
http://researchrepository.murdoch.edu.au/23616/
Title: Longitudinal prevalence, faecal shedding and molecular characterisation of campylobacter spp. and salmonella enterica in sheep

Author: Rongchang Yang, Caroline Jacobson, Graham Gardner, Ian Carmichael, Angus J.D. Campbell, Una Ryan

PII: S1090-0233(14)00322-0
DOI: http://dx.doi.org/doi:10.1016/j.tvjl.2014.08.001
Reference: YTVJL 4234

To appear in: The Veterinary Journal

Accepted date: 3-8-2014

Please cite this article as: Rongchang Yang, Caroline Jacobson, Graham Gardner, Ian Carmichael, Angus J.D. Campbell, Una Ryan, Longitudinal prevalence, faecal shedding and molecular characterisation of campylobacter spp. and salmonella enterica in sheep, The Veterinary Journal (2014), http://dx.doi.org/doi:10.1016/j.tvjl.2014.08.001.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Longitudinal prevalence, faecal shedding and molecular characterisation of Campylobacter spp. and Salmonella enterica in sheep

Rongchang Yang a, Caroline Jacobson a, Graham Gardner a, Ian Carmichael b, Angus J. D. Campbell c and Una Ryan a,*

a School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia
b South Australian Research and Development Institute, 33 Flemington Street, Glenside, South Australia 5065, Australia
c Faculty of Veterinary Science, University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia

* Corresponding author. Tel.: +61 8 93602482.
E-mail address: Una.Ryan@murdoch.edu.au (U. Ryan).
Highlights

- This is the first comprehensive study of faecal excretion of *Campylobacter* and *Salmonella* spp. by sheep across Australia.
- A multiplex qPCR for detection of *Campylobacter* and *Salmonella* spp. in faecal samples from sheep was developed and the prevalence, bacterial shedding and species were analysed.
- In a longitudinal study, 3,412 faecal samples from sheep were tested over three sampling times.
- The overall prevalences of *Campylobacter* spp. and *S. enterica* were 13.3% and 5.0%, respectively, with the highest prevalence for *Campylobacter* spp. in South Australia and the highest prevalence for *S. enterica* in New South Wales.
- *Campylobacter jejuni* was the only *Campylobacter* sp. identified from a subset of 120 positive samples sequenced at the 16S locus and *S. enterica* serovar Typhimurium were the only serovar of *S. enterica* identified from a subset of 120 positive samples sequenced at the *ompF* locus.
- *Campylobacter* spp. had the highest median bacterial concentration in faeces at weaning and post-weaning (medians of $3.4 \times 10^6$ and $1.1 \times 10^5$, respectively), whereas *S. enterica* had the highest median bacterial concentration at pre-slaughter ($1.8 \times 10^5$/g faeces).

Abstract

Faecal excretion of *Campylobacter* spp. and *Salmonella enterica* in sheep in Australia was determined using a quantitative multiplex PCR (qPCR) targeting the *Campylobacter* spp. purine biosynthesis gene (*PurA*) and the *S. enterica* outer membrane protein (*ompF*). The multiplex qPCR was specific and *Campylobacter* spp. and *S. enterica* were each detected with a sensitivity of 5 organisms/µL faecal DNA extract. This multiplex qPCR was used to determine the prevalence and concentration of *Campylobacter* spp. and *S. enterica* in 3412 faecal samples collected from 1189 lambs on eight farms across South Australia ($n = 2$ farms), New South Wales ($n = 1$), Victoria ($n = 2$) and Western Australia ($n = 3$) at three sampling periods (weaning, post-weaning and pre-slaughter). The overall prevalences of *Campylobacter* spp. and *S. enterica* were 13.3% and 5.0%,
respectively, with the highest prevalence for *Campylobacter* spp. in South Australia and the highest prevalence for *S. enterica* in New South Wales. *Campylobacter jejuni* was the only *Campylobacter* sp. identified from a subset of 120 positive samples sequenced at the 16S locus. *S. enterica* serovar Typhimurium were the only serovar of *S. enterica* identified from a subset of 120 positive samples sequenced at the *ompF* locus. Across all states, *Campylobacter* spp. had the highest median bacterial concentration in faeces at weaning and post-weaning (medians of $3.4 \times 10^6$ and $1.1 \times 10^5$, respectively), whereas *S. enterica* had the highest median bacterial concentration at pre-slaughter (1.8 x $10^5$/g faeces).

Keywords: Sheep; *Campylobacter* spp.; 16S rRNA; purA; *Salmonella enterica*; *ompF*; Quantitative PCR; Prevalence
Introduction

Campylobacter spp. remain the most commonly recognised aetiological agents of bacterial gastroenteritis in many developed countries in both human beings and veterinary species, and are the leading causes of bacterial food-borne gastroenteritis worldwide (Blaser, 1997; Allos et al., 2004; Sproston et al., 2011; Sahin et al., 2012). The majority of human infections with Campylobacter spp. are caused by Campylobacter jejuni (~90%), with ~10% caused by Campylobacter coli (Sproston et al., 2011). Epidemiologically, contaminated chicken meat is recognised as a main source of human Campylobacter spp. infections; however, the significance of the ruminant reservoir in zoonotic Campylobacter spp. transmission is increasingly recognised (Wilson et al., 2008; Greig and Ravel, 2009; Sheppard et al., 2009). Campylobacteriosis due to infection with Campylobacter spp. (most commonly C. jejuni) has been identified as a cause of enterocolitis in sheep in Australia and New Zealand (Stephens et al., 1984; Meanger et al., 1989; Glastonbury, 1990; Gumbrell, 2000; Bailey et al., 2003).

Salmonellosis caused by Salmonella enterica is another major cause of gastroenteritis in a range of species in the developed world (Vanselow et al., 2007). There are more than 2,500 serovars of S. enterica, each with a different host range and disease manifestation (Coburn et al., 2007; Sojka et al., 1983; Glastonbury, 1990).

The epidemiology of Campylobacter spp. and Salmonella enterica in sheep and the contamination of both surface water and lamb carcasses with these organisms is poorly understood (Bailey et al., 2003; Stanley and Jones, 2003; Davies et al., 2004; Garcia et al., 2010a; 2010b). These bacteria can be detected by microscopy, culture and immunoassays; however, these assays can lack specificity and are time-consuming (Pawlowski et al., 2009; Leblanc-Maridor et al., 2011a and b; Maciel et al., 2011). Additionally, Campylobacter spp. and S. enterica may enter into a
viable but nonculturable state (VBNC) (Alexandrino et al., 2004; Oliver, 2005; Murphy et al., 2006; Panudaporn et al., 2006).

Quantitative PCR (qPCR) assays have been developed which have the added advantage of being able to enumerate numbers of organisms present (Rothrock et al., 2009; Leblanc-Maridor et al., 2011a and b; Maciel et al., 2011; McAuliffe et al., 2013). The majority of studies that have examined the prevalence and faecal concentrations of these organisms in lambs in Australia have relied on culture and/or immunological methods for detection (Bailey et al., 2003; Duffy et al., 2010). Therefore the aim of the present study was to develop a multiplex qPCR for *Campylobacter* spp. and *S. enterica* and to use the qPCR assay to determine the faecal prevalence and concentrations of *Campylobacter* spp. and *S. enterica* in lambs over a wide geographical area representing the major sheep growing regions of Australia. Specifically Western Australia (WA), New South Wales (NSW), Victoria (Vic) and South Australia (SA), at three sampling periods (weaning, post-weaning and pre-slaughter).

**Materials and methods**

**Animals and collection of faecal samples**

Faecal samples were collected from cross-bred lambs from eight different farms across four states in Australia (Table 1) (Yang et al., 2013, 2014a, b, c and d). Lambs were born and reared in paddocks and were not housed indoors at any stage. The same animals were sampled on each of three occasions at weaning (approximately 12 weeks of age), post-weaning (approximately 19 weeks of age) and pre-slaughter (approximately 29 weeks of age). A total of 3412 faecal samples from approximately 1189 lambs were collected directly from the rectum. All sample collection methods used were approved by the Murdoch University Animal Ethics Committee (approval number R2352/10; date of approval 16 July 2010).
**DNA isolation**

Genomic DNA was extracted from 200 mg of each faecal sample using the Power Soil DNA Kit (MolBio) as described in Sweeny et al. (2011) and Sweeny (2012). A negative control (no faecal sample) was used in each extraction group.

**PCR amplification, quantification and sequencing**

Primers and probes for *Campylobacter* spp. and *S. enterica* were designed using Primer3. A 121 base pair (bp) product was amplified from the *Campylobacter* spp. purine biosynthesis gene *(PurA)* using the forward primer *PurA*F1 5’-CGCCCTTATCCTCAGTAGGAAA-3’, the reverse primer *PurAR1* 5’-TCAGCAGGCGCTTTAAACAG-3’ and the probe 5’- 6-carboxyfluorescein (FAM)-AGCTCCATTTCCACACCGTTGC-3’. A 96 bp product was amplified from the *S. enterica* outer membrane protein (*OmpF*) using the forward primer *OmpF1* 5’-TCGCCGGTCGTTGTCCAT-3’, the reverse primer *OmpR1* 5’-AACCGCAAACGCAGCAAGA-3’ and the probe 5’-2’7’,-dimeth- oxy-4’5’,-dichloro-6-carboxyfluorescein (JOE)-ACGTGACGACGCCAGGCTTTAC-3’.

An internal amplification control (IAC) consisting of a fragment of a coding region from Jembrana disease virus (JDV) cloned into a pGEM-T vector (Promega) and IAC primers were used as described previously (Yang et al., 2013). Each 15 μL PCR mixture contained 1x PCR buffer (10 mM Tris-HCl, 50 mM KCl), 4 mM MgCl₂, 1 mM deoxynucleotide triphosphates, 1.0 U KAPA DNA polymerase (MolBio), 0.2 μM each forward and reverse primer, 0.2 μM each forward and reverse IAC primers, 50 nM probe, 50 nM IAC probe, 10 copies IAC template and 1 μL sample DNA. The PCR cycling conditions consisted of 95 °C for 3 min, then 45 cycles of 95 °C for 20 s and 60 °C for 45 s.

---

¹ See: [http://frodo.wi.mit.edu/](http://frodo.wi.mit.edu/)
A standard curve for quantifying Campylobacter spp. and S. enterica DNA was generated by cloning the purA gene amplicon from Campylobacter spp. and the ompF gene amplicon from S. enterica into pGEM-T (Promega) and transforming the recombinant vector into Escherichia coli competent cells. Plasmid DNA for each bacteria was isolated by alkali sodium dodecyl sulphate lysis, followed by column purification using QIAprep Spin Columns (Qiagen). Plasmid mini-preparations were sequenced using the T7 sequencing primer (Stratagene) and clones with the correct sequence then used as positive controls for generating a standard curve.

Specificity and sensitivity

The analytical specificity of the multiplex qPCR assays was assessed by testing DNA from C. jejuni, C. coli, S. enterica serovar Typhimurium, S. enterica serovar Wandsbek II 21:z10:z6, S. enterica serovar Bredeney, S. enterica serovar Muenchen, S. enterica serovar Adelaide, S. enterica serovar Waycross, S. enterica serovar Infantis, Chlamydia pecorum, Chlamydia abortus, Yersinia enterocolitica, Streptococcus bovis (ATCC 33317), Enterococcus durans (ATCC 11576), Escherichia coli (ATCC 25922), Bacillus subtilis (ATCC 6633), Serratia marcescens (ATCC 14756 pigmented), Citrobacter freundii (NCTC 9750), Enterobacter cloacae (ATCC 13047), Coxiella burnetii, Giardia duodenalis assemblages A and E from sheep, Cryptosporidium sp. (n = 5) Isospora sp., Tenebrio sp., Cyclospora sp., Toxoplasma gondii, Trichostrongylus colubriformis, Teladorsagia circumcincta, Haemonchus contortus and Eimeria sp., as well as human, sheep and cattle genomic DNA.

To determine the sensitivity of the assay, 10-fold serial dilutions of plasmids containing the cloned PCR products amplified from each of the two bacteria (Campylobacter spp. and S. enterica) were prepared from 1,000,000 copies to 10 copies. These were then ‘spiked’ into faecal samples and the DNA was extracted and amplified as described above. Mean detection limits, the coefficient of determination R squared (RSQ) values and % relative standard deviation (RDS) were calculated.
Template copy numbers were converted to numbers of organism present on the basis that purA (Campylobacter spp.) and ompF (S. enterica) are single copy genes (Pearson et al., 2007; Tatavarthy and Cannons, 2010; GenBank CP000814) and bacterial genomes are haploid. Therefore the detected plasmid numbers were equivalent to the numbers of Campylobacter spp. and S. enterica.

Inhibition and efficiency

Equal amounts of the IAC template (10 copies) were added to all faecal DNA samples to detect any PCR inhibitors present in the extracted DNA. If inhibition was evident, then the sample was diluted and re-amplified. Amplification efficiency ($E$, a measure of inhibition), was estimated by using the slope of the standard curve and the formula $E = 1 + 10^{(-1/slope)}$ (Nybo, 2011). To estimate amplification efficiency on faecal samples, serial dilutions of five individual DNA samples (neat, 1:10, 1:100) were performed and multiple qPCR reactions were conducted on each dilution. The $Ct$ values were then plotted vs. the log base 10 of the dilution and a linear regression was performed using the Rotor-Gene 6.0 software.

Molecular typing

A subset of five samples that were positive for Campylobacter spp. from each sampling on each farm ($n = 120$) were subjected to PCR for the Campylobacter spp. 16S rRNA gene (287 bp amplicon) using primers and PCR conditions described by Lubeck et al. (2003). A subset of five samples that were positive for S. enterica from each sampling on each farm ($n = 120$) were subjected to PCR for the S. enterica ompF gene (578 bp amplicon) using primers and PCR conditions described by Tatavarthy and Cannons (2010). PCR products were separated by gel electrophoresis and purified using an in-house filter tip method (Yang et al., 2013). Purified PCR products were sequenced using an ABI Prism Dye Terminator Cycle Sequencing kit.
Biosystems) using an annealing temperature of 58 °C. Nucleotide sequences were analysed using Chromas Lite version 2.0\(^2\) and aligned with reference sequences from GenBank using Clustal W\(^3\).

**Statistical analysis**

Prevalences were expressed as the percentage of samples positive by PCR, with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa et al., 2000). \(\chi^2\) and analysis of variance (ANOVA) were performed using SPSS 21.0 for Windows. A \(P\) value of 0.05 was used to declare statistical significance when determining if there was any association between the prevalence and concentration (median value from the positives in the individual population) of bacteria at different sampling times and across states.

**Results**

**Specificity, sensitivity and efficiency**

Evaluation of specificity of the multiplex bacterial qPCR revealed no cross-reactions with other genera; the qPCR only amplified the relevant bacterial species. Sensitivity analysis revealed that the mean minimum detection for both *Campylobacter* spp. and *S. enterica* was 5 organisms/µL, which equates to \(\sim\)1250 organisms/g faeces. The mean RSQ values for *Campylobacter* spp. and *S. enterica* were 0.99 and 0.99, respectively. The % RDS for *Campylobacter* spp. and *S. enterica* were 3.5% and 4.5%, respectively. The frequency of PCR inhibition, as determined by IAC amplification, was \(\sim\)2%. The mean efficiencies for *Campylobacter* spp. and *S. enterica* were 103.8% and 97.4%, respectively.

**Prevalence of Campylobacter spp. and Salmonella enterica**

---


\(^3\) See: [http://www.clustalw.genome.jp](http://www.clustalw.genome.jp).
The overall prevalences of *Campylobacter* spp. and *S. enterica* in faecal samples from lambs were 13.3% and 5.0%, respectively (Table 2). The highest prevalence of *Campylobacter* spp. was in faecal samples from lambs in SA (59% for SA2 and 44.6% for SA1, both from post-weaning samplings). Overall, there were significant differences between the prevalence of bacterial pathogens between states (*P* < 0.01 for *Campylobacter* spp.; *P* < 0.05 for *S. enterica*). The highest prevalence of *S. enterica* in faecal samples from lambs was in NSW post-weaning (23.8%), which was significantly higher than all three sampling times from the other farms and the pre-slaughter periods from the NSW farm (*P* < 0.05). In general, the prevalence of *S. enterica* was lower than *Campylobacter* spp. on all eight farms (Table 2). There was no relationship between prevalence and time of sampling (*P* > 0.05).

**Concentration of Campylobacter spp.**

Across all states, the median concentration of *Campylobacter* spp. in lambs was $3.4 \times 10^6$ (range 250 to $3.3 \times 10^{10}$) organisms/g faeces at weaning, $1.1 \times 10^5$ (250 to $8.2 \times 10^{10}$) organisms/g faeces post-weaning and $1.5 \times 10^5$ (range 250 to $1.0 \times 10^7$) organisms/g faeces at pre-slaughter (Table 2). The highest median number of *Campylobacter* spp. (2.1 x $10^8$ organisms/g faeces) was detected on WA3 post-weaning, which coincided with the peak prevalence on this farm. The highest number of *Campylobacter* spp. shed by an individual lamb was also on WA3 post-weaning (8.2 x $10^{10}$ organisms/g). In SA, high median concentrations of *Campylobacter* spp. were detected on SA1, SA2 and Vic2 at weaning ($9.4 \times 10^9$, $1.6 \times 10^9$ and $2.9 \times 10^9$ organisms/g faeces, respectively); this coincided with the peak prevalence on Vic2 (26.7%), whereas the peak prevalence on SA1 and SA2 occurred post-weaning.

**Concentration of Salmonella enterica**

Across all states, the median concentration of *S. enterica* in lambs was $9.3 \times 10^4$ (range 250 to $5 \times 10^8$) organisms/g faeces at weaning, $7.5 \times 10^4$ (250 to $1.5 \times 10^7$) organisms/g faeces post-
weaning and $1.8 \times 10^5$ (range 250 to $1.1 \times 10^5$) organisms/g faeces at pre-slaughter (Table 2). The highest median number of *S. enterica* ($7.4 \times 10^7$ organisms/g faeces) was detected on SA2 at weaning (Table 2).

**Identification of Campylobacter spp. and serovars of Salmonella enterica**

All *Campylobacter* spp. sequences ($n = 120$) were identified as *C. jejuni* (16S rRNA sequence identical to GenBank CP001876) and all *S. enterica* sequences ($n = 120$) were identified as *S. enterica* serovar Typhimurium (*ompF* sequence identical to GenBank Z31594).

**Discussion**

In this longitudinal study, a multiplex qPCR was used to determine the prevalence, concentration and species or serovars of *Campylobacter* spp. and *S. enterica* in faecal samples collected from lambs at weaning, post-weaning and pre-slaughter from eight farms across four states in Australia. The multiplex qPCR assay was specific for *Campylobacter* spp. and *S. enterica*. In addition, the *ompF* gene used as the qPCR target in *S. enterica* is thought to be restricted to this bacterial species, which should increase the specificity of the assay (Tatavarthy and Cannons, 2010). However, qPCR does not differentiate between viable and non-viable organisms, which would require culture.

The detection limits in this study (5 organisms/μL faecal DNA extract) are similar to or better than published studies on qPCR detection without pre-enrichment. Previous studies on *Campylobacter* spp. have reported detection limits of 10 genome copies for *C. jejuni* in porcine faeces (Leblanc-Maridor et al., 2011a; 2011b) and five bacteria for *C. jejuni* in environmental water samples (Rothrock et al., 2009). A qPCR based on the *SdfI* gene detected 32 genome copies of *S. enterica* in faeces (Maciel et al., 2011). PCR is sometimes hindered by inhibitors in faecal samples, (Wilson, 1997); however, in the present study, PCR inhibition (as determined by the IAC
amplification) occurred in only ~2% of the examined samples during the qPCR assay. Using qPCR, chronic shedders of high concentrations of bacteria can be identified, isolated and/or treated, and slaughter postponed, preventing cross-contamination. The sensitivity of the assay could also be increased with a pre-enrichment step.

In the present study, the overall prevalence of *Campylobacter* spp. and *S. enterica* in faecal samples from lambs was 13.3% and 5.0%, respectively. However, the prevalence varied widely among states and at different sampling points. As an example, the prevalence of *Campylobacter* spp. was 15.4% at weaning in SA2, but 59% post-weaning. Similarly the prevalence of *S. enterica* peaked at 23.8% in NSW post-weaning, but was only 3.6% during the pre-slaughter period. In a study of slaughter age lambs in NSW and Queensland, 14/19 (73.7%) of all flocks tested were positive for *C. jejuni* by culture and the prevalence in individual lambs was 8% (Bailey et al., 2003). In Scotland, the prevalence of *Campylobacter* spp. by culture in ovine faeces was 14-49% (Garcia et al., 2010a; Sproston et al., 2011).

In a national baseline microbiological survey of Australian sheep carcasses and frozen boneless sheep meat conducted in 2004, *S. enterica* was isolated from 0/1117 carcases and from 3/560 (5.4%) samples of boneless product. *Campylobacter* spp. were isolated from 4/1117 (0.4%) carcases and from 1/560 (0.2%) boneless samples (Phillips et al., 2006). However, it is important to note that low or no recovery rates of these organisms in faeces and carcasses during slaughter may be due to the limited sensitivity of culture methods (Fredriksson-Ahomaa and Korkeala, 2003). In a recent study, which compared qPCR and culture methods for the detection of *S. enterica* in faeces, 45 samples that were positive using the PCR assay were negative on culture (Maciel et al., 2011).

In the present study, the prevalence of *S. enterica* was low across all states (0-10.7%), with the exception of NSW, where prevalences peaked at 23.8 and 18.1% at post-weaning and weaning,
respectively. The prevalence of *S. enterica* in the faeces of healthy slaughtered sheep appears to vary widely. In a recent study of 486 samples from 164 sheep and lambs at two abattoirs in Australia, *S. enterica* was isolated from 20% of faeces, 13% of fleeces and 1.3% of pre-chill carcasses (Duffy et al., 2010). Previous studies have reported prevalences ranging from 0.1% in the UK (Davies et al., 2004) to 42% in Australia (Samuel et al., 1981).

Bacterial concentrations in faeces were also determined by qPCR. For *S. enterica*, the pathogen load was relatively low across all farms, with the exception of SA2 at weaning, where, although the shedding was high, the prevalence was low (1.7%). The highest median number of *Campylobacter* spp. detected was at WA3 post-weaning (2.1 x 10^8 organisms/g faeces), although individuals at SA2 shed up to 1.6 x 10^9 organisms/g faeces. Little is known about bacterial shedding of *Campylobacter* spp. in sheep; the reasons for the high *Campylobacter* spp. output in some lambs are unknown, but may be due to stress or to multiple infections reducing host immunity, since these sheep were known to be co-infected with *Giardia* spp., *Cryptosporidium* spp., *Eimeria* spp., *Chlamydia pecorum*, *Yersinia* spp. and strongyles (Yang et al., 2014a, b, c, d).

*Campylobacter jejuni* and *S. enterica* serovar Typhimurium were the only species identified from a subset of 120 positive samples for each bacteria. However, since only a subset of positive samples was sequenced, other species and serovars may also be present. A previous study of 55 *S. enterica* isolates from Australian sheep at slaughter identified 11 different serovars (Duffy et al., 2010). In Scotland, *C. jejuni* accounted for 75% of all the positive samples from sheep sent for slaughter, followed by *C. coli* (16%), *Campylobacter upsaliensis* (2.52%) and *Campylobacter fetus* (1.26%) (Garcia et al., 2010a).

**Conclusions**
Campylobacter spp. and, to a lesser extent, S. enterica, are prevalent in lambs in Australia and high levels of bacterial shedding are present in some animals. Further studies are required to determine the extent of production losses associated with these bacterial infections in sheep.

Conflict of interest statement

The study was financed by Meat and Livestock Australia (MLA), Australian Wool Innovation Limited (AWI) and the Australian Government, which had no influence on study design, data evaluation or manuscript preparation. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was funded by Meat and Livestock Australia (MLA), Australian Wool Innovation Limited (AWI) and the Australian Government. We thank the participating farmers for their support and for providing access to sheep for sample collection. We thank Justin Hoad for providing faecal samples from NSW. Samples from the WA farms were collected and DNA extracted by Joshua Sweeny. Thanks also go to Josephine Ng-Hublin for DNA extraction of faecal samples collected from the eastern states.

References


Table 1 Sheep farms sampled during the present study.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Farm location</th>
<th>Mean annual rainfall (mm)</th>
<th>Farm size (Ha)</th>
<th>Number of sheep</th>
<th>Breed</th>
<th>Commencement of lambing</th>
<th>Winter stocking rate (DSE/Ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA1</td>
<td>Wirrega, SA</td>
<td>430</td>
<td>1040</td>
<td>1800</td>
<td>Suffolk</td>
<td>April</td>
<td>10</td>
</tr>
<tr>
<td>SA2</td>
<td>Struan, SA</td>
<td>550</td>
<td>1500</td>
<td>5500</td>
<td>BL/Merino x Suffolk</td>
<td>June</td>
<td>15</td>
</tr>
<tr>
<td>Vic1</td>
<td>Rosedale, Vic</td>
<td>620</td>
<td>300</td>
<td>300*</td>
<td>BL/Merino x Dorset and Southdown</td>
<td>July</td>
<td>10</td>
</tr>
<tr>
<td>Vic2</td>
<td>Ballarat, Vic</td>
<td>750</td>
<td>1960</td>
<td>7000</td>
<td>Merino x Suffolk</td>
<td>August</td>
<td>13</td>
</tr>
<tr>
<td>NSW</td>
<td>Armidale, NSW</td>
<td>495</td>
<td>2958</td>
<td>1000</td>
<td>BL/Merino</td>
<td>May-August</td>
<td>20</td>
</tr>
<tr>
<td>WA1</td>
<td>Pingelly, WA</td>
<td>450</td>
<td>1500</td>
<td>1350</td>
<td>Merino x Suffolk</td>
<td>July</td>
<td>12</td>
</tr>
<tr>
<td>WA2</td>
<td>West Arthur, WA</td>
<td>500</td>
<td>1250</td>
<td>1750</td>
<td>Merino x Suffolk</td>
<td>August</td>
<td>10</td>
</tr>
<tr>
<td>WA3</td>
<td>Frankland, WA</td>
<td>550</td>
<td>560</td>
<td>3300</td>
<td>Merino x Suffolk</td>
<td>July</td>
<td>21</td>
</tr>
</tbody>
</table>

DSE, dry sheep equivalent; BL, Border Leicester; SA, South Australia; Vic, Victoria; NSW, New South Wales, WA, Western Australia.

* Number of ewes.
Table 2  Prevalence (%) and quantity (organisms/g) of *Campylobacter* spp. and *Salmonella enterica* in faeces collected from sheep on eight farms in four states of Australia over three sampling periods as determined by qPCR.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Sampling period</th>
<th><em>Campylobacter</em> spp. Prevalence (%)</th>
<th><em>Campylobacter</em> spp. Range (organisms/g)</th>
<th><em>Campylobacter</em> spp. Median (organisms/g)</th>
<th><em>Salmonella enterica</em> Prevalence (%)</th>
<th><em>Salmonella enterica</em> Range (organisms/g)</th>
<th><em>Salmonella enterica</em> Median (organisms/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA1</td>
<td>Weaning</td>
<td>16.4 (10.7-22.0)</td>
<td>3.1 x 10^6-6.2 x 10^6</td>
<td>9.4 x 10^5</td>
<td>2.4 (0.1-4.8)</td>
<td>6.3 x 10^7-1.7 x 10^7</td>
<td>2.1 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>44.6 (36.6-52.6)</td>
<td>750-9.4 x 10^6</td>
<td>1.6 x 10^5</td>
<td>1.4 (0.0-3.2)</td>
<td>4.5 x 10^7-2.5 x 10^7</td>
<td>8.8 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>27.7 (20.7-34.6)</td>
<td>3.8 x 10^5-2.4 x 10^6</td>
<td>1.7 x 10^5</td>
<td>1.3 (0.3-3.0)</td>
<td>4.5 x 10^5-2.8 x 10^5</td>
<td>1.4 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Weaning</td>
<td>15.4 (9.9-20.8)</td>
<td>3.0 x 10^5-1.6 x 10^5</td>
<td>6.5 x 10^5</td>
<td>1.9 (0.0-4.0)</td>
<td>6.3 x 10^5-1.7 x 10^5</td>
<td>7.4 x 10^4</td>
</tr>
<tr>
<td>SA2</td>
<td>Post-weaning</td>
<td>59.0 (51.3-66.7)</td>
<td>250-1.1 x 10^5</td>
<td>1.9 x 10^4</td>
<td>9.6 (5.0-14.2)</td>
<td>250-1.5 x 10^5</td>
<td>4.4 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>31.3 (23.8-38.8)</td>
<td>750-1.0 x 10^5</td>
<td>2.1 x 10^4</td>
<td>2.7 (0.1-5.4)</td>
<td>250-6.9 x 10^5</td>
<td>2.1 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Weaning</td>
<td>6.6 (0.0-1.6)</td>
<td>1.3 x 10^5-1.3 x 10^5</td>
<td>1.3 x 10^4</td>
<td>2.2 (0.1-4.4)</td>
<td>250-1.2 x 10^5</td>
<td>500</td>
</tr>
<tr>
<td>Vic1</td>
<td>Post-weaning</td>
<td>2.3 (1.0-4.6)</td>
<td>8.1 x 10^5-4.3 x 10^5</td>
<td>1.3 x 10^4</td>
<td>2.3 (0.1-4.6)</td>
<td>1.3 x 10^5-6.3 x 10^5</td>
<td>6.3 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>3.8 (0.8-6.7)</td>
<td>250-8.1 x 10^4</td>
<td>1.4 x 10^4</td>
<td>3.1 (0.4-5.8)</td>
<td>3.8 x 10^5-3.7 x 10^5</td>
<td>2.4 x 10^4</td>
</tr>
<tr>
<td>Vic2</td>
<td>Weaning</td>
<td>26.7 (20.2-33.2)</td>
<td>250-3.4 x 10^8</td>
<td>2.9 x 10^4</td>
<td>5.7 (2.3-9.1)</td>
<td>250-7.8 x 10^7</td>
<td>1.3 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>2.3 (1.0-4.6)</td>
<td>6.9 x 10^5-6.3 x 10^5</td>
<td>2.5 x 10^4</td>
<td>5.2 (1.9-8.5)</td>
<td>250-7.5 x 10^7</td>
<td>1.9 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.1 (0.1-6.1)</td>
<td>3.8 x 10^5-3.7 x 10^5</td>
<td>2.4 x 10^4</td>
</tr>
<tr>
<td>NSW</td>
<td>Weaning</td>
<td>4.4 (1.2-7.5)</td>
<td>4.8 x 10^5-6.8 x 10^5</td>
<td>3.4 x 10^4</td>
<td>18.1 (12.2-24.1)</td>
<td>5.5 x 10^6-4.5 x 10^6</td>
<td>7.8 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>4.4 (1.2-7.5)</td>
<td>3.0 x 10^5-7.2 x 10^5</td>
<td>2.1 x 10^4</td>
<td>23.8 (17.2-30.3)</td>
<td>250-7.3 x 10^7</td>
<td>1.2 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>1.2 (0.0-2.8)</td>
<td>931-1.1 x 10^3</td>
<td>6.1 x 10^3</td>
<td>3.6 (0.8-6.4)</td>
<td>1.8 x 10^4-1.6 x 10^3</td>
<td>3.0 x 10^3</td>
</tr>
<tr>
<td></td>
<td>Weaning</td>
<td>10.5 (5.1-15.9)</td>
<td>2.0 x 10^5-5.7 x 10^5</td>
<td>7.1 x 10^3</td>
<td>4.0 (0.6-7.5)</td>
<td>250-1.2 x 10^5</td>
<td>750</td>
</tr>
<tr>
<td>WA1</td>
<td>Post-weaning</td>
<td>4.1 (0.6-7.6)</td>
<td>6.3 x 10^5-1.4 x 10^5</td>
<td>3.2 x 10^4</td>
<td>6.6 (2.2-10.9)</td>
<td>1.4 x 10^6-9.2 x 10^5</td>
<td>2.3 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>5.8 (1.6-9.9)</td>
<td>4.0 x 10^5-5.0 x 10^5</td>
<td>1.7 x 10^4</td>
<td>10.7 (5.2-16.3)</td>
<td>1.5 x 10^6-1.1 x 10^6</td>
<td>6.6 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Weaning</td>
<td>12.1 (6.0-18.3)</td>
<td>475-2.9 x 10^5</td>
<td>5.4 x 10^4</td>
<td>4.7 (0.7-8.7)</td>
<td>1.8 x 10^6-5.0 x 10^6</td>
<td>7.4 x 10^4</td>
</tr>
<tr>
<td>WA2</td>
<td>Post-weaning</td>
<td>4.6 (0.7-8.5)</td>
<td>1.3 x 10^5-7.5 x 10^5</td>
<td>1.7 x 10^4</td>
<td>2.8 (0.0-5.8)</td>
<td>5.6 x 10^5-7.1 x 10^5</td>
<td>1.3 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>9.3 (3.8-14.9)</td>
<td>4.0 x 10^5-5.0 x 10^5</td>
<td>1.7 x 10^4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Weaning</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>4.0 (2.7-8.7)</td>
<td>5.8 x 10^4-8.3 x 10^5</td>
<td>1.6 x 10^4</td>
</tr>
<tr>
<td>WA3</td>
<td>Post-weaning</td>
<td>15.8 (8.7-23.0)</td>
<td>4.5 x 10^5-8.2 x 10^5</td>
<td>2.1 x 10^4</td>
<td>2.0 (0.4-4.7)</td>
<td>2.1 x 10^5-3.7 x 10^4</td>
<td>3.3 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>5.0 (0.7-9.3)</td>
<td>750-6.4 x 10^5</td>
<td>1.1 x 10^5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>13.3 (12.1-14.4)</td>
<td>0.8 x 10^5-3.9 x 10^5</td>
<td>3.9 x 10^4</td>
<td>5.0 (4.3-5.7)</td>
<td>6.5 x 10^4</td>
<td>9.4 x 10^4</td>
</tr>
</tbody>
</table>

ND, not detected.