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Title: Longitudinal prevalence, faecal shedding and molecular characterisation of *campylobacter* spp. and *salmonella enterica* in sheep

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1 **Longitudinal prevalence, faecal shedding and molecular characterisation of *Campylobacter***
2 **spp. and *Salmonella enterica* in sheep**
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4
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21 **Highlights**

22 • This is the first comprehensive study of faecal excretion of *Campylobacter* and *Salmonella* spp.
23 by sheep across Australia.

24 • A multiplex qPCR for detection of *Campylobacter* and *Salmonella* spp. in faecal samples from
25 sheep was developed and the prevalence, bacterial shedding and species were analysed.

26 • In a longitudinal study, 3,412 faecal samples from sheep were tested over three sampling times.

27 • The overall prevalences of *Campylobacter* spp. and *S. enterica* were 13.3% and 5.0%,
28 respectively, with the highest prevalence for *Campylobacter* spp. in South Australia and the
29 highest prevalence for *S. enterica* in New South Wales.

30 • *Campylobacter jejuni* was the only *Campylobacter* sp. identified from a subset of 120 positive
31 samples sequenced at the 16S locus and *S. enterica* serovar Typhimurium were the only serovar
32 of *S. enterica* identified from a subset of 120 positive samples sequenced at the *ompF* locus.

33 • *Campylobacter* spp. had the highest median bacterial concentration in faeces at weaning and
34 post-weaning (medians of 3.4 x $10^6$ and 1.1 x $10^5$, respectively), whereas *S. enterica* had the
35 highest median bacterial concentration at pre-slaughter (1.8 x $10^5$/g faeces).

36

37 **Abstract**

38 Faecal excretion of *Campylobacter* spp. and *Salmonella enterica* in sheep in Australia was
39 determined using a quantitative multiplex PCR (qPCR) targeting the *Campylobacter* spp. purine
40 biosynthesis gene (*PurA*) and the *S. enterica* outer membrane protein (*ompF*). The mutiplex qPCR
41 was specific and *Campylobacter* spp. and *S. enterica* were each detected with a sensitivity of 5
42 organisms/µL faecal DNA extract. This multiplex qPCR was used to determine the prevalence and
43 concentration of *Campylobacter* spp. and *S. enterica* in 3412 faecal samples collected from 1189
44 lambs on eight farms across South Australia (*n* = 2 farms), New South Wales (*n* = 1), Victoria (*n* =
45 2) and Western Australia (*n* = 3) at three sampling periods (weaning, post-weaning and pre-
46 slaughter). The overall prevalences of *Campylobacter* spp. and *S. enterica* were 13.3% and 5.0%,

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47 respectively, with the highest prevalence for *Campylobacter* spp. in South Australia and the highest
48 prevalence for *S. enterica* in New South Wales. *Campylobacter jejuni* was the only *Campylobacter*
49 sp. identified from a subset of 120 positive samples sequenced at the 16S locus. *S. enterica* serovar
50 Typhimurium were the only serovar of *S. enterica* identified from a subset of 120 positive samples
51 sequenced at the *ompF* locus. Across all states, *Campylobacter* spp. had the highest median
52 bacterial concentration in faeces at weaning and post-weaning (medians of 3.4 x $10^6$ and 1.1 x $10^5$,
53 respectively), whereas *S. enterica* had the highest median bacterial concentration at pre-slaughter
54 (1.8 x $10^5$/g faeces).

55

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

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59 **Introduction**

60 *Campylobacter* spp. remain the most commonly recognised aetiological agents of bacterial
61 gastroenteritis in many developed countries in both human beings and veterinary species, and are
62 the leading causes of bacterial food-borne gastroenteritis worldwide (Blaser, 1997; Allos et al.,
63 2004; Sproston et al., 2011; Sahin et al., 2012). The majority of human infections with
64 *Campylobacter* spp. are caused by *Campylobacter jejuni* (~90%), with ~10% caused
65 by *Campylobacter coli* (Sproston et al., 2011). Epidemiologically, contaminated chicken meat is
66 recognised as a main source of human *Campylobacter* spp. infections; however, the significance of
67 the ruminant reservoir in zoonotic *Campylobacter* spp. transmission is increasingly recognised
68 (Wilson et al., 2008; Greig and Ravel, 2009; Sheppard et al., 2009). Campylobacteriosis due to
69 infection with *Campylobacter* spp. (most commonly *C. jejuni)* has been identified as a cause of
70 enterocolitis in sheep in Australia and New Zealand (Stephens et al., 1984; Meanger et al., 1989;
71 Glastonbury, 1990; Gumbrell, 2000; Bailey et al., 2003).
72

73 Salmonellosis caused by *Salmonella enterica* is another major cause of gastroenteritis in a
74 range of species in the developed world (Vanselow et al., 2007). There are more than 2,500
75 serovars of *S. enterica*, each with a different host range and disease manifestation (Coburn et al.,
76 2007; Sojka et al., 1983; Glastonbury, 1990).
77

78 The epidemiology of *Campylobacter* spp. and *Salmonella enterica* in sheep and the
79 contamination of both surface water and lamb carcasses with these organisms is poorly understood
80 (Bailey et al., 2003; Stanley and Jones, 2003; Davies et al., 2004; Garcia et al., 2010a; 2010b).
81 These bacteria can be detected by microscopy, culture and immunoassays; however, these assays
82 can lack specificity and are time-consuming (Pawlowski et al., 2009; Leblanc-Maridor et al., 2011a
83 and b; Maciel et al., 2011). Additionally, *Campylobacter* spp. and *S. enterica* may enter into a

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84 viable but nonculturable state (VBNC) (Alexandrino et al., 2004; Oliver, 2005; Murphy et al., 2006;
85 Panutdaporn et al., 2006).
86
87 Quantitative PCR (qPCR) assays have been developed which have the added advantage of
88 being able to enumerate numbers of organisms present (Rothrock et al., 2009; Leblanc-Maridor et
89 al., 2011a and b; Maciel et al., 2011; McAuliffe et al., 2013). The majority of studies that have
90 examined the prevalence and faecal concentrations of these organisms in lambs in Australia have
91 relied on culture and/or immunological methods for detection (Bailey et al., 2003; Duffy et al.,
92 2010). Therefore the aim of the present study was to develop a multiplex qPCR for *Campylobacter*
93 spp. and *S. enterica* and to use the qPCR assay to determine the faecal prevalence and
94 concentrations of *Campylobacter* spp. and *S. enterica* in lambs over a wide geographical area
95 representing the major sheep growing regions of Australia. Specifically Western Australia (WA),
96 New South Wales (NSW), Victoria (Vic) and South Australia (SA), at three sampling periods
97 (weaning, post-weaning and pre-slaughter).
98

99 **Materials and methods**

100 *Animals and collection of faecal samples*

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

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

114

115 *PCR amplification, quantification and sequencing*

116 Primers and probes for *Campylobacter* spp. and *S. enterica* were designed using Primer 3[1].
117 A 121 base pair (bp) product was amplified from the *Campylobacter* spp. purine biosynthesis gene
118 (*PurA*) using the forward primer *PurA*F1 5'-CGCCCTTATCCTCAGTAGGAAA-3', the reverse
119 primer *PurA*R1 5'-TCAGCAGGCGCTTTAACAG-3' and the probe 5'- 6-carboxyfluorescein
120 (FAM)-AGCTCCATTTCCCACACGCGTTGC-3'. A 96 bp product was amplified from the *S.*
121 *enterica* outer membrane protein (*OmpF*) using the forward primer *OmpF*1 5'-
122 TCGCCGGTCGTTGTCCAT-3', the reverse primer *Omp*R1 5'-AACCGCAAACGCAGCAGAA-
123 3' and the probe 5'-2'7,'-dimeth-oxy-4'5,'-dichloro-6-carboxyfluorescein (JOE)-
124 ACGTGACGACCCACGGCTTTAC-3'.

125

126 An internal amplification control (IAC) consisting of a fragment of a coding region from
127 Jembrana disease virus (JDV) cloned into a pGEM-T vector (Promega) and IAC primers were used
128 as described previously (Yang et al., 2013). Each 15 µL PCR mixture contained 1x PCR buffer (10
129 mM Tris-HCl, 50 mM KCI), 4 mM $MgCl_2$, 1 mM deoxynucleotide triphosphates, 1.0 U KAPA
130 DNA polymerase (MolBio), 0.2 µM each forward and reverse primer, 0.2 µM each forward and
131 reverse IAC primers, 50 nM probe, 50 nM IAC probe, 10 copies IAC template and 1 µL sample
132 DNA. The PCR cycling conditions consisted of 95 °C for 3 min, then 45 cycles of 95 °C for 20 s
133 and 60 °C for 45 s.

134

[1] See: http://frodo.wi.mit.edu/

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135 A standard curve for quantifying *Campylobacter* spp. and *S. enterica* DNA was generated
136 by cloning the *purA* gene amplicon from *Campylobacter* spp. and the *ompF* gene amplicon from *S.*
137 *enterica* into pGEM-T (Promega) and transforming the recombinant vector into *Escherichia coli*
138 competent cells. Plasmid DNA for each bacteria was isolated by alkali sodium dodecyl sulphate
139 lysis, followed by column purification using QIAprep Spin Columns (Qiagen). Plasmid mini-
140 preparations were sequenced using the T7 sequencing primer (Stratagene) and clones with the
141 correct sequence then used as positive controls for generating a standard curve.
142
143 *Specificity and sensitivity*
144 The analytical specificity of the multiplex qPCR assays was assessed by testing DNA from
145 *C. jejuni, C. coli, S. enterica* serovar Typhimurium, *S. enterica* serovar Wandsbek II $21:_{z10:z6}$, *S.*
146 *enterica* serovar Bredeney, *S. enterica* serovar Muenchen, *S. enterica* serovar Adelaide, *S. enterica*
147 serovar Waycross, *S. enterica* serovar Infantis, *Chlamydia pecorum*, *Chlamydia abortus*, *Yersinia*
148 *enterocolitica*, *Streptococcus bovis* (ATCC 33317), *Enterococcus durans* (ATCC 11576),
149 *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Serratia marcescens* (ATCC
150 14756 pigmented), *Citrobacter freundii* (NCTC 9750), *Enterobacter cloacae* (ATCC 13047),
151 *Coxiella burnetii*, *Giardia duodenalis* assemblages A and E from sheep, *Cryptosporidium* sp. (*n* =
152 5) *Isospora* sp., *Tenebrio* sp., *Cyclospora* sp., *Toxoplasma gondii*, *Trichostrongylus colubriformis*,
153 *Teladorsagia circumcincta*, *Haemonchus contortus* and *Eimeria* sp., as well as human, sheep and
154 cattle genomic DNA.
155
156 To determine the sensitivity of the assay, 10-fold serial dilutions of plasmids containing the
157 cloned PCR products amplified from each of the two bacteria (*Campylobacter* spp. and *S. enterica*)
158 were prepared from 1,000,000 copies to 10 copies. These were then 'spiked' into faecal samples
159 and the DNA was extracted and amplified as described above. Mean detection limits, the coefficient
160 of determination R squared (RSQ) values and % relative standard deviation (RDS) were calculated.

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161 Template copy numbers were converted to numbers of organism present on the basis that *purA*
162 (*Campylobacter* spp.) and *ompF* (*S. enterica*) are single copy genes (Pearson et al., 2007;
163 Tatavarthy and Cannons, 2010; GenBank CP000814) and bacterial genomes are haploid. Therefore
164 the detected plasmid numbers were equivalent to the numbers of *Campylobacter* spp. and *S.*
165 *enterica*.
166
167 *Inhibition and efficiency*
168 Equal amounts of the IAC template (10 copies) were added to all faecal DNA samples to
169 detect any PCR inhibitors present in the extracted DNA. If inhibition was evident, then the sample
170 was diluted and re-amplified. Amplification efficiency (*E*, a measure of inhibition), was estimated
171 by using the slope of the standard curve and the formula $E = -1+10^{(-1/slope)}$ (Nybo, 2011). To
172 estimate amplification efficiency on faecal samples, serial dilutions of five individual DNA samples
173 (neat, 1:10, 1:100) were performed and multiple qPCR reactions were conducted on each dilution.
174 The *Ct* values were then plotted vs. the log base 10 of the dilution and a linear regression was
175 performed using the Rotor-Gene 6.0 software.
176
177 *Molecular typing*
178 A subset of five samples that were positive for *Campylobacter* spp. from each sampling on
179 each farm ($n$ = 120) were subjected to PCR for the *Campylobacter* spp. 16S rRNA gene (287 bp
180 amplicon) using primers and PCR conditions described by Lubeck et al. (2003). A subset of five
181 samples that were positive for *S. enterica* from each sampling on each farm ($n$ = 120) were
182 subjected to PCR for the *S. enterica ompF* gene (578 bp amplicon) using primers and PCR
183 conditions described by Tatavarthy and Cannons (2010). PCR products were separated by gel
184 electrophoresis and purified using an in-house filter tip method (Yang et al., 2013). Purified PCR
185 products were sequenced using an ABI Prism Dye Terminator Cycle Sequencing kit (Applied

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186 Biosystems) using an annealing temperature of 58 ºC. Nucleotide sequences were analysed using
187 Chromas Lite version 2.0[2] and aligned with reference sequences from GenBank using Clustal W[3].
188
189 *Statistical analysis*
190 Prevalences were expressed as the percentage of samples positive by PCR, with 95%
191 confidence intervals calculated assuming a binomial distribution, using the software Quantitative
192 Parasitology 3.0 (Rózsa et al., 2000). $\chi^2$ and analysis of variance (ANOVA) were performed using
193 SPSS 21.0 for Windows. A *P* value of 0.05 was used to declare statistical significance when
194 determining if there was any association between the prevalence and concentration (median value
195 from the positives in the individual population) of bacteria at different sampling times and across
196 states.
197
198 **Results**
199 *Specificity, sensitivity and efficiency*
200 Evaluation of specificity of the multiplex bacterial qPCR revealed no cross-reactions with
201 other genera; the qPCR only amplified the relevant bacterial species. Sensitivity analysis revealed
202 that the mean minimum detection for both *Campylobacter* spp. and *S. enterica* was 5 organisms/µL,
203 which equates to ~1250 organisms/g faeces. The mean RSQ values for *Campylobacter* spp. and *S.*
204 *enterica* were 0.99 and 0.99, respectively. The % RDS for *Campylobacter* spp. and *S. enterica* were
205 3.5% and 4.5%, respectively. The frequency of PCR inhibition, as determined by IAC
206 amplification, was ~2%. The mean efficiencies for *Campylobacter* spp. and *S. enterica* were
207 103.8% and 97.4%, respectively.
208
209 *Prevalence of Campylobacter spp. and Salmonella enterica*

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[2] See: http://www.technelysium.com.au.
[3] See: http://www.clustalw.genome.jp.

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210 The overall prevalences of *Campylobacter* spp. and *S. enterica* in faecal samples from lambs
211 were 13.3% and 5.0%, respectively (Table 2). The highest prevalence of *Campylobacter* spp. was in
212 faecal samples from lambs in SA (59% for SA2 and 44.6% for SA1, both from post-weaning
213 samplings). Overall, there were significant differences between the prevalence of bacterial
214 pathogens between states ($P < 0.01$ for *Campylobacter* spp.; $P < 0.05$ for *S. enterica*). The highest
215 prevalence of *S. enterica* in faecal samples from lambs was in NSW post-weaning (23.8%), which
216 was significantly higher than all three sampling times from the other farms and the pre-slaughter
217 periods from the NSW farm ($P < 0.05$). In general, the prevalence of *S. enterica* was lower than
218 *Campylobacter* spp. on all eight farms (Table 2). There was no relationship between prevalence and
219 time of sampling ($P > 0.05$).
220
221 *Concentration of Campylobacter spp.*
222 Across all states, the median concentration of *Campylobacter* spp. in lambs was $3.4 \times 10^6$
223 (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
224 faeces post-weaning and $1.5 \times 10^5$ (range 250 to $1.0 \times 10^7$) organisms/g faeces at pre-slaughter
225 (Table 2). The highest median number of *Campylobacter* spp. ($2.1 \times 10^8$ organisms/g faeces) was
226 detected on WA3 post-weaning, which coincided with the peak prevalence on this farm. The
227 highest number of *Campylobacter* spp. shed by an individual lamb was also on WA3 post-weaning
228 ($8.2 \times 10^{10}$ organisms/g). In SA, high median concentrations of *Campylobacter* spp. were detected
229 on SA1, SA2 and Vic2 at weaning ($9.4 \times 10^6$, $1.6 \times 10^9$ and $2.9 \times 10^6$ organisms/g faeces,
230 respectively); this coincided with the peak prevalence on Vic2 (26.7%), whereas the peak
231 prevalence on SA1 and SA2 occurred post-weaning.
232
233 *Concentration of Salmonella enterica*
234 Across all states, the median concentration of *S. enterica* in lambs was $9.3 \times 10^4$ (range 250
235 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-

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236 weaning and 1.8 x $10^5$ (range 250 to 1.1 x $10^8$) organisms/g faeces at pre-slaughter (Table 2). The
237 highest median number of *S. enterica* (7.4 x $10^7$ organisms/g faeces) was detected on SA2 at
238 weaning (Table 2).
239
240 *Identification of Campylobacter spp. and serovars of Salmonella enterica*
241 All *Campylobacter* spp. sequences (*n* = 120) were identified as *C. jejuni* (16S rRNA
242 sequence identical to GenBank CP001876) and all *S. enterica* sequences (*n* = 120) were identified
243 as *S. enterica* serovar Typhimurium (*ompF* sequence identical to GenBank Z31594).
244
245 **Discussion**
246 In this longitudinal study, a multiplex qPCR was used to determine the prevalence,
247 concentration and species or serovars of *Campylobacter* spp. and *S. enterica* in faecal samples
248 collected from lambs at weaning, post-weaning and pre-slaughter from eight farms across four
249 states in Australia. The multiplex qPCR assay was specific for *Campylobacter* spp. and *S. enterica*.
250 In addition, the *ompF* gene used as the qPCR target in *S. enterica* is thought to be restricted to this
251 bacterial species, which should increase the specificity of the assay (Tatavarthy and Cannons,
252 2010). However, qPCR does not differentiate between viable and non-viable organisms, which
253 would require culture.
254
255 The detection limits in this study (5 organisms/μL faecal DNA extract) are similar to or
256 better than published studies on qPCR detection without pre-enrichment. Previous studies on
257 *Campylobacter* spp. have reported detection limits of 10 genome copies for *C. jejuni* in porcine
258 faeces (Leblanc-Maridor et al., 2011a; 2011b) and five bacteria for *C. jejuni* in environmental water
259 samples (Rothrock et al., 2009). A qPCR based on the *SdfI* gene detected 32 genome copies of *S.*
260 *enterica* in faeces (Maciel et al., 2011). PCR is sometimes hindered by inhibitors in faecal samples,
261 (Wilson, 1997); however, in the present study, PCR inhibition (as determined by the IAC

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262 amplification) occurred in only ~2% of the examined samples during the qPCR assay. Using qPCR,
263 chronic shedders of high concentrations of bacteria can be identified, isolated and/or treated, and
264 slaughter postponed, preventing cross-contamination. The sensitivity of the assay could also be
265 increased with a pre-enrichment step.
266

267 In the present study, the overall prevalence of *Campylobacter* spp. and *S. enterica* in faecal
268 samples from lambs was 13.3% and 5.0%, respectively. However, the prevalence varied widely
269 among states and at different sampling points. As an example, the prevalence of *Campylobacter*
270 spp. was 15.4% at weaning in SA2, but 59% post-weaning. Similarly the prevalence of *S. enterica*
271 peaked at 23.8% in NSW post-weaning, but was only 3.6% during the pre-slaughter period. In a
272 study of slaughter age lambs in NSW and Queensland, 14/19 (73.7%) of all flocks tested were
273 positive for *C. jejuni* by culture and the prevalence in individual lambs was 8% (Bailey et al., 2003).
274 In Scotland, the prevalence of *Campylobacter* spp. by culture in ovine faeces was 14-49% (Garcia
275 et al., 2010a; Sproston et al., 2011).
276

277 In a national baseline microbiological survey of Australian sheep carcasses and frozen
278 boneless sheep meat conducted in 2004, *S. enterica* was isolated from 0/1117 carcases and from
279 3/560 (5.4%) samples of boneless product. *Campylobacter* spp. were isolated from 4/1117 (0.4%)
280 carcases and from 1/560 (0.2%) boneless samples (Phillips et al., 2006). However, it is important to
281 note that low or no recovery rates of these organisms in faeces and carcasses during slaughter may
282 be due to the limited sensitivity of culture methods (Fredriksson-Ahomaa and Korkeala, 2003). In a
283 recent study, which compared qPCR and culture methods for the detection of *S. enterica* in faeces,
284 45 samples that were positive using the PCR assay were negative on culture (Maciel et al., 2011).
285

286 In the present study, the prevalence of *S. enterica* was low across all states (0-10.7%), with
287 the exception of NSW, where prevalences peaked at 23.8 and 18.1% at post-weaning and weaning,

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288 respectively. The prevalence of *S. enterica* in the faeces of healthy slaughtered sheep appears to
289 vary widely. In a recent study of 486 samples from 164 sheep and lambs at two abattoirs in
290 Australia, *S. enterica* was isolated from 20% of faeces, 13% of fleeces and 1.3% of pre-chill
291 carcasses (Duffy et al., 2010). Previous studies have reported prevalences ranging from 0.1% in the
292 UK (Davies et al., 2004) to 42% in Australia (Samuel et al., 1981).
293
294 Bacterial concentrations in faeces were also determined by qPCR. For *S. enterica*, the
295 pathogen load was relatively low across all farms, with the exception of SA2 at weaning, where,
296 although the shedding was high, the prevalence was low (1.7%). The highest median number of
297 *Campylobacter* spp. detected was at WA3 post-weaning (2.1 x $10^8$ organisms/g faeces), although
298 individuals at SA2 shed up to 1.6 x $10^9$ organisms/g faeces. Little is known about bacterial shedding
299 of *Campylobacter* spp. in sheep; the reasons for the high *Campylobacter* spp. output in some lambs
300 are unknown, but may be due to stress or to multiple infections reducing host immunity, since these
301 sheep were known to be co-infected with *Giardia* spp., *Cryptosporidium* spp., *Eimeria* spp.,
302 *Chlamydia pecorum, Yersinia* spp. and strongyles (Yang et al., 2014a, b, c, d).
303
304 *Campylobacter jejuni* and *S. enterica* serovar Typhimurium were the only species identified
305 from a subset of 120 positive samples for each bacteria. However, since only a subset of positive
306 samples was sequenced, other species and serovars may also be present. A previous study of 55 *S.*
307 *enterica* isolates from Australian sheep at slaughter identified 11 different serovars (Duffy et al.,
308 2010). In Scotland, *C. jejuni* accounted for 75% of all the positive samples from sheep sent for
309 slaughter, followed by *C. coli* (16%), *Campylobacter upsaliensis* (2.52%) and *Campylobacter fetus*
310 (1.26%) (Garcia et al., 2010a).
311
312 **Conclusions**

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313 *Campylobacter* spp. and, to a lesser extent, *S. enterica*, are prevalent in lambs in Australia
314 and high levels of bacterial shedding are present in some animals. Further studies are required to
315 determine the extent of production losses associated with these bacterial infections in sheep.
316

317 **Conflict of interest statement**

318 The study was financed by Meat and Livestock Australia (MLA), Australian Wool
319 Innovation Limited (AWI) and the Australian Government, which had no influence on study design,
320 data evaluation or manuscript preparation. None of the authors has any other financial or personal
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322

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329 samples collected from the eastern states.
330

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509 **Table 1** Sheep farms sampled during the present study.
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| Farm | Farm location | Mean annual rainfall (mm) | Farm size (Ha) | Number of sheep | Breed | Commencement of lambing | Winter stocking rate (DSE/Ha) |
|---|---|---|---|---|---|---|---|
| SA1 | Wirrega, SA | 430 | 1040 | 1800 | Suffolk | April | 10 |
| SA2 | Struan, SA | 550 | 1500 | 5500 | BL/Merino x Suffolk | June | 15 |
| Vic1 | Rosedale, Vic | 620 | 300 | 300 [a] | BL/Merino x Dorset and Southdown | July | 10 |
| Vic2 | Ballarat, Vic | 750 | 1960 | 7000 | Merino x Suffolk | August | 13 |
| NSW | Armidale, NSW | 495 | 2958 | 1000 | BL/Merino | May-August | 20 |
| WA1 | Pingelly, WA | 450 | 1500 | 1350 | Merino x Suffolk | July | 12 |
| WA2 | West Arthur, WA | 500 | 1250 | 1750 | Merino x Suffolk | August | 10 |
| WA3 | Frankland, WA | 550 | 560 | 3300 | Merino x Suffolk | July | 21 |

512
513 DSE, dry sheep equivalent; BL, Border Leicester; SA, South Australia; Vic, Victoria; NSW, New South Wales, WA, Western Australia.
514 [a] Number of ewes.
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516 **Table 2** Prevalence (%) and quantity (organisms/g) of *Campylobacter* spp. and *Salmonella enterica* in faeces collected
517 from sheep on eight farms in four states of Australia over three sampling periods as determined by qPCR.

| Farm | Sampling period | *Campylobacter* spp. Prevalence (%) | *Campylobacter* spp. Range (organisms/g) | *Campylobacter* spp. Median (organisms/g) | *Salmonella enterica* Prevalence (%) | *Salmonella enterica* Range (organisms/g) | *Salmonella enterica* Median (organisms/g) |
|---|---|---|---|---|---|---|---|
| | Weaning | 16.4 (10.7-22.0) | $3.1 \times 10^4$-$6.2 \times 10^9$ | $9.4 \times 10^6$ | 2.4 (0.1-4.8) | $6.3 \times 10^3$-$1.7 \times 10^7$ | $2.1 \times 10^5$ |
| SA1 | Post-weaning | 44.6 (36.6-52.6) | 750-$9.4 \times 10^7$ | $1.6 \times 10^5$ | 1.4 (0.0-3.2) | $4.5 \times 10^3$-$2.5 \times 10^6$ | $8.8 \times 10^3$ |
| | Pre-slaughter | 27.7 (20.7-34.6) | $3.8 \times 10^3$-$2.4 \times 10^6$ | $1.7 \times 10^5$ | 1.3 (0.0-3.0) | $4.5 \times 10^3$-$2.8 \times 10^5$ | $1.4 \times 10^5$ |
| | Weaning | 15.4 (9.9-20.8) | $3.0 \times 10^3$-$1.6 \times 10^9$ | $6.5 \times 10^6$ | 1.9 (0.0-4.0) | $6.3 \times 10^3$-$1.7 \times 10^7$ | $7.4 \times 10^7$ |
| SA2 | Post-weaning | 59.0 (51.3-66.7) | 250-$1.1 \times 10^8$ | $1.9 \times 10^4$ | 9.6 (5.0-14.2) | 250-$1.5 \times 10^7$ | $4.4 \times 10^4$ |
| | Pre-slaughter | 31.3 (23.8-38.8) | 750-$1.0 \times 10^7$ | $2.1 \times 10^5$ | 2.7 (0.1-5.4) | 250-$6.9 \times 10^4$ | $2.1 \times 10^3$ |
| | Weaning | 0.6 (0.0-1.6) | $1.3 \times 10^3$-$1.3 \times 10^3$ | $1.3 \times 10^3$ | 2.2 (0.1-4.4) | 250-$1.2x10^3$ | 500 |
| Vic1 | Post-weaning | 2.3 (0.1-4.6) | $8.1 \times 10^5$-$4.3 \times 10^8$ | $1.3 \times 10^6$ | 2.3 (0.1-4.6) | $1.3 \times 10^3$-$6.3 \times 10^3$ | $6.3 \times 10^3$ |
| | Pre-slaughter | 3.8 (0.8-6.7) | 250-$8.1 \times 10^4$ | $1.4 \times 10^4$ | 3.1 (0.4-5.8) | $3.8 \times 10^3$-$3.7 \times 10^5$ | $2.4 \times 10^4$ |
| | Weaning | 26.7 (20.2-33.2) | 250-$3.4 \times 10^{10}$ | $2.9 \times 10^6$ | 5.7 (2.3-9.1) | 250-$7.8 \times 10^5$ | $1.3 \times 10^4$ |
| Vic2 | Post-weaning | 2.3 (0.1-4.6) | $6.9 \times 10^4$-$6.3 \times 10^6$ | $2.5 \times 10^5$ | 5.2 (1.9-8.5) | 250-$7.5 \times 10^6$ | $1.9 \times 10^4$ |
| | Pre-slaughter | ND | ND | ND | 3.1 (0.1-6.1) | $3.8x10^3$-$3.7x10^5$ | $2.4x10^4$ |
| | Weaning | 4.4 (1.2-7.5) | $4.8 \times 10^3$-$6.8 \times 10^6$ | $3.4 \times 10^5$ | 18.1 (12.2-24.1) | $5.5 \times 10^3$-$4.5 \times 10^6$ | $7.8 \times 10^4$ |
| NSW | Post-weaning | 4.4 (1.2-7.5) | $3.0 \times 10^3$-$7.2 \times 10^6$ | $2.1 \times 10^4$ | 23.8 (17.2-30.3) | 250-$7.3 \times 10^6$ | $1.2 \times 10^5$ |
| | Pre-slaughter | 1.2 (0.0-2.8) | 931-$1.1 \times 10^7$ | $6.1 \times 10^3$ | 3.6 (0.8-6.4) | $1.8 \times 10^3$-$1.6 \times 10^7$ | $3.0 \times 10^5$ |
| | Weaning | 10.5 (5.1-15.9) | $2.0 \times 10^3$-$5.7 \times 10^8$ | $7.1 \times 10^5$ | 4.0 (0.6-7.5) | 250-$1.2x10^3$ | 750 |
| WA1 | Post-weaning | 4.1 (0.6-7.6) | $6.3 \times 10^3$-$1.4 \times 10^5$ | $3.2 \times 10^4$ | 6.6 (2.2-10.9) | $1.4 \times 10^4$-$9.2 \times 10^5$ | $2.3 \times 10^5$ |
| | Pre-slaughter | 5.8 (1.6-9.9) | $4.0 \times 10^3$-$5.0 \times 10^6$ | $1.7 \times 10^5$ | 10.7 (5.2-16.3) | $1.5 \times 10^3$-$1.1 \times 10^8$ | $6.6 \times 10^6$ |
| | Weaning | 12.1 (6.0-18.3) | 475-$2.9 \times 10^5$ | $5.4 \times 10^4$ | 4.7 (0.7-8.7) | $1.8 \times 10^5$-$5.0 \times 10^8$ | $7.4 \times 10^6$ |
| WA2 | Post-weaning | 4.6 (0.7-8.5) | $1.3 \times 10^3$-$7.5 \times 10^7$ | $1.7 \times 10^5$ | 2.8 (0.0-5.8) | $5.6 \times 10^4$-$7.1 \times 10^5$ | $1.3 \times 10^5$ |
| | Pre-slaughter | 9.3 (3.8-14.9) | $4.0 \times 10^3$-$5.0 \times 10^6$ | $1.7 \times 10^5$ | ND | ND | ND |
| | Weaning | ND | ND | ND | 4.0 (0.2-7.8) | $5.8 \times 10^5$-$8.3 \times 10^6$ | $1.6 \times 10^6$ |
| WA3 | Post-weaning | 15.8 (8.7-23.0) | $4.5 \times 10^3$-$8.2 \times 10^{10}$ | $2.1 \times 10^8$ | 2.0 (0.0-4.7) | $2.1 \times 10^4$-$3.7 \times 10^6$ | $3.3 \times 10^5$ |
| | Pre-slaughter | 5.0 (0.7-9.3) | 750-$6.4 \times 10^4$ | $1.1 \times 10^4$ | ND | ND | ND |
| Total | | 13.3 (12.1-14.4) | 0-$8.2 \times 10^{10}$ | $3.9 \times 10^5$ | 5.0 (4.3-5.7) | 0-$5.0 \times 10^8$ | $9.4 \times 10^4$ |

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519 ND, not detected.

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