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# Faecal shedding of pathogenic *Yersinia enterocolitica* determined by qPCR for *yst* virulence gene is associated with reduced live weight but not diarrhoea in prime lambs

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ABSTRACT

Associations between faecal shedding of pathogenic *Yersinia enterocolitica* (based on the *yst* virulence gene) with growth, carcass weight and diarrhoea were investigated using an observational longitudinal study of 1200 crossbred prime (meat) lambs on eight Australian farms. Live weight, breech faecal soiling score (scale 1–5) and faecal consistency score (FCS; scale 1–5) were recorded, and faecal samples collected from each lamb on three sampling occasions; weaning (≈12 weeks of age), post-weaning (≈19 weeks) and pre-slaughter (≈29 weeks). Hot standard carcass weight was measured at slaughter. Faecal samples were screened for presence and concentration of pathogenic *Y. enterocolitica* using quantitative PCR. Associations of pathogenic *Y. enterocolitica* detection and shedding intensity with lamb health and production were assessed using general linear models (carcass weight), linear mixed effects models (live weight, FCS and breech soiling score) and non-parametric tests (FCS and breech soiling score). Prevalence for non-pelleted faeces (FCS ≥ 3.0) and diarrhoea (FCS ≥ 4.0) were compared with the two-tailed z-test, odds ratios and relative risk. Lambs shedding pathogenic *Y. enterocolitica* were 3.78 kg lighter post-weaning ($P < 0.001$) and 2.61 kg lighter pre-slaughter ($P = 0.035$) compared to lambs in which pathogenic *Y. enterocolitica* was not detected. Higher faecal concentration of pathogenic *Y. enterocolitica* was associated with lower live weight ($P < 0.001$). There was no association between pathogenic *Y. enterocolitica* detection and carcass weight. Overall, there was no evidence of association between pathogenic *Y. enterocolitica* detection and diarrhoea (higher FCS, higher risk for non-pelleted faeces or diarrhoea, or higher breech soiling score). Only one flock had increased relative risk for non-pelleted faeces associated with pathogenic *Y. enterocolitica* detection, and one other flock had increased relative risk for diarrhoea associated with pathogenic *Y. enterocolitica* detection. This is the first report of an association between reduced sheep live weight and pathogenic *Y. enterocolitica* based on the presence of the *yst* gene for heat stable enterotoxin determined by qPCR in sheep. Notably, impacts on live weight were observed in the absence of diarrhoea.

## 1. Introduction

Yersiniosis causes gastrointestinal disease in sheep characterised by diarrhoea, depression, ill thrift and deaths (McSporran et al., 1984; Slee and Button, 1990). Clinical manifestation of disease associated with *Y. enterocolitica* has been reported in Australia (Slee and Button, 1990; Slee and Skilbeck, 1992; Stanger, 2017; Stanger et al., in press), and worldwide (Bin-Kun et al., 2004; Gill, 1996).

The chromosomal *yst* gene encodes a low-molecular-weight, heat-stable enterotoxin belonging to a family of structurally and functionally related enterotoxins produced by several species of diarrheagenic bacteria (Robins-Browne et al., 1979; Delor et al., 1990; Kechagia et al., 2007). As *yst* is confined to pathogenic bioserotypes of *Y. enterocolitica,* it is considered a useful marker of potential virulence (Ibrahim et al., 1997). Quantitative PCR (qPCR) using primers and probe sequences specific to the *Y. enterocolitica yst* virulence gene have been used to quantify "pathogenic *Y. enterocolitica*" in faecal samples from humans (Ibrahim et al., 1997; Zheng et al., 2007) and sheep (Yang et al., 2016). A longitudinal study of prime (meat breed) lambs between weaning and slaughter on eight Australian farms identified pathogenic *Y.*

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*enterocolitica* (determined by *yst* virulence gene) in all eight flocks with point prevalence ranging 0–49% (Yang et al., 2016).

Faecal carriage of *Y. enterocolitica* is reported in young sheep in the absence of clinical disease (Slee and Button, 1990; Slee and Skilbeck, 1992; Stanger, 2017), but it has not been determined whether faecal shedding of pathogenic *Y. enterocolitica* is associated with impacts on health and production of lambs in sheep meat production systems. Live weight and carcass weight are important profit drivers for sheep meat production, and live weight is an important determinant of lamb survival and welfare during the post-weaning period (Hatcher et al., 2008; Campbell et al., 2009; Hatcher et al., 2010). Diarrhoea and subsequent faecal soiling of the fleece also have adverse impacts on lamb welfare and productivity, including increasing predisposition to fly strike (Wardhaugh et al., 1989) and risk of carcass contamination with faecal pathogens at slaughter (Hadley et al., 1997).

This study aimed to determine whether faecal shedding of pathogenic *Y. enterocolitica* (determined by *yst* virulence gene using qPCR) was associated with diarrhoea, reduced growth (live weight) or reduced carcass weight in prime lambs.

## 2. Materials and methods

### 2.1. Animals, sample collection and measurements

The study conforms to international cohort study reporting guidelines for strengthening the reporting of observational studies in epidemiology (STROBE) described by von Elm et al. (2008). All procedures were approved by relevant animal ethics committees in each state, with the overall methodology approved and monitored by the Murdoch University Animal Ethics Committee (approval no. R2352/10).

A prospective observational (cohort) investigation of cross-bred prime (meat) lamb flocks was conducted on eight different farms (one flock per farm) located across four states of Australia (Table 1). Farms were located in states of Western Australia (WA), South Australia (SA), Victoria (VIC) and New South Wales (NSW). Characteristics of the farms (including size of property, total number of sheep on the property, annual rainfall, time of lambing and presence of other livestock on the farm) have been described by Yang et al. (2016). Observations for farms WA1 and WA2 were made for lambs born in 2009, and for all other farms observations were made for lambs born in 2011 (Table 1). Farms were recruited into the study on the basis that at least 100 crossbred female lambs would be available in a single flock, and that lambs would be managed under conditions typical for commercial prime (meat) lamb production in Australia. The sample size (minimum 100 lambs per farm) was determined on the basis of establishing prevalence for a range of protozoan and bacterial pathogens, including *Yersinia* spp. (Yang et al., 2016). Lambs were identified with numbered ear tags. Female lambs were recruited into the study at either marking (approximately 4–6 weeks of age) or weaning (Table 1), and were managed as a single cohort with ewe and wether lamb flockmates until slaughter. Within cohorts (flocks), lamb age was estimated to range up to 8 weeks, depending on duration of lambing.

Each lamb was sampled on three separate occasions, specifically weaning (approximately 12 weeks old), post-weaning (approximately 19 weeks old) and pre-slaughter (approximately 29 weeks old). Lambs were weighed (live weight), assessed for breech faecal soiling and faecal samples were collected at each sampling occasion. Breech faecal soiling score was measured using a scale of 1 (no evidence of breech faecal soiling) to 5 (very severe breech faecal soiling extending down the hind legs to, or below the hocks) as previously described (Sweeny et al., 2012; Australian Wool Innovation, Meat and Livestock Australia, 2013). Some lambs were lost to follow-up at one or more sample occasions due to incomplete mustering from the paddock or mortality (Table 1).

A total of 3343 faecal samples were collected directly from the rectum using a sterile swab (weaning sample SA1, SA2 and NSW only) or gloved hand (all other samples) from 1200 cross-bred lambs (Table 1). Faecal consistency score (FCS) and faecal worm egg counts

**Table 1**
Prevalence (with Jeffrey's 95% confidence interval) and faecal shedding of pathogenic *Y. enterocolitica* (*yst* virulence gene by qPCR) in sheep faecal samples collected from 8 Australian farms.

| | | | | Shedding concentration in positive samples (organisms/g faeces) | |
|---|---|---|---|---|---|
| Farm | Sampling occasion | Samples (n) | Prevalence[a]% (95% CI) | Range | Median |
| NSW | Weaning | 156 | 50.0 (42.2–57.8) | $4.2 \times 10^3$–$7.2 \times 10^7$ | $1.08 \times 10^5$ |
| | Post-weaning | 160 | 29.4 (22.7–36.8) | $8.5 \times 10^3$–$3.6 \times 10^6$ | $6.68 \times 10^4$ |
| | Pre-slaughter | 140 | 0.0 (0.0–1.8) | – | – |
| SA1 | Weaning | 165 | 5.4 (2.7–9.7) | $4.2 \times 10^3$–$8.6 \times 10^5$ | $3.08 \times 10^4$ |
| | Post-weaning | 154 | 3.9 (1.6–7.8) | $1.1 \times 10^4$–$1.4 \times 10^5$ | $2.74 \times 10^4$ |
| | Pre-slaughter | 158 | 0.6 (0.0–2.9) | – | $4.38 \times 10^4$ |
| SA2 | Weaning | 158 | 5.7 (2.9–10.1) | $1.1 \times 10^4$–$1.4 \times 10^6$ | $4.14 \times 10^5$ |
| | Post-weaning | 151 | 2.0 (0.6–5.2) | $6.4 \times 10^5$–$9.4 \times 10^6$ | $1.86 \times 10^6$ |
| | Pre-slaughter | 123 | 0.0 (0.0–3.0) | – | – |
| Vic1 | Weaning | 177 | 8.5 (5.0–13.2) | $1.4 \times 10^4$–$1.4 \times 10^6$ | $8.10 \times 10^4$ |
| | Post-weaning | 172 | 0.0 (0.0–1.4) | – | – |
| | Pre-slaughter | 159 | 0.0 (0.0–1.6) | – | – |
| Vic2 | Weaning | 176 | 6.2 (3.4–10.6) | $3.6 \times 10^4$–$1.4 \times 10^6$ | $5.92 \times 10^4$ |
| | Post-weaning | 175 | 0.0 (0.0–1.4) | – | – |
| | Pre-slaughter | 128 | 0.0 (0.0–1.9) | – | – |
| WA1 | Weaning | 123 | 0.8 (0.0 − 3.7) | – | $4.58 \times 10^4$ |
| | Post-weaning | 122 | 0.0 (0.0–2.0) | – | – |
| | Pre-slaughter | 121 | 0.0 (0.0–2.0) | – | – |
| WA2 | Weaning | 107 | 0.0 (0.0–2.3) | – | – |
| | Post-weaning | 109 | 0.0 (0.0–2.3) | – | – |
| | Pre-slaughter | 107 | 7.5 (3.6–13.6) | $3.4 \times 10^4$–$15.6 \times 10^5$ | $8.94 \times 10^4$ |
| WA3 | Weaning | 101 | 2.0 (0.4–6.2) | $2.9 \times 10^4$–$4.5 \times 10^4$ | $3.71 \times 10^4$ |
| | Post-weaning | 101 | 1.0 (0.1–4.5) | – | $8.95 \times 10^4$ |
| | Pre-slaughter | 100 | 0.0 (0.0–2.5) | – | – |
| All farms | Total (n) | 3343 | 5.7 (5.0–6.5) | $4.2 \times 10^3$–$7.2 \times 10^7$ | $8.55 \times 10^4$ |
| | Period prevalence | **1200** | **14.3 (12.4–16.4)** | | |

NSW: New South Wales SA: South Australia Vic: Victoria WA: Western Australia.
[a] Adapted from Yang et al. (2016).