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Research paper

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

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ABSTRACT

Associations between intensity and frequency of *Cryptosporidium* and *Giardia* shedding with growth, carcase weight and dressing% were investigated using a longitudinal study of 1182 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 carcase 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 2.31–4.52 kg over the 3 sampling occasions. *Cryptosporidium parvum* shedding was associated with less HSCW in high (3.22 kg less) and low (3.22 kg less) shedding lambs post-weaning, and high (2.21 kg less) and low (2.60 kg 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 0.59 kg 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, carcase weight and dressing efficiency beyond the neonatal period in sheep representing a range of genetic backgrounds and different sheep production environments.

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.

Abbreviations: HSCW, hot standard carcase weight; WEC, faecal worm egg count.

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Table 1
Sheep farms sampled.

Farm	District	Mean rainfall (mm/annum)	Rainfall pattern	Lamb breed	Commencement of lambing	Winter stocking rate
SA1	Wirrega	430	Winter	Suffolk	Mid April	10 DSE/ha
SA2	Struan	550	Winter	Suffolk × BL/Merino	June	15 DSE/ha
Vic1	Rosedale	620	Winter	Dorset & Southdown × BL/Merino	Mid July	10 DSE/ha
Vic2	Ballarat	750	Winter	Suffolk × Merino	Early August	13 DSE/ha
NSW	Armidale	495	Summer	BL × Merino	May–August	20 DSE/ha
WA1	Arthur River	500	Mediterranean (winter rainfall)	Suffolk × Merino	Early August	10 DSE/ha
WA2	Pingelly	450	Mediterranean (winter rainfall)	Suffolk × Merino	Mid July	12 DSE/ha
WA3	Frankland	550	Mediterranean (winter rainfall)	Suffolk × 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.

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, 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 (Aliosio 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,b). 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, 3412 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 carcase 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 × 100.

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

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 250 mg 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.