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Research paper

Longitudinal study of *Salmonella* 1,4,[5],12:i:- shedding in five Australian pig herds

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ABSTRACT

The shedding patterns of *Salmonella* spp. and MLVA profiles of *Salmonella enterica* subspecies *enterica* (I) serotype 1,4,[5],12:i:- were monitored in a 12-month longitudinal observational study of five pig herds to inform management; provide indications of potential hazard load at slaughter; and assist evaluation of MLVA for use by animal and public health practitioners. Twenty pooled faecal samples, stratified by age group, were collected quarterly. When *Salmonella* was cultured, multiple colonies were characterized by serotyping and where *S.* Typhimurium-like serovars were confirmed, isolates were further characterized by phage typing and multiple locus variable number tandem repeat analysis (MLVA). *Salmonella* was detected in 43% of samples. *Salmonella* 1,4,[5],12:i- was one of several serovars that persisted within the herds and was found among colonies from each production stage. Virtually all *Salmonella* 1,4,[5],12:i:- isolates were phage type 193, but exhibited 12 different, closely-related MLVA profiles. *Salmonella* 1,4,[5],12:i:- diversity within herds was low and MLVA profiles were stable indicating colonization throughout the herds and suggesting each farm had an endemic strain. High prevalence of *S.* 1,4,[5],12:i:- specific shedding among terminal animals indicated high hazard load at slaughter, suggesting that primary production may be an important pathway of *S.* 1,4,[5],12:i:- into the human food chain, this has implications for on-farm management and the application and targeting control measures and further evidence of the need for effective process control procedures to be in place during slaughter and in pork boning rooms. These findings have implications for animal health and food safety risk mitigation and risk management.

1. Introduction

Non-typhoidal salmonellosis in humans and animals represents a substantial global public health burden. *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S.* Typhimurium) is a serovar commonly isolated from livestock and poses a risk of zoonotic disease for humans. Monophasic variants of *S.* Typhimurium with the serotype 1,4,[5],12:i:- have emerged in US, Europe, Latin America and Asia since the mid-1990s (Moreno Switt et al., 2009; Hauser et al., 2010). This serotype has risen to international prominence due to increasing isolation and implication in human disease (Mossong et al., 2007; CDC, 2011; Gossner et al., 2012; Nguyen, 2013). Pigs have been identified as a major reservoir of *S.* 1,4,[5],12:i:- in Europe (Hauser et al., 2010; Hopkins et al., 2010).

The most common serotype that causes human salmonellosis in Australia is *S.* Typhimurium. serotype *S.* 1,4,[5],12:i:-Phage Type (PT) 193 appears to have emerged only recently and to date has been implicated in few outbreaks (OzFoodNet, 2012, 2013, 2015). However, Australian pigs have low rates of *Salmonella* infection and *S.* 1,4,[5],12:i:- has only recently been identified in an Australian pig herd (Hamilton et al., 2015). Hamilton et al. (2015) found that their study herd harboured a persistent, single serotype, 341/353 isolates serotyped were *S.* 1,4,[5],12:i:- PT193 with 12 exceptions, 11 non-motile *S.* Typhimurium (*S.* 1,4,[5],12:-:-) isolates and a single *S.* rough:i:- isolate. Although 13 different multiple locus variable number tandem repeat (VNTR) analysis (MLVA) pro-

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files were detected, only two MLVA profiles (04-15-11-00-490 and 04-16-11-00-490) persisted throughout the study.

In this longitudinal study, we aimed to further investigate the occurrence and possible persistence of *S.* 1,4,[5],12:i:- in five Australian farrow-to-finish herds with suspected *S.* 1,4,[5],12:i:- colonization. No live animal movement had occurred between the herds prior to or during the study. The objectives of the study were to: monitor rates of detection of *Salmonella* and *S.* 1,4,[5],12:i:- shedding within herds and between production stages in these herds, indicative of extent and persistence of colonization; describe *Salmonella* serotype populations shed; and monitor *S.* 1,4,[5],12:i:- MLVA profiles over an extended period.

2. Material and methods

2.1. Study design and sampling

A prospective longitudinal design was used to investigate *Salmonella* shedding in five farrow-to-finish pig herds located in two southern states of Australia, operating in a Mediterranean-like climate. Samples were collected at three-month intervals in 2014 and 2015. *Salmonella* 1,4,[5],12:i:- had been detected in each herd on at least one occasion prior to the first sampling of this study.

2.1.1. Herd selection

Herds were selected to provide comparable case studies of Australian commercial pig herds and comprised sow herd sizes from 400 to 600 head. Three herds were multi-site production systems (Herds 1, 2. 3) and two employed single-site operations (Herds 4, 5). The herds were geographically isolated, had no live animal linkages and all market-destined weaners were produced by the herds themselves. Herds 1 and 2 and Herds 3, 4 and 5 shared a feed supply company, but only Herds 1 and 2 received feed from the same mill. Each herd employed their own vehicle to transport pigs to the abattoir. Each herd had a high health status (< 3% average post-wean mortality) and employed conventional enclosed sheds and deep bedding systems. The herd profiles are summarized in Table S1.

2.1.2. Sampling design

Herds were sampled at three-monthly intervals over one year to monitor persistence over an extended time period. Herds were stratified by age group. On each sampling occasion, five pooled faecal samples (6 pats per sample) were each collected from gestating sows, lactating sows and litters, weaners and finisher stock. Dry sows were defined as gestating sows—empty sows and sows in-pig prior to move to the farrowing house; weaners were 3–4 weeks to 10 weeks old; finishers were 15 weeks to finish (22–24 weeks); samples from farrowing crates included faeces from lactating sows and suckling piglets. The collection of 20 pooled samples per herd sampling occasion, representing approximately six pigs per sample (n = 120 in total) and was designed to maximize the likelihood of *Salmonella* detection, providing 95% confidence of detecting *Salmonella* or *S.* 1,4,[5],12:i:- in at least one sample if herd shedding prevalence was above approximately 8%, respectively, assuming perfect test sensitivity (Cannon and Roe, 1982; Humphry et al., 2004). Likewise, collection of five pooled samples per production stage per sampling occasion (n = 30) provided 95% confidence of detecting at least one positive per sampling event per production stage at a minimum production stage shedding prevalence of approximately 10%; assuming perfect test sensitivity, or approximately 25% per sampling occasion with test sensitivity of 69% (Funk et al., 2000).

2.1.3. Sampling methods

Portions of six undisturbed faecal pats, each weighing approximately 5 g, were sampled from a single pen floor and aggregated (pooled) in a sterile 120 ml pot. The samples were de-identified and stored in iceboxes at approximately 4 °C. If a herd employed less than five pens to house a single production stage at the time of sampling (i.e. employing large pens housing high numbers of animals, at least 200 head) a second pooled sample was collected from a distant location in the pen housing the largest population to ensure five pooled samples were collected per sampling occasion. Sow crates were treated as a single unit, contributions from both sow and suckling pigs were collected from six non-sequential crates. Farrowing shed sampling was designed to maximize the likelihood of detection and representation of the *Salmonella* population diversity present as a potentially important linkage, and point of transmission, between breeder and finisher animals. A *Salmonella* or *S.* 1,4,[5],12:i:- detection from a sample equated to at least one pig in the sampled pen shedding *Salmonella* or *S.* 1,4,[5],12:i:-, with the exception of farrowing shed samples in which case at least one crate could be deemed positive.

2.1.4. Microbiological analysis

The samples were chilled and transported to the laboratory within four days of sample collection and refrigerated upon arrival. *Salmonella* were isolated and characterized as previously described (Hamilton et al., 2015) with the exception that if the first *Salmonella* colony from each sample was found to conform with *S.* 1,4,[5],12:i:- the isolate was further characterized, however, if it was not *S.* 1,4,[5],12:i:- the four to nine remaining colonies were partially serotyped. If the partially serotyped isolates indicated *S.* 1,4,[5],12:i:- or another serotype that differed from the first isolate a representative was fully serotyped. All isolates confirmed to be *S.* 1,4,[5],12:i:- (n = 73) or *S.* Typhimurium (n = 1) were phage typed (Anderson et al., 1977; Rabsch, 2007) and were tested for antimicrobial susceptibility (Clinical Laboratory Standards Institute, 2011). MLVA analysis was performed as described previously (Lindstedt et al. (2004), Larsson et al. (2009)) and analysed using GeneMapper software (Applied Biosystems) MLVA profiles are presented in the Australian nomenclature (Gilbert, 2008).

2.2. Statistical analysis

Data were collated in Excel (Microsoft Excel, 2011, Microsoft Corporation, Redmond, WA, USA). Data exploration and statistical analyses were conducted in R (R Core Team, 2016).

Estimation of *Salmonella* serovars and *S.* 1,4,[5],12:i:- MLVA profile diversity was conducted by aggregating sampling and calculating Shannon (H) and inverse Simpson (D) diversity indices (Hurlbert, 1971; Kim et al., 2011). A geometric laboratory testing protocol was proved to be equivalent, in terms of serovar representativeness, to a binomial testing protocol, confirmed by simulation (J. Tuke, personal communication).

3. Results

3.1. Descriptive results and univariable analysis

Salmonella was detected in 171/400 pooled samples, which equates to approximately 224 samples in which *Salmonella* was present assuming test sensitivity of 69% (Funk et al., 2000). In total, 181 isolates were fully serotyped and 18 serovars were identified. Three hundred and seventy-five isolates were partially serotyped and found not to be *S.* 1,4,[5],12:i:-. *S.* 1,4,[5],12:i:- was detected in 95/400 samples. All *S.* 1,4,[5],12:i:- isolates were PT193, with the exception of one PT6 isolate and five isolates that did not react with the phages and were deemed PT untypable. A single *S.*

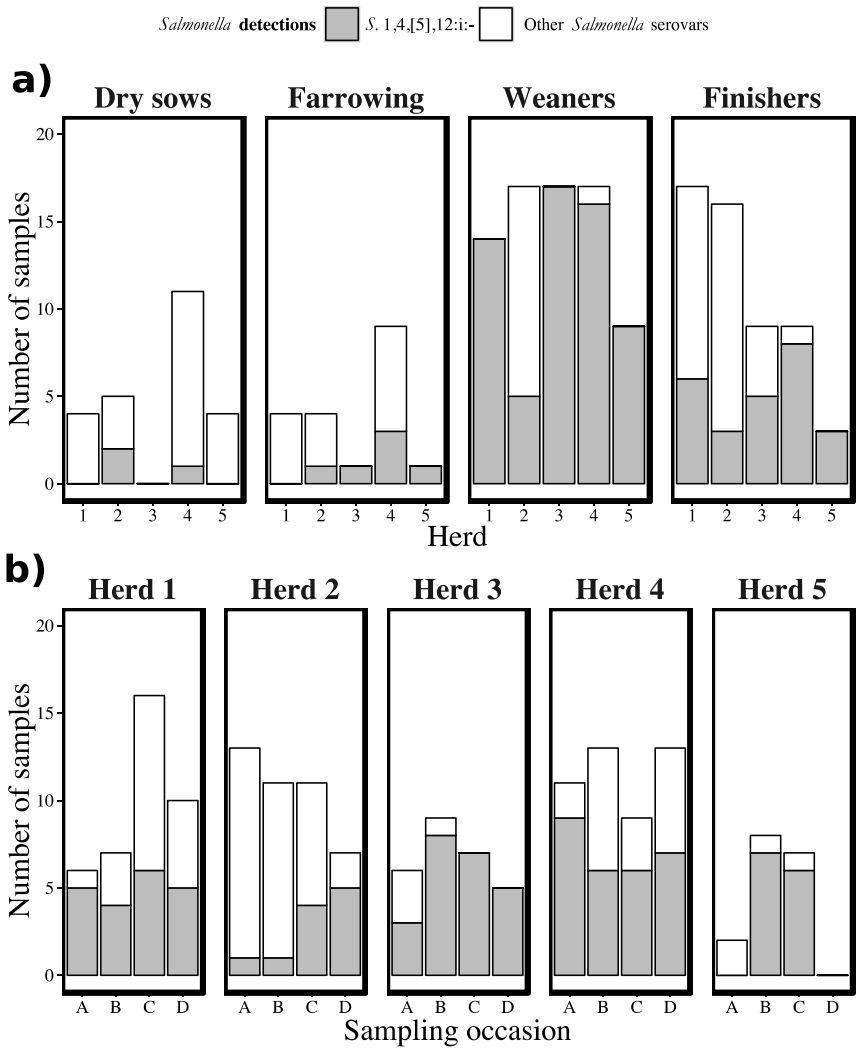

Fig. 1. Number of pools (pooled samples, each consisting of contributions from six individual faecal pats) in which S. 1,4,[5],12:i:- and other *Salmonella* serovars were detected by: a) production stage (facet) and Herd 1–5 (x-axis); and b) herd (facet) and sampling occasion A-D (x-axis). Total 80 pools per herd; 20 pools per production stage/sampling occasion per herd.

Typhimurium isolate was identified from Herd 1 samples and was also PT untypable.

3.2. *Salmonella* spp. and *S.* 1,4,[5],12:i:- detections by herd, production stage and sampling occasion

The number of *Salmonella* detections in each herd ranged from 17/80 in Herd 5 and 46/80 in Herd 4, while *S.* 1,4,[5],12:i:- detections ranged from 11/80 in Herd 2 to 28/80 in Herd 4 (Table S2). *Salmonella* 1,4,[5],12:i:- was detected in a very high proportion of *Salmonella* positive samples were from weaner and finisher pigs, 84.3% (86/102), but a much lower proportion of *Salmonella* positive sow and farrowing shed samples, 20.9% (9/43) (Fig. 2).

Salmonella detections from farrowing crate samples ranged from 1/20 in Herd 3 to 9/20 in Herd 4; *S.* 1,4,[5],12:i:- detections from none in Herd 1 to three among Herd 4 samples. Detection rates were considerably higher among weaner and finisher stock, particularly weaners, when compared to sows and farrowing crates (Fig. 1a). Herd weaner detections ranged from 9/20 to 17/20 for *Salmonella* and 5/20 to 17/20 for *S.* 1,4,[5],12:i:- shedding (Table S2). Shedding rates appeared to be lower among finishers, from 3/20 to 17/20 for *Salmonella* and 3/20 to 8/20 for *S.* 1,4,[5],12:i:-. Gestating sows shedding detections were: 0/20 to 11/20 for *Salmonella* and 0 to 2/20 for *S.* 1,4,[5],12:i:-.

Salmonella and *S.* 1,4,[5],12:i:- shedding persisted in four of the five herds (Fig. 1b). In two herds, Herds 2 and 3, the proportion of *S.* 1,4,[5],12:i:- detections relative to other serovars increased over time. *Salmonella* detections by sampling occasion in Herd 1 ranged from 6/20-16/20, Herd 2: 7/20-13/20, Herd 3: 5/20-9/20, Herd 4: 9/20-13/20, and Herd 5: 0/20-8/20 (Table S3). Detection of *S.* 1,4,[5],12:i:- shedding for Herd 1 ranged from 4/20-6/20, Herd 2: 1/20-5/20, Herd 3: 3/20-8/20, Herd 4: 6/20-9/20, and Herd 5: 0/20-7/20. A possible seasonal or climate-related effect was observed—relatively higher numbers of detections on cooler, wetter sampling occasions—however, results of mixed effects modeling were not considered robust due to the low herd numbers and a one year sampling period (data not presented).

The detection of *Salmonella* and *S.* 1,4,[5],12:i:- are provided by herd and production stage and herd and sampling occasion in supplementary Tables S2 and S3, respectively.

3.3. *Salmonella* serovar distribution

Relatively diverse *Salmonella* populations were present in each of the five herds with 4–8 serovars circulating on each farm. At

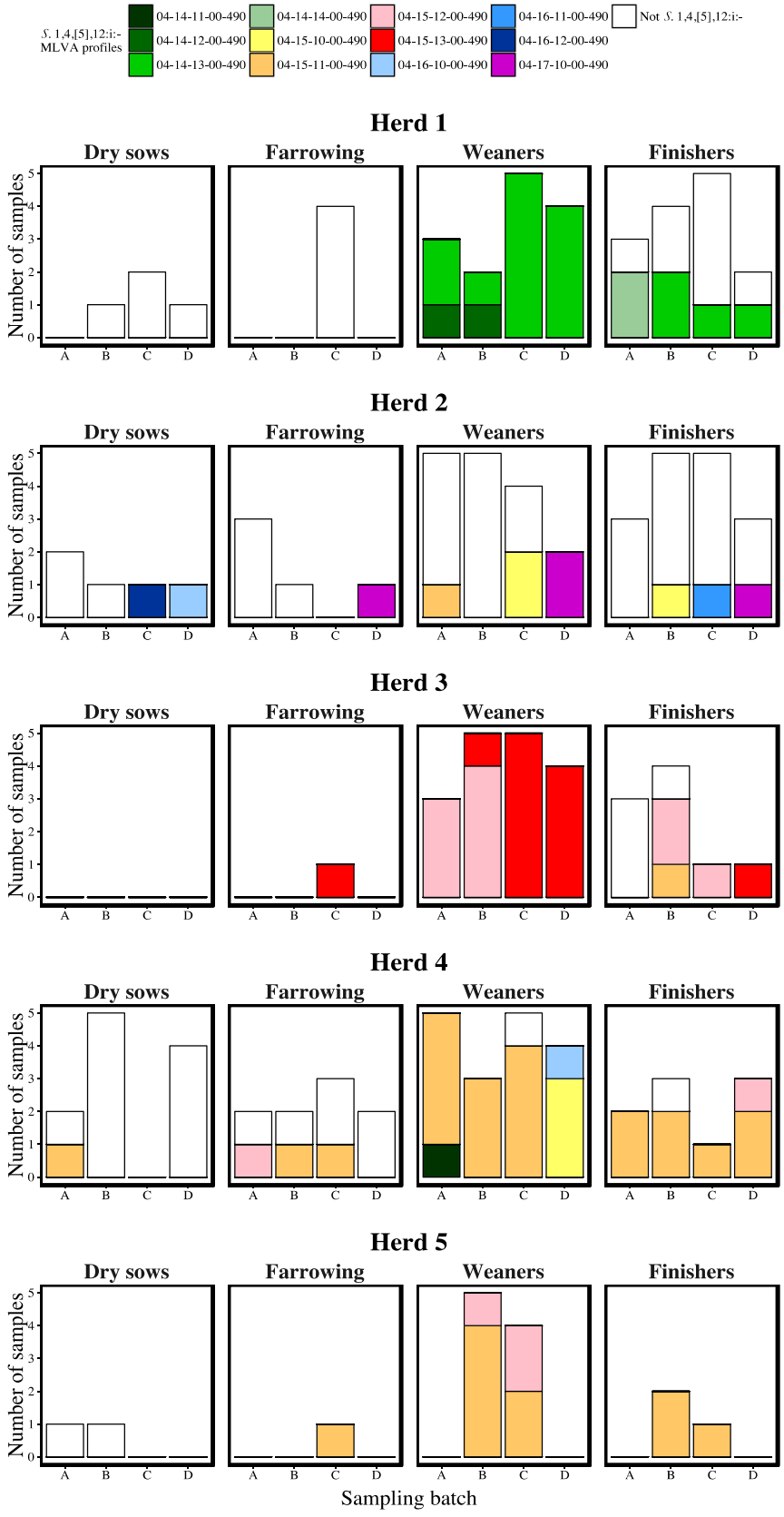

Fig. 2. S. 1,4,[5],12:i:- MLVA profiles by production stage and sampling occasion. Rows show results by herd, columns by production stage and sampling occasion (x-axis). The bar fill identifies the MLVA profiles per S.1,4,[5],12:i:- positive sample; white fill indicates samples in which a *Salmonella* serovar that was not S. 1,4,[5],12:i:- was detected. MLVA types are colour coded.

Table 1
Salmonella serovar detection frequency and estimated diversity by herd.

Serovar	Herd					Total
S. 1,4,[5],12:i:-	1	2	3	4	5	
	20	11	23	30	13	97
S. Adelaide	5			8		13
S. Agona			1			1
S. Anatum[a]					2	2
S. Bovismorbificans	1	3		2		6
S. Give	1					1
S. Havana					1	1
S. Hofit		1				1
S. London				10		10
S. Mbandaka	1	1	1			3
S. Ohio[b]		10			1	11
S. Oranienburg			2			2
S. Rissen		22				22
S. Typhimurium	1					1
S. Worthington	12	2				14
S. I rough:z_{10}:e,n,x	1					1
Number of serovars	8	7	4	4	5	18
Shannon (H) index	1.49	1.60	0.84	1.24	1.15	1.76
Simpson (D) index	3.22	3.91	1.65	2.69	2.42	3.32

[a] Includes an *S.* Anatum var 15+ isolate.
[b] Includes *S.* Ohio var 14+.

least one *S.* 1,4,[5],12:i:- colony was identified in 55.6% (95/171) *Salmonella* positive samples (Table 1).

3.4. *Salmonella* 1,4,[5],12:i:- MLVA profile distribution

Twelve MLVA profiles were identified among the study *S.* 1,4,[5],12:i:- isolates. *Salmonella* 1,4,[5],12:i:- MLVA VNTR copy number variation was found at loci STTR5 and STTR6 only; all study isolates were identified by VNTR copy numbers 04, 00 and 490 at loci STTR9, STTR10 and STTR3, respectively. The most frequently isolated *S.* 1,4,[5],12:i:- MLVA profiles for STTR5 and STTR6 were (in parentheses: number of herds; percentage of study isolates): 15-11 (4; 35.6%), 14-13 (1; 16.7%) and 15-12 (3; 14.4%). The results of *S.* 1,4,[5],12:i:- MLVA typing by herd are summarized in Table 2. The single *S.* Typhimurium isolate from Herd 1 was identified by MLVA profile 04-14-13-00-490. The only PT6 *S.* 1,4,[5],12:i:- isolated from a Herd 2 sample was identified by MLVA profile 04-17-10-00-490.

3.5. Herd *Salmonella* spp. and *Salmonella* 1,4,[5],12:i:- ecology

3.5.1. Herd 1

The herd was managed as a multi-site operation employing isolated breeder and grow-out sites; sow numbers remained stable throughout the study. Low level scouring was observed among weaners during sampling, however, no outbreak of clinical salmonellosis occurred during the study period. *Salmonella* detections varied considerably between sampling occasions, however, *S.* 1,4,[5],12:i:- detections were consistent, ranging from four to six detections per sampling occasion (Fig. 1b). The single *S.* Typhimurium colony isolated in the study was from Herd 1 and had an MLVA profile identified among *S.* 1,4,[5],12:i:- PT193 isolates from Herd 1, 04-14-13-00-490; the isolate was PT untypable.

3.5.2. Herd 2

The herd was a multi-site operation under single ownership and management employing a grow-out site and, initially (sampling occasions A, B and C), two farrowing sites; the sow herd was moved to a single new farrowing site between sampling occasions C and D. The sow herd was expanding throughout the study period, through increased import of gilts and self-replacement. Prior to the first sampling, the herd suffered a laboratory- confirmed outbreak of salmonellosis, which the consulting veterinarian associated with a porcine circovirus type 2 outbreak, affecting approximately 80 pigs in a single cohort. The herd suffered a localised mouse plague at the time of the second sampling occasion (B) followed by a major outbreak of leptospirosis, affecting approximately 50% of the sow herd, between sampling occasions C and D. Biosecurity and cleaning and disinfection standards were high initially and stringency was noticeably increased in response to the leptospirosis outbreak. A decreasing trend in *Salmonella* detections was observed over time, however, *S.* 1,4,[5],12:i:- detections increased as the study progressed (Table S2, Fig. 1b). All *S.* 1,4,[5],12:i:- isolates tested were PT 193 except two untypable isolates and one PT6 isolate.

3.5.3. Herd 3

The herd consisted of two independently owned and managed sites: a farrow to wean enterprise and a grow-out enterprise. Sow numbers remained stable throughout the study. A severe outbreak of confirmed salmonellosis in weaners, in which approximately 100 pigs died, occurred at the breeder site three months prior to the first sampling occassion. No major health problems were observed or reported at the breeder site during the study; hygiene levels were low at the grow-out site and ongoing respiratory issues were observed and reported by the producer. A change in management at the breeder site occurred between sampling occasions B and C and hygiene standards were noticeably improved. *Salmonella* 1,4,[5],12:i:- persisted among weaners and was identified among finisher isolates in sampling occasions B to D.

3.5.4. Herd 4

The herd was a single farrow to finish production site, and sow numbers remained stable throughout the study. Some scouring was observed among weaners during sampling visits, but no other health problems were noted. Hygiene was variable; all weaner-grower cohorts came into close contact in a low hygiene shed from 8 to 12 weeks of age, furthermore, there was an ongoing rodent problem throughout the study period. The herd experienced a major outbreak of laboratory confirmed salmonellosis approximately 12 months prior to the study. *Salmonella* 1,4,[5],12:i:- detections persisted throughout the study and all were PT193.

Table 2
Salmonella 1,4,[5],12:i:- MLVA profile detections in the five herds.

MLVA profile	Herd					Total
	1	2	3	4	5	
04–14-11-00-490				1		1
04–14-12-00-490	2					2
04–14-13-00-490	16					16
04–14–14-00-490	2					2
04–15-10-00-490		3		4		7
04–15-11-00-490		1	1	22	10	33
04–15-12-00-490			10	2	3	15
04–15-13-00-490			12			12
04–16-10-00-490		1		1		2
04–16-11-00-490		1				1
04–16-12-00-490		1				1
04–17-10-00-490		4				4
Number of MLVA profiles	3	6	3	5	2	12
Shannon (H) index	0.80	1.78	1.10	1.16	0.98	1.96
Simpson (D) index	1.66	5.12	2.66	2.21	2.42	5.24

3.5.5. Herd 5

The herd was managed as a single farrow to finish site, sow numbers remained stable during the study period. Limited scouring was observed among weaners, there were no notable disease outbreaks during the period of observation. *Salmonella* was not detected among samples from sample occasion D and *S.* 1,4,[5],12:i:- was not detected on sampling occasions A nor D. All *S.* 1,4,[5],12:i:- were PT193 with one exception, a single untypable isolate from sampling occasion B.

4. Discussion

Many studies have investigated *Salmonella* shedding in pig herds (Funk et al., 2001; Raji· et al., 2005; Pires et al., 2013, 2014). *Salmonella* 1,4,[5],12:i:- emergence, distribution and persistence within pig production systems have also been explored in Europe (Davies et al., 2011; Niemann et al., 2015). This exploratory study sought to monitor and compare the persistence and characteristics of emergent *S.* 1,4,[5],12:i:- and contemporary *Salmonella* serovars over time in five independent farrow-to-finish herds operating in the context of the Australian pig industry.

Multiple *Salmonella* serovars were detected in each of the study herds, as has commonly been reported elsewhere (Funk et al., 2001; Gebreyes et al., 2004; Raji· et al., 2005; Farzan et al., 2008; Mueller-Doblies et al., 2013; Pires et al., 2014). The highest level of serovar diversity was observed in Herd 2 (H = 1.60). Multiple serovars were isolated on each sampling occasion in Herd 2, demonstrating that the herd harboured a persistent *Salmonella* population comprised of multiple serovars. The variation in Herd serovar diversity and the specific serovars from herds suggests that each herd's resident *Salmonella* population may have derived from a different source and/or were introduced via different pathways. *Salmonella* 1,4,[5],12:i:- PT193 was detected in all five herds and persisted for up to a year. Results show considerably higher *S.* 1,4,[5],12:i:- shedding relative to other serovars in four of five herds. This differs from Hamilton et al. (2015) findings in that each herd in this study concurrently harboured multiple *Salmonella* serovars non-*Salmonella* group B serovars, reflected in the level of diversity identified in each herd, but resembles findings elsewhere (Niemann et al., 2015). This suggests that the Hamilton et al. (2015) herd was *Salmonella*-free prior to the introduction of *S.* 1,4,[5],12:i:- and that no other serovars became established during their study. Whereas the herds in this study may have maintained a resident *Salmonella* population prior to the introduction of *S.* 1,4,[5],12:i:-. This also suggests that *Salmonella* was introduced to these study herds via a pathway, or pathways, that were either not present or were not contaminated with *Salmonella* in the Hamilton et al. (2015) study herd. Although the Hamilton et al. (2015) herd was a grow-out site, receiving weaned pigs, operating in a different location there were no obvious explanations for the differences in *Salmonella* populations detected. In each herd the rate of detection of *Salmonella* per sampling occasion fluctuated only moderately over the sampling period, with the exception of Herd 5 in which no *Salmonella* were detected on the final sampling occasion.

Variability in the level of *Salmonella* shedding between age groups within herds was observed as has been reported by other studies (Funk et al., 2001; Raji· et al., 2005; Pires et al., 2013, 2014). While higher detection of *Salmonella* shedding among weaned terminal stock have been reported (Kranker et al., 2003; Nollet et al., 2005a; Molla et al., 2010), our study identified considerably greater escalation in *S.* 1,4,[5],12:i:- specific shedding among weaners and, to a lesser extent, finishers, relative to other serovars. Studies have shown variation in shedding between serovars (Van Winsen et al., 2001; Österberg and Wallgren, 2008; Osterberg et al., 2009; Österberg et al., 2010; Pires et al., 2014); our findings indicate that *S.* 1,4,[5],12:i:- may cause high shedding rates amongst colonized pigs, particularly in younger animals. Higher shedding leading to higher transmission and subsequent prevalence could be associated with the emergence of the serovar on-farm. The observed escalation could be associated with greater *S.* 1,4,[5],12:i:- colonization in younger stock correlated with higher shedding rates or longer duration of shedding (Pires et al., 2014). This supports further investigation of serovar specific shedding and the development of control strategies targeted at serovars of importance to animal and human health, serovars such as *S.* 1,4,[5],12:i:- PT193 (Clothier et al., 2010; Hauser et al., 2010; Correia-Gomes et al., 2012; Keelara et al., 2013; Pires et al., 2014).

Almost all of the *S.* 1,4,[5],12:i:- were PT193. The level of *S.* 1,4,[5],12:i:- MLVA profile diversity per herd was very low (H = 0.80-1.16), with the exception of Herd 2 (H = 1.78). However, populations in each of the five herds were distinguishable, mirroring recent findings in Germany (Niemann et al., 2015). In this study, seven *S.* 1,4,[5],12:i:- PT193 MLVA profiles not previously associated with pigs or pork in Australia were identified. In total 12 MLVA profiles were identified. The MLVA profile variation was restricted to polymorphism at STTR5 and STTR6 loci only. Variation at these loci is relatively common and most authors suggest that isolates with single locus variations at these loci be considered related (Hopkins et al., 2007; Larsson et al., 2009; Dimovski et al., 2014). These findings suggest a clonal *S.* 1,4,[5],12:i:- population may be circulating among Australian pigs unlike reports elsewhere where multiple *S.* 1,4,[5],12:i:- clones have been identified (Moreno Switt et al., 2009; EFSA, 2010; Hopkins et al., 2010). The most widespread *S.* 1,4,[5],12:i:- MLVA profiles, 04-15-11-00-

490 and 04-15-12-00-490, were identified in four and three herds, respectively. These and very similar *S.* 1,4,[5],12:i:- MLVA profiles have been identified in Australian passive surveillance data (NEPSS, 1996). The passive surveillance isolate sources were diverse and included both human and non-porcine animals. These findings do not suggest a pig-specific *S.* 1,4,[5],12:i:- association in Australia, rather, that a highly related *S.* 1,4,[5],12:i:- population may be circulating within the Australian pig industry and possibly other Australian domestic livestock industries and people.

Niemann et al. (2015) considered single or double locus variants at loci STTR5 and STTR6 to be clonal variants. If we applied the principle of STTR5 and/or STTR6 single or double locus variants with maximum VNTR copy number differences of ≤2, then the number of *S.* 1,4,[5],12:i:- PT193 MLVA types on each farm collapsed to one. Herd 1 (MLVA 04-14-(12/13/14)-00-490) was distinct from Herds 3 and 5, which had closely related MLVA profiles 04-15-(11/12/13)-00-490; each exhibited STTR6 variation, only. Additional variation at locus STTR5 was observed in isolates from Herds 2 and 4, herds with higher *S.* 1,4,[5],12:i:- MLVA profile diversity (Table 2). In Herd 2 *S.* 1,4,[5],12:i:- MLVA profiles 04-(15/16/17)-(10/11/12)-00-490; in Herd 4 *S.* 1,4,[5],12:i:- MLVA profiles 04-(14/15/16)-(10/11/12)-00-490. In Herd 2 we observed high levels of detection of *Salmonella* serovars other than *S.* 1,4,[5],12:i:- and a diverse serovar population. However, in Herd 4, levels of detection of other serovars and *S.* 1,4,[5],12:i:- PT193 were higher, but serovar diversity was lower, relative to Herd 2. Interestingly the *Salmonella* serovar population diversity in Herd 2 was also the highest. This could indicate that *S.* 1,4,[5],12:i:- and other *Salmonella* serovars were introduced to Herd 2 from multiple sources and/or on multiple occasions, perhaps unlike the other herds.

Although other *Salmonella* serovars were detected in this study, only one biphasic *S.* Typhimurium was identified among 181 isolates fully serotyped. In addition, 365 isolates were partially serotyped and the first phase flagellar antigen (H1) ≠ i, indicating no serovar *S.* Typhimurium was present among the wider group of isolates. The sole *S.* Typhimurium isolate from Herd 1 was described by an MLVA profile also identified among *S.* 1,4,[5],12:i:- PT193 isolates from the same herd, indicating this isolate may also be closely related. Due to the similarity in all other characteristics we sequenced the *fljAB* region of this strain and found it was missing, indicating that the isolate should be classified monophasic (data not shown.)

The characteristics of the study *S.* 1,4,[5],12:i:- isolates indicated that stable, highly related populations were circulating in each of the five herds. Over the course of the study more than 30 cohorts of growing pigs were sampled within each herd, the apparent high relatedness of *S.* 1,4,[5],12:i:- isolates indicates that multiple cohorts were exposed to a persistent population, presumably cycling within the herd. This suggests that a single point source introduction and subsequent establishment of the serovar occurred in each herd or multiple introductions from a stable source population. Identification of the likely pathways through which *S.* 1,4,[5],12:i:- is or was being transmitted between herds would have considerable implications for the Australian pig industry and merits further investigation. Identifying and eliminating these pathways would aid control of the spread of this serovar, other *Salmonella* serovars and potentially unrelated pathogens within the industry. Given no live animal movements between the herds occurred, future investigations might consider breeder herds, feed, humans, vehicles and wildlife as other potential pathways of introduction. There are a limited number of genetic suppliers in Australia and few nucleus herds. The same genetic company supplied herds 2 and 3, while a separate breeder supplied both Herds 4 and 5. The serovar *S.* 1,4,[5],12:i:- has been isolated from animal feed samples in Australia (NEPSS, 1996). Several herds shared feed supply companies, however, only Herds 1 and 2 were sourced feed from the same mill. The *S.* 1,4,[5],12:i:- MLVA profiles identified among Herd 1 and 2 isolates differed but reflected the level of similarity in *S.* 1,4,[5],12:i:- MLVA profiles across all five herds. None of the herds used the same company or vehicles to move stock though they did share some specific service providers and each of the herds was to some degree accessible to birds and other potential vectors.

Salmonella 1,4,[5],12:i:- had been isolated from each of the herds prior to the study. In each case samples were collected in response to outbreaks of scouring amongst grow-out pigs. The outbreaks varied in scale and acuteness. In each herd they were controlled and only mild scouring among weaned pigs was observed during the sampling period. The clinical disease outbreaks could have coincided with the introduction of the serovar to each herd or may have been associated with other host or environmental factors, such as other disease issues affecting the herds. It is likely that shedding rates increased during the outbreaks, however, it was interesting to note the high detection rates of the serovar observed some time after the disease outbreaks had subsided, particularly among grow-out pigs. These results show that the serovar was endemic in the herds during the sampling period, carriage and/or ongoing transmission between and within cohorts may explain the persistent shedding of the serovar observed. Further research on rates of *S.* 1,4,[5],12:i:- shedding during and post-outbreaks would be informative. Furthermore, the effects of introduction of the serovar on pig health and subsequent shedding among naïve pigs might provide an indication as to whether clinical disease outbreaks in these herds likely coincided with *S.* 1,4,[5],12:i:- introduction. This would help to identify possible pathways of introduction.

Salmonella 1,4,[5],12:i:- was identified among isolates sourced from weaners and finishers in all five study herds. In each herd we routinely observed higher *S.* 1,4,[5],12:i:- shedding among weaners and, to a lesser extent, finisher pigs. These findings align with observation and veterinary reports of clinical enteritis among younger pigs in these herds prior to and during the study. The very high *S.* 1,4,[5],12:i:- detection rates among weaner samples in each herd, and over multiple sampling occasions, demonstrates routine exposure of terminal batches despite high hygiene standards and all-in all-out systems in three of the four herds. *Salmonella* 1,4,[5],12:i:- colonized weaners then appear to have shed the serovar more persistently and/or in greater relative numbers than other serovars. However, observation of clinical disease, or lack thereof, proved to be a poor indicator of hazard burden within herds. These findings indicate that maintaining batch integrity may not be effective in the control of *S.* 1,4,[5],12:i:- exposure and colonization of pigs.

In each herd *S.* 1,4,[5],12:i:- MLVA profiles identified in weaner-derived samples were also identified in finisher samples. This could indicate persistence within cohorts, via carriage and/or repeat exposure and/or exposure to 'herd' strains from other sources such as pigs, the environment or (mechanical) vectors. In addition, isolates obtained from samples from farrowing sheds and/or gestating sows in Herds 2, 3, 4 and 5 described MLVA profiles that were also identified among weaners and finishers in the same herd consistent with transmission between breeding and terminal animals within herds.

We suspect that sows may play a role in maintaining *S.* 1,4,[5],12:i:- within these herds despite low *S.* 1,4,[5],12:i:- detection rates. Studies have shown that sporadic *Salmonella* shedding by sows and suckling piglets can make detection challenging, but colonization of young piglets has been demonstrated (Davies et al., 1999; Funk et al., 2001; Kranker et al., 2003; Vigo et al., 2009; Davies et al., 2011). Hill et al. (2015) developed a farm transmission model using data from the EU and concluded that if sow *Salmonella* prevalence was greater than 10% sows would account for the majority of *Salmonella* transmission within the herd, below this prevalence feed became the dominant contributor to slaughter pig *Salmonella* status. The authors noted the high variability in individual shedding

of organisms, and thereby exposure, of pigs within herds and that to predict a high incidence within a cohort sow shedding must be at very high numbers. Sporadic shedding rates may account for the low sow *Salmonella* detections in this study, potentially masking relatively higher prevalence of colonization and numbers shed at key points in production. Therefore, sows could present an important node in the maintenance of the serovar within these herds despite the low detection rates observed. Exposure of young pigs may be occurring via the sow post-partum or via some alternate source(s) in the rearing environment as demonstrated in longitudinal studies of farrow-to-finish herds conducted by Nollet et al. (2005a); Nollet et al. (2005b). As described, numerous studies of risk factors for *Salmonella* have been conducted in relation to pigs and other food animal production indicating the potential of sows to maintain *Salmonella* in herds, these findings support the assertion that control measures should target sows as well as market destined pigs, which was not the case in the herds observed. However, more research into the economic efficacy and efficiency of control measures, such as dietary supplementation with organic acids, employed in weaner diets in Herds 1, 3 and 4, are needed to inform expenditure on *Salmonella* control strategies within herds.

The potential pathways by which *Salmonella* in primary pig production may reach end-consumers have been well described and the implication of domestic pork products in human *S.* 1,4,[5],12:i:- cases indicates that domestic pork presents some level of *S.* 1,4,[5],12:i:- food safety risk (Botteldoorn et al., 2004; Ball et al., 2011; Kirchner et al., 2011; OzFoodNet, 2012; Anon., 2013). Some *S.* 1,4,[5],12:i:- isolates from this study described the same MLVA profiles as available isolates derived from pig carcasses and domestic human salmonellosis cases (Anon., 2014). This study has shown persistent, high levels of *S.* 1,4,[5],12:i:- PT193 shedding among terminal pigs to market weight in five independent production systems, thereby identifying a potential hazard source in the Australian food chain. Observed variation in *S.* 1,4,[5],12:i:- detections from finishers between herds may be reflected in herd associated food safety risk. However, studies conducted by Swanenburg et al., (2001a), Swanenburg et al., (2001b) found that the though many factors may affect Salmonella hazard load on carcass the level of Salmon*ella* colonization within herds had no bearing on carcass contamination, only *Salmonella*-free status. Consequently, as advocated elsewhere, the *Salmonella* or *Salmonella* serovar status of herds may be the most important information to inform effective process control and verification systems during slaughter and in pork boning rooms to mitigate *Salmonella* food safety risks and improve the industry's ability to manage potential risks associated with products.

The recent recognition of the presence of *S.* 1,4,[5],12:i:- in Australia, the apparent high level of relatedness of the population observed in this study, and the similarities of strains reported by national passive surveillance systems suggests the organism emerged recently, and has spread widely (NEPSS, 1996). The nature of emergence, via parallel evolution or introduction, and timeframe requires further investigation of the relatedness of Australian *S.* 1,4,[5],12:i:- strains and comparison with other domestic Group B *Salmonella* serovars and strains reported or having originated overseas. Further comparisons of sequences will contribute to this discussion.

The Australian pig industry enjoys the natural biosecurity advantages of operating within an island continent, further enhanced by stringent quarantine restrictions and industry specific protections such as the prohibition of live pig and fresh pork imports. The pandemic *S.* Typhimurium DT104 has never been isolated in Australian livestock. Yet this study describes an emergent infectious agent that appears to have spread to widely dispersed pig herds with no live animal connections, animal transportation links and minimal overlap in terms of feed supply. This suggests the risk of spread within the industry may be considerable, reaffirming the importance of effective individual herd biosecurity and the need to investigate the means and pathways by which the pathogens does or has spread, such as via breeding stock, feed, people or wildlife.

Most previously published Australian porcine *Salmonella* studies primarily employed serological sampling methods, cross-sectional study design and/or have focused beyond the farmgate (Hamilton et al., 2000, 2003, 2005). This study reiterates the value of longitudinal sampling and extensive characterization to effectively describe *Salmonella* populations and population dynamics within animal production systems (Lo Fo Wong et al., 2004).

Collecting pooled samples reduced the costs associated with sampling and bacterial culture, demonstrated by the high number of detections relative to calculation of estimated shedding prevalence across the five subject herds (data not shown). Throughout this study it was common to identify multiple serovars, phenotypes and MLVA profiles among isolates sourced from a single pen sample. Typing a single isolate from a culture positive sample does not provide adequate information in relation to the *Salmonella* population within a pen, production stage or herd. This has implications for laboratory investigations where only single colony picks are submitted for typing. The high rates of *Salmonella* and *S.* 1,4,[5],12:i:- detection and estimated proportion of pigs shedding among terminal stock, particularly weaners, indicates that surveillance and research would obtain efficiency benefits in targeting these production stages.

Phage typing proved to be of little value in differentiating *S.* 1,4,[5],12:i:- in this study as virtually all *S.* 1,4,[5],12:i:- isolates were identified as PT193, consistent with the low diversity within the collection. This is unlike *Salmonella* studies conducted elsewhere that found the application of both phage typing and MLVA advantageous (Prendergast et al., 2011). Available Australian surveillance data indicates that this is likely to be the case across Australian food industries (NEPSS, 1996). Given the high costs and idiosyncrasies associated with phage typing, further evaluation of the utility of MLVA and sequence-based typing methodologies for Group B and other *Salmonella* serovars in relation to surveillance systems and research may be warranted. This study provides further empirical evidence for the use of MLVA in outbreak strain identification of *S.* 1,4,[5],12:i:- on the basis of discriminatory power and relative stability over extended periods. However, the current uncertainty in relation to the clustering of MLVA profiles hampers interpretation (Barco et al., 2013). On the basis of our findings we advocate the published recommendations in Dimovski et al. (2014), whereby small VNTR copy number changes at loci STTR5 and STTR6 are clustered. The increasing use and publication of whole genome sequencing studies will further enhance understanding of the ecology of *Salmonella* in pig herds and more broadly.

Declaration of interest

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2016.11.010.

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