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# Sequence analysis of Salmonella enterica isolates obtained from shelter dogs throughout Texas

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# Abstract

Dogs are a potential source of zoonotic Salmonella transmission. We had previously estimated the prevalence of Salmonella shedding among shelter dogs throughout Texas using a repeated cross-sectional study design. Our current objectives were to fully characterize the Salmonella isolates and to assess their relatedness, using whole-genome sequencing. Antimicrobial resistance (AMR) genes were detected in 4/27 (15%) of the isolates. The fosfomycin resistance gene fosA7 was identified in two isolates; to our knowledge, there are no published reports of this gene in canine Salmonella isolates. The biocide resistance gene gacEdelta1, conferring resistance to quaternary ammonium compounds, was detected in an isolate that had four additional AMR genes. The most frequently identified serotypes were Newport (6/27, 22%) and Javiana (4/27, 15%), both of which were widespread among animal shelters. For these serotypes, there was evidence of both transmission of Salmonella within the shelter environment and separate introductions of Salmonella into a shelter. Several canine Salmonella isolates were closely related to human clinical isolates (four canine isolates within 10 SNPs and six more within 20 SNPs), suggesting a shared pathogen population. Educational outreach programmes targeting animal shelter workers would be useful for optimizing knowledge of Salmonella and other canine-associated zoonotic pathogens.

#### KEYWORDS

Antimicrobial resistance, Dog, Epidemiology, Public health, Salmonella spp., Zoonoses

# **1** | INTRODUCTION

Salmonella enterica is estimated to cause approximately 1.2 million illnesses, 22,000 hospitalizations and 425 deaths annually in the United States, at an annual cost of \$3.3 billion (Hale et al., 2012; Hoffmann, Batz, & Morris, 2012; Scallan et al., 2011). Although generally associated with self-limiting acute enteritis, Salmonella can also cause invasive infections that may be fatal. Children under the

age of 5 years, elderly adults and immunocompromised persons are particularly susceptible to severe disease (Crump, Sjolund-Karlsson, Gordon, & Parry, 2015). Exposure is typically foodborne (Scallan et al., 2011), but Salmonella can also be transmitted through direct contact with an infected animal's faeces (Hoelzer, Moreno Switt, & Wiedmann, 2011). Dogs are a potential source of zoonotic Salmonella transmission (Morse, Duncan, Estep, Riggs, & Blackburn, 1976; Sato, Mori, Koyama, & Nagase, 2000) and can harbour other bacterial

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species with mobile resistance elements of severe consequence to humans (Cole et al., 2020).

Clinical signs of salmonellosis in adult dogs and puppies can include vomiting, diarrhoea, fever, lethargy and abdominal pain (Marks, Rankin, Byrne, & Weese, 2011). However, many canine *Salmonella* infections remain subclinical. The prevalence of *Salmonella* shedding among dogs is extremely variable, ranging from approximately 1%–20% for pet dogs (Hackett & Lappin, 2003; Leonard et al., 2011; Procter et al., 2014) to 70%–90% for racing Greyhounds and sled dogs (McKenzie et al., 2010; Morley et al., 2006). Risk factors for canine *Salmonella* shedding include ingestion of a raw meat diet, receipt of an antibiotic or probiotic, living in a rural environment and contact with livestock (Lefebvre, Reid-Smith, Boerlin, & Weese, 2008; Leonard et al., 2011; Reimschuessel et al., 2017).

Salmonella shedding among dogs in animal shelters could pose a zoonotic threat to shelter workers and adoptive families, as well as a risk of within-shelter transmission to other animals (Leahy, Cummings, Rodriguez-Rivera, Rankin, & Hamer, 2016). The objectives of this study were to fully characterize *Salmonella* isolates obtained from shelter dogs throughout Texas and to assess their relatedness, using whole-genome sequencing.

#### 2 | MATERIALS AND METHODS

#### 2.1 | Study design

We sampled dogs in seven animal shelters across Texas between May 2013 and December 2014 using a repeated cross-sectional study design, as previously described (Leahy et al., 2016). Shelters were located in College Station, Dallas, Edinburg, El Paso, Fort Worth, Houston and San Antonio. Dogs eligible for sample collection were those that were caged individually and appeared healthy during a brief physical examination. Relevant data were compiled from shelter records or directly noted by the research team during sample collection. Voided faecal samples were collected from the kennel floor or during defecation. Faecal samples were placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI) and maintained at approximately 4°C during transport to the research laboratory for processing. Those samples not immediately transported to the laboratory were placed into a Para-Pak C & S bottle (Meridian Bioscience, Inc., Cincinnati, OH) and maintained at room temperature until transport the next day. The sampling protocol was approved by the Texas A&M University Institutional Animal Care and Use Committee, and informed consent was obtained from shelter directors.

# 2.2 | Microbiological procedure for *Salmonella* detection

Bacteriological culture methods from Leahy et al. (2016) were used to isolate *Salmonella* from faecal samples. Briefly, each sample

(approximately 10 grams) was enriched in 90 ml of tetrathionate broth (Becton, Dickinson and Company, Franklin Lakes, NJ) containing 1.8 ml of iodine solution. Following incubation, the mixture was streaked onto Brilliant Green agar with novobiocin (BGN) and Xylose Lysine Tergitol 4 (XLT-4) selective media (Northeast Laboratory Services, Winslow, ME). Presumptive *Salmonella* colonies were inoculated into Kligler Iron Agar (KIA) slants, and colonies exhibiting the biochemical properties of *Salmonella* were streaked onto Tryptic Soy agar with 5% sheep blood (Becton, Dickinson and Company). An isolated colony was inoculated into Brain Heart Infusion (BHI) broth (Becton, Dickinson and Company) and then frozen in 15% glycerol for subsequent characterization. Presumptive *Salmonella* isolates were confirmed by amplification and detection of the *invA* gene using PCR (Kim et al., 2007).

#### 2.3 | Whole-genome sequencing

DNA was extracted from pure colonies using an automated magnetic bead-based process (MagMAX CORE; Thermo Fisher Scientific, Waltham, MA) and quantified with fluorometry (Qubit 2.0; Thermo Fisher Scientific). Genomic libraries were prepared and barcoded using the Nextera XT DNA Library Preparation Kit (Illumina, Inc.), and they were sequenced on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 with 2 x 250 bp chemistry.

#### 2.4 | Sequence analysis

Sequencing reads for each isolate were assembled using SKESA v. 2.3.0 (Souvorov, Agarwala, & Lipman, 2018). The resulting assemblies were screened for antimicrobial resistance (AMR) genes using NCBI AMRFinderPlus v. 3.0.12 (Feldgarden et al., 2019). Serotypes were predicted using sistr\_cmd v. 1.0.2 (Yoshida et al., 2016), and multilocus sequence typing (MLST) profiles were identified using mlst v. 2.16.1 (https://github.com/tseemann/ mlst) (Jolley & Maiden, 2010). A core genome phylogeny of the assemblies was constructed using Parsnp v. 1.2 (Treangen, Ondov, Koren, & Phillippy, 2014) and annotated using iTOL v. 4 (Letunic & Bork, 2019). Single nucleotide polymorphism (SNP) distances between isolates sharing a MLST profile were calculated using the CFSAN SNP pipeline (Davis et al., 2015). Sequence data were also submitted to the NCBI Pathogen Detection database (https:// www.ncbi.nlm.nih.gov/pathogens) for comparison with other genomes. In this database, isolates are classified as either clinical or environmental/other. We assumed clinical isolates to be from human cases if a non-human host was not specified.

# 3 | RESULTS

Individual faecal samples were collected from 554 dogs in seven shelters across Texas, and the median number of samples per shelter

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was 85 (range, 48–107). Among sampled dogs, 263 (47.5%) were females and 290 (52.3%) were males; sex was not recorded for 1 (0.2%) dog. Age group classification was as follows: 474 (85.6%) adults ( $\geq$ 1 year old), 71 (12.8%) puppies (< 1 year old) and 9 (1.6%) unknown. As previously reported (Leahy et al., 2016), the prevalence of *Salmonella* shedding as detected by culture was 4.9% (27/554; 95% Cl, 3.2%–7.0%).

No AMR genes were detected in 23/27 (85%) of the isolates (Figure 1). Two isolates carried one AMR gene each (*fosA7* and *tet*(*C*)), one carried two AMR genes (*fosA7* and *tet*(*B*)), and one carried five AMR genes (*aaA1*, *floR2*, *qacEdelta1*, *sul1* and *tet*(*G*)).

The most frequently identified serotypes were Newport (n = 6, 22%) and Javiana (n = 4, 15%), isolated from dogs in four and three of the shelters, respectively (Figure 1). Serotypes Braenderup, Infantis and Rubislaw were represented by two isolates each. Serotype Newport isolates included two different MLST profiles (ST45, n = 3; ST118, n = 3). All other serotypes included only one MLST profile.

Two of the Javiana isolates were very closely related, with zero SNP differences identified between them. These were obtained from two dogs in the same shelter, both of which had been admitted 2 days before sampling. All other pairwise comparisons between Javiana isolates showed much more distant genetic relationships, with greater than 1,000 SNPs identified between each pair. Among the ST45 Newport isolates, the closest pair was separated by 32 SNPs; both other pairwise comparisons showed separation by more than 200 SNPs. For the ST118 isolates, the closest pair was separated by 94 SNPs and other pairs by greater than 400 SNPs. The Braenderup, Infantis and Rubislaw isolate pairs were separated by 94, 35 and 56 SNPs, respectively. As of 4 February 2020, 17/27 (63%) isolates were included in SNP clusters in the NCBI Pathogen Detection database. The two identical Javiana isolates were in a SNP cluster with a total of 77 isolates, while the remaining 15 isolates were in 13 distinct clusters ranging in size from 4 to 2,531 isolates. Using the values provided by the Isolate Browser, four isolates (15%) were within 10 SNPs of a human clinical isolate and six more (total of 37%) were within 20 SNPs.

# 4 | DISCUSSION

Identification of known AMR genes was uncommon among the study isolates. Similarly, 80% (96/120) of Salmonella isolates obtained from apparently healthy pet dogs were found to be pan-susceptible, based on a panel of 15 antimicrobial agents (Leonard et al., 2012). The fosfomycin resistance gene fosA7 was identified in two Salmonella isolates (serotypes Derby and Heidelberg). To our knowledge, there are no published reports documenting this gene in Salmonella isolated from dogs, although it has been previously identified in canine Salmonella isolates according to the NCBI Pathogen Detection database. The fosA7 gene was recently detected in Salmonella Heidelberg isolates from broiler chickens in Canada (Rehman, Yin, Persaud-Lachhman, & Diarra, 2017). The Heidelberg isolate from this study is closely related (within 5 SNPs) to nine isolates from chickens and one human clinical isolate in the NCBI Pathogen Detection database. The Derby isolate is most closely related (within 20 SNPs) to isolates from pigs in Texas and Georgia. In the United States, fosfomycin is approved for use in people for the treatment of urinary



**FIGURE 1** Core genome phylogeny of *Salmonella enterica* isolates annotated with NCBI Sequencing Read Archive accession number, in silico serotype prediction, multilocus sequence typing (MLST) profile, and antimicrobial resistance (AMR) genes detected. *Salmonella* isolates were obtained from shelter dogs throughout Texas during a repeated cross-sectional study of seven animal shelters between May 2013 and December 2014

tract infections. Fosfomycin is used very rarely in dogs, primarily as therapy for recurrent or resistant urinary tract infections. A biocide resistance gene of note was *qacEdelta1*, conferring resistance to quaternary ammonium compounds, in the Duesseldorf isolate that had four additional AMR genes. No other isolates cluster with this one as of the submission of this study.

Serotype diversity was high among the study isolates. The most common serotypes were Newport and Javiana, both of which were widespread among Texas animal shelters. Newport has previously been detected in dogs (McKenzie et al., 2010; Morley et al., 2006; Reimschuessel et al., 2017) and has been isolated from raw meat diets that are fed to dogs (Chengappa, Staats, Oberst, Gabbert, & McVey, 1993; Morley et al., 2006). To our knowledge, however, Javiana had not been reported among dogs in the United States prior to the study period; this serotype is typically associated with amphibians and reptiles (Clarkson et al., 2010; Srikantiah et al., 2004). Javiana was recently identified in a small number of dogs in Texas, Georgia and North Carolina (Reimschuessel et al., 2017), and other investigators have reported Javiana among dogs in Caribbean nations (Amadi et al., 2017; Seepersadsingh, Adesiyun, & Seebaransingh, 2004). We are not aware of other reports of Rubislaw shedding among dogs in the United States, although this serotype was isolated from one dog in a study of apparently healthy dogs in Grenada (Amadi et al., 2017). Salmonella Infantis was identified in dry dog food in 2012 and subsequently implicated in a multistate outbreak of human disease; cases of canine illness linked to this food were also reported (Imanishi et al., 2014).

Assessment of SNP distances between isolates sharing a serotype provided evidence of both transmission of Salmonella within the shelter environment (two Javiana isolates from dogs in the same shelter were genetically very similar) and separate introductions of Salmonella into a shelter (three Newport isolates from dogs in the same shelter were genetically very distinct from each other). Gaps in knowledge of canine-associated zoonotic diseases have been identified among animal shelter workers (Steneroden, Hill, & Salman, 2011), and educational outreach programmes would be useful for filling these gaps and promoting shelter hygiene and biosecurity efforts. Comparison with other genomes in the NCBI Pathogen Detection database revealed that several canine Salmonella isolates were closely related to human clinical isolates, demonstrating the overlap between canine and human pathogens. Additional epidemiologic data would be necessary to explore the possibilities of either zoonotic transmission or exposure to a common source of infection.

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#### CONFLICT OF INTEREST

No conflict of interest exists.

#### AUTHOR CONTRIBUTION

Kevin J. Cummings: Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Project administration; Supervision; Writing-original draft. Patrick K. Mitchell: Formal analysis; Methodology; Writing-review & editing. Lorraine D. Rodriguez-Rivera: Methodology; Writing-review & editing. Laura B. Goodman: Formal analysis; Funding acquisition; Methodology; Supervision; Writing-review & editing.

#### PEER REVIEW

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