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Short Communication

Recovery of *Salmonella enterica* from Australian Layer and Processing Environments Following Outbreaks Linked to Eggs

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Introduction

Salmonella enterica are a significant cause of foodborne disease worldwide, with eggs being the most important food vehicles for infection (Threlfall *et al.*, 2014). In Australia, the most common serovar infecting humans is Typhimurium (*Salmonella* Typhimurium) with thousands of cases resulting from numerous outbreaks linked to eggs (Moffatt *et al.*, 2016). To highlight *Salmonella* Typhimurium's capacity to navigate the farm to fork continuum we undertook an examination of the farm-derived microbiological and trace back evidence of *Salmonella* spp. gathered in response to outbreaks linked to eggs.

Materials and Methods

We reviewed investigation findings for Australian *S. enterica* outbreaks linked to eggs between 2001 and 2011, with our methods detailed elsewhere (Moffatt *et al.*, 2016). Trace back data relating to outbreaks were obtained from records and correspondence with food safety regulators and investigators from state and territory primary industry departments (Moffatt *et al.*, 2016). This included detail on the egg type and quality, extent of trace back, production systems, farm inspections, and *Salmonella* serovars recovered during sampling.

Results

Trace back and housing systems

Commercially produced eggs were implicated in 159 (96%) of 166 outbreaks with the remainder involving backyard (noncommercial) eggs. For outbreaks involving commercial production, housing system detail was only available for 70 (44%) events. Of these 36 (52%) were cage, 33 (47%) free range, and 1 (1%) barn laid. Trace back was conducted for 106 (64%) outbreaks, identifying a specific farm on 72 (68%) occasions. From the available data, no difference between the proportion of outbreaks linked to eggs produced in cage and free range housing systems was found.

Environmental sampling and egg testing

Of the 72 farms identified, 63 (88%) underwent environmental sampling and egg testing. *Salmonella* spp. were detected on 41 (63%) farms via environmental testing (including a *Salmonella* Enteritidis positive quality assurance isolate), with 30 of 41 (73%) farms having the outbreak strain. Among 49 farms where whole eggs were sampled, 16 (33%) had *Salmonella* spp. isolated, with 12 of 16 (75%) farms yielding the outbreak strain. While specific sampling details for on-farm investigations were incomplete, available data showed drag swabbing resulted more frequently in *Salmonella spp.* being isolated (40 farms), followed by fecal sampling (25 farms), equipment swabbing (22 farms), and feed sampling (21 farms).

Salmonella *serovars*

The Table 1 shows the recovery of *Salmonella* spp. from farms and processing environments following outbreak investigations. *Salmonella* Typhimurium was recovered from environmental samples and eggs sampled in response to 30

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TABLE 1. *SALMONELLA* SPP. RECOVERED FROM OUTBREAK-ASSOCIATED FARM AND LAYER ENVIRONMENTS

Year	*Outbreak agent*	*Environmental sampling results*	*Egg sampling results*
2001	***Salmonella* Typhimurium PT 135**	***Salmonella* Typhimurium PT 135**	Not sampled
2002	*Salmonella* Potsdam	*Salmonella* Agona, *Salmonella* Broughton, *Salmonella* Infantis	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 135a**	***Salmonella* Typhimurium PT 135a**, *Salmonella* subspecies I	*Salmonella* spp. not detected
2003	*Salmonella* Typhimurium PT 135a	*Salmonella* Infantis, *Salmonella* Mbandaka, *Salmonella* Singapore	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 135	*Salmonella* spp. not detected	*Salmonella* spp. not detected
2004	*Salmonella* Typhimurium PT 126	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 135a	*Salmonella* Mbandaka	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 9	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 126**	***Salmonella* Typhimurium PT 126**	*Salmonella* spp. not detected
2005	***Salmonella* Typhimurium PT 9**	***Salmonella* Typhimurium PT 9**	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 9**	*Salmonella* spp. not detected	***Salmonella* Typhimurium PT 9**
	***Salmonella* Typhimurium PT 135a**	***Salmonella* Typhimurium PT 135a**	***Salmonella* Typhimurium PT 135a**
	***Salmonella* Enteritidis PT 26**	***Salmonella* Enteritidis PT 26** (from quality assurance sampling)	Not sampled
	***Salmonella* Typhimurium PT 9**	*Salmonella* spp. not detected	***Salmonella* Typhimurium 9**
	Salmonella Typhimurium PT 44	*Salmonella* Infantis, *Salmonella* Livingstone, *Salmonella* Orion var15, *Salmonella* Orion var34, *Salmonella* Worthington	Not sampled
	Salmonella Typhimurium PT 135a	*Salmonella* spp. not detected	Not sampled
	Salmonella Typhimurium PT 9	*Salmonella* spp. not detected	Not sampled
2006	***Salmonella* Typhimurium PT 170**	***Salmonella* Typhimurium PT 170**, *Salmonella* subspecies I ser 16:I,v:-	*Salmonella* spp. not detected
	Salmonella Montevideo	*Salmonella* Oranienburg, *Salmonella* Singapore	Not sampled
	***Salmonella* Typhimurium PT 197**	***Salmonella* Typhimurium PT 197**, *Salmonella* Singapore, *Salmonella* Zanzibar	***Salmonella* Typhimurium PT 197**
	***Salmonella* Livingstone**	***Salmonella* Livingstone**, *Salmonella* Cerro	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 170**	***Salmonella* Typhimurium PT 170**	***Salmonella* Typhimurium PT 170**
	Salmonella Typhimurium PT 170	*Salmonella* Agona, *Salmonella* Havana, *Salmonella* Ohio, *Salmonella* Seftenberg	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 170**	***Salmonella* Typhimurium PT 170**	***Salmonella* Typhimurium PT 170**
2007	***Salmonella* Typhimurium PT 197**	***Salmonella* Typhimurium PT 197**, *Salmonella* Singapore, *Salmonella* Zanzibar	***Salmonella* Typhimurium PT 197**
	***Salmonella* Typhimurium PT 197**	***Salmonella* Typhimurium PT 197**, *Salmonella* Singapore, *Salmonella* Zanzibar	***Salmonella* Typhimurium PT 197**
	***Salmonella* Typhimurium PT 197**	***Salmonella* Typhimurium PT 197**, *Salmonella* Singapore, *Salmonella* Zanzibar	***Salmonella* Typhimurium PT 197**
	***Salmonella* Typhimurium PT 44**	***Salmonella* Typhimurium PT 44**	***Salmonella* Typhimurium PT 44**, *Salmonella* Typhimurium PT 29
	Salmonella Typhimurium PT 44	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 197**	***Salmonella* Typhimurium PT 197**, *Salmonella* Singapore, *Salmonella* Zanzibar	***Salmonella* Typhimurium PT 197**
	***Salmonella* Typhimurium PT 29**	***Salmonella* Typhimurium PT 29**	***Salmonella* Typhimurium PT 29**, *Salmonella* Typhimurium PT 44

(*continued*)

Table 1. (Continued)

Year	Outbreak agent	Environmental sampling results	Egg sampling results
	Salmonella Typhimurium PT 9	*Salmonella* Typhimurium (reportedly not matching outbreak agent)	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 135a	Not sampled	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 9**	***Salmonella* Typhimurium PT 9**	*Salmonella* spp. not detected
2008	***Salmonella* Typhimurium PT 126**	***Salmonella* Typhimurium PT 126** (MLVA 03-17-16-13-523)	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 170	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 44	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 170	*Salmonella* Infantis	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 126**	***Salmonella* Typhimurium PT 126** (MLVA 03-17-16-13-523)	Not sampled
	Salmonella Typhimurium PT 170	*Salmonella* Anatum, *Salmonella* Infantis, *Salmonella* Montevideo, *Salmonella* Orion, *Salmonella* Singapore	*Salmonella* Singapore
	***Salmonella* Typhimurium PT 126**	***Salmonella* Typhimurium PT 126** (MLVA 03-17-16-13-523)	Not sampled
2009	*Salmonella* Typhimurium PT 170	*Salmonella* spp. not detected	*Salmonella* spp. not detected
2009	*Salmonella* Typhimurium PT 170	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 170	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	***Salmonella* Singapore**	***Salmonella* Singapore**, *Salmonella* Havana, *Salmonella* Montevideo, *Salmonella* Orion	*Salmonella* Orion, *Salmonella* Montevideo
	Salmonella Typhimurium PT 170	*Salmonella* Agona, *Salmonella* Infantis	*Salmonella* spp. not detected
2010	*Salmonella* Typhimurium PT 170	*Salmonella* spp. not detected	Not sampled
	***Salmonella* Typhimurium PT 89**	***Salmonella* Typhimurium PT 89**, *Salmonella* Infantis, *Salmonella* Montevideo, *Salmonella* Orion, *Salmonella* subspecies I	Not sampled
	Salmonella Typhimurium PT 170	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 9	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	***Salmonella* Singapore**	***Salmonella* Singapore**, *Salmonella* Havana, *Salmonella* Montevideo, *Salmonella* Orion	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 135a**	***Salmonella* Typhimurium PT 135a**, *Salmonella* Anatum, *Salmonella* Mbandaka, *Salmonella* Montevideo, *Salmonella* Tennessee, *Salmonella* subspecies I, *Salmonella* Typhimurium PT 135	***Salmonella* Typhimurium PT 135a**, *Salmonella* Anatum, *Salmonella* Mbandaka, *Salmonella* Montevideo, *Salmonella* Tennessee, *Salmonella* subspecies I
	Salmonella Typhimurium PT 9	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 9	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 9	*Salmonella* spp. not detected	*Salmonella* spp. not detected
2011	***Salmonella* Typhimurium PT 9**	***Salmonella* Typhimurium PT 9** (MLVA 03-24-11-11-523)	Not sampled
	Salmonella Typhimurium PT 170	*Salmonella* spp. not detected	*Salmonella* spp. not detected
	Salmonella Typhimurium PT 170	*Salmonella* Typhimurium PT 170 (different MLVA to outbreak agent), *S*, Havana, *Salmonella* Infantis, *Salmonella* Saintpaul	Not sampled
	Salmonella Typhimurium PT 9	*Salmonella* spp. not detected	Not sampled
	***Salmonella* Typhimurium PT 9**	***Salmonella* Typhimurium PT 9**	Not sampled
	***Salmonella* Typhimurium PT 170**	***Salmonella* Typhimurium PT 170** (MLVA 03-09-07-14-523)	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 170**	***Salmonella* Typhimurium PT 170** (MLVA 03-09-07-13-523)	*Salmonella* spp. not detected
	***Salmonella* Typhimurium PT 135a**	***Salmonella* Typhimurium PT 135a**, *Salmonella* Anatum, *Salmonella* Kottbus, *Salmonella* Montevideo	*Salmonella* Anatum, *Salmonella* Montevideo

Bold parts indicate a match between the outbreak agent and *Salmonella* spp recovered via environmental sampling or from sampled eggs.
MLVA, multiple-locus variable number tandem repeats analysis.

outbreaks, including 28 (93%) where the outbreak case isolates and farm-derived isolates matched. Non-Typhimurium serovars, including *Salmonella* Infantis, *Salmonella* Montevideo, and *Salmonella* Orion, were recovered on multiple occasions. However, on only four occasions did a farm-derived non-Typhimurium serovar match an outbreak strain, including *Salmonella* Singapore (twice), *Salmonella* Enteritidis, and *Salmonella* Livingstone.

Discussion

Our report shows a high detection rate for outbreak associated *Salmonella* spp., particularly *Salmonella* Typhimurium, following microbiological investigation of layer farms and processing environments in Australia. The frequency of detection of this serovar over the study period highlights ongoing issues with environmental contamination and layer colonization, carrying significant implications for food safety in Australia.

Recovery of multiple *Salmonella* serovars from layer environments is not unusual. A state-wide survey of Australian farms revealed nearly half were positive for *Salmonella* spp. with 20% having *Salmonella* Typhimurium detected (New South Wales Food Authority [NSWFA], 2013). Prevalence between and within layer flocks also varies with *Salmonella* Typhimurium being shed for up to 15 weeks postinfection (Pande *et al.*, 2016). Our data also show high detection rates for *Salmonella* spp., particularly *Salmonella* Typhimurium, reflecting the targeted nature of outbreak testing. *Salmonella* Typhimurium was not isolated from all investigated farms, which may reflect intermittent shedding of *Salmonella* Typhimurium (Pande *et al.*, 2016) or differences in sampling strategies employed for outbreak events and between states.

In the European Union (EU), characteristics are assessed to aid in determining whether a *Salmonella* serovar is of public health significance. These include its frequency in causing human salmonellosis, its prevalence in an animal population, its ability to spread rapidly, and evidence of its increased virulence (European Commission [EC], 2003). Australian public health data have shown significant increases in *Salmonella* Typhimurium infections and outbreaks, and this serovar is responsible for the majority of human outbreak cases, with eggs the most important source of infection (Moffatt *et al.*, 2016). Furthermore, the prevalence in the Australian population of serovars other than *Salmonella* Typhimurium is low, while the pathogenicity and virulence of *Salmonella* Typhimurium relative to these other serovars are demonstrated (McWhorter *et al.*, 2015).

Layer farm contamination with *Salmonella* Typhimurium is a significant problem, with good agreement between the levels of environmental contamination, egg shell contamination, and human disease (Wales and Davies, 2011). From our data, of the 16 outbreaks where *S. enterica* was isolated from farm-sampled eggs, 14 (88%) had the same serovar(s) isolated from environmental samples. The presence of eggshell contamination without contamination of the internal contents, together with a low frequency of reproductive tract infection, suggests horizontal infection via feces is the major route for contamination with *Salmonella* Typhimurium (Pande *et al.*, 2016).

In Australia most commercially produced eggs are washed to reduce microbial contamination (Gole *et al.*, 2014). Considerable debate exists around egg washing with suggestions it may facilitate shell penetration by *Salmonella* Typhimurium (Gole *et al.*, 2014), which may offer insight into its capacity for evading postcollection controls and causing outbreaks.

Salmonella contamination of flocks and shell eggs is multifactorial (Denagamage *et al.*, 2015) with thorough outbreak investigation essential to understanding egg-associated disease and guiding mitigation strategies. Our effort to collate trace back findings in a uniform manner by retrospectively compiling investigative data from multiple sources outside of health departments was challenging. Data collection was not standardized across jurisdictions, with a lack of detail on sampling methods and the types of production systems implicated in outbreaks being notable limitations. However since 2011, guidelines for trace back investigation for egg-associated outbreaks have been developed (Biosecurity South Australia and New South Wales Food Authority [NSWFA], 2013), and we support their adoption and routine use. These should include the collection of a core data set to be shared with public health investigators. Notwithstanding, our evidence continues to highlight the need for a national regulatory response and review into the issue of *Salmonella* Typhimurium and eggs in Australia, with policy makers and stakeholders needing to consider international approaches in addressing egg-associated salmonellosis.

Conclusion

Egg-associated salmonellosis is an important public health issue in Australia, with the detection of *Salmonella* Typhimurium within layer environments and flocks representing a risk to human health. Reducing this risk will require ongoing and high level engagement between the human health sector, regulators, and industry.

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