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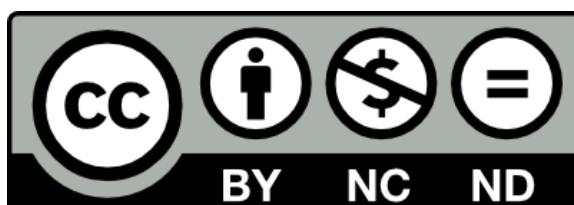
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Greater intensity and frequency of *Cryptosporidium* and *Giardia* oocyst shedding beyond the neonatal period is associated with reductions in growth, carcass weight and dressing efficiency in sheep

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Highlights

High shedding intensity associated with reduced live weight and carcass productivity
Increased frequency of detection associated with reduced carcass productivity *Giardia* and *Cryptosporidium* associated with reduced production after weaning Intensity and timing of shedding relative to slaughter impact carcass outcomes

Abstract

Associations between intensity and frequency of *Cryptosporidium* and *Giardia* shedding with growth, carcass weight and dressing % were investigated using a longitudinal study of 1,182 lambs on eight Australian farms. Live weight was recorded and faecal samples were collected on three sampling occasions; weaning (approximately 12 weeks of age), post-weaning (approximately 19 weeks) and pre-slaughter (approximately 29 weeks). Hot standard carcass weight (HSCW) and dressing % were measured at slaughter. Faecal samples were screened for presence and concentration of *Cryptosporidium*, *Giardia* and *Haemonchus* oocysts using a quantitative PCR. Trichostrongylid eggs were quantified with modified McMaster faecal worm egg count (WEC). Protozoan shedding intensity was categorised as high (above median oocyst concentration in positive sheep), low (below median oocyst concentration in positive sheep) or not detected. Shedding was also categorised for shedding type (no shedding, single *Giardia* infection, single *Cryptosporidium* infection, concurrent *Giardia* and *Cryptosporidium* infection) and lambs were categorised for frequency of shedding (shedding identified on 0, 1, 2 or 3 occasions). Associations of parasite shedding intensity category, shedding type, shedding frequency, WEC and *Haemonchus* status (positive or negative) with lamb production were assessed using general linear models (HSCW and dressing %) and linear mixed effects models (live weight). High *Cryptosporidium parvum* shedding was associated with lower live weight, ranging 2.31-4.52kg over the 3 sampling occasions. *Cryptosporidium parvum* shedding was associated

with less HSCW in high (3.22kg less) and low (3.22kg less) shedding lambs post-weaning, and high (2.21kg less) and low (2.60kg less) shedding lambs pre-slaughter as well as lower dressing % (2.7% lower in high shedding lambs post-weaning). *Cryptosporidium* (all species) shedding pre-slaughter was associated with reduced dressing % in both high (1.25% lower) and low (1.21% lower) shedding lambs. *Giardia* shedding pre-slaughter was associated with 0.59kg less HSCW in high shedding lambs. Increased frequency of *C. parvum* and *Giardia* shedding in a specific animal (repeated detection) were associated with reduced HSCW and dressing %. Concurrent *Giardia* and *Cryptosporidium* shedding pre-slaughter was associated with reduced dressing %. No statistically significant main effects for either WEC ($P>0.05$) or *Haemonchus* status ($P>0.05$) were identified for any of the sheep meat productivity measures (live weight, HSCW and dressing %). The findings suggest naturally acquired *Cryptosporidium* and *Giardia* infections in grazing sheep are associated with depressed growth, carcass weight and dressing efficiency beyond the neonatal period in sheep representing a range of genetic backgrounds and different sheep production environments.

Abbreviations:

HSCW: hot standard carcass weight

WEC: Faecal worm egg count

Keywords:

Cryptosporidium; *Giardia*; lambs; qPCR; production parameters; hot standard carcass weight (HSCW); dressing percentage

1. Introduction

Intestinal parasitism is widely recognised as an important cause of reduced production in livestock, including sheep, worldwide. The production and welfare impacts of helminthosis are relatively well understood, but the role of protozoan intestinal parasites in livestock productivity is not well described.

Cryptosporidium spp. and *Giardia* spp. are gastro-intestinal protozoa that affect a wide range of mammals (Geurden et al., 2008; Feng and Xiao, 2011), including sheep. The prevalence of *Cryptosporidium* and *Giardia* in sheep varies between studies conducted worldwide but for *Cryptosporidium* generally ranges from 15%-27% in lambs (Santín et al., 2007; Robertson et al., 2010; Ye et al., 2013; Yang et al., 2014a) and for *Giardia* generally ranges from 1.5–55.6% (Feng and Xiao, 2011). The genus *Cryptosporidium* consists of 26 valid species and more than 50 genotypes with *C. xiaoi*, *C. ubiquitum* and *C. parvum* most frequently identified in sheep (Ryan et al., 2005; Santín et al., 2007; Soltane et al., 2007, Geurden et al., 2008, Mueller-Doblies et al., 2008, Quílez et al., 2008, Fayer and Santín, 2009; Giles et al., 2009; Paoletti et al., 2009, Yang et al., 2009; Díaz et al., 2010; Robertson et al., 2010; Wang et al., 2010; Fiuza et al., 2011; Shen et al., 2011; Sweeny et al., 2011; Sweeny et al., 2012a; Cacciò et al., 2013; Connelly et al., 2013; Imre et al., 2013; Yang et al., 2014a). *Giardia duodenalis* is the species infecting mammals and consists of eight major genetic groups (assemblages), two of which (A and B) are found in both humans and animals (including sheep) and are considered zoonotic, whereas the remaining six (C–H) are host-specific and do not infect humans (Feng and Xiao, 2011; Ryan and Caccio, 2013). The most commonly reported genotypes in sheep are assemblage E (livestock genotype) and assemblage A (van Keulen et al., 2002; Lalle et al., 2005; Ryan et al., 2005; Yang et al., 2009). Assemblage B has been less commonly reported in sheep (Aloisio et al., 2006).

As sheep may contribute significantly to contamination of watercourses, the majority of studies conducted on *Cryptosporidium* and *Giardia* in sheep to date have focused on the

prevalence of zoonotic species to better understand the public health risk posed by infections in sheep. However, little research has been conducted on the clinical and production impacts of *Cryptosporidium* or *Giardia* in sheep. Cryptosporidiosis and giardiasis in lambs has been associated with clinical symptoms such as severe diarrhoea, depression, weight loss and mortality (O’Handley and Olson, 2006; Geurden et al., 2008). Sweeny et al. (2012b) previously identified associations between *Cryptosporidium* and *Giardia* with reduced lamb carcase productivity in flocks on two farms in Western Australia, but an important limitation of that study was that only qualitative (not quantitative) data on presence of *Cryptosporidium* and *Giardia* in faeces (shedding) was available. The relationship between both presence and magnitude of protozoan parasite infection with sheep productivity across a wider geographical area has not been described. Therefore the aim of the present study was to investigate associations between intensity of *Cryptosporidium* and *Giardia* shedding (between weaning and slaughter) and growth and carcase productivity in lambs across four different sheep producing regions in Australia.

2. Materials and Methods

2.1 Animals, faecal sample collection and production parameters

The selection of animals included in this experiment and sample collection methods have been previously described (Yang et al., 2014a; 2014b). In brief, lambs from eight farms were included in this study (Table 1). Farms were located in Western Australia (WA), New South Wales (NSW), Victoria (Vic) and South Australia (SA). Sheep were kept in paddocks (i.e. not confined to feedlot or indoor housing) and were managed under normal conditions for commercial sheep meat production in each district. Lambs were mixed breed, with the exception of SA1 (Table 1). Sires of lambs were British breeds (Suffolk, Dorset, Southdown

or Border Leicester) and dams were either Merino, Border Leicester-Merino or Suffolk (Table 1).

Sampling was based on a longitudinal study design with each lamb sampled on three occasions (i.e the same animals were sampled on each occasion), specifically; weaning (approximately 12 weeks old), post-weaning (approximately 19 weeks old) and pre-slaughter (approximately 29 weeks old). Within cohorts, age was estimated to range up to 8 weeks depending on duration of lambing. Lambs were weighed (live weight) and faecal samples were collected directly from the rectum at each sampling occasion. Over these three sampling occasions, 3,412 faecal samples were collected from 1189 lambs. Faecal samples were collected using a gloved hand or a sterile swab (weaning sample SA1, SA2 and NSW only). Faecal samples were chilled (on ice) during storage and transport to the laboratory, and then stored in the refrigerator (4.0°C).

Sheep were slaughtered at commercial abattoirs. All sheep were classified as “lamb” at slaughter with no eruption of permanent incisor teeth (AUS-MEAT 2005). Hot standard carcass weight (HSCW) was measured for all lambs at slaughter based on AUS-MEAT definition (AUS-MEAT 2005). Dressing percentage (%) was calculated using HSCW divided by pre-slaughter live weight x 100.

All procedures were approved and monitored by Murdoch University Animal Ethics Committee (approval number R2352/10).

[INSERT TABLE 1]

2.2. Faecal worm egg counts

Where sufficient quantity of faecal material was available, faecal worm egg counts (WEC) were performed using a modified McMaster technique (Lyndal-Murphy, 1993). Two grams of faeces were used from each sample and each egg counted represented 50 eggs/g

(epg) of faeces. Insufficient faecal material was available for WEC for some samples, predominantly at the first sampling (weaning).

2.3 DNA isolation

Genomic DNA was extracted from 250mg of each faecal sample using a Power Soil DNA Kit (MolBio, Carlsbad, California). A negative control (no faecal sample) was used in each extraction group.

2.4 PCR amplification - *Cryptosporidium* and *Giardia*

All samples were screened at the actin locus for *Cryptosporidium* and *Giardia* at the glutamate dehydrogenase (*gdh*) locus using quantitative PCR (qPCR) previously described (Yang et al., 2014a; 2014b). An internal amplification control (IAC) which consisted of a fragment of a coding region from Jembrana Disease Virus cloned into a pGEM-T vector (Promega, USA), was used as previously described (Yang et al., 2013). All *Cryptosporidium* positives were screened using a *C. parvum* and *C. hominis* specific qPCR at a unique *Cryptosporidium* specific gene (Clec) that codes for a novel mucin-like glycoprotein that contains a C-type lectin domain (CTLTD) previously described (Morgan et al., 1996; Yang et al., 2009; Bhalchandra et al., 2013). *Cryptosporidium* and *Giardia* oocysts per gram of faeces (g^{-1}) were measured by qPCR as previously described (Yang et al., 2014a; 2014b). PCR contamination controls were used including negative controls and separation of preparation and amplification areas. All *Cryptosporidium* positives were sequenced for confirmation as described (Yang et al., 2014a). *Giardia* positives were identified to assemblage level using assemblage specific primers at the triose phosphate isomerase (*tpi*) locus as previously described (Geurden et al., 2008).

Sensitivity analysis for the *Cryptosporidium* qPCR revealed that the assay could reliably detected eight copies of the cloned *C. xiaoi* amplicon per μ l of faecal DNA extract

which is equivalent to a sensitivity of two *Cryptosporidium* oocysts per μl of faecal DNA extract. The mean RSQ (R squared) was 0.99 and the % Relative Standard Deviation (RDS) = 1.5% (Yang et al., 2014a). The *Cryptosporidium* actin qPCR described was also evaluated using haemocytometer counted oocysts and also flow-cytometry counted oocysts on clinical cryptosporidiosis samples and again found to be both precise and reliable (Yang et al., 2014c).

Sensitivity analysis for the *Giardia* qPCR revealed that the assay reliably detected four copies of the cloned Assemblage E amplicon per μl of faecal DNA extract which is equivalent to a sensitivity of one *Giardia* cyst per μl of faecal DNA extract. The mean RSQ (R squared) was 0.98 and the % Relative Standard Deviation (RDS) = 5.5% (Yang et al., 2014b).

DNA from samples from Western Australia was previously analysed using different primers as described in Sweeny et al. (2011).

2.5 PCR amplification – *Haemonchus contortus*

The presence of *Haemonchus contortus* DNA in faecal samples was determined using a multiplex qPCR that included *H. contortus*, *Teladorsagia circumcincta* and *Trichostrongylus* spp.

The primers and probe for *Haemonchus* were modified from Harmon et al. (2007). A fragment (92 bp) of the *Haemonchus* rRNA ITS 1 region was amplified using the forward primer HC-ITS F1 5' CATATACATGCAACGTGATGTTATGAA 3', the reverse primer HC-ITS R1 5' GCTCAGGTTGCATTATACAAATGATAAA 3' and the probe 5' FAM-ATGGCGACGATGTTC - BHQ-1' (Biosearch Technologies, Novato, CA, USA). Each 15 μl PCR mixture contained 1 \times PCR Buffer, 3 mM MgCl_2 , 1 μl of 2.5nM dNTP's, 1.0 U Kapa DNA polymerase (MolBio, Carlsbad, California), 0.2 μM each of forward and reverse primers, 0.1mM probe, 0.2 μM each of forward and reverse IAC primers, 50 nM of the

probe, 10 copies of IAC template and 1 µl of sample DNA. The PCR cycling conditions consisted of a pre-melt at 95°C for 3 min and then 45 cycles of 95°C for 30 sec, and a combined annealing and extension step of 60°C for 45 sec.

The analytical specificity of the multiplex qPCR assay was assessed by testing DNA from a range of nematode, protozoan and bacterial species as well as human, sheep and cattle DNA and revealed no cross-reaction with other genera and only amplified the relevant strongyle species. Sensitivity analysis was conducted as described in section 2.4 and equated to a minimum detection of 10 epg. The mean R squared for *H. contortus* qPCR was 0.98 and the % relative standard deviation for *H. contortus* qPCR was 0.93. The mean efficiency for the *H. contortus* qPCR was 107.3%.

2.6 Statistical analysis

Protozoan parasite shedding for each lamb at each timepoint was categorised as high (faecal oocyst shedding above median for positive samples for the farm/timepoint), low (faecal oocyst shedding below median for positive samples for the farm/timepoint) or not detected (no evidence of faecal oocyst shedding) separately for *Cryptosporidium* spp. (including *C. parvum*), *C. parvum* and *Giardia* spp. Lambs were categorized by frequency of parasite detection (parasite detected on 0, 1, 2 or 3 occasions) separately for *Cryptosporidium* spp. (including *C. parvum*), *C. parvum* and *Giardia* spp. Lambs were also categorised for parasite status (no infection, *Giardia* only, *Cryptosporidium* only, mixed *Giardia* and *Cryptosporidium*) at each sampling occasion. Worm egg count was log transformed for analyses using Log₁₀(WEC+25). Individual sheep were categorised for *Haemonchus* presence/absence for each occasion based on qPCR.

Associations between protozoan parasites with HSCW and dressing % were analysed using general linear models (SAS Version 9.2, SAS Institute, Cary, NC, USA), with site (ie farm locations) and kill group (where sheep in each flock were consigned for slaughter over

more than one date) included as fixed effects and WEC included as a covariate. Within this base model parasite shedding category (high, low or not detected) of either *Cryptosporidium* spp, *C. parvum* or *Giardia* spp. within each of the three different time points (weaning, post-weaning and pre-slaughter) was incorporated as a fixed effect equating to a total of 9 separate models (i.e. 3 parasites x 3 time points). The WEC covariate was not significant ($P>0.100$) for any model so this was excluded from subsequent models presented in this study.

The associations between trichostrongylid parasites with HSCW and dressing % were analysed using general linear models (SAS Version 9.2, SAS Institute, Cary, NC, USA) separately for each of the three different time points (weaning, post-weaning and pre-slaughter), with log transformed WEC, *Haemonchus* status (presence/absence), site and kill group included as fixed effects.

Live weight measurements were recorded for each animal at each sampling occasion, so this was analysed in a single linear mixed effects model (SAS Version 9.2, SAS Institute, Cary, NC, USA) for each parasite (i.e. each model was for a specific parasite but included all 3 time points). For protozoan parasites, the linear mixed effects models included the shedding category (high, low or not detected), site (farm location) and sampling occasion (weaning, post-weaning or pre-slaughter) each included as fixed effects, WEC included as a covariate, and animal identification (ID) included as a random term to account for the multiple sampling of individuals over time. The WEC covariate was not significant ($P>0.100$) so this was excluded from subsequent models presented in this study. For trichostrongylid parasites, the linear mixed effects models included log transformed WEC, *Haemonchus* status (presence/absence), site and sampling occasion included as fixed effects and animal identification as a random term.

Associations between HSCW and dressing % with frequency of parasite detection were assessed separately for each parasite using linear mixed effects models with frequency

of detection (0, 1, 2 or 3 positive occasions) and site (i.e. farm locations) included as a fixed effects. Least square means were generated for all linear mixed effects models.

Associations between HSCW and dressing % with parasite status were assessed separately for each timepoint using general linear models with parasite status (no infection, *Giardia* only, *Cryptosporidium* only, mixed *Giardia* and *Cryptosporidium*), site (i.e. farm locations) and kill group included as a fixed effects. Associations between liveweight with parasite status were assessed in a single linear mixed effects model with parasite status (no infection, *Giardia* only, *Cryptosporidium* only, mixed *Giardia* and *Cryptosporidium*), site (i.e. farm locations) and timepoint included as a fixed effects, and animal ID included as a random term. Least square means were generated for all general linear models.

3 Results

3.1 Parasite prevalence and faecal shedding

Cryptosporidium spp. were identified in 16.9% of faecal samples (Table 2) with prevalence and faecal oocyst concentration previously reported in greater detail (Yang et al., 2014a). *Cryptosporidium parvum* was identified in 9.8% (49/500) of *Cryptosporidium*-positive samples, and mixed *C. parvum* and *C. xiaoi* infections were identified in 2.4% (12/500) of positive samples (Yang et al., 2014a). *Giardia* spp. were identified in 20.2% of faecal samples (Table 3), with prevalence and faecal oocyst concentration previously reported in greater detail (Yang et al., 2014b).

[INSERT TABLE 2]

[INSERT TABLE 3]

3.2 Associations of *Cryptosporidium* (all species) with lamb growth, carcass weight and dressing percentage

Cryptosporidium spp. shedding at pre-slaughter was associated with about 1.2% lower dressing %, within both high shedding ($P < 0.01$) and low shedding ($P < 0.01$) lambs compared to non-shedding lambs (Table 4). The impact of high and low shedding categories on dressing % did not differ from each other. In contrast there was no association between *Cryptosporidium* spp. shedding category and dressing % at either weaning or post-weaning. There was also no association between *Cryptosporidium* spp. shedding category and either HSCW (at any of the sampling time points) or live weight.

[INSERT TABLE 4]

3.3 Associations of *C. parvum* with lamb growth, carcase weight and dressing percentage

Cryptosporidium parvum shedding at pre-slaughter was associated with between 2.2 – 2.6kg lower HSCW, in both high shedding ($P<0.01$) and low shedding lambs ($P<0.01$) compared to non-shedding lambs (Table 4). The impact on HSCW did not differ between these two groups.

This HSCW effect was also reflected at the post-weaning time point, but was only evident in the high shedding group where high *C. parvum* shedding was associated with about 3.2kg lower HSCW ($P<0.01$) compared to non-shedding lambs (Table 4). At weaning there was no association between *C. parvum* shedding category and HSCW.

For dressing %, the only association evident with *C. parvum* shedding category was observed at post-weaning where the high shedding group had 2.7% lower dressing ($P<0.01$) than the non-shedding lambs (Table 4).

Cryptosporidium parvum shedding category was associated with lower live weight (Table 4). Compared with non-shedding lambs, high shedding lambs were 2.78kg lighter at weaning ($P<0.01$), 4.52kg lighter at post-weaning ($P<0.01$) and 2.31kg lighter at slaughter ($P<0.01$; Table 4). Live weight in low shedding lambs were 2.78kg lighter at slaughter than non-shedding lambs at weaning ($P=0.058$).

3.4 Associations of *Giardia* spp. with lamb growth, carcase weight and dressing percentage

Giardia shedding at post-weaning was associated with lower HSCW, with HSCW reductions of 0.59kg in high shedding lambs relative to non-shedding lambs ($P<0.05$; Table 4). High *Giardia* shedding lambs had 0.66kg lower HSCW relative to low shedding lambs ($P=0.053$) at post-weaning.

There were no associations between *Giardia* spp. shedding category and HSCW at weaning or pre-slaughter, nor between *Giardia* spp. shedding category and either dressing % (any time point) or live weight.

3.5 Associations of frequency of parasite detection with carcass weight and dressing percentage

Increased frequency of detection of *C. parvum* and *Giardia* were both associated with reductions in HSCW and dressing % (Table 5). Specifically, detection of *C. parvum* on three occasions was associated with 4.02kg lower HSCW ($P<0.001$) and 3.85% lower dressing % ($P<0.05$) compared with lambs in which *C. parvum* was never detected. Detection of *C. parvum* on two occasions was associated with 2.59kg lower HSCW ($P<0.05$) compared with detection on one occasion, and *C. parvum* detection on one occasion was associated with 1.43kg lower HSCW ($P<0.001$) compared with lambs in which *C. parvum* was never detected. A similar pattern (albeit with smaller magnitude of difference) was observed for *Giardia* spp. with detection on three occasions associated with 0.93kg lower HSCW ($P=0.065$) and 1.41% lower dressing % ($P<0.05$) compared with lambs in which *Giardia* was never detected, and detection on two occasions was associated with 0.82kg lower HSCW ($P<0.01$) and 0.63% lower dressing % ($P=0.086$) compared with lambs in which *Giardia* was never detected.

[INSERT TABLE 5]

3.5 Association of mixed infections with carcass weight, dressing percentage and live weight

There was no significant main effect ($P>0.100$) of infection type (no infection, single *Giardia* infection, single *Cryptosporidium* infection, mixed *Giardia* and *Cryptosporidium* infection) on live weight, HSCW (for any sampling occasion) or dressing % at weaning or

post-weaning. There was an association between infection type at pre-slaughter and dressing % ($P < 0.01$) whereby lambs with shedding *Cryptosporidium* only (44.79%) or mixed *Giardia* and *Cryptosporidium* (44.29%) had lower dressing % than lambs with no infection (45.77%). Similarly, lambs shedding mixed *Giardia* and *Cryptosporidium* (44.29%) had lower dressing % than lambs shedding *Giardia* only (45.36%).

3.6 Association between WEC and *Haemonchus* status with carcass weight, dressing percentage and live weight

Faecal WEC and prevalence of *H. contortus* (by qPCR) are shown in Table 6. The mean WEC for all farms and timepoints were below 1000 eggs per gram, suggesting that whilst *H. contortus* was identified by qPCR on all 8 farms included in the study, clinical haemonchosis was unlikely at a flock level.

No statistically significant main effects for either WEC ($P > 0.05$) or *Haemonchus* status ($P > 0.05$) were identified for any of the sheep meat productivity measures (liveweight, HSCW and dressing %). Trends were observed towards a negative association between WEC and liveweight ($P = 0.086$), WEC at weaning and dressing % ($P = 0.069$) and *Haemonchus* status at slaughter and dressing % (no *Haemonchus* 45.4% vs *Haemonchus* present 46.1%; $P = 0.057$).

[INSERT TABLE 6]

4 Discussion

This is the first report of associations for intensity and frequency of faecal protozoan shedding with growth and carcass productivity in sheep. High *C. parvum* shedding was associated with reduced live weight, HSCW and dressing %. High *Cryptosporidium* (all

species) shedding was associated with reduced dressing % and high *Giardia* shedding was associated with reduced HSCW. Relationships between protozoan shedding intensity and productivity were observed at specific time points that varied between the parasites. Importantly, high shedding post-weaning and pre-slaughter were associated with reduced productivity, suggesting that *Cryptosporidium* and *Giardia* infections have sub-clinical consequences for ruminants beyond the neonatal period and challenges the notion that *Cryptosporidium* and *Giardia* are of relevance for ruminants only as a cause of neonatal diarrhoea. Apart from intensity of protozoan shedding, repeated detection of *C. parvum* and *Giardia* in individual animals was associated with reduced HSCW and dressing %, again suggesting that infection beyond the neonatal period has consequences for sheep meat productivity, and both intensity and timing of shedding relative to slaughter may impact carcass outcomes.

Sheep flocks included in this study were distributed across a wide geographical area, representing a range of sheep production environments. The distance between WA3 and NSW was approximately 3300km and there was considerable variation in rainfall pattern between farms (Table 1). This was an observational study with natural parasite infections, and sheep were managed under extensive grazing conditions typical for commercial sheep meat production. Both carcass weight and dressing % are important profit drivers for the sheep meat industry, and the reductions in HSCW and dressing % observed would be expected to reduce efficiency and increase costs of processing.

The present study has extended findings from the previous reports identifying reduced HSCW and dressing % associated with detection of *Cryptosporidium* and *Giardia* (Sweeny et al., 2012b; Sweeny 2012) by examining protozoan shedding intensity (determined by qPCR) rather than simply presence of organism (by PCR). Apart from demonstrating that magnitude and not simply presence of shedding was associated with carcass productivity, the present study included a much larger number of sheep located over

a wider geographical area, was able to identify associations at specific sampling timepoints and identified that relationships between parasite shedding and HSCW existed only for *C. parvum* (but not all *Cryptosporidium* spp.).

Cryptosporidium (including *C. parvum*) and *Giardia* were identified in all eight flocks included in the present study, and have been widely reported in sheep in Australia (Ryan et al., 2005; Yang et al., 2009; Sweeny et al., 2011; Sweeny et al., 2012a; Yang et al., 2014a; Yang et al., 2014b) and worldwide (Xiao 2010). Therefore the observations of reduced HSCW and dressing % associated with these organisms have important implications for sheep meat industries worldwide. The productivity consequences of greatest magnitude were for lambs categorized as high shedding for *C. parvum* at post-weaning (3.2kg lower HSCW than non-shedding lambs) and pre-slaughter (2.2kg lower HSCW). *Cryptosporidium parvum* was identified on all eight farms at either post-weaning or pre-slaughter, albeit at low prevalences (below 6%). A recent review by Ryan et al. (2014) reported geographic differences in prevalence and predominant *Cryptosporidium* species and genotypes in sheep, with *C. parvum* apparently the dominant species in Europe based on available epidemiological studies. It is likely that host age influences distribution of *Cryptosporidium* species, but further evidence derived from longitudinal studies is required to confirm this.

Lambs shedding *Cryptosporidium* pre-slaughter had lower dressing % than non-shedding lambs, but no differences in HSCW or live weight were observed. Dressing % is the ratio of HSCW divided by live weight, and differences in dressing % may be reflecting variation in either HSCW or the visceral (and non-carcass tissue) of the animal. Although not significant, HSCW was 0.3kg lighter in lambs shedding *Cryptosporidium* pre-slaughter, whilst their visceral tissue (live weight -HSCW) was 0.8kg heavier, implying that impact on visceral (non-carcass) tissue weight was the key driver of the reduced dressing % response observed for these lambs. This observation was consistent with previous studies showing dressing % (but not necessarily live weight) was reduced in sheep infected with nematodes

(Liu et al., 2005; Jacobson et al., 2009) and protozoan parasites (Sweeny 2012; Sweeny et al., 2012b), and supports the suggestion that live weight may underestimate carcass productivity losses in sheep associated with parasitism.

Increased frequency of detection of both *C. parvum* and *Giardia* was associated with greater magnitude of reductions in HSCW and dressing %. Whether the repeated observations of shedding were due to ongoing (chronic) infection, separate infections (different genotype) or re-infection (same genotype) could not be determined. Both *Cryptosporidium* and *Giardia* infections are thought to be mostly self-limiting although chronic infection and reinfections have been reported at least in humans (Cama et al., 2008; Halliez and Buret, 2013). Cama et al. (2008) reported that reinfection with both the same and different genotypes were common. In cattle, reinfection after treatment is common (O'Handley et al., 2000; Guerden et al., 2006; Geurden et al., 2010). Little is known about the situation in sheep.

Observations in this study highlighted the importance of longitudinal sampling to identify relationships between protozoan shedding and productivity outcomes. Shedding of *C. parvum* or *Giardia* post-weaning were associated with reduced HSCW at slaughter (approximately 10 weeks later). Importantly, the reductions in HSCW associated with high shedding post-weaning were both statistically significant and of a magnitude relevant for commercial sheep meat production. Relationships between protozoan shedding and subsequent HSCW and dressing % were not significant at the weaning sampling, possibly due to the greater time period between sampling and slaughter.

Thresholds for “high” (or pathological) oocyst shedding concentration have not been established for either *Cryptosporidium* or *Giardia*. We used the median oocyst shedding concentration in positive animals for each parasite/farm/timepoint as the cutoff for categorising shedding animals as low or high shedding, and significant effects were observed using this methodology despite the range in median shedding intensity observed across farms

and timepoints (Tables 2 and 3). This is the first study to our knowledge that has used longitudinal sampling and quantitative molecular measures of shedding to determine impacts on growth and carcass productivity in sheep, so there was little evidence available to support or refute the approach using median values when determining appropriate methods for analyses. Larger data sets may be able to identify thresholds for shedding intensity where future impacts on carcass productivity are more likely to be observed.

Interactions between protozoan parasites and a range of host factors determining disease outcomes have been reported in sheep, although these are less well described than nematode parasite-host factor interactions. *Eimeria* shedding is likely to have a genetic component (Yvone et al., 1992; Reeg et al., 2005) and factors including stress, litter size, stocking intensity, poor hygiene and concurrent infections have been implicated as risk factors for clinical coccidiosis and cryptosporidiosis in young lambs (de Graaf et al., 1999; Taylor, 2008; Chartier and Paraud, 2012). In the present study, mixed (concurrent) infections were not associated with reduced live weight, HSCW or dressing %, except at pre-slaughter where lambs with mixed infections had lower dressing %.

It should be noted that associations between infection and reduced growth (*C. parvum*) or HSCW (*C. parvum* and *Giardia* spp.) in this observational study could not be confirmed as causative (i.e. parasitism causing reduced growth or carcass size). It may be the case that sheep which otherwise had restricted growth were more susceptible to infection and high shedding intensity. Despite evidence that *Cryptosporidium* and *Giardia* are common and widespread in livestock, the effects of infection on livestock productivity and the mechanisms by which deleterious effects may occur are surprisingly poorly understood (O'Handley and Olson 2006), but are likely related to alterations in gut epithelial structure and function (Buret 2007). Olson et al. (1995) attributed reduced carcass weight in lambs to reduced feed conversion efficiency in specific pathogen-free barn raised lambs subsequent to surgical infection with *Giardia*. Ralston et al. (2003) observed associations between reduced

feed intake and *Giardia* in steers, but a review by Geurden et al. (2010) concluded that there was no experimental data “to conclusively indicate an economical impact” of *Giardia* on production in calves. Similarly, whilst abomasal *Cryptosporidium andersoni* has been associated with reduced weight gain (Anderson 1987; Ralston et al., 2010) and reduced feed efficiency (Ralston et al., 2003) in feedlot cattle, and *C. muris* has been associated with reduced milk production in dairy cows (Esteban et al., 1995), reviews by O’Handley and Olsen (2006) and Santin (2013) concluded there was a lack of evidence to indicate intestinal cryptosporidiosis results in any long-term production effects in ruminants. Feed intake was not recorded in the present study, so it was not possible to determine whether differences in HSCW were associated with reduced appetite and/or feed conversion efficiency.

The study was conducted using flocks of sheep being raised for meat production under commercial farming conditions. There were potential biases due to factors independent of parasitism impacting growth/weight such as date of birth (lambing periods extended up to 8 weeks), sex, parity and litter size. Whilst the size of the data set was likely to have addressed some of these biases, assumptions of similarity of factors (such as distribution of litter size) between flocks was made. Future studies accounting for wider range of factors with potential to impact growth independent of parasitism could be used to better define associations between infection and growth in sheep.

This study was not designed to investigate the effect of nematodes on live weight or carcass productivity, so farmers employed their normal strategic anthelmintic treatments and grazing management. Treatment with benzimidazoles (BZ) may impact shedding intensity (Xiao et al., 1996; O’Handley et al., 1997; O’Handley et al., 2000; Geurden et al., 2006). Records showed that BZ treatments were not used for WA1, WA2, WA3, SA1 or VIC1. Records for SA2, VIC2 and NSW were incomplete. The BZ dosage required to reduce cyst excretion in sheep is not known, but a review by Geurden et al. (2010) suggests that for calves the total dosage (5-20mg/kg fenbendazole or albendazole daily for 3 consecutive days) is higher compared with helminth treatment. No sheep in this study were treated with 3 consecutive doses of any anthelmintic. Furthermore, studies in calves have shown that cyst suppression after 3 days of BZ treatment was either not complete or short acting in field

conditions, and it has been proposed that this may reflect rapid re-infection in contaminated environments (O'Handley et al., 2000; Guerden et al., 2006; Geurden et al., 2010). Therefore any BZ treatments used in the present study were likely sub-therapeutic, and reinfection rapid. The results reported for this study therefore reflect production losses in sheep with oocyst excretion observed under field conditions (i.e. contaminated environment and reinfection likely) and with anthelmintic treatment protocols typical for lambs being raised for slaughter. Further studies are required to determine the effect of BZ on cyst excretion by sheep at doses recommended for nematode control and under field conditions where reinfection is likely.

No significant main effects for WEC or *Haemonchus* status for live weight, HSCW or dressing % were observed in this study. Resilience to trichostrongylid parasites by prime lambs with nutrition adequate to support growth rates of 200g/d has been reported in the same regions included in this study, specifically southern Australia in prime lambs up to slaughter (Carmichael et al., 2011; Carmichael et al., 2013) and northern NSW in prime lambs up to weaning (Dever et al., 2016). Furthermore, the effect of infection is likely to have been complicated by variations in resistance both between and within flocks which may impact on lamb growth during the acquisition of immunity (Greer 2008). It should also be noted that lambs in the present study were being raised for slaughter using management typical for each property, including anthelmintic treatments and grazing management. It is possible that the nematode infection pressure and range of WEC observed in these flocks was not sufficient for effects on live weight, HSCW and dressing % to be observed.

5. Conclusion

This study is, to the best of our knowledge, the first to report associations between increased intensity of faecal shedding of protozoan parasites and reduced sheep meat productivity, specifically carcass weight (*C. parvum* and *Giardia*), dressing % (*C. parvum* and *Cryptosporidium* spp.) and live weight (*C. parvum*) in sheep. The results suggest *Cryptosporidium* and *Giardia* infections have consequences for ruminant productivity beyond the neonatal period. Repeated detection of *C. parvum* and *Giardia* shedding in individual animals was also associated with reduced HSCW and dressing %. Mixed *Giardia* and *Cryptosporidium* shedding pre-slaughter was associated with reduced dressing %. The study incorporated a large number of animals of varying genetic background located over wide geographical area, representing a range of sheep production environments and natural protozoan infection scenarios. *Cryptosporidium* and *Giardia* have been widely reported in sheep flocks throughout Australia and around the world, therefore the associations with

reduced carcass weight and dressing % reported by this study have important implications for sheep meat industries worldwide.

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References

- Aliosio, F., Filippini, G., Antenucci, P., Lepri, E., Pezzotti, G., Cacciò, S.M. Pozio, E. 2006. Severe Weight loss in lambs infected with *Giardia duodenalis* assemblage B. *Vet. Parasitol.* 142, 154-158.
- Anderson B.C. 1987. Abomasal cryptosporidiosis in cattle. *Vet. Pathol.* 24, 235-238
- AUS-MEAT 2005. Handbook of Australian Meat (International Red Meat Manual). 7th ed. (AUS-MEAT: Brisbane, Australia) ISBN 0 9578793 69
- Bhalchandra, S., Ludington, J., Coppens, I., Ward, H.D., 2013. Identification and characterization of *Cryptosporidium parvum* Clec, a novel C-type lectin domain-containing mucin-like glycoprotein. *Infect. Immun.* 81(9), 3356-3365.
- Buret A.G. 2007. Mechanisms of epithelial dysfunction in giardiasis. *Gut.* 56(3): 316-317.
- Cacciò, S.M., Sannella, A.R., Mariano, V., Valentini, S., Berti, F., Tosini, F., Pozio, E., 2013. A rare *Cryptosporidium parvum* genotype associated with infection of lambs and zoonotic transmission in Italy. *Vet. Parasitol.* 191, 128-131.
- Cama, V.A., Bern, C., Roberts, J., Cabrera, L., Sterling, C.R., Ortega, Y., Gilman, R.H., Xiao, L., 2008. *Cryptosporidium* species and subtypes and clinical manifestations in children, Peru. *Emerg Infect Dis.* 14(10), 1567-1574.
- Carmichael, I.H., Blackburn, P.J., Martin, R.R. 2011. The influence of gastro-intestinal worms and nutrition on performance of prime lambs in south-eastern Australia. In: *Proceedings of the Australian Sheep Veterinarians 2011 Conference (Barossa Valley and Adelaide)*. 2011: 55-58.
- Carmichael, I.H., Blackburn, P.J., Martin, R.R. 2013. Worm burdens and egg counts in South Australian prime lambs. In: *Proceedings of the Australian Sheep Veterinarians 2013 Conference (Albany)*. 2013: 20-28.
- Chartier, C., Paraud C., 2012. Coccidiosis due to *Eimeria* in sheep and goats, a review. *Small Ruminant Research.* 103(1)84-92.

- Connelly, L., Craig, B.H., Jones, B., Alexander, C.L., 2013. Genetic diversity of *Cryptosporidium* spp. within a remote population of Soay Sheep on St. Kilda Islands, Scotland. *Appl Environ Microbiol.* 79, 2240-2246.
- deGraaf, D.C., Vanopdenbosch E., Ortega-Mora L.M., Abbassi H., Peeters J.E., 1999. A review of the importance of cryptosporidiosis in farm animals. *Int. J. Parasitol.* 29(8):1269-87.
- Dever, M.L., Kahn, L.P., Doyle, E.K. 2016. Growth is impeded by gastrointestinal nematodes in weaned rather than suckling meat-breed lambs in a high summer rainfall region, on the Northern Tablelands, New South Wales. *Anim. Prod. Sci.* In press - online 8 June 2016. DOI: org/10.1071/AN15806
- Díaz, P., Quílez, J., Chalmers, R.M., Panadero, R., López, C., Sánchez-Acedo, C., Morrondo, P., Díez-Baños, P., 2010. Genotype and subtype analysis of *Cryptosporidium* isolates from calves and lambs in Galicia (NW Spain). *Parasitol.* 137, 1187-1193.
- Esteban, E., Anderson, B.C. 1995. *Cryptosporidium muris*: prevalence, persistency, and detrimental effect on milk production in a drylot dairy. *J. Dairy Sci.* 78, 1068-1072.
- Fayer, R., Santín, M., 2009. *Cryptosporidium xiaoi* n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (*Ovis aries*). *Vet. Parasitol.* 164, 192-200.
- Feng, Y., Xiao L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clin Microbiol Rev.* 24(1):110-40
- Fiuza, V.R., Cosendey, R.I., Frazão-Teixeira, E., Santín, M., Fayer, R., de Oliveira, F.C., 2011. Molecular characterization of *Cryptosporidium* in Brazilian sheep. *Vet. Parasitol.* 175, 360-362.
- Geurden, T., Thomas, P., Casaert, S., Vercruyssen, J., Claerebout, E., 2008. Prevalence and molecular characterisation of *Cryptosporidium* and *Giardia* in lambs and goat kids in Belgium. *Vet Parasitol.* 155:142-5.

- Geurden, T., Vercruyssen, J., Claerebout, E., 2006. Field testing of fenbendazole treatment combined with hygienic and management measures against a natural *Giardia* infection in calves. *Vet. Parasitol.* 142, 367-371.
- Geurden, T., Vercruyssen, J., Claerebout, E., 2010. Is *Giardia* a significant pathogen in production animals? *Exp. Parasitol.* 124:98-106.
- Giles, M., Chalmers, R., Pritchard, G., Elwin, K., Mueller-Doblies, D., Clifton-Hadley, F., 2009. *Cryptosporidium hominis* in a goat and a sheep in the UK. *Vet. Rec.* 164, 24-25.
- Greer, A.W. 2008. Trade-offs and benefits: implications of promoting a strong immunity to gastrointestinal parasites in sheep. *Parasite Immunol.* 30, 123-132.
- Harmon, A.F., Williams, Z.B., Holler, L.D., Hildreth, M.B., 2007. Comparison of three different preservatives for morphological and real-time PCR analyses of *Haemonchus contortus* eggs. *Vet. Parasitol.* 145, 361-365.
- Halliez, M.C., Buret, A.G., 2013. Extra-intestinal and long term consequences of *Giardia duodenalis* infections. *World J Gastroenterol.* 19(47), 8974-8985.
- Harmon, A.F., Williams, Z.B., Zarlenga, D.S., Hildreth, M.B., 2007. Real-time PCR for quantifying *Haemonchus contortus* eggs and potential limiting factors. *Parasitol. Res.* 101, 71-76.
- Imre, K., Luca, C., Costache, M., Sala, C., Morar, A., Morariu, S., Ilie, M.S., Imre, M., Dărăbuș, G., 2013. Zoonotic *Cryptosporidium parvum* in Romanian newborn lambs (*Ovis aries*). *Vet Parasitol.* 191, 119-122.
- Jacobson, C., Pluske, J., Besier, R.B., Bell K., Pethick, D., 2009. Associations between nematode larval challenge and gastrointestinal tract size that affect carcass productivity in sheep. *Vet. Parasitol.* 161, 248-254.
- Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D., Cacciò, S.M. 2005. Genetic heterogeneity at the β -giardin locus among human and animal isolates of *Giardia*

- duodenalis* and identification of new, potentially zoonotic, genotypes. *Int. J. Parasitol.* 35, 207–213
- Liu, S.M., Smith, T.L., Briegel, J., Murray, A., Masters, D.G., Karlsson, L.J.E., Palmer, D.G., Greeff, J.C., Besier, R.B., Gao, S.B., 2005. Comparing productive performance of nematode resistant Merino sheep with non-selected control. *Livest. Prod. Sci.* 97, 117-129.
- Lyndal-Murphy, M., 1993, Anthelmintic resistance in sheep, In: L.A., Corner, Bagust, T.J. (Eds.) *Australian Standard Diagnostic Techniques for Animal Diseases*. CSIRO for the standing Committee on Agriculture and Resource Management, Melbourne.
- Morgan, U.M., O'Brien, P.A., Thompson, R.C., 1996. The development of diagnostic PCR primers for *Cryptosporidium* using RAPD-PCR. *Mol. Biochem. Parasitol.* 77, 103-108.
- Mueller-Doblies, D., Giles, M., Elwin, K., Smith, R.P., Clifton-Hadley, F.A., Chalmers, R.M., 2008. Distribution of *Cryptosporidium* species in sheep in the UK. *Vet. Parasitol.* 154, 214-219.
- O'Handley, R.M., Cockwill, C., Jelinski, M., McAllister, T.A., Olson, M.E. 2000. Effects of repeat fenbendazole treatment in dairy calves with giardiasis on cyst excretion, clinical signs and production. *Vet. Parasitol.* 89, 209-218.
- O'Handley, R.M., Olson, M.E., 2006. Giardiasis and cryptosporidiosis in ruminants. *Vet. Clin. North Am. Food Anim. Pract.* 22, 623–643.
- O'Handley, R.M., Olson, M.E., McAllister, T.A., Morck, D.W., Jelinski, M., Royan, G., Cheng, K.J. 1997. Efficacy of fenbendazole for treatment of giardiasis in calves. *Am. J. Vet. Res.* 58, 384-388.
- Olson, M.E., McAllister, T.A., Deselliers, L., Morck, D.W., Cheng, K.J., Buret, A.G., Ceri, H., 1995. Effects of giardiasis on production in a domestic ruminant (lamb) model. *Am. J. Vet. Res.* 56, 1470-1474.

- Paoletti, B., Giangaspero, A., Gatti, A., Iorio, R., Cembalo, D., Milillo, P., Traversa, D. 2009. Immunoenzymatic analysis and genetic detection of *Cryptosporidium parvum* in lambs from Italy. *Exp. Parasitol.* 122, 349-352.
- Quílez, J., Torres, E., Chalmers, R.M., Hadfield, S.J., Del Cacho, E., Sánchez-Acedo, C., 2008. *Cryptosporidium* genotypes and subtypes in lambs and goat kids in Spain. *Appl. Environ. Microbiol.* 74, 6026-6031.
- Ralston, B.J., Cockwill, C.L., Guselle, N., Van Herk, F.H., McAllister, T.A., Olson, M.E., 2003. Prevalence of *Giardia* and *Cryptosporidium andersoni* and their effects on performance in feedlot beef cattle. *Canadian Journal of Animal Science* 83, 153-159.
- Ralston, B., Thompson, R.C.A., Pethick, D., McAlister, T.A., Olsen, M.E. 2010. *Cryptosporidium andersoni* in Western Australian feedlot cattle. *Aust. Vet. J.* 88, 458-460.
- Reeg, K.J., Gauly, M., Bauer, C., Mertens, C., Erhardt, G., Zahner, H., 2005. Coccidial infections in housed lambs; oocyst excretion, antibody levels and genetic influences on the infection. *Vet. Parasitol.* 127(3-4):209-19.
- Robertson, L.J., Gjerde, B.K., Furuseth Hansen, E., 2010. The zoonotic potential of *Giardia* and *Cryptosporidium* in Norwegian sheep: a longitudinal investigation of 6 flocks of lambs. *Vet. Parasitol.* 171, 140-145.
- Ryan, U., Caccio, S.M., 2013. Zoonotic potential of *Giardia*. *Int J Parasitol.* 43(12-13):943-56
- Ryan, U.M., Bath, C., Robertson, I., Read, C., Elliot, A., McInnes, L., Traub, R., Besier, B., 2005. Sheep may not be an important zoonotic reservoir for *Cryptosporidium* and *Giardia* parasites. *Appl. Environ. Microbiol.* 71, 4992-4997.
- Ryan, U., Fayer, R., Xiao L., 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology.* 141(13):1667-85.

- Santin, M. 2013. Clinical and subclinical infections with *Cryptosporidium* in animals. N. Z. Vet. J. 61, 1-10.
- Santín, M., Trout, J.M., Fayer, R., 2007. Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* species and genotypes in sheep in Maryland. Vet Parasitol. 146:17-24.
- Shen, Y., Yin, J., Yuan, Z., Lu, W., Xu, Y., Xiao, L., Cao, J., 2011. The identification of the *Cryptosporidium ubiquitum* in pre-weaned Ovines from Aba Tibetan and Qiang autonomous prefecture in China. Biomed. Environ. Sci. 24, 315-320.
- Soltane, R., Guyot, K., Dei-Cas, E., Ayadi, A., 2007. Prevalence of *Cryptosporidium* spp. (*Eucoccidiorida: Cryptosporiidae*) in seven species of farm animals in Tunisia. Parasite. 14, 335-338.
- Sweeny, J.P., Ryan, U.M., Robertson, I.D., Yang, R., Bell, K., Jacobson, C., 2011. Longitudinal investigation of protozoan parasites in meat lamb farms in southern Western Australia. Prev. Vet. Med. 101, 192-203.
- Sweeny, J.P.A., Robertson, I.D., Ryan, U.M., Jacobson, C., Woodgate, R.G., 2012a. Impacts of naturally acquired protozoa and strongylid nematode infections on growth and faecal attributes in lambs. Vet. Para. 184, 298-308.
- Sweeny, J.P.A., Ryan, U.M., Robertson, I.D., Jacobson, C., 2012b. *Cryptosporidium* and *Giardia* associated with reduced lamb carcass productivity. Vet. Parasit. 182: 127-139.
- Sweeny J.P.A., 2012. Determining the impact of protozoan and strongylid parasites on meat lamb productivity. PhD thesis. Murdoch University.
- Taylor, M., 2008. Changing patterns of parasitism in sheep. In Practice. 31, 474-483.
- Van Keulen, H., Macechko, P.T., Wade, S., Schaaf, S., Wallis, P.M., Erlandsen, S.L. 2002. Presence of human *Giardia* in domestic, farm and wild animals, and environmental samples suggests a zoonotic potential for giardiasis. Vet. Parasitol. 108, 97-107.

- Wang, Y., Feng, Y., Cui, B., Jian, F., Ning, C., Wang, R., Zhang, L., Xiao, L., 2010. Cervine genotype is the major *Cryptosporidium* genotype in sheep in China. *Parasitol. Res.* 106, 341-347.
- Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: an update. *Exp Parasitol.* 124:80-9.
- Xiao, L., Saeed, K., Herd, R.P., 1996. Efficacy of Albendazole and fenbendazole against *Giardia* infection in cattle. *Vet. Parasitol.* 61, 165-170.
- Yang, R., Jacobson, C., Gordon, C., Ryan, U., 2009. Prevalence and molecular characterisation of *Cryptosporidium* and *Giardia* species in pre-weaned sheep in Australia. *Vet. Parasitol.* 161, 19-24.
- Yang, R., Murphy, C., Song, Y., Ng-Hublin, J., Estcourt, A., Hijjawi, N., Chalmers, R., Hadfield, S., Bath, A., Gordon C., Ryan, U.M., 2013. Specific and quantitative detection and identification of *Cryptosporidium hominis* and *C. parvum* in clinical and environmental samples. *Exp. Parasitol.* 135, 142-147.
- Yang, R., Jacobson, C., Gardner, G., Carmichael, I., Campbell, A.J.D., Ng-Hublin, J., Ryan, U., 2014a. Longitudinal prevalence, oocyst shedding and molecular characterisation of *Cryptosporidium* species in sheep across four states in Australia. *Vet. Parasitol.* 200, 50-58.
- Yang, R., Jacobson, C., Gardner, G., Carmichael, I., Campbell, A.J.D., Ryan, U., 2014b. Development of a quantitative PCR (qPCR) for *Giardia* and analysis of the prevalence, cyst shedding and genotypes of *Giardia* present in sheep across four states in Australia. *Exp. Parasitol.* 137, 46-52.
- Yang, R.C., Papparini, A., Monis, P., Ryan, U. 2014c. Comparison of next-generation droplet digital PCR (ddPCR) with quantitative PCR (qPCR) for enumeration of *Cryptosporidium* oocysts in faecal samples. *Int. J. Parasitol.* 44, 1105-1113.

- Ye, J., Xiao, L., Wang, Y., Wang, L., Amer, S., Roellig, D.M., Guo Y., Feng, Y., 2013
Periparturient transmission of *Cryptosporidium xiaoi* from ewes to lambs. *Vet
Parasitol.* 197:627-33.
- Yvone, P., Cabaret, J., Solon S., 1992. Repeatability of ovine faecal oocyst counts in natural
infections with *Eimeria* spp. *Int. J. Parasitol.* 22,151-8.

Table 1. Sheep farms sampled

Far m	District	Mean rainfall (mm/ann um)	Rainfall pattern	Lamb breed	Commence ment of lambing	Winter stocking rate
SA1	Wirrega	430	Winter	Suffolk	Mid April	10DSE/ha
SA2	Struan	550	Winter	Suffolk x BL/Merino	June	15DSE/ha
Vic 1	Rosedale	620	Winter	Dorset & Southdown x BL/Merino	Mid July	10DSE/ha
Vic 2	Ballarat	750	Winter	Suffolk x Merino	Early August	13DSE/ha
NS W	Armidale	495	Summer	BL x Merino	May -August	20 DSE/ha
WA 1	Arthur River	500	Mediterranean (winter rainfall)	Suffolk x Merino	Early August	10DSE/ha
WA 2	Pingelly	450	Mediterranean (winter rainfall)	Suffolk x Merino	Mid July	12DSE/ha
WA 3	Franklan d	550	Mediterranean (winter rainfall)	Suffolk x Merino	Mid July	21 DSE/Ha

DSE/ha: dry sheep equivalent (standard unit used to compare livestock carrying capacity) per hectare

SA - South Australia; Vic – Victoria; NSW – New South Wales; WA – Western Australia

BL: Border Leicester

Table 2. Prevalence and faecal shedding of *Cryptosporidium* (all species) and *C. parvum* in faecal samples. Data adapted from Yang et al. (2014a).

Farm	Sampling occasion	Samples (n)	<i>Cryptosporidium</i> spp.			<i>C. parvum</i>		
			Prevalence % (95% CI)	Range	Median	Prevalence % (95% CI)	Range	Median
SA1	Weaning	165	8.5 (4.2-12.7)	938-1.8 x 10 ⁶	4.8 x 10 ⁴	1.8 (0-3.9)	938-1.8 x 10 ⁶	4.8 x 10 ⁴
	Post-weaning	148	6.1 (2.2-9.9)	3.9 x 10 ³ -9.8 x 10 ⁵	2.3 x 10 ⁴	0.7 (0-2.0)	3.9 x 10 ³ -9.8 x 10 ⁵	2.3 x 10 ⁴
	Pre-slaughter	159	9.4 (4.9-14.0)	7.3 x 10 ³ -1.7 x 10 ⁵	1.4 x 10 ⁵	1.8 (0-3.8)	7.3 x 10 ³ -1.7 x 10 ⁵	1.4 x 10 ⁵
SA2	Weaning	169	17.8 (12.0-23.5)	375-7.9 x 10 ⁶	3.1 x 10 ³	0	375-7.9 x 10 ⁶	3.1 x 10 ³
	Post-weaning	156	19.2 (13.0-25.4)	313-3.0 x 10 ⁵	4.7 x 10 ³	0.6 (0-1.9)	313-3.0 x 10 ⁵	4.7 x 10 ³
	Pre-slaughter	147	6.1 (2.2-10.0)	4.7 x 10 ³ -1.7 x 10 ⁶	8.0 x 10 ⁴	1.4 (0-3.2)	4.7 x 10 ³ -1.7 x 10 ⁶	8.0 x 10 ⁴
Vic1	Weaning	180	3.3 (0.7-6.0)	125-8.7 x 10 ⁵	1.6 x 10 ⁴	0.6 (0-1.6)	125-8.7 x 10 ⁵	1.6 x 10 ⁴
	Post-weaning	172	18.6 (12.8-24.4)	390-3.7 x 10 ⁹	5.1 x 10 ³	1.7 (0-3.7)	390-3.7 x 10 ⁹	5.1 x 10 ³
	Pre-slaughter	160	8.8 (4.4-13.1)	1.6 x 10 ³ -7.8 x 10 ⁴	1.6 x 10 ³	0	1.6 x 10 ³ -7.8 x 10 ⁴	1.6 x 10 ³
Vic2	Weaning	176	21 (15.0-27.0)	313-4.8 x 10 ⁵	7.8 x 10 ³	0.6 (0-1.7)	313-4.8 x 10 ⁵	7.8 x 10 ³
	Post-weaning	173	9.2 (4.9-13.6)	1.0 x 10 ³ -7.1 x 10 ⁶	1.8 x 10 ³	0	1.0 x 10 ³ -7.1 x 10 ⁶	1.8 x 10 ³
	Pre-slaughter	128	9.4 (4.3-14.4)	937-6.0 x 10 ⁶	9.0 x 10 ⁴	1.6 (0-3.7)	937-6.0 x 10 ⁶	9.0 x 10 ⁴
NSW	Weaning	160	22.5 (16.0-29.0)	313-1.1 x 10 ⁶	6.1 x 10 ³	1.9 (0-4.0)	313-1.1 x 10 ⁶	6.1 x 10 ³
	Post-weaning	160	27.5 (20.6-43.3)	563-2.1 x 10 ⁸	1.7 x 10 ⁴	0.6 (0-1.8)	563-2.1 x 10 ⁸	1.7 x 10 ⁴
	Pre-slaughter	167	12.5 (7.5-17.6)	262-1.4 x 10 ⁷	1.2 x 10 ⁴	1.8 (0-3.8)	262-1.4 x 10 ⁷	1.2 x 10 ⁴
WA1	Weaning	124	37.1 (28.6-45.6)	125-2.6 x 10 ⁶	1.6 x 10 ⁴	0	125-2.6 x 10 ⁶	1.6 x 10 ⁴
	Post-weaning	122	14.8 (8.5-21.0)	313-1.1 x 10 ⁵	4.5 x 10 ³	1.6 (0-3.9)	313-1.1 x 10 ⁵	4.5 x 10 ³
	Pre-slaughter	121	24.0 (16.4-31.6)	375-1.6 x 10 ⁷	5.8 x 10 ⁴	0.8 (0-2.4)	375-1.6 x 10 ⁷	5.8 x 10 ⁴
WA2	Weaning	107	43.9 (34.5-53.3)	63-5.3 x 10 ³	4.0 x 10 ¹	5.6 (1.2-10.0)	63-5.3 x 10 ³	400
	Post-weaning	109	26.6 (18.3-34.9)	313-2.4 x 10 ⁷	1.5 x 10 ⁵	2.8 (0-5.8)	313-2.4 x 10 ⁷	1.5 x 10 ⁵
	Pre-slaughter	107	36.4 (27.3-45.6)	1.6 x 10 ³ -2.9 x 10 ⁷	2.0 x 10 ⁴	2.8 (0-5.9)	1.6 x 10 ³ -2.9 x 10 ⁷	2.0 x 10 ⁴
WA3	Weaning	101	18.8 (11.2-26.4)	313-4.7 x 10 ⁵	2.3 x 10 ⁴	5.9 (1.3-10.6)	313-4.7 x 10 ⁵	2.3 x 10 ⁴
	Post-weaning	101	6.9 (2.0-11.9)	313-3.7 x 10 ⁶	5.9 x 10 ³	1.0 (0-2.9)	313-3.7 x 10 ⁶	5.9 x 10 ³
	Pre-slaughter	100	14 (7.2-22.8)	2.0 x 10 ³ -4.8 x 10 ⁷	1.0 x 10 ⁵	0	2.0 x 10 ³ -4.8 x 10 ⁷	1.0 x 10 ⁵
Total		3412	16.9 (15.6-18.1)	63 – 3.7 x 10⁹	2.6 x 10⁴	1.3 (0.9-1.6)	63 – 3.7 x 10⁹	2.6 x 10⁴

CI: confidence interval

Table 3. Prevalence and faecal shedding of *Giardia* in faecal samples. Data adapted from Yang et al. (2014b).

Farm	Sampling occasion	Samples (n)	Prevalence % (95% CI)	Oocyst concentration (oocysts g ⁻¹) in positive samples	
				Range	Median
SA1	Weaning	165	16.4 (10.7-22.0)	63-3.7 x10 ⁵	3.9 x 10 ³
	Post-weaning	148	20.3 (13.8-26.7)	390-1.9 x10 ⁸	8.3 x 10 ⁵
	Pre-slaughter	159	11.9 (6.9-17.0)	1.4 x10 ³ -1.7 x10 ⁵	1.5 x 10 ⁵
SA2	Weaning	169	14.8 (9.4-20.1)	1.1 x10 ³ -1.2 x10 ⁵	1.1 x 10 ⁵
	Post-weaning	156	19.9 (13.6-26.1)	188-2.1 x 10 ⁹	9.8 x 10 ³
	Pre-slaughter	147	17.7 (11.5-23.9)	438-9.5 x 10 ⁵	8.2 x10 ⁴
Vic1	Weaning	180	7.2 (3.4-11.0)	390-1.3 x 10 ⁹	2.3 x 10 ⁴
	Post-weaning	172	23.3 (16.9-29.6)	63-1.0 x10 ⁹	4.2 x 10 ⁴
	Pre-slaughter	160	9.4 (4.9-13.9)	1.6 x10 ³ -4.8 x10 ⁵	1.2 x 10 ⁴
Vic2	Weaning	176	13.6 (8.6-18.7)	63-1.8 x 10 ⁵	9.8 x10 ³
	Post-weaning	173	20.8 (14.8-26.9)	313-2.0 x 10 ⁸	1.8 x 10 ³
	Pre-slaughter	128	30.5 (22.5-28.4)	125-4.7 x 10 ⁵	9.0 x10 ⁴
NSW	Weaning	160	16.9 (11.1-22.7)	63-4.5 x 10 ⁵	2.0 x 10 ³
	Post-weaning	160	17.5 (11.6-23.4)	313-2.2 x 10 ⁵	3.9 x 10 ³
	Pre-slaughter	167	34.7 (27.5-42.0)	125-2.0 x 10 ⁵	4.3 x 10 ³
WA1	Weaning	124	27.4 (19.6-35.3)	313-5.0 x 10 ⁶	2.6 x10 ⁴
	Post-weaning	122	35.2 (26.8-43.7)	63-1.2 x 10 ⁵	7.6 x 10 ³
	Pre-slaughter	121	42.1 (33.4-50.9)	813-9.5 x 10 ³	7.5 x10 ⁴
WA2	Weaning	107	28.0 (19.5-36.5)	63-6.9 x 10 ⁵	4.2 x 10 ³
	Post-weaning	109	25.7 (17.5-33.9)	63-1.1 x 10 ⁷	2.8 x 10 ⁴
	Pre-slaughter	107	29.9 (21.1-38.6)	125-2.4 x10 ⁶	5.2 x 10 ⁴
WA3	Weaning	101	7.9 (2.7-13.2)	1.9 x10 ³ -1.5 x10 ⁶	3.7 x 10 ⁵
	Post-weaning	101	10.9 (4.8-17.0)	63-7.4 x10 ⁸	3.1 x 10 ⁴
	Pre-slaughter	100	15.0 (8.0-22.1)	63-4.7 x10 ⁹	5.2 x 10 ⁵
Total		3412	20.2 (18.9-21.6)	63-4.7 x 10⁹	1.6 x 10⁴

CI: confidence interval

Table 4: Association of parasite shedding category with lamb carcase weight, dressing % and live weight with least square means \pm standard error and F value for parasite main effect. F values significant <0.05 are shown in bold.

	Sampling occasion	Shedding category and main effect	Parasite		
			<i>C. parvum</i>	<i>Cryptosporidium</i> spp.	<i>Giardia</i> spp.
HSCW (kg) #	Weaning	High shedding	20.550 \pm 0.726	21.333 \pm 0.248	21.307 \pm 0.285
		Low shedding	20.164 \pm 0.800	21.573 \pm 0.250	21.159 \pm 0.288
		Not detected	21.614 \pm 0.079	21.621 \pm 0.090	21.663 \pm 0.086
		Parasite F value	2.61	0.58	1.92
	Post-weaning	High shedding	18.404 \pm 0.848 ^a	21.361 \pm 0.284	21.068 \pm 0.221 ^a
		Low shedding	21.619 \pm 1.204 ^b	21.13 \pm 0.293	21.727 \pm 0.259 ^b
		Not detected	21.622 \pm 0.079 ^b	21.612 \pm 0.086	21.61 \pm 0.089 ^b
		Parasite F value	7.14**	0.45	3.3*
	Pre-slaughter	High shedding	19.468 \pm 0.798 ^a	21.488 \pm 0.284	21.557 \pm 0.219
		Low shedding	19.074 \pm 1.069 ^a	21.324 \pm 0.308	21.345 \pm 0.228
		Not detected	21.676 \pm 0.077 ^b	21.690 \pm 0.084	21.704 \pm 0.090
		Parasite F value	6.69**	0.8	1.12
Dressing % #	Weaning	High shedding	44.62 \pm 1.02	45.56 \pm 0.35	45.52 \pm 0.401
		Low shedding	43.76 \pm 1.12	45.26 \pm 0.35	45.07 \pm 0.406
		Not detected	45.52 \pm 0.11	45.51 \pm 0.13	45.52 \pm 0.121
		Parasite F value	1.56	0.25	0.56
	Post-weaning	High shedding	42.81 \pm 1.21 ^a	45.14 \pm 0.41	45.28 \pm 0.316
		Low shedding	47.42 \pm 1.72 ^b	45.90 \pm 0.42	45.43 \pm 0.369
		Not detected	45.52 \pm 0.11 ^b	45.51 \pm 0.12	45.55 \pm 0.128
		Parasite F value	3.12*	0.86	0.35
	Pre-slaughter	High shedding	44.35 \pm 1.14	44.43 \pm 0.40 ^a	45.15 \pm 0.309
		Low shedding	44.66 \pm 1.52	44.47 \pm 0.43 ^a	45.09 \pm 0.323
		Not detected	45.53 \pm 0.11	45.68 \pm 0.12 ^b	45.65 \pm 0.127
		Parasite F value	0.7	7.27**	2.02
Live weight (kg)##	Weaning	High shedding	28.00 \pm 0.95 ^a	31.52 \pm 0.34	30.91 \pm 0.38
		Low shedding	30.78 \pm 1.13 ^{ab}	31.02 \pm 0.35	32.19 \pm 0.39
		Not detected	31.58 \pm 0.14 ^b	31.59 \pm 0.15	31.52 \pm 0.15
		Parasite F value			
	Post-weaning	High shedding	35.31 \pm 1.16 ^a	39.69 \pm 0.39	39.62 \pm 0.31
		Low shedding	38.20 \pm 1.51 ^{ab}	39.27 \pm 0.39	39.62 \pm 0.36
		Not detected	39.84 \pm 0.14 ^b	39.83 \pm 0.15	39.83 \pm 0.15
		Parasite F value			
	Pre-slaughter	High shedding	44.72 \pm 1.13 ^a	47.15 \pm 0.41	47.04 \pm 0.32
		Low shedding	46.02 \pm 1.38 ^{ab}	47.79 \pm 0.44	47.21 \pm 0.34
		Not detected	47.03 \pm 0.15 ^b	46.94 \pm 0.16	46.96 \pm 0.16
		Parasite F value	15.39**	0.37	1.23

HSCW: hot standard carcase weight

^{ab} values within time points and parasite with different superscripts are significantly different ($P < 0.05$). Note – post hoc tests were performed for all parasites/sample occasions. Values within parasites/sample occasions without superscripts are not significantly different ($P > 0.05$)

** F value < 0.01

* F value < 0.05

General linear model (separate model for each parasite and timepoint)

Linear mixed effects model (separate model for each parasite, each model includes all 3 timepoints)

Table 5: Association of frequency of parasite detection with lamb carcass weight and dressing % with least square means \pm standard error, and F value for parasite main effect. F values significant <0.05 are shown in bold

	Frequency of detection and main effect	Parasite		
		<i>C. parvum</i>	<i>Cryptosporidium</i> spp.	<i>Giardia</i> spp.
HSCW (kg)	0 positive occasions	21.652 \pm 0.081 ^a	21.629 \pm 0.105	21.699 \pm 0.109 ^a
	1 positive occasions	20.225 \pm 0.389 ^b	21.579 \pm 0.144	21.686 \pm 0.143 ^a
	2 positive occasions	17.637 \pm 1.195 ^c	21.401 \pm 0.291	20.882 \pm 0.237 ^b
	3 positive occasions	n/a	20.694 \pm 0.589	20.771 \pm 0.489 ^{ab}
	Frequency F value	11.72**	0.9	4.23**
Dressing %	0 positive occasions	45.59 \pm 0.12 ^a	45.59 \pm 0.15 ^a	45.60 \pm 0.15 ^a
	1 positive occasions	44.86 \pm 0.55 ^a	45.55 \pm 0.20 ^a	45.62 \pm 0.20 ^a
	2 positive occasions	41.68 \pm 1.70 ^b	45.06 \pm 0.41 ^{ab}	44.97 \pm 0.33 ^{ab}
	3 positive occasions	n/a	43.58 \pm 0.83 ^b	44.19 \pm 0.69 ^{bc}
	Frequency F value	3.2*	2.2	2.2*

HSCW: hot standard carcass weight

^{abc} values within parasite and carcass parameter with different superscripts are significantly different ($p<0.05$)

** F value <0.01

* F value <0.05

n/a: insufficient numbers

Table 6. Worm egg count and prevalence of *Haemonchus contortus* in faecal samples

Farm	Sampling occasion	WEC (epg)		qPCR <i>H. contortus</i>	
		Mean \pm SE	Range	Valid samples (n)	Prevalence % (95% CI)
SA1	Weaning	n/a	n/a	165	7.9 (4.5, 12.7)
	Post-weaning	282 \pm 35	0 - 2600	154	19.5 (13.8, 26.3)
	Pre-slaughter	372 \pm 37	0 - 2500	158	14.6 (9.7, 20.7)
SA2	Weaning	n/a	n/a	158	8.2 (4.7, 13.3)
	Post-weaning	1 \pm 1	0 - 1500	151	8.6 (4.9, 13.9)
	Pre-slaughter	210 - 21	0 - 1350	123	13.0 (7.9, 19.8)
Vic1	Weaning	853 \pm 117	0 - 12 000	176	9.7 (6.0, 14.7)
	Post-weaning	164 \pm 24	0 - 3100	170	7.1 (3.9, 11.6)
	Pre-slaughter	315 \pm 62	0 - 6500	159	50.3 (42.6, 58.0)
Vic2	Weaning	124 \pm 20	0 - 1500	175	13.1 (8.8, 18.7)
	Post-weaning	198 \pm 32	0 - 2500	175	2.9 (1.1, 6.1)
	Pre-slaughter	219 \pm 33	0 - 3000	127	2.4 (0.7, 6.2)
NSW	Weaning	n/a	n/a	146	63.7 (55.7, 71.2)
	Post-weaning	625 \pm 125	0 - 15 000	146	56.8 (48.7, 64.7)
	Pre-slaughter	0 \pm 0	0 - 50	131	7.6 (4.0, 13.1)
WA1	Weaning	239 \pm 17	0 - 800	123	0 (0, 2.0)
	Post-weaning	50 \pm 7	0 - 400	122	0.8 (0.1, 3.8)
	Pre-slaughter	205 \pm 40	0 - 3200	121	0.8 (0.1, 3.8)
WA2	Weaning	183 \pm 44	0 - 3150	107	5.6 (2.4, 11.2)
	Post-weaning	133 \pm 46	0 - 2500	109	0.9 (0.1, 4.2)
	Pre-slaughter	575 \pm 87	50 - 3800	107	(0, 2.3)
WA3	Weaning	172 \pm 20	0 - 1200	101	10.9 (5.9, 18.1)
	Post-weaning	41 \pm 11	0 - 650	101	2.0 (0.4, 6.2)
	Pre-slaughter	41 \pm 11	0 - 650	100	0 (0, 2.5)

WEC: McMaster worm egg count

epg: eggs per gram

CI: confidence interval

SE: standard error

n/a: not available (insufficient faecal material)