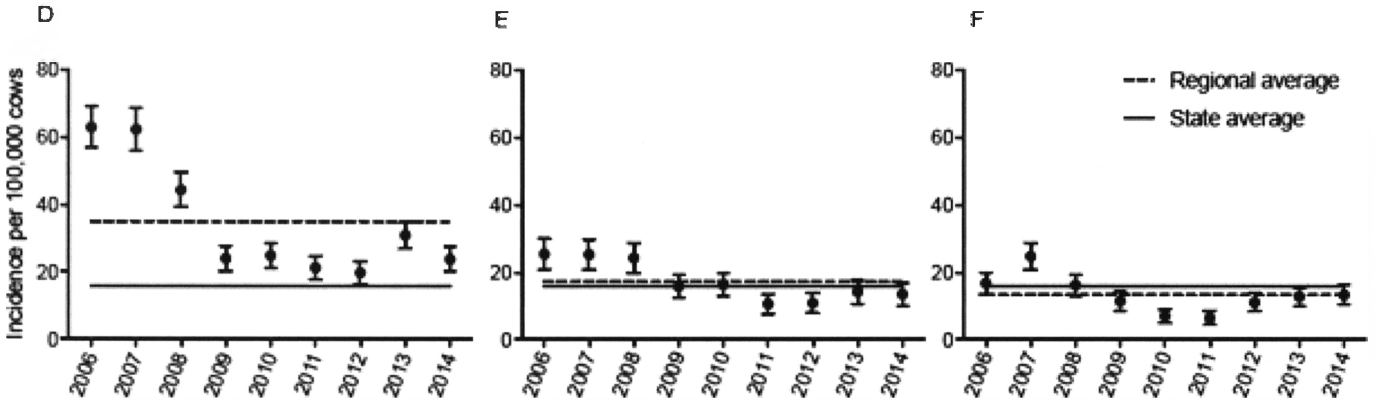
From 2006 to 2014, 4,829 accessions and 100 different serovars were isolated, including 12 accessions from PCR diagnostic testing of which the diagnostic laboratory imple-

Figures 2A, 2B, 2C. Distribution of bovine *Salmonella enterica* isolates from 2006 to 2014.



Three-year averages incidence of bovine *Salmonella* isolates per 100,000 cows in each region. (A) is for 2006 to 2008, and non-solid border is used for improved readability only in (A). (B) for 2009-2011, and (C) for 2012-2014. The legend for the map is; from lighter color to darker; 0.00 to 2.00, 2.01 to 10.00, 10.01 to 25.00, 25.01 to 70.00, and 70.01 to 137.93 per 100,000 cows.

Figures 2D, 2E, 2F. Distribution of bovine *Salmonella enterica* isolates from 2006 to 2014.



Average incidence of bovine *Salmonella* isolates per 100,000 cows in the 3 highest-incidence regions of Wisconsin; (D) for East-central, (E) for South-central, and (F) for South-west regions; are shown with Poisson regression and 95% confidence interval. State average is depicted with a solid line and regional average is the dotted line.

mented in July 2014. Of these accessions, 96.9% of *Salmonella* serovars were isolated from animals reported as dairy breeds, whereas 2.9% of animals were unspecified breeds and 0.2% was beef breeds. No information on the age of the animal was available. Although the 5 top serovars were Dublin (1,104), Cerro (747), Newport (692), Kentucky (428), and Montevideo (411) accessions, 185 isolates of Typhimurium were detected (Table 1). The state average for combined serovar incidence over the 9-year study period was 15.86 cases per 100,000 cows. This average was obtained by dividing 4,829 accessions by total cow number of 30,449,000 over 9 years and normalizing by 100,000 cows. This data was a convenience

sample and did not represent the true incidence because it relied upon practicing veterinarians to submit samples to the diagnostic laboratory. It is possible that other diagnostic laboratories may have been used, shedding cattle could be showing no clinical signs of the disease and therefore were not sampled, or as is often the case, animals were treated presumptively based on herd history and clinical signs.

Three-year averages for all *Salmonella enterica* serovars were broken down into incidence in each region (Table 2). The overall temporal trend we observed was a decrease in the incidence of all serovars over the period from 2006 to 2014. Region F, the East-central region, had the overall high-

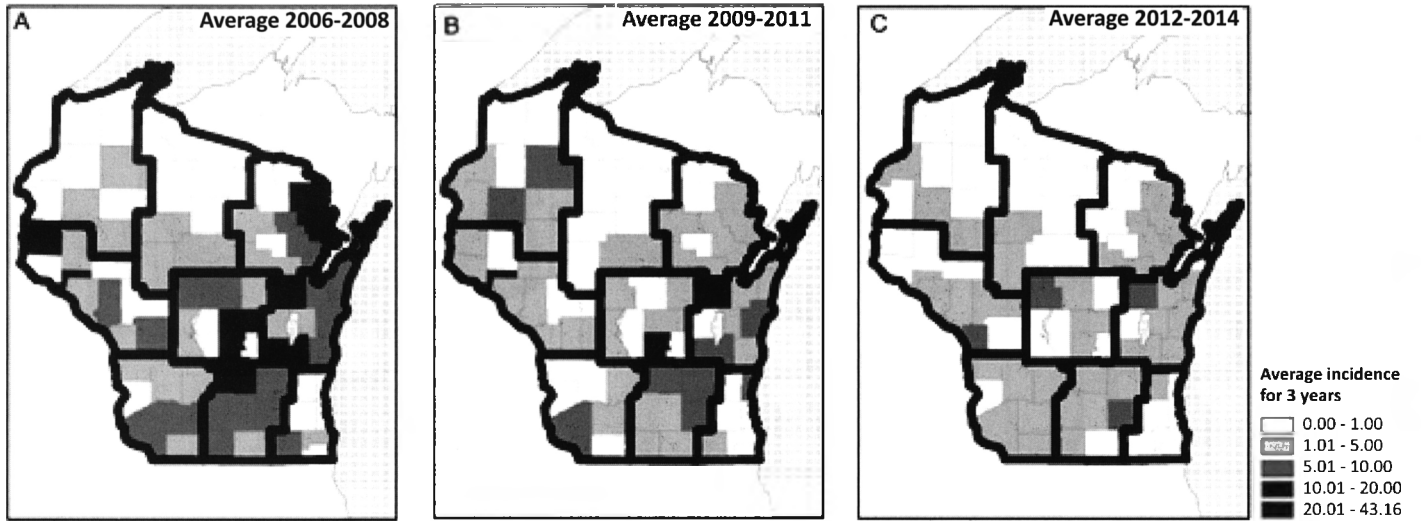
Table 1. Incidence of ranked top 6 bovine *Salmonella enterica* subspecies *enterica* serovars in Wisconsin from 2006 until 2014 serotyped at WVDL, out of 4,829 total accessions represented in numbers and percentage of the total *Salmonella* serovars isolated.

	Rank 1	2	3	4	5	6	
Serovar	Dublin	Cerro	Newport	Kentucky	Montevideo	Typhimurium	Others
Numbers	1,104	747	692	428	411	185	1,262
Percent	23%	15%	14%	9%	9%	4%	26%

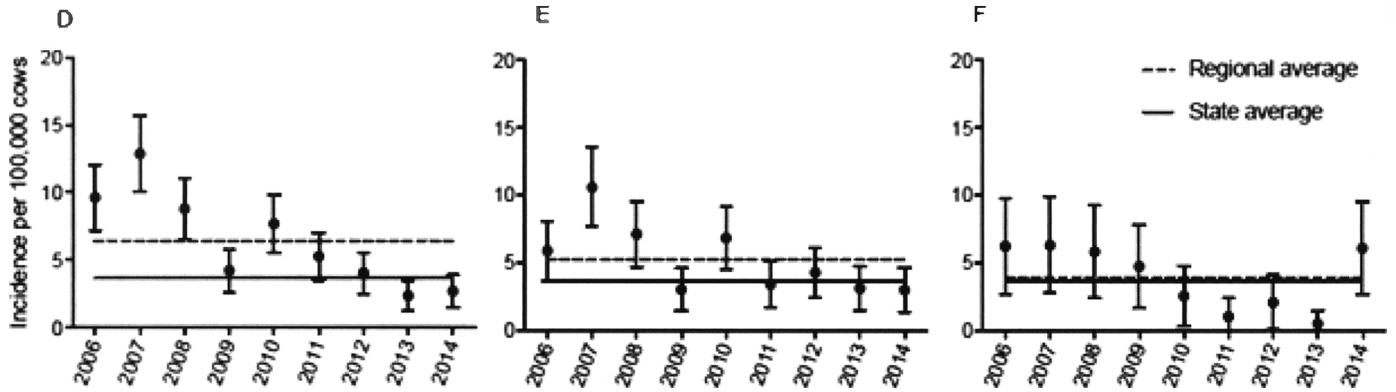
Table 2. Regional averages of isolated bovine salmonella all serovars combined in Wisconsin for the period of 2006 – 2008, 2009 – 2011, 2012 – 2014 per 100,000 cows, and total average of incidence for the 9-year study period. Region A is North-west, B: North-central, C: North-east, D: West-central, E: Central, F: East-central, G: South-west, H: South-central, and I: South-east region, respectively.

Region	2006 - 2008	2009 - 2011	2012 - 2014	Total average
A	11.44	8.85	6.76	9.02
B	7.52	4.47	4.99	5.66
C	16.56	7.29	9.71	11.19
D	14.88	5.08	6.87	8.95
E	21.67	7.39	7.44	12.17
F	56.54	23.40	24.89	34.95
G	19.25	8.33	12.41	13.33
H	25.00	14.28	12.84	17.38
I	11.86	3.66	7.31	7.61

Figure 3. Distribution of bovine *Salmonella enterica* serovar Dublin isolates from 2006 to 2014.



Three-year averages incidence of bovine *Salmonella* Dublin isolates per 100,000 cows in each region. (A) is for 2006 to 2008, (B) for 2009-2011, and (C) for 2012-2014. The legend for the map is; from lighter color to darker; 0.00 to 1.00, 1.01 to 5.00, 5.01 to 10.00, 10.01 to 20.00, and 20.01 to 43.16 per 100,000 cows.



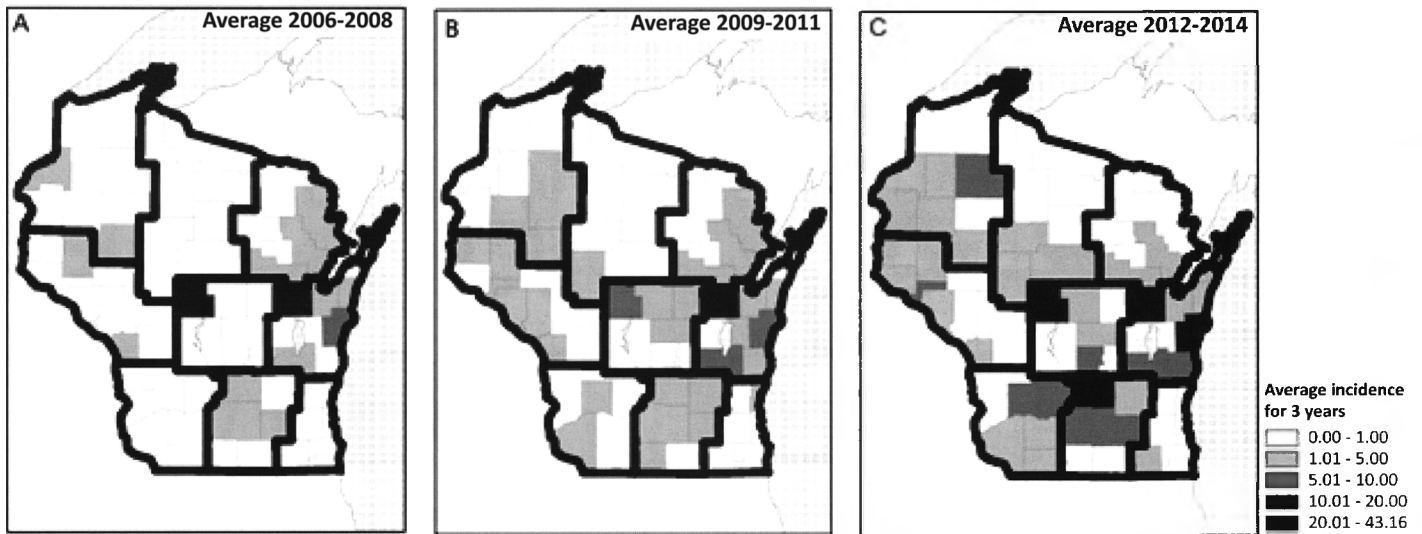
Average incidence of bovine *Salmonella* Dublin isolates per 100,000 cows in the 3 highest-incidence regions of Wisconsin; (D) for East-central, (E) for South-central, and (F) for North-east regions; are shown with Poisson regression and 95% confidence interval. For comparison, state average is depicted with a solid line and regional average is the dotted line.

est incidence of *Salmonella* serovars, whereas region B, the North-central region, had the lowest incidence. All regions experienced a reduction in *Salmonella* incidence from the study ranges of 2006 to 2008 as compared to 2012 to 2014. However, several counties had an increase in incidence between the study ranges of 2009 to 2011 and 2012 to 2014 (Regions B, C, D, E, F, G and I). The reason for the increases, although slight for some regions (B, E and F), was unknown. One possibility could have been the economic recession in 2009 that caused a reduction in diagnostic sample submission. Geographically, the East-central region (Figure 2D, region F) and the South-central region (Figure 2E, Region H) had the highest incidence of 34.95 and 17.38 per 100,000 cows (Table 2), respectively, compared to the state average of 15.86. This was also observed in Figure 1C. These 2 regional high-incidence ratios also showed a significant decrease in

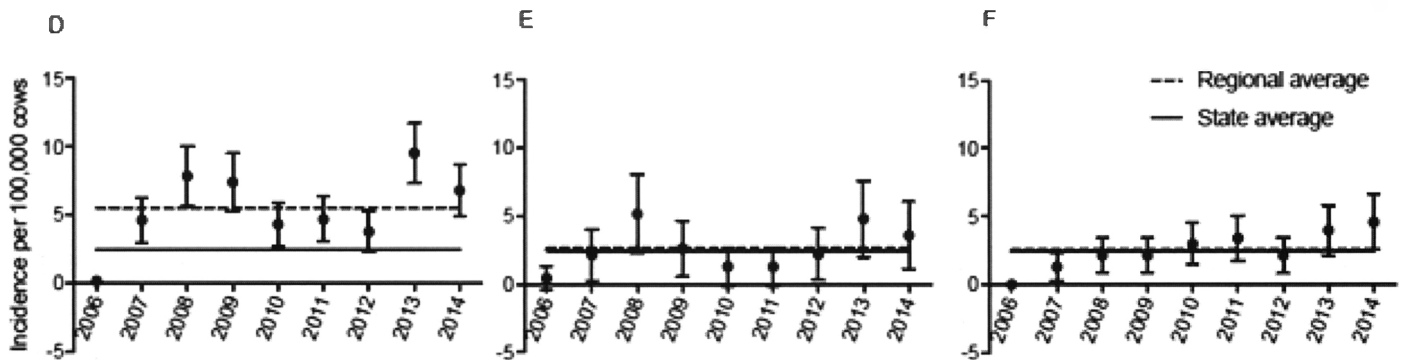
the total *Salmonella* serovar incidence during 2009 and 2011. The East-central region rebounded during the 2012 to 2014 period. In Wisconsin, the majority of farms are located in the southern two-thirds of the state (Figure 1B). Areas of high dairy farm density coincided with high incidences in this dataset. High *Salmonella* incidence suggests that risks for uninfected cows to acquire salmonellosis are high. In addition to high *Salmonella* incidence, higher density of cows may have resulted in spread of salmonellosis.^{21,22,25}

The South-west region (G) recorded total average incidence of 13.33 per 100,000 cows (Figure 2F and Table 2), lower than the South-central and East-central regions. The Central regions had total average incidence of 12.17, similar to that of the South-west region. At the start of the study time period (2006 and 2007), there was significantly more salmonellosis in the South-west region compared to the state

Figure 4. Distribution of bovine *Salmonella enterica* serovar Cerro isolates from 2006 to 2014.



Three year averages incidence of bovine *Salmonella* Cerro isolates per 100,000 cows in each region. (A) is for 2006 to 2008, (B) for 2009-2011, and (C) for 2012-2014. The legend for the map is; from lighter color to darker; 0.00 to 1.00, 1.01 to 5.00, 5.01 to 10.00, 10.01 to 20.00, and 20.01 to 43.16 per 100,000 cows.



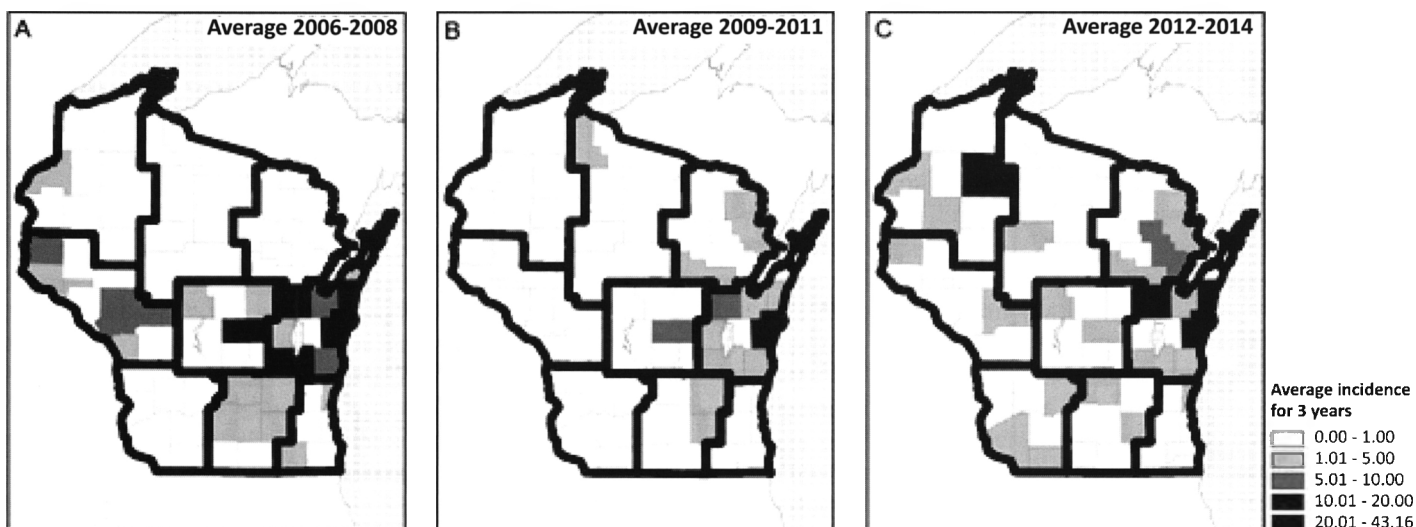
Average incidence of bovine *Salmonella* Cerro isolates per 100,000 cows in the 3 highest-incidence regions of Wisconsin; (D) for East-central, (E) for Central, and (F) South-central regions; are shown with Poisson regression and 95% confidence interval. For comparison, state average is depicted with a solid line and regional average is the dotted line.

or regional average (Figure 2F). The 9-year averages of the remaining 5 regions of the state were lower than the normalized state average including the North-west (9.02), North-central (5.66), North-east (11.19), West-central (8.95), and South-east (7.61) (Table 2). These regions have smaller dairy cattle populations and on average, smaller herd size. The northern part of the state is dominated by national forests, bogs and small lakes, which are not ideal for dairy farming (Figure 1B). The average incidence of salmonellosis in the northern regions (Figure 1A, Regions A, B, C, and Figure 1B) combined for all serovars during the study period was 8.69, almost half of that for the regions representing the southern two-thirds of the state (Figure 1A, Regions D-I, and Figure 1B), which showed average incidence of 15.73, which was obtained by averaging total average numbers of regions D-I in Table 2. The average incidence in the northern regions was also much less

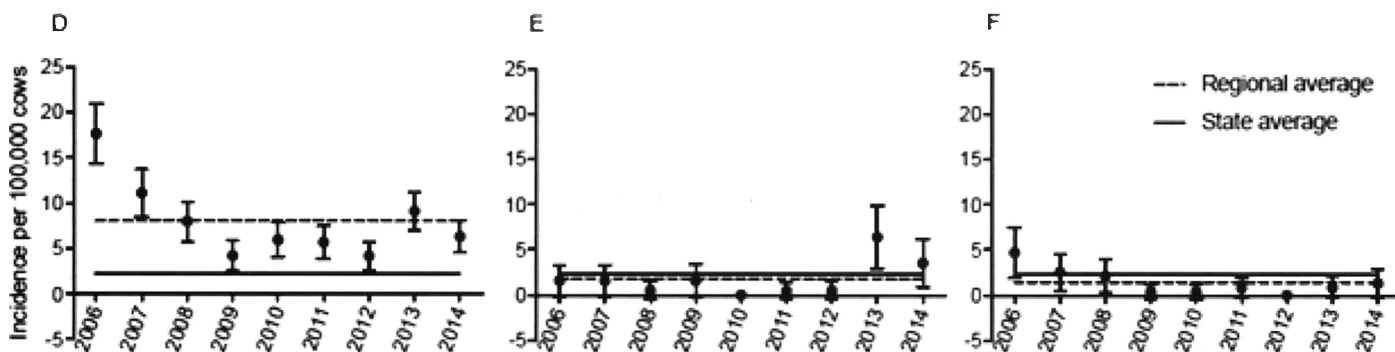
than the overall statewide average of 15.86, although the P-value was between 0.1 and 0.05, hence the difference was not statistically significant. The central area of the state (Figure 1A, Region E) has sandy soil and the agricultural economy is focused on crops such as potatoes, cranberries, corn, and peas rather than dairy production. The average incidence for this region during the examined period was 12.17 (Table 2, Region E). The South-east region (Region I) had the fewest accessions of 7.61 (Table 2, Region I) for the study period, likely due to the landscape that is heavily populated by the Milwaukee metropolitan area and the majority of the state's manufacturing industries (Figure 1C).

When looking at individual serovar data, *Salmonella* ser. Dublin occurred with the highest frequency in the state compared to other serovars during the 2006 thru 2014 time period. State average for the entire period was 3.67 cases per

Figure 5. Distribution of bovine *Salmonella enterica* serovar Newport isolates from 2006 to 2014.



Three-year averages incidence of bovine *Salmonella* Newport isolates per 100,000 cows in each region. (A) is for 2006 to 2008, (B) for 2009-2011, and (C) for 2012-2014. The legend for the map is; from lighter color to darker; 0.00 to 1.00, 1.01 to 5.00, 5.01 to 10.00, 10.01 to 20.00, and 20.01 to 43.16 per 100,000 cows.



Average incidence of bovine *Salmonella* Newport isolates per 100,000 cows in the 3 highest-incidence regions of Wisconsin; (D) for East-central, (E) for North-east, and (F) for Central regions; are shown with Poisson regression and 95% confidence interval. For comparison, state average is depicted with a solid line and regional average is the dotted line.

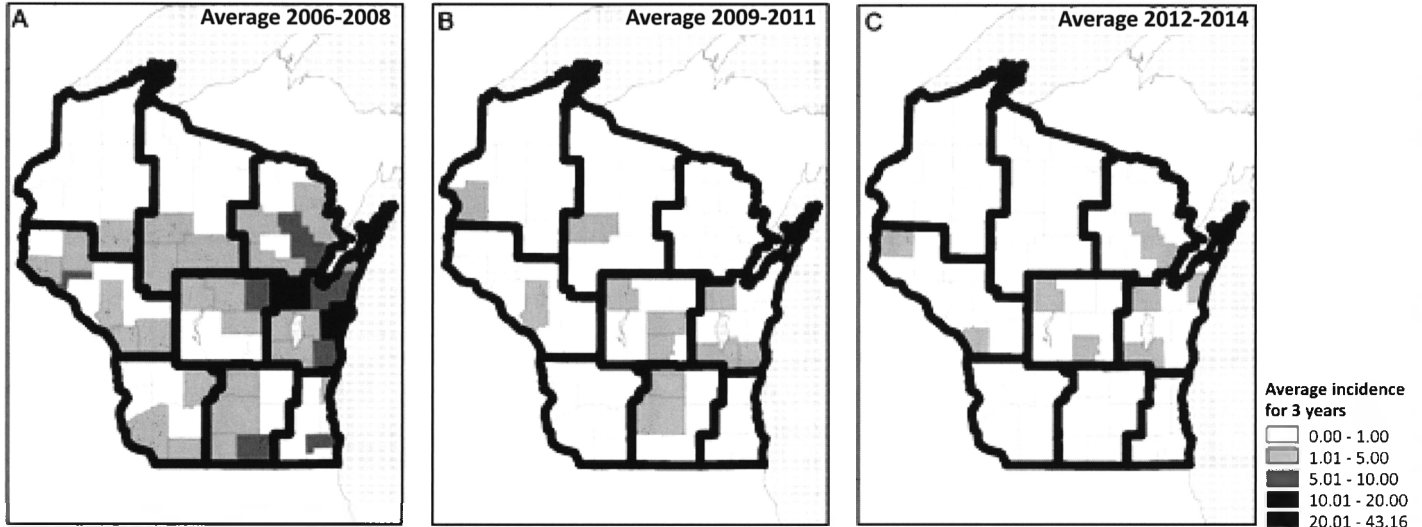
100,000 cows (Figure 3D, 3E, and 3F). Regionally, East-central (Figure 3D, Region F), South-central (Figure 3E, Region H), and North-east (Figure 3F, Region C) regions had higher than the state average, with 6.41, 5.23, and 3.91, respectively. Interestingly, the incidence for South-central (H) region in 2007 was 10.58, which was different from other years. In 2007, Dane County had 19 positive accessions and Columbia County had 12 positive accessions, which contributed to this high number in the South-central (H) region. In 2008, both counties saw a reduction in *Salmonella* ser. Dublin incidence, which led to a return to the 2006 incidence level. Overall, a reduction in the incidence of *Salmonella* ser. Dublin was observed throughout the study period (Figure 3A, 3B, and 3C).

Salmonella ser. Cerro showed overall increase in incidence across the state during the study period (Figure 4A, 4B, and 4C). In East-central region (F), Central region (E), and South-central region (H), the 9-year average incidences were 5.45, 2.59, and 2.52 per 100,000 cows respectively, which

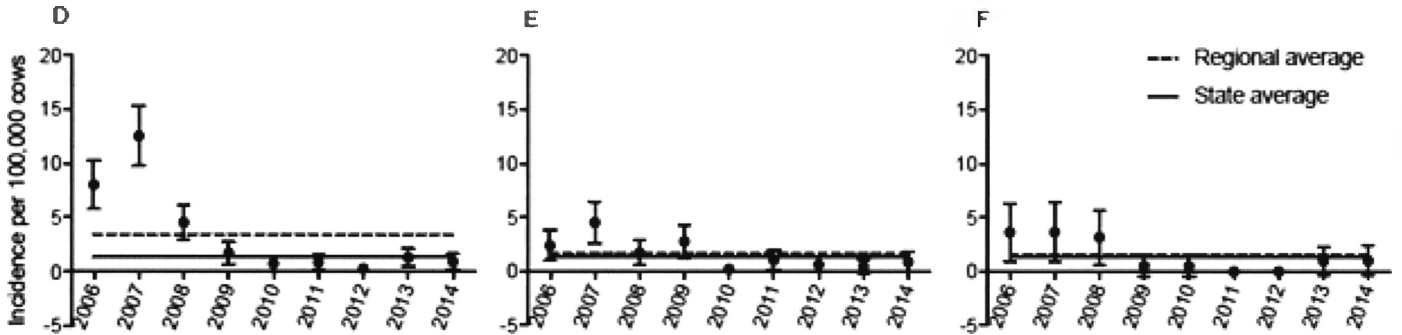
were higher than the state average of 2.45 (Figure 4D, 4E, and 4F). Other regions had much lower numbers, including 1.46 in the North-west region (A), 1.27 in the North-central region (B), 1.66 in the North-east region (C), 1.16 in the West-central region (D), 1.77 in the South-west region (G), and 0.46 in the South-east region (I) (Figure 4A, 4B, and 4C). It is important to note that the incidence of *Salmonella* ser. Cerro increased from 2006 to 2014 (Figure 4A, 4B, and 4C), whereas all other serovars showed overall decline during the same period.

In 2006, *Salmonella* ser. Cerro was observed in only 3 counties with ratio of 1.19, 1.20 and 2.17 per 100,000 cows. By 2014, the entire statewide average was 3.34, with the highest incidence of 29.41 in Sawyer County (Figure 4C, Region A). The East-central region (6.78) (Figure 4D), South-central region (4.58) (Figure 4E), Central (3.56) (Figures 4F), and South-west (4.96) regions (figure not shown) of the state had high incidence in 2014. The incidences of *Salmonella* ser. Cerro and Newport, another high-incidence serovar, from

Figure 6. Distribution of bovine *Salmonella enterica* serovar Kentucky isolates from 2006 to 2014.



Three-year averages incidence of bovine *Salmonella* Kentucky isolates per 100,000 cows in each region. (A) is for 2006 to 2008, (B) for 2009-2011, and (C) for 2012-2014. The legend for the map is; from lighter color to darker; 0.00 to 1.00, 1.01 to 5.00, 5.01 to 10.00, 10.01 to 20.00, and 20.01 to 43.16 per 100,000 cows.



Average incidence of bovine *Salmonella* Kentucky isolates per 100,000 cows in the 3 highest-incidence regions of Wisconsin; (D) for East-central, (E) for South-central, and (F) for North-east regions; are shown with Poisson regression and 95% confidence interval. For comparison, state average is depicted with a solid line and regional average is the dotted line.

2012 to 2014 contributed to the overall high *Salmonella* incidence for the last 3 years of the study period in Sawyer County (Figure 2C, Region A).

Wisconsin is not the only state to observe the increase in *Salmonella ser. Cerro* incidence. *Salmonella ser. Cerro* was also reported to be increasing in the state of New York where 77% (44 of 57) of study herds were *Salmonella* positive, and 46% of the *Salmonella*-positive herds were infected with *Salmonella ser. Cerro* or 56% of the *Salmonella*-positive isolates were identified as *Salmonella ser. Cerro*.^{8,9} In this study, pulsed-field gel electrophoresis (PFGE) found 8 distinct pattern types among the 116 *Salmonella ser. Cerro* isolates tested, and 89% of these isolates presented with the identical and dominant PFGE pattern.⁹ This was a change from previous data collected between 2004 and 2005 in the northeastern United States, including the state of New York, where *Salmonella ser. Cerro* was very rarely isolated.⁶ Factors that contributed to the rapid increase of the dominant

PFGE pattern were unknown, and it was hypothesized that isolation of the predominant PFGE pattern of *Salmonella ser. Cerro* from cattle throughout New York could mean the rapid spread of a successful phenotype.⁹

Several possible factors could have contributed to the increase of *Salmonella ser. Cerro* that we observed in this study. In a study conducted from March 2004 until January 2006 among dairy farms in Pennsylvania, *Salmonella ser. Cerro* superseded *Salmonella ser. Typhimurium var. Copenhagen* and *Salmonella ser. Kentucky* in prevalence.³⁷ The study suggested that *Salmonella ser. Cerro* adapted to the bovine environment well, and either out-competed *Salmonella ser. Typhimurium var. Copenhagen* and *Salmonella ser. Kentucky* or independently established a long-term infection in the bovine herds.³⁷ The mathematical model developed from this study supports the observation that *Salmonella ser. Cerro* was associated with long duration of infection without obvious clinical signs, similar to a carrier state.⁵ These

results matched the data reported here where a decrease in *Salmonella* ser. Kentucky incidence, along with the other serovars examined in this study, was observed with a concurrent increase in *Salmonella* ser. Cerro incidence. Whether this same mechanism was responsible for the emergence of the serovar in Wisconsin warrants future study.

Another potential factor for increase in *Salmonella* ser. Cerro incidence and a concurrent decrease in *Salmonella* ser. Dublin incidence could be the common use of vaccination against *Salmonella* ser. Dublin.^f Live-attenuated vaccine for *Salmonella* ser. Dublin has been marketed commercially since the 1980s. The *Salmonella* ser. Dublin live-attenuated vaccine was proven to be more effective than killed bacterins vaccines, and had led to significant protection in a study against the virulent *Salmonella* ser. Dublin strain SL1367 inoculum that was given orally with a syringe.³¹ It is possible that this vaccine has led to a reduction in *Salmonella* ser. Dublin carriage and shedding and thereby infections, which led to an increased prevalence of *Salmonella* ser. Cerro.³⁴ There was evidence that selection pressure from interventions, including vaccination, has occurred in humans where *Salmonella* ser. Typhi decreased in incidence upon the introduction of a vaccine, which caused an increase in the incidence of *Salmonella* ser. Paratyphi.³³ In our study, *Salmonella* ser. Dublin and *Salmonella* ser. Kentucky decreased over the examined period while *Salmonella* ser. Cerro increased. These could be natural fluctuations or could be a result of a selection pressure, which will require future studies.

As seen with other *Salmonella* serovars, except Cerro, the *Salmonella* ser. Newport incidence decreased slightly (Figure 5A, 5C) from the state average of 3.19 in 2006 to 1.84 in 2014. The East-central region (Region F) was the only region where regional average (8.05) was higher than the state average (2.27) for *Salmonella* ser. Newport (Figure 5D). The Northeast region (Figure 5E, Region C) recorded the next highest average incidence, in which case numbers went up to 6.41 in 2013 and 3.54 in 2014 from the previous averages ranging from 0.00 to 1.58 in years between 2006 and 2012. Waushara County, which is in Central region (Region E in Figure 1A), recorded a high incidence of 41.38 in 2006, 20.69 in 2007, and 35.71 in 2008 of *Salmonella* ser. Newport, but then decreased gradually and stabilized between 7.0 and 8.0 since 2009. This downward trend is shown in Figure 5F. This increase was responsible for the high incidence in the 2006-2008 average (Figure 5A). Sawyer County in the North-west region (A) recorded no positive cases each year until 2011, but spiked to have 13.70 in 2012, 28.57 in 2013, and 14.71 in 2014. This contributed to the incidence of all serovars combined for 2014 to be 44.12, because *Salmonella* ser. Cerro also recorded 29.41 in Sawyer County in 2014, as mentioned above.

Currently, there is little understanding of why *Salmonella* serovars cycle in a population. Besides *Salmonella* ser. Cerro, the other serovars examined here were all decreasing in incidence (Figures A, B, and C in 2-6). During this study

period, the populations of dairy cows in the state were generally stable as demonstrated from the county cow number data.³⁵ Most large or expanding farms cannot maintain a closed herd, requiring purchase of replacement animals. Also, many dairies have off-site (often out of state) rearing of replacement heifers. Procurement and movement of replacement animals represents a risk of introducing new pathogens to the herd, which might account for the increase in *Salmonella* ser. Cerro.³⁷ Similar observations were made in Pennsylvania from 2005 until 2010 when prevalence of *Salmonella* ser. Cerro increased and the 2 dominant strains, *Salmonella* ser. Typhimurium and *Salmonella* ser. Newport, decreased.³⁴ Severe enteritis in both cattle and humans was associated with *Salmonella* ser. Newport before the emergence of *Salmonella* ser. Cerro.³⁴ *Salmonella* ser. Newport remains a prominent and significant human pathogen despite a reduction in its prevalence in cattle.³⁴ It is possible that changes in the dominant serovar in Wisconsin were caused by natural cycling or selection pressures, such as vaccination for *Salmonella* ser. Dublin. Tewari et al suggest the decline in *Salmonella* ser. Newport in Pennsylvania was caused by an increase in *Salmonella* vaccine usage leading to increase prevalence of *Salmonella* ser. Cerro.³⁴

Continued surveillance of *Salmonella* ser. Newport is important due to its ability to adapt to various hosts and to develop multidrug resistance. This serovar is responsible for many human foodborne infections and is monitored closely by public health and animal health agencies.^{17,18} In Wisconsin, ongoing collaboration between the WVDL and the Wisconsin State Laboratory of Hygiene compares human and animal *Salmonella* ser. Newport isolates via PFGE. In addition to PFGE analysis, susceptibilities of each bovine isolate are also examined using the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) antimicrobial susceptibility panel.⁸ Further research should utilize GIS mapping and antimicrobial resistance monitoring for both human and bovine *Salmonella* ser. Newport isolates to understand the transmission of these isolates.

The incidence for *Salmonella* ser. Kentucky (9%) and Montevideo (9%) was significantly less than Dublin (23%), Cerro (15%) and Newport (14%), which resulted in more annual variability due to a low incidence of cases (Table 1). *Salmonella* ser. Kentucky incidence decreased gradually during the study period of 2006-2014 (Figure 6A, 6B, and 6C). The top 3 counties with high incidence in 2006 were 18.95 in Manitowoc, 15.38 in Pepin, and 14.04 in Waupaca. All 3 had zero incidences in 2014. The top 3 counties in 2014 were Oconto (3.71), Outagamie (3.09), and Polk (2.41) counties. This serovar's overall state average was 1.40 per 100,000 cows. The East-central region's incidence average for this serovar was 3.46, with higher average incidence of 8.03 for 2006, 12.56 for 2007, and 4.55 for 2008. From 2009 to 2014, the regional average was consistent between 1.73 and 0.30 (Figure 6D). For the South-central region, which had the second highest regional average of 1.63, *Salmonella* ser.

Kentucky recorded averages of 4.54 (2007), 1.72 (2008), and 2.78 (2009). The subsequent years, however, recorded less than the regional average, ranging from 0.21 to 1.06 (Figure 6E). The North-east region had an average of 1.50, which was slightly over the state average of 1.40. The first 3 years (2006 thru 2008) recorded averages of 3.63, 3.66, and 3.17 respectively, but dropped down to 0.51 in 2009, and remained low between 0.0 and 1.01 (Figure 6F).

Sawyer County (Region A; North-west), which is outside of the 2 high-incidence regions of East-central and South-central, showed unique incidence patterns. In 2014, it recorded 44.12 per 100,000 cows for all *Salmonella* serovars combined (Figure 2C). Sawyer County's incidence history was 0.00 (2006 - 2008), 30.30 (2009), 0.00 (2010), 14.93 (2011), 27.40 (2012), 28.57 (2013), and 44.12 (2014). As for *Salmonella* ser. Cerro, the county recorded zero cases from 2006 until 2010. In 2011, incidence went up to 14.93, but then zeroed again in 2012 and 2013. In 2014, it recorded a high incidence of 29.41. *Salmonella* ser. Newport recorded zero incidences from 2006 until 2011, but then increased to 13.70 (2012), 28.57 (2013), and 14.71 (2014) in the county. *Salmonella* ser. Dublin recorded 15.15 in 2009, but zero for the remaining years in this dataset. *Salmonella* ser. Montevideo was 15.15 in 2009, but zero for other years in the county whereas *Salmonella* ser. Kentucky was not reported in any years in the county. The 2 serovars *Salmonella* ser. Cerro and Newport contributed to the spike of 44.12 incidences in 2014. Sawyer County suggests multiple events might have taken place to the herds located there such as purchasing or moving animals in herd consolidation as the population decreased from 7,500 cows in 2006 to 6,800 cows in 2014.³⁶ Purchase or movement of contaminated feedstuff or a wildlife vector from nearby commercial poultry farms could also have been a factor. Although the inciting cause of individual county or region incidence was outside of the scope of this study, investigating reasons of the relatively outstanding changes in incidence of specific serovars in limited geographic area may hold a key for the control and management efforts of bovine *Salmonella*. Because *Salmonella* ser. Montevideo did not show remarkable features, figures on this serovar were omitted for space limitation reasons.

There are limitations to this study. First, it was possible that disease cases were unidentified due to no or little clinical signs. This is more likely to happen to serovars such as Cerro and Dublin, which could be subclinical.⁵ Due to the nature of the submission process, there may be some bias in the number and nature of isolates obtained, thereby affecting our estimated incidence rates. There are several other veterinary diagnostic laboratories that veterinarians and farmers can send samples in the region, and no collaborative data sharing system has been established to gather data comprehensively. Furthermore, the decision on whether to submit samples is at the discretion of veterinarians, and the WVDL does not have information on the submission of repeat samples or the subsequent length of time in between submissions unless it

is noted on the submission form. Furthermore, the samples were convenience samples sent in to the diagnostic laboratory, and not random selections from the animal population of interest; therefore, inference to the general population based on our conclusions are limited.

Another point that has to be noted is that the WVDL started to use real-time, reverse transcriptase PCR (RT-PCR) for *Salmonella* species and *Salmonella* ser. Dublin isolation starting in July of 2014. This RT-PCR introduction in identification method could have contributed to incidence changes because RT-PCR sensitivity is better than traditional culture.²⁴ However, there was not enough data to compare before and after the PCR introduction. Future study is needed to better understand the impact of molecular testing methods on diagnosed salmonellosis.

Transmission of salmonellosis can occur between farms via contaminated farm equipment and workers, interstate movement of sale cattle, or through contaminated water sources.^{7,13,19} There is no required permanent identification system for cattle, which complicates identification of emerging outbreaks and trace-back to origin farm. Further, it is difficult to put critical control points into the agricultural network to stop the disease cycle. Permanent identification systems do help control disease, as evidenced by the eradication of *Salmonella* ser. Dublin in Denmark by the use of individual cow registration.²³

The authors understand that the amount of serogroups that were not serotyped fully was significant. From the years 2006-2014, the WVDL had 1922 *Salmonella* that were only serogrouped, whereas 4,841 *Salmonella* isolates were serotyped. The WVDL serotypes fully only 1 isolate per accession, which can include several animals and samples. As long as the other *Salmonella* isolated serogroup belongs to the same group (e.g. Group K for Cerro) of the fully serotyped *Salmonella*, we only report 1 serotype. Although this practice could result in an underrepresentation of specific isolates, it is a standard practice within veterinary diagnostic laboratories, and the financial cost of serotyping multiple isolates could not be justified.

Conclusions

Incidences of most *Salmonella* serovars have decreased during the time period of this study with exception of *Salmonella* ser. Cerro, which increased in incidence. Variability in incidence and in location of dairy herds with salmonellosis suggests that continual monitoring is needed for management and potential disease control programs for specific serovars. *Salmonella* management should be emphasized in the East-central and South-central regions of the state.

Endnotes

^a Laboratory Information Management System (LIMS), UVIS, Ross Group Inc., Dayton, OH

- ^b Matrix-Associated Laser Desorption/Ionization Time-of-Flight (MALDI TOF) Mass Spectroscopy (MS), Bruker, Liepzig, Germany
- ^c Excel, Microsoft Corporation, Redmond, WA
- ^d ArcGIS, ESRI, Redlands, CA
- ^e Prism 6.0, Graph Pad Software, Inc., San Diego, CA
- ^f Commercially available vaccine against *Salmonella* ser. Dublin is EnterVene-d by Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
- ^g National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) antimicrobial susceptibility panel, Thermo Fisher Trek Sensititre Diagnostics, Oakwood Village, OH

Acknowledgements

The authors thank Dr. Ronald Gangnon at the University of Wisconsin-Madison for statistical insight and support. The authors declare no conflict of interest.

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