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**Original Research**

**Title:** *Cryptosporidium* infection is associated with reduced growth and diarrhoea in goats beyond weaning

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**Highlights**

- *Cryptosporidium* associated with reduced growth & diarrhoea in goats 9-12 months old
- *C. parvum* and *C. ubiquitum* shedding associated with diarrhoea post-weaning
- *C. xiaoi* associated with reduced growth, but not diarrhoea
- Challenges notion that *Cryptosporidium* infections without diarrhoea are asymptomatic
- First reported production impacts for *C. xiaoi* in goats older than 3 months of age

**Abstract**

*Cryptosporidium* and *Giardia* are common parasites of ruminant livestock worldwide. These parasites are associated with diarrhoea outbreaks in young goats (pre-weaning), but the impacts on health and productivity for older goats (post-weaning) are not well understood. Here we show *Cryptosporidium* faecal shedding is associated with reduced growth and diarrhoea in goats aged approximately 9-15 months. Goats were sampled four times at one-month intervals. Faecal shedding for a range of pathogens were determined using quantitative PCR and sequencing (*Cryptosporidium, Giardia, Eimeria, Salmonella, Campylobacter*), and microscopy (trichostrongylid nematode worm egg count and *Entamoeba*). *Cryptosporidium* faecal shedding was associated with 1.5kg lower growth for the one-month period following sampling. Specifically, *C. xiaoi* was associated with 1.9kg lower growth in the following month. This is the first report of production impacts associated with *C. xiaoi* in ruminants older than 3 months of age. *Cryptosporidium* shedding was associated with an over 4-fold increase in risk of diarrhoea, with *C. parvum* associated with 10-fold and *C. ubiquitum* associated with 16-fold increase in risk of diarrhoea. Notably, *C. xiaoi* shedding was not associated with increased risk of diarrhoea. *Giardia* shedding was associated with looser faecal consistency, but not diarrhoea. Higher *Eimeria* oocyst counts were weakly associated with lower live weight, poorer body condition and looser faecal consistency. Shedding of other enteric pathogens were not associated with impacts on live weight, growth or diarrhoea risk. This study challenges the two notions that *Cryptosporidium* infections only impact health and productivity of goats during the pre-weaning period, and that *Cryptosporidium* (and specifically *C. xiaoi*) infections in the absence of diarrhoea are asymptomatic. Recognising the potential for impacts of *Cryptosporidium* infection on growth rates in the absence of diarrhoea will support improved design for experiments testing

impacts of *Cryptosporidium* on ruminant health and production. Improved understanding of the role of protozoan infections on animal health has implications for the management of goats in order to reduce adverse impacts on farm profitability, animal welfare and public health risk.

**Keywords**

*Cryptosporidium xiaoi; Cryptosporidium parvum; Cryptosporidium ubiquitum;* post-weaning; *Giardia; Salmonella; Campylobacter;* Asymptomatic

## 1. Introduction

*Cryptosporidium* and *Giardia* are widely recognised as a cause of diarrhoea in goat kids, lambs and calves aged up to approximately 3 months of age (de Graaf et al., 1999; Thompson et al., 2008; Noordeen et al., 2012; Paraud and Chartier, 2012; Robertson et al., 2013; Thomson et al., 2017). However, the impacts of *Cryptosporidium* and *Giardia* on productivity of sheep and goats beyond this period (post-weaning) are poorly understood. The general consensus is that infections are commonly asymptomatic, self-limiting, and oocyst shedding can be intermittent (de Graaf et al., 1999; O'Handley and Olson, 2006). Furthermore, there are a number of other parasitic and bacterial infections that may cause diarrhoea and ill thrift (poor growth) in goats (e.g *Eimeria* spp. *Salmonella* spp., *Teladorsagia circumcincta*, *Trichostrongylus* spp.), and these are often identified concurrently with *Cryptosporidium* and *Giardia* infection (de Graaf et al., 1999; Noordeen et al., 2012; Paraud and Chartier, 2012). As such, longitudinal studies (where individual animals are sampled on more than one occasion) that screen for a range of gastrointestinal pathogens (that may be associated with diarrhoea and poor growth) are more likely to establish whether

*Cryptosporidium* or *Giardia* are impacting on goat health and productivity. Recent longitudinal studies demonstrated that *C. parvum* and *Giardia* faecal shedding post-weaning was associated with lower carcass weight and live weight in Australian sheep (Jacobson et al., 2016), but similar studies have not been reported for goats.

Diarrhoea and ill-thrift in goats of post-weaning age are cited as important issues impacting goat meat production, particularly when rangeland goats are confined in goat depots (feedlots) prior to slaughter (Meat and Livestock Australia, 2018). The causes of diarrhoea and ill thrift in goats of post-weaning age are not well understood, but it is suggested that stress related to transport, overcrowding and, for wild goats, capture and domestication predispose goats to disease. Recent investigations identified faecal shedding by captured rangeland goats for a range of infections with potential veterinary and zoonotic significance, including *Cryptosporidium* and *Giardia* (Al-Habsi et al., 2017c), *Eimeria* (Al-Habsi et al., 2017b), *Entamoeba* (Al-Habsi et al., 2017a), and *Salmonella* and *Campylobacter* (Al-Habsi et al., 2018). Elucidating the impact of these infections (and especially *Cryptosporidium* and *Giardia*) on goat productivity, and specifically diarrhoea and ill thrift, for goats raised in commercial production environments would inform recommendations for best-practice management to address impacts on farm profitability and animal welfare, as well as addressing public health risks associated with zoonotic pathogens.

The aim of this study was to investigate whether faecal shedding of protozoan parasites (*Cryptosporidium*, *Giardia, Eimeria*, *Entamoeba*) and/or faecal carriage for bacteria (*Salmonella*, *Campylobacter*) were associated with diarrhoea or ill thrift (reduced growth, live weight or body condition) in captured rangeland goats.

## 2. Materials and methods

*2.1. Animals and sample collection*

The sampling protocol for this study has been previously described (Al-Habsi et al., 2017a; Al-Habsi et al., 2017b; Al-Habsi et al., 2017c; Al-Habsi et al., 2018). Briefly, male rangeland goats (*n*=125) were captured from a grazing property located in the Gascoyne region of Western Australia, and transported by road to a commercial goat depot (feedlot). The mean weight for goats on arrival at the feedlot was 31.4 kg (95% confidence interval 30.4, 32.4). The estimated age of goats was 9–12 months based on dentition.

Goats were weighed and faecal samples collected from each goat on four occasions (S1-S4). The first sample collection (S1) occurred immediately after arrival at the feedlot. Subsequent sampling (S2-S4) occurred monthly thereafter. Faecal samples were collected directly from the rectum and stored on ice or in a refrigerator (4.0°C) until DNA extraction.

Goats were housed in four group pens (30-33 goats per pen) for the duration of the study. Grain-based pellets, hay and water were supplied *ad libitum*. Straw-bedding was provided with bare dirt covering the majority of available pen space. No pasture was available for the duration of the study. Goats were consigned for slaughter after the conclusion of the experiment when they reached acceptable slaughter weight.

All goats were treated with 0.4 mg/kg moxidectin (Cydectin®, Virbac Australia) and 20 mg/kg toltrazuril (Baycox®, Bayer Australia) immediately after the first (S1) and second (S2) sampling as part of the standard management practice for goats being introduced to the feedlot.

*2.2 Measurements*

Live weight was measured on each sampling occasion using electronic scales. Body condition score (BCS) was measured by a single investigator by means of manual palpation of

tissue over the lumbar vertebrae and using a scale of 1 (very thin) to 5 (very fat) as previously described (Russel et al., 1969).

Faecal consistency score (FCS) was measured using a scale of 1 to 5, with 1 = hard faecal pellet; 2 = soft faecal pellet; 3 = soft faeces with loss of pellet structure (firm paste); 4 = pasty diarrhoea (soft paste); 5 = watery diarrhoea (liquid consistency).

Faecal worm egg counts (WEC) were determined with a modified McMaster technique using 2.0 g faeces from each sample, whereby each egg counted represented 50 eggs per gram (epg) of faeces (Lyndal-Murphy, 1993).

*2.3 DNA extraction*

DNA extraction was performed for each faecal sample (*n* = 500) with four freeze–thaw cycles followed by genomic DNA extraction from 200 mg of each faecal sample using a Power Soil DNA Kit (MolBio, Carlsbad, California). A mechanical bead disruption step using glass beads was included to increase the efficiency of DNA extraction. Each extraction group included a negative control (no faecal sample).

*2.4 PCR screening and amplification*

*2.4.1 Cryptosporidium* and *Giardia*

Screening and amplification of faecal samples (*n* = 500) for *Cryptosporidium* and *Giardia* has been described in more detail by Al-Habsi et al. (2017c). Briefly, qPCR screening was performed using methods previously described for *Cryptosporidium* (Yang et al., 2014a) and *Giardia* (Yang et al., 2014b). *Cryptosporidium*-positive samples were amplified at the 18S ribosomal RNA (rRNA) locus using nested PCR (Morgan et al., 1997), with subtyping at the 60 kDa glycoprotein (*gp60*) locus for *C. parvum* (Ng et al., 2008) and *C. ubiquitum* (Li et al., 2014).

*Giardia*-positive samples were amplified at the glutamate dehydrogenase (*gdh*) and β-giardin loci (Read et al., 2004; Lalle et al., 2005). Assemblage E was screened for using assemblage E-specific primers at the triose-phosphate isomerase (*tpi*) gene (Geurden et al., 2008).

*2.4.2 Salmonella* and *Campylobacter*

Screening and amplification of faecal samples (*n* = 500) for *Salmonella* and *Campylobacter* has been described in more detail by Al-Habsi et al. (2018). Briefly, qPCR screening was performed for *Salmonella* targeting the outer membrane protein (*ompF*) and for *Campylobacter* targeting the purine biosynthesis gene (*purA*) using methods previously described (Yang et al., 2014c). *Salmonella*-positive samples were characterised at the outer membrane porin (*ompF*) (Tatavarthy and Cannons, 2010) and invasion A (*invA*) (Swamy et al., 1996) genes as *S. enterica* , and at STM2755 and STM4497 genes as *S.* Typhimurium (Shanmugasundaram et al., 2009). *Campylobacter*-positive samples were characterised at 16S rRNA and hippuricase (*hipO*) genes (Persson and Olsen, 2005).

*2.4.3 Entamoeba*

Faecal samples (*n* = 125) collected at the first sample collection (S1) only were screened for *Entamoeba* cysts using microscopy, and *Entamoeba*-positive samples were confirmed by amplification at 18S rRNA and actin loci using nested PCR described in more detail by (Al-Habsi et al., 2017a).

*2.4.4 Eimeria*

Screening and quantification for *Eimeria* has been described in more detail by Al-Habsi et al. (2017b). Briefly, qPCR at the 18S rRNA locus was used for screening faecal samples and

quantification of oocysts per gram (OPG) was performed using methods previously described by Yang et al. (2014d).

*2.5 Sequencing*

Purified PCR products were sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, California). Nucleotide sequences were analyzed using Chromas lite version 2.0 (http://www. technelysium.com.au) and aligned with reference sequences from GenBank using Clustal W (http://www.clustalw.genome.jp).

*2.6 Worm egg counts*

Faecal worm egg counts (WEC) were performed within 2 days of arrival by microscopy using a modified McMaster method (Lyndal-Murphy, 1993) using 2 g of faeces from each sample whereby each egg counted represented 50 eggs per gram (epg). All eggs counted were trichostrongylid eggs, with no *Nematodirus* eggs observed.

*2.7 Statistical analyses*

The experimental unit for analyses was individual goats. Analyses were performed using SAS (Version 9.2, SAS Institute) for linear mixed effect models and IBM SPSS statistics (version 24, IBM Corporation) for all other analyses. Significance was accepted where $P<0.05$.

Pathogen faecal detection (shedding) was categorised as detected (pathogen detected by qPCR) or absent (not detected) for each goat at each time point (*Cryptospordium*, *Giardia*, *Salmonella, Campylobacter*) or for the first time point only (*Entamoeba*). *Cryptosporidium* faecal detection was further categorised for species detected (e.g. *C. xiaoi* detected, *C. ubiquitum* detected, *C. parvum* detected, *Cryptosporidium* spp. detected but not

sequenced, no *Cryptosporidium* detected). Goats were categorised separately at each sampling occasion for the number of pathogen genera detected (including *Cryptosporidium*, *Giardia*, *Salmonella, Campylobacter*). McMaster WEC and qPCR *Eimeria* OPG were log-transformed for analyses using $Log_{10}$ (x + 25) where x = trichostrongylid eggs (determined by modified McMaster technique) or *Eimeria* oocysts (determined by qPCR) per gram of faeces. Monthly growth rate was determined by calculating weight change (kg) for the sampling interval (approximately one-month period) following faecal sample collection (future growth) for sampling occasions 1-3, and the sampling interval before faecal sample collection (past growth) for sampling occasions 2-4.

Faecal samples were categorised as diarrhoeic (FCS 4 or higher) or pelleted consistency (FCS 1-2). There were no faecal samples with FCS=3. The prevalence of diarrhoea (% samples categorised as diarrhoeic) was determined for faecal samples with and without pathogen faecal shedding detected. Diarrhoea prevalence was determined separately for each of the pathogens (i.e. proportion of diarrhoeic goats with and without evidence of faecal detection determined for each pathogen). The prevalence of diarrhoea for faecal samples with or without pathogen faecal shedding detected were compared using the Chi-squared test with Fishers exact two-sided test for independence. Odds ratios were used to calculate relative risk for diarrhoea associated with pathogen detection category (e.g. risk of diarrhoea associated with pathogen faecal detection). Odds ratios were calculated separately for each pathogen. Odds ratios were also used to calculate relative risk for diarrhoea associated with pathogen number detected (e.g. risk of diarrhoea associated with number of pathogens detected).

Associations between log-transformed oocyst counts (McMaster trichostrongylid WEC and qPCR *Eimeria* OPG) and live weight, past growth, future growth, BCS and FCS were

determined using bivariate correlations with Pearson co-efficient and two-tailed test for significance. Correlations were determined both separately for each time point, and also for all time points combined.

Association between pathogen detection and production parameters (live weight, future growth, past growth, BCS and FCS) were analysed using linear mixed effects models. Initially base models were constructed separately for each dependent variable (live weight, future growth, past growth, BCS and FCS), with pen number (pen 1-4) and sampling occasion (S1, S2, S3 or S4) included as categorical fixed effects, and log transformed WEC and OPG included as co-variates (continuous fixed effects). To account for repeat sampling of individual goats across the four time-points, animal identification code was included as a random term. Within these base models, pathogen detection (detected or absent) was included as a categorical fixed effect, giving a total of 20 models, i.e separate models for 4 pathogens (*Cryptosporidium*, *Giardia, Salmonella, Campylobacter*) for each of 5 dependent variables (live weight, past growth, future growth, BCS and FCS). This process was then repeated with the *Cryptosporidium* species included as a categorical fixed effect (*C. xiaoi* detected*, C. ubiquitum* detected*, C. parvum* detected, *Cryptosporidium* detected but not sequenced, *Cryptosporidium* not detected). Interactions between the pathogen term and other factors in the base model were tested, and non-significant terms ($P>0.05$) were removed in a step-wise manner. Least square means were generated for significant pathogen main effects.

*Entamoeba* screening was only performed for samples from S1, so association between *Entamoeba* detection and production parameters were analysed using univariate general linear models. Models were constructed separately for each dependent variable (live weight, future growth, BCS and FCS), with *Entamoeba* detection (detected or absent) and pen

number (pen 1-4) included as categorical fixed effects, and log transformed WEC and OPG included as co-variates (continuous fixed effects).

Association between number of pathogens detected and production parameters (live weight, future growth, past growth, BCS and FCS) were analysed using linear mixed effects models. Models were constructed separately for each dependent variable (live weight, future growth, past growth, BCS and FCS). Number of pathogens detected (0-4 pathogens), pen number (pen 1-4) and sampling occasion (S1, S2, S3 or S4) were included as fixed effects, and animal identification code was included as a random term. Log transformed WEC and OPG were included as co-variates (continuous fixed effects). Interactions between the number of pathogens term and other factors were tested, and non-significant terms ($P$>0.05) were removed in a step-wise manner. Least square means were generated for significant pathogen number main effects.

## 3. Results

### *3.1 Pathogen prevalence and oocyst counts*

The prevalence for the eight pathogens identified by qPCR screening and sequencing are shown in Table 1, and have been described in more detail by Al-Habsi et al. (2017a); Al-Habsi et al. (2017b); Al-Habsi et al. (2017c); Al-Habsi et al. (2018). Total prevalence (proportion of goats positive for pathogen on at least one sampling occasion) were 27.2% for *Cryptosporidium* spp., 29.6% for *Giardia* (Al-Habsi et al., 2017c), 90.4% for *Eimeria* spp. (Al-Habsi et al., 2017b), 30.4% for *S.* Typhimurium, and 9.6% for *C. jejuni* (Al-Habsi et al., 2018).

McMaster WEC and *Eimeria* oocyst counts are shown in Table 2. With the exception of *Eimeria*, pathogen prevalences were highest at S1, immediately following arrival at the goat depot (Table 1). *Eimeria* prevalence and oocyst counts were highest at S2, approximately one

month after arrival at the goat depot (Table 2). Three *Eimeria* spp. were identified; *E. christenseni*, *E. hirci*, and *E. arloingi* (Al-Habsi et al., 2017b). Overall, 82/125 goats had WEC ≥ 50 epg on at least one sampling occasion. Larval culture and differentiation were not performed, but molecular detection by qPCR identified *H. contortus* and *Trichostrongylus* spp. in samples from S1 only (Al-Habsi, 2017).

*3.2 Live weight, body condition scores and faecal consistency*

Live weight, BCS and FCS at each sampling occasion are shown in Table 3. Live weight increased at each sampling occasion (Table 3). Body condition improved at the second and third sampling occasion relative the previous measurement (Table 3). Faecal samples had looser consistency (higher FCS) at the second sample collection, one month after arrival at the feedlot (Table 3). The prevalence for diarrhoea prevalence was highest at the second sample collection (27.2%), and ranged 4.0-6.4% for the other sampling occasions.

*3.3 Associations for* Cryptosporidium *with goat weight, growth, body condition and faecal consistency*

*Cryptosporidium* faecal shedding was associated with 1.48kg lower growth for the one-month period following sampling compared to *Cryptosporidium*-negative goats (0.71kg/month versus 2.19kg/month; *P*=0.041). *Cryptosporidium xiaoi* shedding was associated with 1.95kg lower growth in the following month compared with *Cryptosporidium*-negative goats (0.24kg/month versus 2.19kg/month; *P*=0.047). There was no association between *Cryptosporidium ubiquitum* shedding and subsequent growth (*P*=0.105). *Cryptosporidium parvum* was only detected at the final sample collection (Table 1), therefore association with subsequent growth rate could not be determined for this species.

There was no association between *Cryptosporidium* and live weight (*P*=0.717), growth in the one-month period prior to sampling (*P*=0.995) or BCS (P=0.977).

*Cryptosporidium* shedding was associated with 0.9 higher FCS relative to *Cryptosporidium*-negative samples (2.34 versus 1.42; *P*<0.001). *Cryptosporidium xiaoi* shedding was associated with 0.49 higher FCS (*P*=0.030), *C. ubiquitum* with 1.45 higher FCS (*P*<0.001) and *C. parvum* with 1.55 higher FCS (*P*<0.001) compared to *Cryptosporidium*-negative samples.

The prevalence of diarrhoea was 21.1% higher for C*ryptosporidium*-positive samples (prevalence 30.3%) compared with *Cryptosporidium*-negative samples (prevalence 9.2%; *P*<0.001). *Cryptosporidium*-positive faecal samples were 4.3 (95% confidence interval 1.9, 9.6; *P*=0.001) times more likely to be classified as diarrhoeic compared with *Cryptosporidium*-negative samples. Compared to *Cryptosporidium*-negative samples, *C. parvum*-positive samples were 9.9 (95% CI 1.4, 71.8; *P*=0.047) times more likely to be classified as diarrhoeic, and *C. ubiquitum*-positive samples were 16.4 (95% CI 3.8, 71.1; *P*<0.001) more likely to be classified as diarrhoeic.

*3.4 Associations for* Giardia *with goat weight, growth, body condition and faecal consistency*

There was no association between *Giardia* and live weight (*P*=0.779), growth in the one-month period prior to sampling (*P*=0.350), growth in one-month period following sampling (*P*=0.173), or BCS (*P*=0.146).

*Giardia* shedding was associated with 0.4 higher FCS compared to *Giardia*-negative samples (1.86 versus 1.46; *P*=0.008), but there was no difference in diarrhoea risk for *Giardia*-positive samples (*P*=0.105).

*3.5 Associations for* Entamoeba *with goat weight, growth, body condition and faecal consistency*

There was no association between *Entamoeba* shedding and live weight (*P*=0.740), growth in the one-month period following sampling (*P*=0.505) or BCS (*P*=0.575). *Entamoeba* prevalence was determined only for S1, so association with previous growth could not be determined. There was no association between *Entamoeba* detection and FCS (*P*=0.977) or diarrhoea risk (*P*=1.000). No goat samples with *Entamoeba* detected were classified as diarrhoeic.

*3.6 Associations for* Salmonella *with goat weight, growth, condition and faecal consistency*

There was no association between *Salmonella* faecal carriage and live weight (*P*=0.081), growth in the one-month period before sampling (*P*=0.827), growth in the one-month period following sampling (*P*=0.088), BCS (*P*=0.952), FCS (*P*=0.219) or diarrhoea risk (*P*=0.294).

*3.7 Associations for* Campylobacter *with goat weight, growth, body condition and faecal consistency*

There was no association between *Campylobacter* faecal carriage and live weight (*P*=0.359), growth in the one-month period before sampling (*P*=0.504), growth in the one-month period following sampling (*P*=0.223), BCS (*P*=0.283), FCS (*P*=0.288) or diarrhoea risk (*P*=0.616).

3.8 *Associations for* Eimeria *with goat weight, growth, body condition and faecal consistency*

There was a weak but significant negative correlation between *Eimeria* oocyst count and live weight (Pearson co-efficient -0.143; *P*=0.001), and between *Eimeria* oocyst count and BCS (Pearson co-efficient -0.096; *P*=0.032). These observations were only evident for data pooled across all time points, with no correlation between *Eimeria* oocyst count and weight or BCS identified at any of the four time points (*P*>0.100). There was no correlation between *Eimeria* oocyst count and growth in the previous or subsequent month either overall or for any of the periods tested (*P*>0.05). There was a positive correlation identified between *Eimeria* oocyst count and FCS at S1 (Pearson co-efficient 0.261; *P*=0.003) and overall (Pearson co-efficient 0.231; *P*<0.001), but not for any of the other sample collections (*P*>0.100).

*3.9 Associations for nematode WEC with goat weight, growth, body condition and faecal consistency*

There was a weak but significant negative correlation between WEC and BCS (Pearson co-efficient -0.096; *P*=0.031). This was only evident for data pooled across all time points, with no correlation between WEC and BCS identified at any of the four time points (*P*>0.100). There was no correlation (*P*>0.100) overall or for any of the sampling periods tested for WEC with live weight, growth in the previous or subsequent month, or FCS.

*3.10 Associations for number of pathogens with goat weight, growth, body condition and faecal consistency*

There was an association between the number of genera identified and faecal consistency score whereby goats with greater number of genera detected had higher FCS (Table 4). Notably, samples with three pathogen genera detected had FCS greater than 3,

suggesting loss of pelleted faecal structure for these goats (Table 4). Faecal samples with three pathogen genera detected were 5.3 (95% CI 1.2, 22.9) times more likely to be classified as diarrhoeic compared to samples with less than three genera detected (*P*=0.043).

There was no association between number of pathogen genera identified and live weight, previous weight change, future weight change or BCS (*P*>0.05).

**4. Discussion**

Our study shows associations for *Cryptosporidium* shedding with lower growth rates and diarrhoea in goats aged approximately 9-15 months old. A number of reviews have indicated a 'paucity of evidence' about clinical impacts of *Cryptosporidium* and *Giardia* infection for ruminants beyond weaning age (2-3 months old) (O'Handley and Olson, 2006; Noordeen et al., 2012; Paraud and Chartier, 2012; Thomson et al., 2017), and long-term impacts of infection on livestock production remain an important research gap. Our study indicates that *Cryptosporidium* spp. shedding by goats of post-weaning age was associated with reduced growth in the month after detection as well as increased diarrhoea risk. *Cryptosporidium xiaoi* was associated with reduced growth, but not diarrhoea. To the best of our knowledge, this study provides the first report of an association between *C. xiaoi* and adverse impacts on goat health and productivity in ruminants beyond weaning age. Importantly, this observation challenges the notion that non-diarrhoeic animals shedding *Cryptosporidium* are asymptomatic, and studies investigating impact of *Cryptosporidium* infections in goats consider monitoring impacts on growth even with apparently asymptomatic infections.

*Cryptosporidium* spp. shedding was associated with 1.5kg lower growth in the month after detection, and an over 4-fold increase in the risk of diarrhoea. Our study was able to

explore associations with health and production parameters for specific *Cryptosporidium* species. The most noteworthy observation in this respect was the association between *C. xiaoi* shedding and 1.9kg reduced growth in the one month period after sampling. Whilst *C. xiaoi* has been reported in diarrhoeic kids less than 3 months of age (Díaz et al., 2010; Rieux et al., 2013; Díaz et al., 2015; Siddiki et al., 2015), infections in goats and sheep post-weaning age are considered to be typically asymptomatic (Robertson et al., 2013). The assumption that infections are typical asymptomatic (or sub-clinical) has been made on the basis that the organism is commonly isolated from non-diarrhoeic goats and sheep (Sweeny et al., 2011; Ye et al., 2013; Hijjawi et al., 2016; Díaz et al., 2018). This is consistent with our observations that *C. xiaoi* shedding was not associated with diarrhoea in 9-12 month-old goats with naturally acquired infections.

Our study indicates *C. xiaoi* can be associated with impacts on goat production, specifically growth rate, in the absence diarrhoea. This implies that the notion that *Cryptosporidium* infections in older goats are typically asymptomatic is flawed. Most *Cryptosporidium* and *Giardia* studies report only associations with diarrhoea. Our study also included measurement of growth rate, live weight and body condition and was therefore able to demonstrate impacts on growth in absence of diarrhoea for *C. xiaoi*. Further, it is likely that measuring growth and live weight underestimates impacts of parasitism on meat production, with adverse impacts on carcass weight and dressing efficiency (carcass weight as proportion of live weight) observed in the absence of measurable impacts on live weight for sheep with gastrointestinal parasitic infections (Jacobson et al., 2009; Sweeny et al., 2011; Jacobson et al., 2016). As such, future studies should explore the impact of *Cryptosporidium* infections on growth and carcass productivity, even for apparently asymptomatic animals (without evidence of diarrhoea).

*Cryptosporidium parvum* and *C. ubiquitum* were both associated with an increased risk of diarrhoea, but the prevalence of both species was too low in the goats to draw meaningful conclusions about the association with growth rate (or lack thereof). *Cryptosporidium parvum* was only detected at the last sample collection, precluding measurement of growth for the following one-month period. Symptomatic *C. parvum* infections appear to be uncommon in goats and sheep beyond weaning age, although recent studies identified associations between *C. parvum* shedding and lower live weight, carcass weight and carcass dressing efficiency in lambs during the post-weaning period (Jacobson et al., 2016). In the present study, *C. ubiquitum*-positive animals appeared to have 2.10kg lower growth rate in the following month, but this difference was not significant ($P$=0.105), suggesting the need for further work to explore impacts on production in goat flocks where this species is evident at higher prevalence. Both *C. parvum* and *C. ubiquitum* are zoonotic, therefore faecal shedding by goats has implications for food safety (Ryan et al., 2018), management of water catchments (Wells et al., 2015), and recommendations for people susceptible to infection that contact goats (Gormley et al., 2011).

*Giardia* shedding was associated with looser faecal consistency (higher FCS), but no increase in the risk of diarrhoea or association with reduced weight, growth or body condition. This was consistent with the general notion that *Giardia* infections are commonly asymptomatic in ruminants of post-weaning age. O'Handley and Olson (2006) noted very few studies describe the long-term impact of giardiosis on ruminant production, and this remains the case for goats and especially with respect to impacts post-weaning. Adverse impacts on growth, feed conversion and carcass productivity have been observed subsequent to experimental *G. duodenalis* infection of specific-pathogen-free lambs at 6 weeks of age (Olson et al., 1995). *Giardia* shedding by Australian lambs with naturally acquired infections was not

associated with any demonstrable impact on live weight, but lambs with higher shedding concentration post-weaning had 0.5kg lighter carcass weight at slaughter (Jacobson et al., 2016).

It was not possible to draw firm conclusions about the impact of *Eimeria* and gastrointestinal nematodes on the health and productivity of the goats in this study. Goats were treated with an anthelmintic (moxidectin) and anticoccidial treatment (toltrazuril) twice during the experiment as part of the feedlot protocol. The relatively low WEC (<500 epg) at S1 suggests worm burdens were not high on arrival at the feedlot, and reflects low level of worm challenge for the wild goats prior to capture. Goats were sourced from a semi-arid region characterised by high temperature and low rainfall. Diet for rangeland goats in this region is predominantly browsing shrubs. Wild rangeland goats are managed at very low stocking densities. These factors all serve to reduce transmission of nematode larvae, and gastrointestinal nematodes typically only impact rangeland goat health following unusually high rainfall or when goats are confined (McGregor et al., 2014; Nogueira et al., 2016; Meat and Livestock Australia, 2018).

Nonetheless, weak but significant correlations between WEC and body condition were noted, suggesting that thinner goats had higher WEC. Similarly, goats with higher *Eimeria* OPG tended to be lighter (negative correlation with live weight), thinner (negative correlation with BCS) and have looser faecal consistency (positive correlation with FCS). This supports the general recommendation that goats, and especially those in poorer body condition or confined at higher stocking densities, should be monitored for evidence of nematodes and *Eimeria* (Meat and Livestock Australia, 2018). The correlations identified, whilst statistically significant, were relatively weak whereby WEC explained less than 10% variation in BCS, and

*Eimeria* OPG explained less that 27% of the variation in FCS, less than 15% of variation in weight and less than 10% variation in BCS.

*Eimeria* and nematode infections were evident one-month after treatment with toltrazuril and moxidectin. This observation has been discussed in more detail (Al-Habsi, 2017; Al-Habsi et al., 2017b), but it was not clear whether this represents reinfection, treatment failure or anthelmintic resistance. *Haemonchus contortus* and *Trichostrongylus* spp. were detected at S1 only (Al-Habsi, 2017). The qPCR was not validated with larval cultures, therefore associations between specific nematode species and goat production were not determined in this study.

High rates of faecal carriage (26%) for *Salmonella* Typhimurium were identified at the first sampling, immediately following capture and transport to the feedlot. There was no evidence of increased diarrhoea risk, nor relationship with live weight or body condition. This suggests that high rates of *Salmonella* faecal carriage may be evident in the absence of diarrhoea, and neither size (weight) or condition would have been useful in predicting faecal carriage by individuals in this cohort of goats. This has implications for assessing *Salmonella* risk, as goats without signs of diarrhoea or ill thrift may act as source of *Salmonella* Typhimurium contamination.

The study had several strengths and weaknesses that impact the conclusions. Diarrhoea is typically a multifactorial disease and mixed infections are common in ruminants (de Graaf et al., 1999). In the present study, faecal samples were screened for a range of pathogens. The statistical analyses for the major pathogens tested (*Cryptosporidium*, *Giardia, Entamoeba, Salmonella, Campylobacter*) also accounted for nematode and *Eimeria* burden (WEC and OPC included as continuous fixed effects). The relatively large sample size (125 goats) and longitudinal design (repeat sampling of individual animals) address some

limitations associated with intermittent shedding of oocysts (Xiao and Herd, 1994). The management of the goats included a stress-inducing event (capture and transport to the feedlot), and goats were managed under conditions typical for rangeland goat meat production, meaning the observations have applicability to goat farms and meat processors (abattoirs).

A major limitation is that causation cannot be confirmed with the observational study design. *Cryptosporidium* shedding was associated with reduced growth and increased diarrhoea risk, but it cannot be determined whether infection resulted in poorer growth and diarrhoea, or whether goats that were otherwise growing poorly and diarrhoeic were more likely to be shedding *Cryptosporidium* or higher concentration of *Eimeria* oocysts. Similarly, animals with concurrent (mixed) infections with three pathogen genera had looser faecal consistency and increased risk of diarrhoea, but it is not clear whether the cumulative effect of pathogen genera identified was causative of diarrhoea, or whether goats that are otherwise diarrhoeic are more likely to be shedding parasites and bacteria. Interestingly, there was no association between faecal shedding for pathogens (*Cryptosporidium*, *Giardia, Entamoeba, Salmonella, Campylobacter*) with live weight, BCS or past growth, suggesting that faecal shedding was not detected only in smaller goats, skinny goats or goats that were otherwise growing poorly.

Other key limitations were related to the duration and location of the study. The experiment was conducted over an approximately three-month period (February – May). The feedlot was located in a region with a Mediterranean climate (hot dry summers, cool wet winters), with colder wetter weather typically experienced in May-September. Only one cohort of goats was included in the study, and further work is required to determine if the observations are replicated in goats sourced over wider geographical area and seasonal

conditions. Finally, as mentioned previously, goats were treated twice with an anthelmintic and anticoccidial treatment. Whilst this reflected normal commercial management for captured rangeland goats, it impacted ability to draw conclusions about the impact of *Eimeria* and nematodes on growth and diarrhoea.

## 5. Conclusion

This study showed that *Cryptosporidium* faecal shedding was associated with lower growth rate and diarrhoea in goats aged approximately 9-15 months old. This challenges the notion that health and production impacts are restricted to the pre-weaning period for goats. *Cryptosporidium xiaoi* was associated with lower growth rate, providing the first report of adverse impacts on health and productivity in ruminants beyond 3 months of age. Importantly, the association between *C. xiaoi* and lower growth was evident in the absence of increased diarrhoea risk, suggesting that *C. xiaoi* infections in the absence of diarrhoea should not be assumed to be asymptomatic. *Cryptosporidium parvum* and *C. ubiquitum* were associated with diarrhoea, and both species are considered zoonotic. Higher *Eimeria* oocyst counts were associated with lower live weight, poorer body condition and looser faeces. Goat health management programmes should incorporate monitoring for *Cryptosporidium* and *Eimeria*, even after weaning and in flocks without evidence of diarrhoea, in order to identify where intervention may be warranted to address adverse impacts of these parasites on the growth of goats.

## Declarations of interest

None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

The funding body (Meat and Livestock Australia and Livecorp) approved the experimental design for the overall project (of which this data formed one portion) as part of the normal project approval process. Meat and Livestock Australia approved the manuscript for submission. Neither funding body was involved in the collection, analysis or interpretation of data, or in the writing of the manuscript other than minor editorial advice on the final manuscript draft to improve clarity of presentation provided by Johann Schroder (Meat and Livestock Australia).

All procedures complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council and Australian Research Council, 2013), and were approved and monitored by the Murdoch University Animal Ethics Committee (approval number R2617/13).

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**Table 1.** Enteric pathogen point prevalence (% with 95% confidence interval in parentheses) for 125 goats sampled on 4 occasions (S1-S4).

| | **Point prevalence** % (95% confidence interval) | | | | | |
|---|---|---|---|---|---|---|
| | **S1** | **S2** | **S3** | **S4** | **Screening method** | **Reference** |
| *Cryptosporidium* spp. | 14.4 (9.1, 21.3) [a] | 4.0 (1.5, 8.5) [b] | 3.2 (1.1, 7.4) [b] | 7.2 (3.6, 12.7) [ab] | qPCR | Al-Habsi et al. (2017c) |
| *C. xiaoi* | 8.8 (4.8, 14.7) [a] | 2.4 (0.7, 6.3) [b] | 0.8 (0.1, 3.7) [b] | 1.6 (0.3, 5.0) [b] | qPCR & sequencing | Al-Habsi et al. (2017c) |
| *C. ubiquitum* | 5.6 (2.5, 10.7) [a] | 0.8 (0.1, 3.7) [b] | 0 (0, 2.0) [b] | 0 (0, 2.0) [b] | qPCR & sequencing | Al-Habsi et al. (2017c) |
| *C. parvum* | 0 (0, 2.0) [a] | 0 (0, 2.0) [a] | 0 (0, 2.0) [a] | 3.2 (1.1, 7.4) [b] | qPCR & sequencing | Al-Habsi et al. (2017c) |
| *Giardia duodenalis* | 12.0 (7.2, 18.5) [a] | 4.0 (1.5, 8.5) [b] | 7.2 (3.6, 12.7) [ab] | 7.2 (3.6, 12.7) [ab] | qPCR | Al-Habsi et al. (2017c) |
| *Salmonella* Typhimurium | 25.6 (18.6, 33.8) [a] | 4.8 (2.0, 9.6) [b] | 1.6 (0.3, 5.0) [b] | 0 (0, 2) [b] | qPCR & sequencing | Al-Habsi et al. (2018) |
| *Campylobacter jejuni* | 8 (4.2, 13.7) [a] | 0.8 (0, 3.7) [b] | 0.8 (0, 3.7) [b] | 0 (0, 2) [b] | qPCR & sequencing | Al-Habsi et al. (2018) |
| *Entamoeba*[#] | 6.4 (3.1, 11.7) | - | - | - | Microscopy & nested PCR | Al-Habsi et al. (2017a) |
| *Eimeria*[*] | 50.4 (41.7, 59.1) [a] | 70.4 (62.0, 77.9) [b] | 44.8 (36.3, 53.6) [a] | 2.4 (0.7, 6.3) [c] | qPCR | Al-Habsi et al. (2017b) |

[#] Determined for S1 only.

[*]Goats were treated with moxidectin and toltrazuril after S1 and S2. [abc] Point prevalence values in rows with different superscripts are significantly different ($P$<0.05).

**Table 2**. Worm egg count (WEC) and *Eimeria* oocyst count (mean ± standard error) for 125 goats sampled on 4 occasions (S1-S4).

| | Sample occasion | | | |
|---|---|---|---|---|
| | S1 | S2[#] | S3[#] | S4[#] |
| **WEC** (eggs per g faeces)[*] | | | | |
| All samples (mean ± SE) | 450 ± 59 [a] | 250 ± 37 [b] | 150 ± 29 [bc] | 100 ± 27 [c] |
| Positive samples only (mean ± SE) | 1308 ± 55 [a] | 893 ± 38 [b] | 815 ± 32 [b] | 893 ± 90 [b] |
| All samples (range) | 0–1850 | 0–1350 | 0–1100 | 0–1600 |
| | | | | |
| ***Eimeria*** (oocysts per g faeces)[**] | | | | |
| All samples (mean ± SE) | 530 ± 183 [a] | 10 525 ± 2496 [b] | 350 ± 88 [a] | 1 ± 1 [c] |
| Positive samples only (mean ± SE) | 1051 ± 352 [a] | 15 851 ± 3627 [b] | 841 ± 193 [a] | 31 ± 21 [a] |
| All samples (range) | 0-15 908 | 0-191 821 | 0-5375 | 0-73 |

[*] Trichostrongylid worm egg count determined by microscopy.

[**] *Eimeria* oocyst count determined by qPCR - adapted from Al-Habsi et al. (2017b). SE: standard error. [abc] Values in rows with different superscripts are significantly different ($P$<0.05).

[#] Note: Goats were treated with moxidectin and toltrazuril after S1 and S2.

**Table 3**. Live weight, body condition score (BCS) and faecal consistency score (FCS) for 125 goats sampled on 4 occasions (S1-S4).

| | **Weight** (kg) | | | **BCS** | | | **FCS** | |
|---|---|---|---|---|---|---|---|---|
| Sampling | Mean ± SE | Range | | Mean ± SE | Range | | Mean ± SE | Range |
| S1 | 31.42 ± 0.49 [a] | 17.0 – 49.0 | | 1.76 ± 0.04 [a] | 1 – 3 | | 1.33 ± 0.81 [a] | 1 - 5 |
| S2 | 32.81 ± 0.50 [b] | 21.0 – 51.0 | | 2.02 ± 0.03 [b] | 1 – 3 | | 2.16 ± 1.36 [b] | 1 - 5 |
| S3 | 34.69 ± 0.45 [c] | 21.5 – 50.5 | | 2.23 ± 0.03 [c] | 1 – 3 | | 1.26 ± 0.70 [a] | 1 - 5 |
| S4 | 37.70 ± 0.44 [d] | 23.5 – 53.5 | | 2.24 ± 0.03 [c] | 1 – 3 | | 1.20 ± 0.67 [a] | 1 - 4 |

[abcd] Values in columns with different superscripts are significantly different ($P$<0.05). SE: Standard error.

**Table 4**. Relationship between number of pathogen genera identified (including *Cryptosporidium*, *Giardia*, *Salmonella* and *Campylobacter*) and faecal consistency score (FCS) for 125 goats sampled on 4 occasions determined using linear mixed effect model (WEC and *Eimeria* OPG included as co-variates).

| Number of genera identified | **FCS** (least square mean ± SE)* | **n** |
|---|---|---|
| 0 | 1.93 ± 0.26 [a] | 301 |
| 1 | 2.25 ± 0.27 [b] | 147 |
| 2 | 2.42± 0.30 [b] | 44 |
| 3 | 3.25 ± 0.43 [c] | 8 |
| Main effect | | |
| *F*-value | 6.11 | |
| *P*-value | <0.001 | |

[abc] Values in columns with different superscripts are significantly different ($P<0.05$). SE: Standard error.