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Short communication

Screening for *Salmonella* in backyard chickens

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ABSTRACT

Salmonellosis is a significant zoonotic disease which has a considerable economic impact on the egg layer industry. There is limited information about the prevalence of *Salmonella* spp. in backyard chickens. The current study was conducted to determine the prevalence of *Salmonella* in backyard chickens, and the associated virulence of any serovars identified. Hundred and fifteen pooled samples from 30 backyard flocks in South Australia were screened. Four flocks tested positive for *Salmonella* spp. The overall *Salmonella* isolation rate in the current study was 10.4%. The estimated prevalence at individual bird level was 0.02% (95% CI 0.025–0.975). The serovars isolated were *Salmonella* Agona, *Salmonella* subsp 2 ser 21:z10:z6 (Wandsbek) and *Salmonella* Bovismorbificans. All *Salmonella* isolates tested positive for the *prgH*, *orfL* and *spiC* genes. The *Salmonella* subsp 2 ser 21:z10:z6 (Wandsbek) had the most antibiotic resistance, being resistant to ampicillin and cephalothin and having intermediate resistance to florphenicol. All of the *Salmonella* Agona had intermediate resistance to the ampicillin, while the *Salmonella* Bovismorbificans were susceptible to all antibiotics tested. With the increased interest of keeping backyard chickens, the current study highlights the zoonotic risk from *Salmonella* spp. associated with home flocks.

1. Introduction

Salmonellosis is a significant veterinary and zoonotic disease, with *Salmonella* Typhimurium being one of the most common zoonotic serovars in Australia (The OzFoodnet Working Group, 2012). Although commercial chicken meat and egg layer flocks are regularly monitored for *Salmonella*, there has been little investigation into backyard chickens as a source. Studies in Iran and Paraguay have estimated the prevalence of *Salmonella* spp. in backyard chickens as 5.8% (Jafari et al., 2007) and 3.5% (Leotta et al., 2010) respectively. Studies conducted overseas investigated the antibiotic resistance of Salmonella in backyard hens (Harsha et al., 2011; Samanta et al., 2014). Eleven percent of *Salmonella* infections in humans can be attributed to animal handling (Hale et al., 2012) and backyard chicken ownership has increased in recent years (Behravesh et al., 2014). Biosecurity practices of small poultry keepers are poor compared to commercial enterprises, in particular hens have high levels of outdoor access, regular contact with wild birds, and frequent movement of poultry between backyard sites (Hernández-Jover et al., 2009). Backyard poultry owners also have limited contact with veterinarians, which could result in a failure to detect a potential disease outbreak in the early stages.

The aims of this study were to determine the prevalence of *Salmonella* spp. in backyard chickens in South Australia, as well as establishing the virulent gene and antibiotic resistance profile of the isolated *Salmonella* spp. It was hypothesized that the prevalence of *Salmonella* spp. in backyard chickens is high and therefore poses a significant zoonotic risk to individuals.

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2. Materials and methods

2.1. Faecal sampling and culture

Fecal samples were collected in Sterile Whirl Pak bags (Thermo Fisher Scientific Australia) from five hundred and seventy five backyard hens (115 pools) from 30 backyard flocks/holdings. Samples collected across various locations near Adelaide, South Australia were tested during March to August 2014. Sample size for this study was calculated using Epi-Tool (Sacks et al., 1989). Epi-Tool was used to calculate the approximate number of pools required for given value of pool size and estimated prevalence. Consequently, samples from 5 hens from each premise were pooled for sample processing (Arnold et al., 2009), i.e. 115 pools, i.e. 1–4 per holding. The flock size of minimum 5 egg laying birds was selected for sampling. The birds reared in the flock were mixed ages and out of 30 properties/holdings, all birds had contact with other animals such as cattle, sheep, dogs or cats. Birds were allowed to range during the day and were locked in a chicken coop/house at night. Ages of the flocks ranged between five months to two years. Flocks had mixed breeds of birds. Flocks were chosen based on willingness of owners to participate in the study.

Faecal samples were collected in a sterile bag and transported back to the lab on ice. Faecal material was homogenised and 1 gram added to 4 mL of buffered peptone water (Oxoid Australia) and incubated at 37 °C overnight. All samples were further processed as previously described by Gole et al. (2014).

Suspected colonies from Xylose Lysine Deoxycholate agar (XLD) were streaked onto Macconkey agar (Oxoid, Australia) and confirmed using biochemical tests. Isolates which were lysine decarboxylase positive and urease and ortho-Nitrophenyl-β-galactoside (ONPG) negative were sent to *Salmonella* Reference Laboratory, Adelaide, Australia for serotyping and phage typing. The estimated prevalence was calculated using Epi-tool. The *Salmonella* culture method used in this study was more sensitive compared to real time PCR (Gole et al., 2014), hence estimated prevalence was calculated assuming a fixed pool size and perfect test. The prevalence was estimated as $P = 1 - (1 - x/m)^{1/k}$ where, P = estimated prevalence; k = pool size, m = number of pool tested; x = number of positive pools (Sacks et al., 1989).

2.2. DNA extraction and polymerase chain reactions (PCR) to test for Salmonella spp. virulence genes

The DNA from each *Salmonella* spp. was purified from the overnight culture using the Promega Wizard Genomic DNA purification Kit (Promega, Wisconsin, USA). The DNA was quantified using spectrophotometer and stored at −20 °C for further use.

Each PCR reaction mixture contained 14.5 μL of Nuclease free water, 1× reaction buffer (Fisher Scientific Australia), 1.8 mM $MgCl_2$, 1 μM of the reverse primer, 1 μM of the forward primer, 200 μM dNTP, 1 U of Taq polymerase and 50 pg of the purified DNA. The samples were then amplified using a Bio-Rad thermal cycler using 2 different protocols. The prgH and sopB genes were initially denatured at 95 °C for 5 min, followed by 30 cycles of amplification (denaturing at 95 °C for 30 s, annealing temperature as per primer in Table 1 for 45 s) and extension at 72 °C for 1 min and 30 s. This was followed by a final extension step at 72 °C for 5 min, and a hold temperature of 10 °C.

The *SpiC*, *OrfL*, *Pef A* and *SpvC* genes used the same protocol except that they were initially denatured at 95 °C for 4 min, and had 35 cycles of amplification with a final hold temperature of 8 °C. The PCR products were then confirmed using a 2% agarose gel electrophoresis.

2.3. Disc diffusion test to investigate antimicrobial resistance

The disk diffusion method was used to determine the antibiotic resistance of all Salmonella isolates against 11 antimicrobial agents: ampicillin 10 μg; chloramphenicol 30 μg; florphenicol 30 μg; streptomycin 10 μg; tetracycline 30 μg; cephalothin 30 μg; cefotaxime 30 μg; neomycin 30 μg; suphamethoxazole/trimethoprim 25 μg; gentamicin 10 μg; ciprofloxacin 5 μg; purchased from Oxoid, UK. Disc diffusion was performed using the Clinical and Labratory Standards Institute (CSLI) document M100-S23 procedure for performing the disk diffusion tests (CLSI standard, 2012). The diameter of the zones of inhibition were measured using a ruler to the nearest mm, and results were then compared to published data for zone interpretation in *Enterobacteriaceae* from the CLIS document M100-S23, and additional information from the

Table 1
List of virulence genes tested during this study using PCR.

Gene	Function of gene	Forward primer (F) (5′-3′) and reverse primer (R) (5′-3′)	Annealing temperature (°C)	Product size (bp)	Reference
prgH	Invasion of macrophages	F-GCCCGAGCAGCCTGAGAAGTTAGAAA R-TGAAATGAGCGCCCCTTGAGCCAGTC	55	755	Hughes et al., 2008
sopB	Invasion of macrophages	F-GAAGACTACCAGGCGCACTT R-TTGTGGATGTCCACGGTGAG	55	804	Gole et al., 2013
Spi C	Survival in macrophages	F-CCTGGATAATGACTATTGAT R-AGTTTATGGTGATTGCGTAT	56	300	Hughes et al., 2008
Orf L	Survival in macrophages/ colonisation	F-GGAGTATCGATAAAGATGTT R-GCGCGTAACGTCAGAATCAA	56	331	Hughes et al., 2008
PefA	Movement across host cell	F-GCGCCGCTCAGCCGAACCAG R-CAGCAGAAGCCCAGGAAACAGTG	58	154	Hughes et al., 2008
spvC	Virulence plasmid	F-TCTCTGCATTTCGCCACCAT R-TGCACAACCAAATGCGGAAG	58	563	Chen et al., 2005