Epidemiology of a *Salmonella enterica* subsp. *enterica* Serovar Typhimurium Strain Associated with a Songbird Outbreak

Sonia M. Hernandez,[a] Kevin Keel,[a] Susan Sanchez,[b] Eija Trees,[c] Peter Gerner-Smidt,[c] Jennifer K. Adams,[d] Ying Cheng,[a] Al Ray III,[a] Gordon Martin,[e] Andrea Presotto,[f] Mark G. Ruder,[a]* Justin Brown,[a] David S. Blehert,[g] Walter Cottrell,[h] and John J. Maurer[a]

Departments of Population Health,[a] Infectious Diseases,[b] Environmental Health Science,[e] and Geography,[f] University of Georgia, Athens, Georgia, USA; Centers for Disease Control and Prevention, Atlanta, Georgia, USA[c]; Association of Public Health Laboratories, Silver Spring, Maryland, USA[d]; U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA[g]; and Pennsylvania Game Commission, University Park, Pennsylvania, USA[h]

Salmonella enterica subsp. *enterica* serovar Typhimurium is responsible for the majority of salmonellosis cases worldwide. This *Salmonella* serovar is also responsible for die-offs in songbird populations. In 2009, there was an *S.* Typhimurium epizootic reported in pine siskins in the eastern United States. At the time, there was also a human outbreak with this serovar that was associated with contaminated peanuts. As peanuts are also used in wild-bird food, it was hypothesized that the pine siskin epizootic was related to this human outbreak. A comparison of songbird and human *S.* Typhimurium pulsed-field gel electrophoresis (PFGE) patterns revealed that the epizootic was attributed not to the peanut-associated strain but, rather, to a songbird strain first characterized from an American goldfinch in 1998. This same *S.* Typhimurium strain (PFGE type A3) was also identified in the PulseNet USA database, accounting for 137 of 77,941 total *S.* Typhimurium PFGE entries. A second molecular typing method, multiple-locus variable-number tandem-repeat analysis (MLVA), confirmed that the same strain was responsible for the pine siskin epizootic in the eastern United States but was distinct from a genetically related strain isolated from pine siskins in Minnesota. The pine siskin A3 strain was first encountered in May 2008 in an American goldfinch and later in a northern cardinal at the start of the pine siskin epizootic. MLVA also confirmed the clonal nature of *S.* Typhimurium in songbirds and established that the pine siskin epizootic strain was unique to the finch family. For 2009, the distribution of PFGE type A3 in passerines and humans mirrored the highest population density of pine siskins for the East Coast.

T he genus *Salmonella* has a worldwide distribution and is one of the most common causes of diarrheal diseases of people and animals. It includes more than 2,400 distinct serotypes, most of which show little specificity for their host species. However, certain *S. enterica* subsp. *enterica* serovars are preferentially found in a particular host in which they cause disease. Examples include *S. enterica* subsp. *enterica* serovar Dublin in cattle and *S. enterica* subsp. *enterica* serovar Pullorum and *S. enterica* subsp. *enterica* serovar Gallinarum in chickens. Other *Salmonella* serovars, such as *S. enterica* subsp. *enterica* serovar Typhimurium, cause enteric disease in a wide range of hosts (7). *Salmonella* Typhimurium is also the causative agent of avian salmonellosis in wild birds, a nonenteric disease (41). *Salmonella* Typhimurium causes the majority of avian salmonellosis outbreaks and can cause high mortality rates in affected songbirds and aquatic birds (11, 15). Among songbirds, avian salmonellosis is a widespread and annual problem in the United States, Canada, and the United Kingdom (41). The repeated and frequent isolation of specific strains suggests that these *S.* Typhimurium strains have become adapted to some species of passerines, may be endemic in those populations, and cause epizootics under specific conditions (11). Although *S.* Typhimurium is typically thought to have a wide host range, strain-host relationships in pine siskins, American goldfinches, and other members of the finch family have been identified. There is still much to learn about the epidemiology of *S.* Typhimurium and the population impacts of salmonellosis for this group of birds; however, reports indicate that incidents of avian salmonellosis are on the rise (11, 12, 19). Of songbirds submitted to the National Wildlife Health Center (NWHC) from 1985 to 2004, 21.5% of mortality events were due to salmonellosis, and the annual trend for all avian *Salmonella*-related mortality events exhib-

ited a 12% increase in proportional mortality (the number of birds that died from salmonellosis divided by the total number of birds presented to the diagnostic lab for necropsy) (19). The increase in outbreaks over the last 25 years has caused some to consider avian salmonellosis an emergent disease that may be directly related to anthropogenic activities such as backyard feeding (15, 41).

Salmonellosis is also a significant source of enteric disease for people in the United States (5) and elsewhere (32), with the majority of cases originating from contaminated food (18). Infections with *S.* Typhimurium are one of the most frequent causes of salmonellosis in humans (22). In some cases, direct transmission of *S.* Typhimurium between birds and people has been reported (33). However, most reports rely on indirect associations, whereby *Salmonella* strain types associated with outbreaks of human cases were concurrent with avian mortality in the same region or the risk of infection was directly related to contact with a wild bird (1, 36, 39). A better understanding of ecological interactions between *S.* Typhimurium, avian hosts, the environment, and

humans will be essential to reduce the burden of human illnesses due to *Salmonella* in the United States.

In 2009, an exceptionally severe and widespread outbreak of avian salmonellosis occurred throughout the United States. This epizootic was coincidental with a human outbreak of *S.* Typhimurium linked to contaminated peanut products (10), and both the general public and the scientific community became concerned that peanut products in wild-bird food were a possible source of the avian infections. This concern led to recalls of products by multiple bird seed manufacturers and to a temporary cessation of feeding activities (35). The objectives of this study were to investigate the geographic, spatial, and genetic relationships of the avian salmonellosis cases submitted to wildlife diagnostic laboratories in the United States during this outbreak, to utilize pulsed-field gel electrophoresis (PFGE) to elucidate important epidemiological details about this outbreak, and to investigate the genetic relationship between this outbreak and non-food-borne human salmonellosis cases. The potential for transmission of *S.* Typhimurium from wild birds to people (1, 21, 26, 33, 40), domestic pets (39), and livestock (1, 17, 31), its dissemination to other wildlife (1, 20), and the potential that backyard feeding may increase the potential for outbreaks (34) illustrate the complexity of the epidemiology of this disease.

MATERIALS AND METHODS

Sample collection. Beginning in January and ending in June 2009, several reports from the public and state agencies of moribund and dead backyard songbirds were received at the Southeastern Cooperative Wildlife Disease Study (SCWDS) and NWHC. Birds were described as lethargic, puffed in appearance, or unaware of human presence or were found dead. Birds were submitted fresh or frozen and were examined by necropsy. Sections of representative organs were fixed in 10% buffered formalin for histopathology. Unfixed samples of liver, esophagus, and crop were submitted to diagnostic laboratories for microbiology analyses. Segments of esophagus and crop with lesions were preferentially selected.

Bacteriology. Tissue samples were macerated, homogenized, and inoculated in dulcitol selenite broth (BD, Franklin Lakes, NJ) overnight at 42°C. Broths were subcultured to selective media, including XLT4 agar, brilliant green novobiocin (BGN) agar, and MacConkey agar (Remel, Lenexa, KS), which were incubated overnight at 37°C. Suspect *Salmonella* colonies from the selective media were picked and streaked again for isolation on tryptic soy agar with 5% sheep blood. Final identification was determined based on biochemical results for citrate, motility-indole-ornithine, and triple-sugar iron media and a whole-cell agglutination test using *Salmonella*-specific poly A-I and Vi antiserum (Fisher Scientific, Pittsburgh, PA) (14). *Salmonella* isolates were submitted to the National Veterinary Services Laboratory (Ames, IA) for serotyping.

Molecular typing of *S.* Typhimurium isolates. PFGE was used to determine the genetic relatedness (25, 28, 37) of *S.* Typhimurium isolates from songbirds to archived songbird isolates, to isolates from other animal sources, and to human isolates represented in the PulseNet national database (16). A master database of *S.* Typhimurium PFGE patterns was generated in BioNumerics (Applied Maths, Austin, TX). This database consisted of 204 total PFGE patterns generated for *S.* Typhimurium isolates obtained from various avian species ($n = 162$), including passerines ($n = 96$), galliformes ($n = 35$), psittacines ($n = 9$), water fowl ($n = 14$), shore birds ($n = 8$), and nonavian species ($n = 42$). Included in this data set were *S.* Typhimurium isolates from the *Salmonella* Reference Collection (SARA isolates 1 to 20) (8) and isolates from past studies (13, 23, 30, 42). Comparisons were made between PFGE patterns using Dice coefficient (42) and unweighted-pair group method using average linkage (UPGMA) clustering. A subset of isolates were characterized using a sequence-based subtyping method, multiple-locus variable-

number tandem-repeat (VNTR) analysis (MLVA), by following the standardized procedure established by PulseNet (3). Comparisons between MLVA profiles were made with BioNumerics software using categorical coefficient and UPGMA clustering (2).

Geographic information system (GIS) analysis. A U.S. map showing the distribution of *Salmonella* bird and human isolates was produced using ArcGIS 10 software (ESRI, Redlands, CA) and a base U.S. physical map. Using ArcGIS 10, we selected the affected counties and exported from the original data set, generating layers of geographical information (mountains, rivers, etc.) for each state on this map. Each county depicted was linked to the type of *Salmonella* isolate (human, bird, or both). Human population density for large urban centers was layered onto this map using information provided by the 2010 U.S. Census (http://2010.census .gov/2010census/).

RESULTS

2009 *S.* Typhimurium outbreak in pine siskins. From January 2009 to November 2009, SCWDS diagnosed *Salmonella* infection as the primary cause of death for 109 birds. Simultaneously, across the country, other labs reported high avian mortalities due to *Salmonella*. For example, the NWHC received 57 cases from 16 states and the Pennsylvania Game Commission received 6 cases. The peak of total case submissions was February for the SCWDS and April for the NWHC. Northern cardinals were the first species to be diagnosed (9 January 2009), but by 30 January 2009, pine siskins became the most frequently diagnosed species.

Nearly all birds diagnosed with salmonellosis had round, firm, yellow to tan, thickened segments of the esophagus and, less commonly, the crop. These foci corresponded to dense infiltrates of mostly degenerate heterophils which expanded and often obliterated the wall of the esophagus. The overlying epithelium was ulcerated, and large colonies of Gram-negative rods were admixed with the necrotic debris and inflammatory infiltrates. In many cases, the spleen was enlarged and mottled due to multifocal infiltrates of heterophils with necrosis of associated tissue and bacterial colonies as previously described. Other tissues, such as liver, testes, and skeletal muscle, were variably affected, consistent with septicemia.

Identification of a single *S.* Typhimurium strain associated with the 2009 pine siskin outbreak in the eastern United States by PFGE. Fifty-seven *S.* Typhimurium isolates retained from the SCWDS cases, 24 isolates from the NWHC, and 6 isolates from the Pennsylvania Game Commission from the 2009 songbird epizootic were compared to determine by PFGE the genetic relatedness of songbird isolates from the recent epizootic to isolates from past cases. Ninety-three passerine isolates were examined by PFGE, and 99% of the isolates clustered tightly in one clonal group (clonal group A) (Fig. 1 and Table 1). Within this clonal group, most subtypes differed from each other by only 1 to 4 DNA fragments (A1 to A12). *Salmonella* Typhimurium PFGE type A3 was responsible for most 2009 cases in pine siskins and American goldfinches (94%). This PFGE type was associated primarily with birds belonging to the finch family Fringillidae that were collected as far back as 1998 (Table 1) (23). *Salmonella* Typhimurium PFGE type A3 was frequently isolated from pine siskins and American goldfinches collected or sampled in the eastern United States (Georgia, South Carolina, North Carolina, Tennessee, Virginia, West Virginia, and Pennsylvania), but it was different from the PFGE type A4 isolated from pine siskins and the common redpoll from the central and western United States (Minnesota and Washington). *Salmonella* Typhimurium PFGE subtype A6 was also isolated in
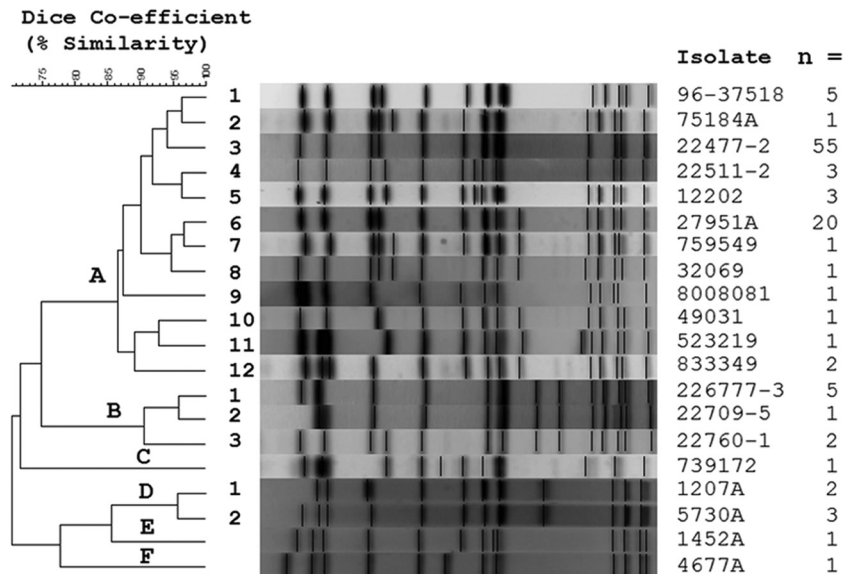
FIG 1 Cluster analysis of avian *S.* Typhimurium pulsed-field gel electrophoresis (PFGE) patterns generated with the restriction enzyme XbaI. Tiff images of *S.* Typhimurium PFGE patterns were compared using DNA pattern recognition software (BioNumerics; Applied Maths, Austin, TX). Levels of similarity were calculated using the band-based Dice similarity coefficient, and clustering of samples was performed using the unweighted-pair group method using average linkages (UPGMA). Six major PFGE clusters, A to F (>85% similarity), were identified among 162 avian *S.* Typhimurium isolates examined. Within PFGE clusters A, B, and D, additional PFGE subtypes, in which patterns differed by only 1 to 4 DNA fragments, were identified. The majority of songbird *S.* Typhimurium isolates produced PFGE A (subtypes 1 to 12) patterns with the restriction enzyme XbaI.

2009 from several songbird species other than finches, most notably the northern cardinal. This same PFGE subtype has been encountered in past years (Table 1). The two dominant *S.* Typhimurium PFGE subtypes identified from songbirds in 2009 were distinctly different from the *S.* Typhimurium subtypes isolated in the same year from water fowl (Fig. 1 and Table 1).

The same *S.* Typhimurium pine siskin strain (A3) is indistinguishable by PFGE and MLVA from human isolates. None of these songbird *Salmonella* PFGE patterns matched the *S.* Typhimurium PFGE pattern associated with the 2009 outbreak in humans, tied to contaminated peanut products (data not shown). However, several passerine PFGE patterns were indistinguishable from (100% similarity) *S.* Typhimurium PFGE patterns in the PulseNet USA database. The songbird PFGE patterns A3 and A6 matched PFGE patterns associated with 137 and 603 *S.* Typhimurium PFGE entries, respectively, of a total of 77,941 *S.* Typhimurium PFGE entries in the PulseNet database (as of 8 March 2012). A minor songbird PFGE type, A5, first described in 1998 (23), matched the *S.* Typhimurium PFGE pattern for 200 entries in the PulseNet database. Another minor PFGE type, A1, matched a PFGE pattern for 592 entries in the PulseNet database. None of these PFGE patterns had been associated with known outbreaks in humans that were identified by PulseNet (16). The minor songbird PFGE types A2, A7, A8, and C did not match any PFGE patterns in the PulseNet database. In comparing passerine *S.* Typhimurium to other animal isolates in our PFGE database, we identified only one isolate with the same passerine A1 PFGE pattern (Table 1).

A second molecular typing method, MLVA, was used to further assess the genetic relatedness among a subset of *S.* Typhimurium songbird isolates, epidemiologically unrelated animal isolates, and human isolates with matching PFGE patterns (Fig. 2). As observed with PFGE, MLVA profiles for passerine isolates clus-

tered together, and within this cluster, these profiles were further segregated into two groups, IIIa and IIIb. MLVA also separated the nonpasserine isolates 192432 and 80008081 with the similar or matching passerine PFGE patterns A1 and A9, respectively, into the separate clusters I and II, respectively. All MLVA profiles for songbirds belonging to the finch family Fringillidae grouped into the IIIb branch. MLVA was also able to discern genetic differences among *S.* Typhimurium isolates with identical PFGE patterns whose isolations were separated temporally (isolate 27951A [2009] versus isolates 6005419, 732619, and 833347 [2006 to 2008] or isolate 22471-1 [2009] versus isolate 98A-33516 [1998]) or spatially (isolate 27951A [Georgia; 2009] versus isolate 22827-1 [New Jersey; 2009]). MLVA also separated epidemiologically unrelated isolates indistinguishable by PFGE (isolate 5730A versus isolates 1448 and 1449A). However, isolates from the same locale and time had the same MLVA profiles (isolates 1207A and 1208A, 1448 and 1449A, and 27951A and 27951B). Although a diversity of MLVA profiles was observed for songbird cluster IIIa, 76% of isolates in MLVA cluster IIIb produced a single profile, T1, despite their spatial separation. Most genetic differences within MLVA cluster IIIb were at one or, at most, two VNTR loci (ST2 and ST6) and usually consisted of one to two repeat differences. Locus ST2 is a highly conserved locus and has never previously been observed to evolve during an outbreak. Locus ST6 is a highly variable locus, so differences seen in that locus are significant (E. Trees and P. Gerner-Schmidt, personal communication). Hence, patterns U and V cannot be regarded as closely related to pattern T1, whereas patterns T2 and T3 are highly related to pattern T1. Human and songbird *S.* Typhimurium isolates with the same MLVA type, T1, and PFGE pattern, A3, were identified in this study.

Temporal and spatial distribution of *S.* Typhimurium strain A3. February was the peak of the 2009 epizootic with *S.* Typhimurium strain A3 in the songbird populations (Fig. 3). Human cases

TABLE 1 *Salmonella* isolates used in this study[i]

Isolate name	No. of isolates	Family[a]	Species[a]	Mo	Yr	State	County	PFGE type	PulseNet match[d]	No. with matching PFGE pattern[e]	No. of matches in yr of isolation[f]	MLVA cluster[g]	MLVA type
22709-5	1	Charadriidae	Piping plover	August	2009	MA	Barnstable	B2	No matches				
22677-3	1	Laridae	Ring-billed gull	July	2009	ND	Mountrail	B1	JPXX01.1261	91	4		
22710-1	1	Laridae	Ring-billed gull	August	2009	ND	Foster	B1	JPXX01.1261	91	4	II	I
22728-2	1	Laridae	Ring-billed gull	August	2009	ND	Mountrail	B1	JPXX01.1261	91	4		
22728-4	1	Laridae	Ring-billed gull	August	2009	ND	Mountrail	B1	JPXX01.1261	91	4		
22791-1	1	Laridae	Herring gull	October	2009	ME	Adroscoggin	B1	JPXX01.1261	91	4		
22716-2	1	Laridae	Franklin's gull	August	2009	SD	Kingsbury	B3	JPXX01.1378	4	0	I	A
22760-1	1	Laridae	Franklin's gull	September	2009	ND	Burleigh	B3	JPXX01.1378	4	0	I	B
192432	1	Gallininae	Chicken	NA	2006	GA	NA	A1	JPXX01.0179	592	40	I	C
1207A	1	Gallininae	Chicken	June	2006	GA	NA	D1	JXXP01.0111	987	140	II	E
1208A	1	Gallininae	Chicken	June	2006	GA	NA	D1	JXXP01.0111	987	140	II	E
5730A	1	Gallininae	Chicken	April	2007	GA	NA	D2	JPXX01.0038	1,368	157	II	F
1452A	1	Gallininae	Chicken	July	2006	GA	NA	E	ND			II	H
4677A	1	Gallininae	Chicken	March	2007	GA	NA	F	JPXX01.0621	4,165	529	I	D
751384A	1	Cardinalidae	Northern cardinal	April	2007	GA	Catoosa	A2	No matches			IIIa	N
751384B	1	Cardinalidae	Northern cardinal	April	2007	GA	Catoosa	A1	JPXX01.0179	592	32		
30052B	1	Cardinalidae	Northern cardinal	January	2009	GA	Spaulding	A3	JPXX01.1043	137	33		
6005419	1	Cardinalidae	Northern cardinal	April	2006	GA	Hall	A6	JPXX01.0150	603	42	IIIa	K2
742217	1	Cardinalidae	Northern cardinal	March	2007	GA	Richmond	A6	JPXX01.0150	603	44		
848065	1	Cardinalidae	Northern cardinal	May	2008	TN	Coffey	A6	JPXX01.0150	603	70	IIIa	O
27437B	6[c]	Cardinalidae	Northern cardinal	January	2009	GA	Spaulding	A6	JPXX01.0150	603	50	IIIa	O
930052	1	Cardinalidae	Northern cardinal	July	2009	GA	Spaulding	A6	JPXX01.0150	603	50		
27457E	1	Cardinalidae	Northern cardinal	February	2009	GA	Spaulding	A6	JPXX01.0150	603	50		
523219	1	Fringillidae	Finch	November	2004	NA	NA	A11	JPXX01.1297	16	1		
98A-33516	1	Fringillidae	American goldfinch	March	1998	KY	McCreary	A3	JPXX01.1043	137	1	IIIb	U
851809	1	Fringillidae	American goldfinch	May	2008	NC	Wilkes	A3	JPXX01.1043	137	13	IIIb	T1[h]
30453	1	Fringillidae	American goldfinch	February	2009	GA	Dawson	A3	JPXX01.1043	137	33		
33463	1	Fringillidae	American goldfinch	February	2009	VA	Floyd	A3	JPXX01.1043	137	33		
31876A	1	Fringillidae	American goldfinch	February	2009	TN	Sevier	A3	JPXX01.1043	137	33	IIIb	T1[h]
31877A	1	Fringillidae	American goldfinch	February	2009	TN	Blount	A3	JPXX01.1043	137	33		
33461A	4[c]	Fringillidae	American goldfinch	February	2009	VA	Rockbridge	A3	JPXX01.1043	137	33		
22477-5	1	Fringillidae	American goldfinch	March	2009	NC	Moore	A3	JPXX01.1043	137	33	IIIb	T1[h]
34332 (liver)	1	Fringillidae	House finch	February	2009	GA	Whitfield	A3	JPXX01.1043	137	33		
731877B	1	Fringillidae	Purple finch	February	2009	TN	Blount	A3	JPXX01.1043	137	33		
22471-1	1	Fringillidae	Purple finch	March	2009	NC	Caldwell	A3	JPXX01.1043	137	33	IIIb	T1[h]
28251B	3[c]	Fringillidae	Pine siskin	January	2009	GA	Rabun	A3	JPXX01.1043	137	33	IIIb	T1[h]
31879	1	Fringillidae	Pine siskin	February	2009	GA	Cherokee	A3	JPXX01.1043	137	33		
31880	1	Fringillidae	Pine siskin	February	2009	GA	Gilmer	A3	JPXX01.1043	137	33		
34331	1	Fringillidae	Pine siskin	February	2009	GA	Whitfield	A3	JPXX01.1043	137	33		
22459-2	1	Fringillidae	Pine siskin	February	2009	MD	Garrett	A3	JPXX01.1043	137	33		
30452C	1	Fringillidae	Pine siskin	February	2009	GA	Dawson	A3	JPXX01.1043	137	33		
31876B	1	Fringillidae	Pine siskin	February	2009	TN	Sevier	A3	JPXX01.1043	137	33	IIIb	T1[h]
31878A	2[c]	Fringillidae	Pine siskin	February	2009	TN	Knox	A3	JPXX01.1043	137	33		
32532A	4[c]	Fringillidae	Pine siskin	February	2009	GA	Oconee	A3	JPXX01.1043	137	33	IIIb	T1[h]
33146C (liver)	2[c]	Fringillidae	Pine siskin	February	2009	SC	Spartanburg	A3	JPXX01.1043	137	33	IIIb	T1[h]
33462A	3[c]	Fringillidae	Pine siskin	February	2009	VA	Rockbridge	A3	JPXX01.1043	137	33		
33464B	3[c]	Fringillidae	Pine siskin	February	2009	VA	Floyd	A3	JPXX01.1043	137	33	IIIb	T1[h]
34113 (liver)	1	Fringillidae	Pine siskin	February	2009	GA	Whitfield	A3	JPXX01.1043	137	33		
P0905345	1	Fringillidae	Pine siskin	February	2009	PA	Huntingdon	A3	JPXX01.1043	137	33	IIIb	T1[h]
22471-9	1	Fringillidae	Pine siskin	March	2009	NC	Caldwell	A3	JPXX01.1043	137	33	IIIb	T2
22477-2	1	Fringillidae	Pine siskin	March	2009	NC	Moore	A3	JPXX01.1043	137	33	IIIb	T1[h]
22487-2	1	Fringillidae	Pine siskin	March	2009	AL	Calhoun	A3	JPXX01.1043	137	33		
34333 (liver)	1	Fringillidae	Pine siskin	March	2009	WV	Randolph	A3	JPXX01.1043	137	33	IIIb	T1[h]
P0906327	1	Fringillidae	Pine siskin	March	2009	PA	Huntingdon	A3	JPXX01.1043	137	33	IIIb	T1[h]
P0907343	1	Fringillidae	Pine siskin	March	2009	PA	Bedford	A3	JPXX01.1043	137	33		
P0907344	1	Fringillidae	Pine siskin	March	2009	PA	Huntingdon	A3	JPXX01.1043	137	33		
P0909859	1	Fringillidae	Pine siskin	March	2009	PA	Franklin	A3	JPXX01.1043	137	33		
22526-4	1	Fringillidae	Pine siskin	April	2009	VT	Orange	A3	JPXX01.1043	137	33		
22528-1	1	Fringillidae	Pine siskin	April	2009	WV	Upshur	A3	JPXX01.1043	137	33		
P0909858	1	Fringillidae	Pine siskin	April	2009	PA	Huntingdon	A3	JPXX01.1043	137	33		
P0912925	1	Fringillidae	Pine siskin	April	2009	PA	Snyder	A3	JPXX01.1043	137	33		
29988	1	Fringillidae	Pine siskin	February	2009	GA	Habersham	A3	JPXX01.1043	137	33		
34504	1	Fringillidae	Pine siskin	March	2009	WV	Randolph	A3	JPXX01.1043	137	33		
P0917357	1	Fringillidae	Pine siskin	June	2009	PA	Somerset	A3	JPXX01.1043	137	33		
22413-1	1	Fringillidae	Pine siskin	January	2009	WA	Thurston	A4	JPXX01.0199	159	22		
22511-2	1	Fringillidae	Pine siskin	April	2009	MN	Carlton	A4	JPXX01.0199	159	22	IIIb	V
22511-4	1	Fringillidae	Common redpoll	April	2009	MN	Carlton	A4	JPXX01.0199	159	22	IIIb	T3[h]
739172	1	Fringillidae	Canary	NA	NA	NA	NA	C	No matches				
96-37518	1	Icteridae	Brown-headed cowbird	March	1996	GA	Clarke	A1	JPXX01.0179	592	5	IIIa	L

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TABLE 1 (Continued)

Isolate name	No. of isolates	Family[a]	Species[a]	Mo	Yr	State	County	PFGE type	PulseNet match[d]	No. with matching PFGE pattern[e]	No. of matches in yr of isolation[f]	MLVA cluster[g]	MLVA type
98A-24966	1	Icteridae	Brown-headed cowbird	January	1998	GA	Camden	A1	JPXX01.0179	592	5	IIIa	Q
98A-28238	1	Icteridae	Brown-headed cowbird	February	1998	GA	Camden	A1	JPXX01.0179	592	5	IIIa	R
833349	1	Icteridae	Brown-headed cowbird	January	2008	GA	Houston	A12	JPXX01.1054	230	26		
11203	1	Icteridae	Rusty blackbird	NA	NA	NA	NA	A12	JPXX01.1054	230	26		
48796	1	Icteridae	Red-winged blackbird	August	2003	GA	Glynn	A6	JPXX01.0150	603	71		
49052	1	Icteridae	Common grackle	August	2003	GA	Cherokee	A6	JPXX01.0150	603	71		
728131	1	Icteridae	Red-winged blackbird	December	2006	GA	Glynn	A6	JPXX01.0150	603	42	IIIa	O
732619	1	Icteridae	Red-winged blackbird	January	2007	GA	Richmond	A6	JPXX01.0150	603	44	IIIa	M2
833347	1	Icteridae	Red-winged blackbird	January	2008	GA	Houston	A6	JPXX01.0150	603	70	IIIa	K4
27951A	1	Icteridae	Red-winged blackbird	February	2009	GA	Burke	A6	JPXX01.0150	603	50	IIIa	K1
27951C	1	Icteridae	Red-winged blackbird	February	2009	GA	Burke	A6	JPXX01.0150	603	50	IIIa	M3
759549	1	Icteridae	Brown-headed cowbird	June	2007	GA	Meriwether	A7	No matches			IIIa	K3
32069	1	Icteridae	Brown-headed cowbird	July	2002	GA	Greene	A8	No matches			IIIa	K2
12202	1	Passeridae	English sparrow	NA	1998	WY	NA	A5	JPXX01.0002	200	2	IIIa	S
A7852	1	Passeridae	English sparrow	NA	1998	WY	NA	A5	JPXX01.0002	200	2		
W12198	1	Passeridae	English sparrow	NA	1998	WY	NA	A5	JPXX01.0002	200	2		
P0907752	1	Passeridae	House sparrow	March	2009	PA	Clearfield	A3	JPXX01.1043	137	33		
22827-1	1	Passeridae	House sparrow	October	2009	NJ	Warren	A6	JPXX01.0150	603	50	IIIa	K1
49051E	1	Cardinalidae	Northern cardinal	August	2003	GA	Bibb	A10	ND			IIIa	M1
231449	1	Icteridae	Red-winged blackbird	January	2002	GA	Richmond	A6	JPXX01.0150	603	50	IIIa	P
8008081	1	Psittacidae	Parakeet	NA	2009	NA	NA	A9	ND			II	J
1448A[b]	1	Muridae	Mouse	June	2006	GA	NA	D2	JPXX01.0038	1,368	112	II	G
1449A[b]	1	Muridae	Mouse	June	2006	GA	NA	D2	JPXX01.0038	1,368	112	II	G

[a] *S.* Typhimurium isolates were organized in this table according to family and species.

[b] Mice were caught in a chicken house.

[c] *S.* Typhimurium was isolated from multiple birds of the same species. All isolates had the same PFGE pattern.

[d] There were a total of 77,941 *S.* Typhimurium entries in the PulseNet database as of 8 March 2012 (accession date).

[e] Total number of entries with a matching PFGE pattern in PulseNet.

[f] Number of matches for that year of isolation.

[g] Major MLVA cluster.

[h] Matches with a human isolate.

[i] NA, not available; ND, not done.

associated with this *S.* Typhimurium strain were scattered throughout 2008 and 2009. Most human illnesses due to PFGE type A3 were reported for the Atlantic states (76%; *n* = 34) (Fig. 4) from December 2008 to November 2009. There was spatial (Orange County, VT) and temporal (February 2009, Knox County, TN) overlap in the identification of PFGE type A3 for songbird and human *S.* Typhimurium isolates (Fig. 4). The distribution of this *S.* Typhimurium strain mirrored the highest-density distribution of pine siskins for the East Coast (4).

DISCUSSION

In 2009, a single *S.* Typhimurium strain A3, as defined by PFGE, was observed and accounted for all infections and deaths of pine siskins and American goldfinches along the Atlantic coast. MLVA confirmed that a single *Salmonella* strain was responsible for this epizootic in pine siskins. This *Salmonella* strain was distinct from 2009 isolates from other bird families and pine siskin/common redpoll isolates obtained from the central/western United States. MLVA was able to discern differences between epidemiologically unrelated isolates with the same or similar PFGE patterns, illustrating the finer

resolving power of sequencing-based subtyping methods for identifying subtler genetic differences than PFGE (6, 9).

An archived American goldfinch isolate from 1998 had the same A3 PFGE pattern (23) and clustered with 2009 pine siskin isolates by MLVA, with the only difference observed in one of the seven VNTR loci. This pine siskin strain appears to have been circulating in songbirds for some time. Songbird *S.* Typhimurium isolates were distinctly different by MLVA from other avian isolates and belonged to one of two major clades, with finch isolates forming their own clade (IIIb). *S.* Typhimurium strains infecting passerines appear to be adapted to their avian host (24, 29), but unlike other host-adapted *S. enterica* serovars (e.g., *S.* Gallinarum and *S.* Pullorum [7]), these avian strains appear to be capable of causing human illnesses (27).

There have been several reported human cases of salmonellosis linked to exposure to wild birds. Penfold and coworkers (33) described an outbreak of *Salmonella* gastroenteritis in a hospital that was linked to sparrows present in the kitchen facility. In New Zealand, after an avian epizootic, *S.* Typhimurium phage type DT160 was found in domestic livestock and humans. Interviews with patients suffering from salmonellosis (DT160) showed that
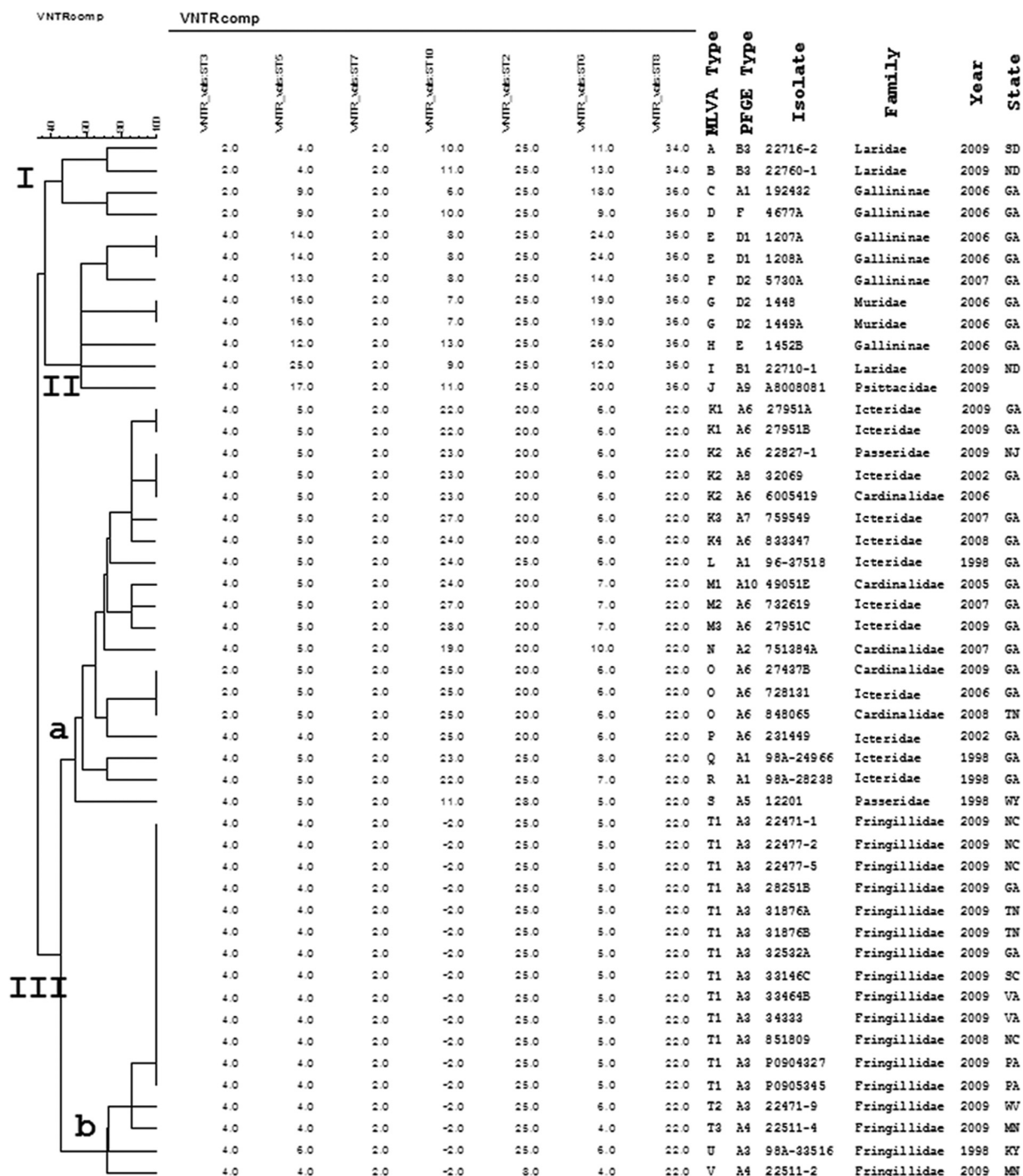
VNTRoomp   VNTRcomp

VNTR_vds:ST3	VNTR_vds:ST5	VNTR_vds:ST7	VNTR_vds:ST10	VNTR_vds:ST2	VNTR_vds:ST6	VNTR_vds:ST8	MLVA Type	PFGE Type	Isolate	Family	Year	State
2.0	4.0	2.0	10.0	25.0	11.0	34.0	A	B3	22716-2	Laridae	2009	SD
2.0	4.0	2.0	11.0	25.0	13.0	34.0	B	B3	22760-1	Laridae	2009	ND
2.0	9.0	2.0	6.0	25.0	18.0	36.0	C	A1	192432	Gallininae	2006	GA
2.0	9.0	2.0	10.0	25.0	9.0	36.0	D	F	4677A	Gallininae	2006	GA
4.0	14.0	2.0	8.0	25.0	24.0	36.0	E	D1	1207A	Gallininae	2006	GA
4.0	14.0	2.0	8.0	25.0	24.0	36.0	E	D1	1208A	Gallininae	2006	GA
4.0	13.0	2.0	8.0	25.0	14.0	36.0	F	D2	5730A	Gallininae	2007	GA
4.0	16.0	2.0	7.0	25.0	19.0	36.0	G	D2	1448	Muridae	2006	GA
4.0	16.0	2.0	7.0	25.0	19.0	36.0	G	D2	1449A	Muridae	2006	GA
4.0	12.0	2.0	13.0	25.0	26.0	36.0	H	E	1452B	Gallininae	2006	GA
4.0	25.0	2.0	9.0	25.0	12.0	36.0	I	B1	22710-1	Laridae	2009	ND
4.0	17.0	2.0	11.0	25.0	20.0	36.0	J	A9	A8008081	Psittacidae	2009	
4.0	5.0	2.0	22.0	20.0	6.0	22.0	K1	A6	27951A	Icteridae	2009	GA
4.0	5.0	2.0	22.0	20.0	6.0	22.0	K1	A6	27951B	Icteridae	2009	GA
4.0	5.0	2.0	23.0	20.0	6.0	22.0	K2	A6	22827-1	Passeridae	2009	NJ
4.0	5.0	2.0	23.0	20.0	6.0	22.0	K2	A8	32069	Icteridae	2002	GA
4.0	5.0	2.0	23.0	20.0	6.0	22.0	K2	A6	6005419	Cardinalidae	2006	
4.0	5.0	2.0	27.0	20.0	6.0	22.0	K3	A7	759549	Icteridae	2007	GA
4.0	5.0	2.0	24.0	20.0	6.0	22.0	K4	A6	833347	Icteridae	2008	GA
4.0	5.0	2.0	24.0	25.0	6.0	22.0	L	A1	96-37518	Icteridae	1998	GA
4.0	5.0	2.0	24.0	20.0	7.0	22.0	M1	A10	49051E	Cardinalidae	2005	GA
4.0	5.0	2.0	27.0	20.0	7.0	22.0	M2	A6	732619	Icteridae	2007	GA
4.0	5.0	2.0	28.0	20.0	7.0	22.0	M3	A6	27951C	Icteridae	2009	GA
4.0	5.0	2.0	19.0	20.0	10.0	22.0	N	A2	751384A	Cardinalidae	2007	GA
2.0	5.0	2.0	25.0	20.0	6.0	22.0	O	A6	27437B	Cardinalidae	2009	GA
2.0	5.0	2.0	25.0	20.0	6.0	22.0	O	A6	728131	Icteridae	2006	GA
2.0	5.0	2.0	25.0	20.0	6.0	22.0	O	A6	848065	Cardinalidae	2008	TN
4.0	4.0	2.0	25.0	20.0	6.0	22.0	P	A6	231449	Icteridae	2002	GA
4.0	5.0	2.0	23.0	25.0	8.0	22.0	Q	A1	98A-24966	Icteridae	1998	GA
4.0	5.0	2.0	22.0	25.0	7.0	22.0	R	A1	98A-28238	Icteridae	1998	GA
4.0	5.0	2.0	11.0	28.0	5.0	22.0	S	A5	12201	Passeridae	1998	WY
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	22471-1	Fringillidae	2009	NC
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	22477-2	Fringillidae	2009	NC
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	22477-5	Fringillidae	2009	NC
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	28251B	Fringillidae	2009	GA
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	31876A	Fringillidae	2009	TN
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	31876B	Fringillidae	2009	TN
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	32532A	Fringillidae	2009	GA
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	33146C	Fringillidae	2009	SC
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	33464B	Fringillidae	2009	VA
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	34333	Fringillidae	2009	VA
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	851809	Fringillidae	2008	NC
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	P0904327	Fringillidae	2009	PA
4.0	4.0	2.0	<2.0	25.0	5.0	22.0	T1	A3	P0905345	Fringillidae	2009	PA
4.0	4.0	2.0	<2.0	25.0	6.0	22.0	T2	A3	22471-9	Fringillidae	2009	WV
4.0	4.0	2.0	<2.0	25.0	4.0	22.0	T3	A4	22511-4	Fringillidae	2009	MN
4.0	6.0	2.0	<2.0	25.0	6.0	22.0	U	A3	98A-33516	Fringillidae	1998	KY
4.0	4.0	2.0	<2.0	8.0	4.0	22.0	V	A4	22511-2	Fringillidae	2009	MN

Dendrogram cluster labels (left margin): I, II, III, a, b

FIG 2 Cluster analysis of avian *S.* Typhimurium isolates by MLVA. The dendrogram was generated in BioNumerics (Applied Maths) using the categorical coefficient and unweighted-pair group method using average linkages (UPGMA). Avian *S.* Typhimurium isolates subtyped by MLVA belong to one of three major clusters (I to III). Of the songbird isolates typed by MLVA, those belonging to the bird families Cardinalidae (cardinals), Fringillidae (American goldfinch, common redpoll, pine siskin, and purple finch), Icteridae (brown-headed cowbird and red-winged blackbird), and Passeridae (English sparrow and house sparrow) grouped into cluster III. Within cluster III, two additional branches were identified. All *S.* Typhimurium isolates typed by MLVA that belonged to cluster IIIb were isolated from birds belonging to the finch family Fringillidae.

direct handling of wild birds was a significant risk factor ([1]). In Norway, sporadic human outbreaks (1966 to 1996) were analyzed and found to be seasonal, with 78% of the cases occurring in January to April, a time corresponding to annual avian salmonel-losis outbreaks, and people known to have direct contact with wild birds or their droppings had a statistically significant increased risk of infection ([36]). Spatial and temporal overlaps were identified between a few avian and human cases in this report; however,
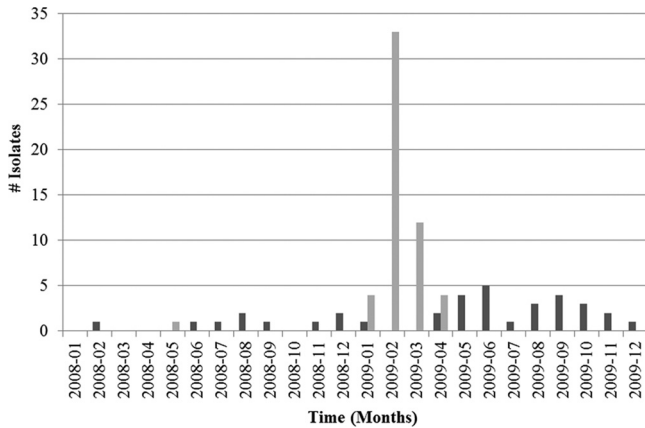
FIG 3 Temporal distribution of *S.* Typhimurium strains with the PFGE pattern A3 in birds and humans for the years 2008 and 2009. Light gray, birds; dark gray, humans.

the peak of human cases associated with this and other passerine *S.* Typhimurium strains was in late spring and early summer months, a period in which the epizootics have generally subsided. These sporadic cases in humans may reflect transmission resulting from either human contact with birds actively shedding *Salmonella* or, more likely, contact with a common, contaminated environmental source.

Since the 1980s, epizootics have caused tens of thousands of mortalities in songbirds in the United States, Europe, and New Zealand. In the United Kingdom, avian salmonellosis appears to be contributing to the decline of some songbird species, particularly in combination with other infectious diseases (38). It is unclear whether the same scenario is likely in the United States, although several large outbreaks, likely totaling more than tens of thousands of birds each, have occurred. Investigations that describe the spatial, temporal, and molecular relationships of *Salmonella* outbreaks are paramount for piecing together how these outbreaks affect songbird populations. For example, a recent review of salmonellosis of garden birds in England showed that there are currently two host-adapted *Salmonella* phage types, *S.* Typhimurium DT40 and DT56v, that are circulating widely in British garden birds and that the reservoir of infection is maintained within wild bird populations (29). Based on these findings, it also appears that host-adapted *Salmonella* strains circulate among wild birds and are conserved for long periods of time. Additionally, two clonal types (A3 and A4) were circulating among pine siskins from different flyways (eastern and western, respectively), and they could be distinguished from other songbird *S.* Typhimurium strains.
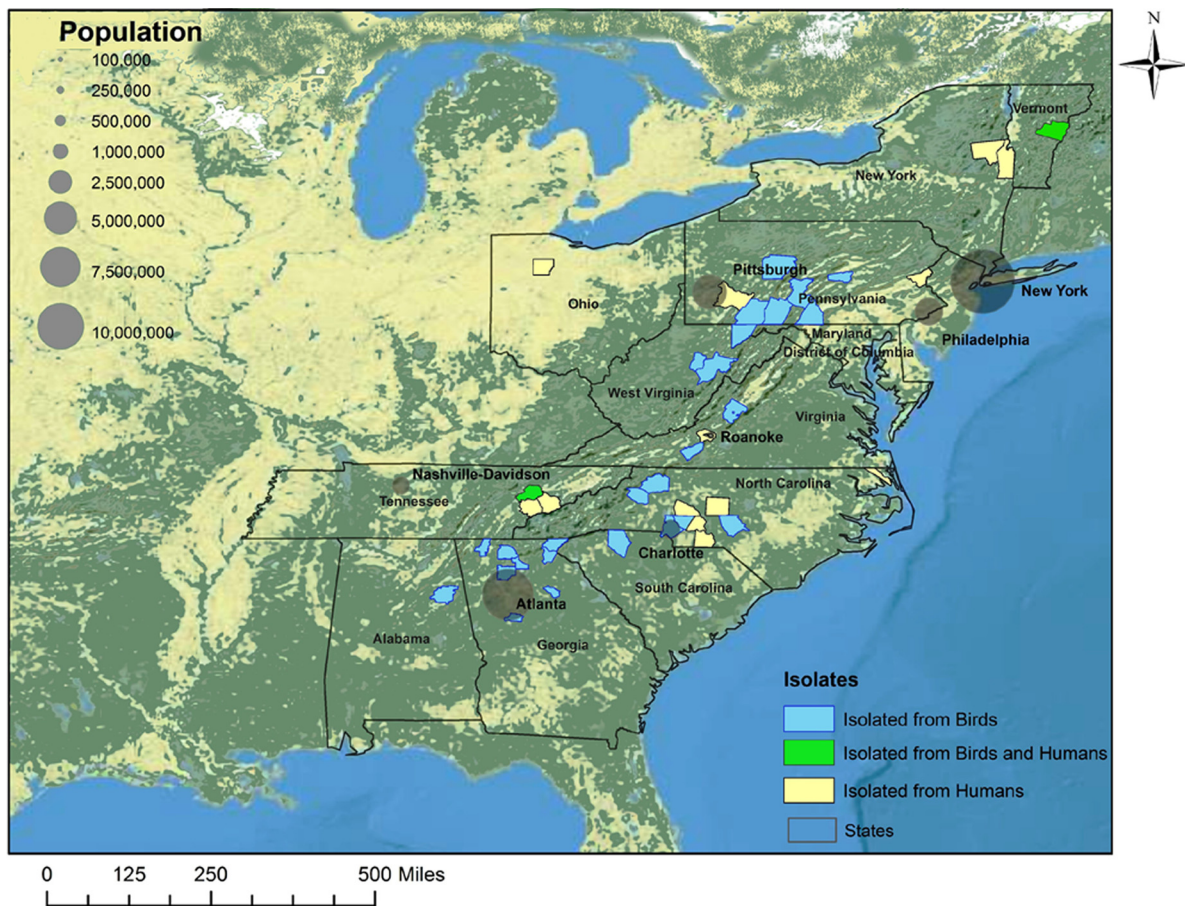
FIG 4 Spatial distribution of *S.* Typhimurium strains with the PFGE pattern A3 in the eastern United States. Human (yellow) and bird (light blue) isolates of PFGE type A3 that were obtained between May 2008 and November 2009 were mapped to the county level. Counties from which both bird and human isolates were obtained are colored green. Major urban centers on the Atlantic coast are depicted along with their population size, shown in gray. The scale for the human population is shown in the upper left, ranging from 100,000 to 10 million. (Map used by permission. Copyright © 2012 Esri and its data providers. All rights reserved.)

Ideally, coordinated nationwide surveillance and standardization of methods will further our understanding of salmonellosis for wild birds. Ultimately, studies that simultaneously investigate avian, human, and environmental sources of *Salmonella* and describe potential mechanisms of movement of this pathogen among all three compartments by utilizing both empirical data and predictive modeling will lead to a more complete picture of the epidemiology of *Salmonella*. In addition, whole-genome sequencing of the avian *S.* Typhimurium isolates described in this study will provide insight into the evolution of these songbird strains in North America and the adaptations to their songbird hosts.

ACKNOWLEDGMENTS

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