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Research paper

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

T. Weaver[a], M. Valcanis[b], K. Mercoulia[b], M. Sait[b], J. Tuke[c], A. Kiermeier[d], G. Hogg[a], A. Pointon[d], D. Hamilton[d], H. Billman-Jacobe[a,*]

[a] *Faculty of Veterinary and Agricultural Science, The University of Melbourne, Victoria, 3010, Australia*
[b] *Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology and Immunology, at the Doherty Institute for Infection and Immunity, The University of Melbourne, Victoria, 3010, Australia*
[c] *School of Mathematical Sciences, University of Adelaide, South Australia, 5005, Australia*
[d] *South Australian Research and Development Institute, South Australia, 5064, Australia*

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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-

* Correspondence to: Faculty of Veterinary and Agricultural Science, University of Melbourne, Parkville, Victoria, 3010, Australia.
E-mail address: hbj@unimelb.edu.au (H. Billman-Jacobe).

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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.*