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Frequency, serotype distribution, and antimicrobial susceptibility patterns of *Salmonella* in small poultry flocks in California

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Abstract. Backyard poultry operations are increasingly popular and commonplace in both rural and suburban locations. Although *Salmonella* surveillance programs are well established for large commercial poultry systems, information on smaller operations is lacking. We identified the occurrence and serotype distribution of *Salmonella* spp. recovered from backyard flock cases submitted to the California Animal Health and Food Safety Laboratory System (Davis, CA) in 2012–2015, and evaluated minimum inhibitory concentration for 12 antimicrobials as well as the lesions associated with *Salmonella* spp. in these cases. From records of 2,347 backyard flock cases with 2,627 samples, 44 samples (1.7%) were positive for *Salmonella* spp. DNA by PCR, and 41 (1.6%) of these samples yielded a *Salmonella* isolate by culture for further characterization. Seventeen different serotypes, including 3 isolates identified to the serogroup level, were identified from these isolates. Antimicrobial resistance was infrequent; however, 2 multidrug–resistant isolates were identified. Enteric or systemic lesions associated with *Salmonella* recovery were uncommon, with 77.3% of cases having no disease attributable to *Salmonella*. Recovered serotypes overlap with those seen in commercial poultry as well as in foodborne outbreaks reported by the Centers for Disease Control and Prevention in humans. Zoonotic risks via contact and food product contamination make monitoring of backyard flocks for *Salmonella* a critical part of flock surveillance programs, and we propose a potential sampling scheme.

Key words: Antimicrobial resistance; backyard poultry; pathology; *Salmonella*; serotype.

Backyard poultry operations are increasingly popular and commonplace in both rural and suburban locations.[7,17,19] Birds are kept for a variety of reasons, from home consumption to niche market production. According to the USDA Economic and Research Service, U.S. farmers' markets have increased from <2,000 in 1994 to >8,000 in 2014 (https://goo.gl/tTT367), and are common locations for local egg and bird sales. This expanded interest in backyard poultry production has led to new public health concerns about the risks of disease transmission between domestic birds, wild avian species, and humans.[15]

Contact with poultry and contaminated poultry products without established biosecurity practices represents a risk for transmission of zoonotic pathogens such as *Salmonella*.[12,17] According to the Centers for Disease Control and Prevention (CDC) 2012 Surveillance for Foodborne Disease Outbreaks in the United States Annual Report, 20.7% (120 of 579) of single-agent outbreaks and 65.2% (15 of 23) of multi-state foodborne outbreaks were caused by *Salmonella*. Among the most commonly implicated pathogen-food pairs resulting in outbreaks, the *Salmonella* poultry product (meat and eggs) grouping was responsible for 39.4% (403 of 1,022) of total illnesses (https://goo.gl/kHT3si). In 2016, 8 multi-state *Salmonella* outbreaks in humans were associated with live poultry in backyard flocks (https://goo.gl/8NQ1h6). Although *Salmonella* surveillance and intervention strategies are well established for large commercial poultry systems, data from smaller operations are lacking.[12,14] Non-traditional housing systems such as small coops and pasture operations facilitate contact of housed poultry with vectors known to harbor *Salmonella*.[8,13] Information regarding potential pathogens such as *Salmonella* in these operations is necessary to assess transmission risks and pathogen dynamics.[8]

We identified the frequency and serotype distribution as well as the minimum inhibitory concentrations (MICs) of antimicrobials for *Salmonella* spp. recovered from small poultry flock cases at the California Animal Health and Food Safety Laboratory System (CAHFS; Davis, CA) and evaluated lesions associated with *Salmonella* in these cases. Diagnostic reports of small poultry flock cases submitted to

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CAHFS, Davis from January 2012 through December 2015 were reviewed for inclusion in our study. A case was included if the submitting flock contained <1,000 birds in a single operation. Reasons for exclusion included those with birds that were not owned (e.g., rescues), birds from commercial operations, and poultry used for research purposes. Diagnostic workups into cause of death included evaluation of clinical history, gross and histologic examinations; bacterial cultures of lung, liver, or any diseased tissues identified at autopsy; *Salmonella* testing of intestinal contents and diseased tissues; PCR testing for avian influenza; screening for heavy metal abnormalities; and additional testing based on the autopsy examination including PCR for *Mycoplasma gallisepticum, M. synoviae*, and infectious bronchitis virus; *M. gallisepticum* and *M. synoviae* serology; fecal flotation and/or mucosal scraping for evidence of intestinal parasites; and electron microscopy of affected tissues. Determination of lesions associated with *Salmonella* was based on the pathologist's diagnosis of cause of death and interpretive comments along with results of ancillary testing.

Samples submitted for *Salmonella* testing were placed into a tetrathionate selective enrichment broth containing 0.01% brilliant green and 0.02% iodine at a 1:10 sample-to-broth ratio, incubated for 18–24 h at 37 ± 2°C, and subjected to PCR testing for *Salmonella* spp. based on the *invA* gene target as described previously.[3,20] Broth samples that were positive by PCR were subcultured onto 3 selective media (MacConkey agar, Hektoen enteric agar, and brilliant green with 0.002% novobiocin agar). After incubation for 18–24 h at 37 ± 2°C, plates were examined for *Salmonella*-suspect colonies, which were confirmed by biochemical testing and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Individual colonies were serogrouped (Difco antiserum, BD Diagnostics, Sparks, MD) and serotyped (*Salmonella* antisera, Statens Serum Institut, Denmark) using agglutination as described using the White–Kauffmann–Le Minor scheme.[11]

The MIC determination was undertaken on the 25 available isolates by microbroth dilution (Trek Sensititre, Trek Diagnostic Systems, Thermo Fisher Scientific, Waltham, MA) and was performed in accordance with criteria provided by the Clinical and Laboratory Standards Institute.[4,5] Briefly, bacterial isolates were subcultured on 0.5% sheep blood agar and incubated for 18–24 h at 35°C. Each isolate was suspended in phosphate-buffered saline to a concentration equivalent to a 0.5 McFarland standard, and 10 µL of this standard was added to 10 mL of cation-adjusted Mueller–Hinton broth, inoculated into a susceptibility plate, and incubated for 18 h at 35°C. The MIC for each isolate was determined as the lowest concentration of antimicrobial that prevented visible growth. Isolates were tested against 12 antimicrobials at the listed dilutions: amoxicillin (0.25–16 µg/mL), ceftiofur (0.25–4 µg/mL), enrofloxacin (0.12–2 µg/mL), florfenicol (1–8 µg/mL), gentamicin (0.5–8 µg/mL), neomycin (2–32 µg/mL), oxytetracycline (0.5–8 µg/mL), streptomycin (8–1024 µg/mL), sulfadimethoxine (32–256 µg/mL), sulfathiazole (32–256 µg/mL), tetracycline (0.25–8 µg/mL), and trimethoprim–sulfamethoxazole (0.5/9.5–2/38 µg/mL). The following quality control organisms were used: *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Enterococcus faecalis* ATTC 29212, and *Staphylococcus aureus* ATTC 29213.

A total of 2,347 accessions consisting of 2,627 individual samples were included in our study and were recovered from 2,521 chickens, 47 turkeys, 44 ducks, 10 geese, and 5 squabs. Samples collected for *Salmonella* testing included feces or cloacal swab (2,188), small intestinal contents (6), large intestinal contents (415), celomic or ovarian tissues (7), liver (6), bursa of Fabricius (2), cerebellum (2), and yolk sac material (1). Birds were submitted from 49 of the 58 California counties, the vast majority of which were from urban and suburban Sacramento, San Francisco, and Los Angeles areas.

Forty-four samples from 37 (32 chicken, 3 turkey, 3 duck) accessions were positive by PCR, 41 of which had viable *Salmonella* recovered, demonstrating a detection frequency of 1.7%. No significant differences in frequency were detected over time (2.0% in 2012, 1.7% in 2013, 2.1% in 2014, 1.7% in 2015). Sites with non-recoverable *Salmonella* were all from chicken fecal samples: one diagnosed with lead toxicosis in 2012, one diagnosed with Marek's disease in 2013, and one identified with renal gout, respiratory disease, and celomitis in 2015.

Culture-positive sample sites included feces (34), pooled intestine (6), and bursa of Fabricius (1) from chicken (36), turkey (3), and duck (2) specimens. Identified *Salmonella* (*S. enterica* subsp. *enterica*) serotypes included Agona (1), Braenderup (1) Dublin (1), Enteritidis (4), Heidelberg (3), I 4,5,12:i:- (1), Infantis (3), Kentucky (1), Montevideo (6), Muenchen (1), Ohio (1), Saintpaul (2), Senftenberg (3), Thompson (2), Typhimurium (5), and 2 untypeable isolates (1 that only agglutinated with poly A-I antisera, and 1 that was monophasic); additionally, one isolate of *S. enterica* subsp. *arizonae* IIIb (*S.* Arizonae) was recovered. Three additional positive samples had *Salmonella* identified to the group level (1 serogroup C2 from chicken feces, 1 serogroup C2 from turkey feces, and 1 serogroup B from chicken feces) but were not recoverable for serotype evaluation. Five accessions that had multiple animals sampled yielded the same serotype from each individual animal tested (Infantis, Kentucky, Montevideo, Senftenberg, Thompson). One accession had 2 different serotypes (Infantis, Kentucky) recovered from 2 layers in the same flock.

Overall, high MICs and resistance were sporadic in these isolates (Table 1). The most notable exception was sulfadimethoxine: 92% (23/25) of isolates tested were classified as resistant. Two multidrug–resistant isolates (defined as resistant to ≥3 antimicrobial classes) were identified. A *Salmonella* Dublin isolate recovered from the feces of a juvenile chicken diagnosed with poxvirus infection showed resistance to amoxicillin, ceftiofur, oxytetracycline, streptomycin, sulfadimethoxine,

Table 1. Summary statistics of minimum inhibitory concentrations (MICs) and antimicrobial resistance for 25 *Salmonella* spp. isolates recovered from backyard flock submissions.

	Mode MIC (μg/mL)	MIC50 (μg/mL)	MIC90 (μg/mL)	% resistant	Serotype demonstrating resistance
Amoxicillin	1	1	>16	12	Heidelberg (2), Dublin (1)
Ceftiofur	1	1	2	4	Dublin (1)
Enrofloxacin	≤0.12	≤0.12	≤0.12	4	Untypeable (1)
Florfenicol	4	4	8	8	Senftenberg (1), Ohio (1)
Gentamicin	≤0.5	≤0.5	≤0.5	4	Montevideo (1)
Neomycin	≤2	≤2	≤2	0	None
Oxytetracycline	2	2	4	8	Senftenberg (1), Dublin (1)
Streptomycin	≤8	≤8	16	8	Senftenberg (1), Dublin (1)
Sulfadimethoxine	>256	>256	>256	92	All except Agona (1) and untypeable (1)
Sulfathiazole	64	≤32	128	4	Dublin (1)
Tetracycline	2	1	2	8	Senftenberg (1), Dublin (1)
Trimethoprim–sulfadimethoxine	<0.5/9.5	<0.5/9.5	<0.5/9.5	0	None

Number in parentheses is the number of isolates of each serotype demonstrating resistance.

sulfathiazole, and tetracycline; a *Salmonella* Senftenberg isolate from a chick with Marek's disease was resistant to florfenicol, oxytetracycline, streptomycin, sulfadimethoxine, and tetracycline.

Autopsy findings recorded by the pathologist in the reports along with the *Salmonella* serotype recovered from backyard chickens, ducks, and turkeys are presented in Table 2. Primary lesions associated with a positive *Salmonella* isolation were uncommon in the birds of our study and consisted mostly of localized or disseminated infections accompanied by conditions associated with immune dysfunction: 3 chickens, 2 with *Salmonella* Enteritidis and 1 with *Salmonella* Typhimurium, had concurrent Marek's disease; 2 neonatal chicks with *Salmonella* Senftenberg had bursal and yolk sac atrophy; 1 duck with *Salmonella* Saintpaul was infected with duck viral enteritis (DVE) (anatid alphaherpesvirus 1); and 1 duck with *Salmonella* Heidelberg–associated enteritis had acute hepatic necrosis and hepatocellular iron overload. Disseminated infections were found in 1 turkey with osteomyelitis and systemic spread of *S.* Arizonae; and 2 chickens with salpingitis and celomitis (1 with a rectal tear and 1 with pyelonephritis) caused by *Salmonella* Montevideo.

Records of birds with lesions not associated with *Salmonella* colonization on histologic examination had a wide range of clinical diagnoses, including Marek's disease (7), neoplasia consisting of carcinomatosis and lymphoid leukosis (6), dermatitis or cellulitis (4), cloacal cannibalism (3), airsacculitis or pneumonia (2), tremovirus A (avian encephalomyelitis virus; 2), salpingitis or celomitis (2), and individual cases of liver hemorrhagic syndrome, DVE, poxvirus infection, proventriculitis or ingluvitis, histomoniasis, renal disease with urate stasis, lead toxicosis, and 1 bird with stunted growth but no other lesions at autopsy.

A program that offers low- or no-cost autopsy and ancillary testing to small poultry operations for determining a cause of death and performing surveillance for high-consequence diseases is provided by CAHFS. Backyard poultry accessions rose from 401 cases in 2010 to 1,459 cases in 2015, with submissions to the Davis branch increasing 289% during this time period. As the number of backyard poultry flocks increases, opportunities for exposure to zoonotic pathogens such as *Salmonella* via contaminated food products or via direct contact with birds increase.[6,17]

The Davis branch of the laboratory system performs the majority of evaluations for small poultry operations in California. In comparison with other CAHFS locations during the same 4-y period, the remaining 3 branches performed a total of 1,036 (San Bernardino), 468 (Turlock), and 315 (Tulare) compared with the >2,400 accessions evaluated in Davis. Additionally, birds were submitted to Davis from throughout the state including northern, north-central, south-central, and southern areas of the state.

Backyard operations frequently have animals housed with at least partial access to outdoor environments, facilitating exposure to high-risk sources including wild birds, rodents, and wildlife species.[2,8,10] Management and surveillance procedures to prevent *Salmonella*, particularly *Salmonella* Enteritidis, in commercial layer flocks have been well established.[13,21] State and federal regulatory programs such as the Food and Drug Administration Egg Rule apply to larger operations; however, small operations are not required to adhere to these standards.[2,21] The need to ensure that products are free from bacterial pathogens is just as important for backyard operations as for commercial ones.[2] A study published by the USDA Center for Epidemiology and Animal Health found that only 30.2% of urban poultry owners in the Los Angeles area and 40.0% in the Miami area were aware of

Table 2. Autopsy findings in 44 backyard poultry cases with samples positive for *Salmonella*.

Pathology diagnosis	Species	Concurrent conditions	*Salmonella* serotype identified
Lesions associated with *Salmonella*			
Marek's disease	CH	Celomitis, splenitis, serositis, myocardial necrosis, bacterial bursitis	Enteritidis (2), Typhimurium (1)
Bursal atrophy	CH	Septicemia	Senftenberg (2)
Duck viral enteritis	DU	Enteritis, hepatitis	Saintpaul (1)
Acute hepatic necrosis	DU	Hepatocellular iron overload, enteritis	Heidelberg (1)
Multi-organ infections			
Osteomyelitis	TU	Septic dissemination	*S. enterica* subsp. *arizonae* IIIb (1)
Peritonitis/celomitis	CH	Rectal tear (1); pyelonephritis (1)	Montevideo (2)
Lesions not associated with *Salmonella*			
Marek's disease	CH	Oropharyngitis, *E. coli* celomitis, myocardial necrosis, hepatic & renal disease	Typhimurium (2), Muenchen (1), Ohio (1), Montevideo (1), Senftenberg (1), culture negative (1)
Neoplasia (carcinomatosis, lymphoid leucosis)	CH	Yolk peritonitis, celomitis	Montevideo (2), Enteritidis (2), Typhimurium (1), monophasic untypeable group C2 (1)
Dermatitis/trauma/feather loss	CH	Rhinitis, bronchitis, cellulitis	Kentucky (1), Infantis (1), Heidelberg (1), group C2 (1)
Cloacal cannibalism	CH	Splenic lymphoid hyperplasia, nephritis, hepatitis, intestinal parasites	Thompson (2), Saintpaul (1)
Airsacculitis/pneumonia	CH, TU		Group B (1), group C2 (1)
Avian encephalomyelitis virus	CH		Infantis (2)
Salpingitis/celomitis	CH	Hepatitis, splenomegaly	Heidelberg (1), untypeable; no group determined (1)
Liver hemorrhagic syndrome	CH		Braenderup (1)
Duck viral enteritis	DU	Serosal trematodes	Typhimurium (1)
Poxvirus infection	CH	Bursitis	Dublin (1)
Proventriculitis/ingluvitis	CH		I 4,5,12:i:- (1)
Histomoniasis	TU		Agona (1)
Renal disease with urate stasis	CH	Pulmonary necrosis, sinusitis, glandular mineralization, celomitis	Culture negative (1)
Lead toxicosis	DU		Culture negative (1)
None	CH	Stunted	Montevideo (1)

CH = chicken; DU = duck; TU = turkey. Number in parentheses is the number of isolates of each serotype associated with the pathology diagnosis.

the connection between contact with poultry and *Salmonella* infections in people.[1]

Because only sick or dead animals were evaluated, our study population is inherently biased, and true prevalence on an individual or flock basis cannot be determined. However, valuable surveillance information about *Salmonella* dynamics and risks can be determined by studying these types of convenience samples.[8] On an individual bird level, the frequency of detection was 1.67% (44 of 2,627), and on the individual case submission level, the frequency of detection was 1.58% (37 of 2,347). Although these estimates cannot be considered as true prevalence figures, they do indicate a low but measurable presence of *Salmonella* spp. in backyard poultry operations. In addition, the frequency of detection remained consistent over the 4 y evaluated in our study (1.7–2.1%.)

In small flock populations, collecting cloacal swabs from individual birds is the preferred way to detect such a low frequency of *Salmonella*.[9] Individual sampling may be difficult to accomplish and cost prohibitive for small poultry operations. Because *Salmonella* is shed intermittently, periodic sampling of fresh feces in the housing environment can provide a sensitive and cost effective way of determining if *Salmonella* is present in a group of birds.[16,22] Testing of 5 pairs of boot swabs collected from walking around the housing environment has been shown to have a sensitivity in detecting the presence of *Salmonella* similar to sampling 300 individual fecal samples in pools of 5.[18] Collection of environmental samples would not require restraint of individual birds for cloacal testing and may be more easily accomplished by backyard producers.[2] We propose that the use of a sterile swab or boot pair that is used to gather fecal material

where birds congregate (near feeders or under roosts) and sampling every 3–4 mo will provide valuable information about *Salmonella* presence in backyard flocks without being cost- or labor-prohibitive. Future studies utilizing this type of sampling scheme could provide better estimates of true prevalence in these populations and provide greater estimates of risk from this zoonotic agent, particularly given that multi-antimicrobial–resistant isolates were detected in these small poultry flocks.

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