1 A comparison of seasonal nematode egg outputs from co-grazed weaner sheep

2 derived from the foundational Camden Park Estate and Australian Meat Merino

3 flocks

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Table of Contents

1. Abstract 3
2. Introduction 5
3. Materials and Methods 7
  3.1 Animals, experimental design and parasite management 7
  3.2 Faecal sample collection and egg counts 8
  3.3 Larval Culture 9
  3.4 PCR for worm identification 10
    3.4.1 Isolation of DNA 10
    3.4.2 PCR Assay 10
  3.5 Body and Fleece Weights 11
  3.6 Statistical Analysis 12
4. Results 12
  4. 1 Comparison of faecal egg counts and larval cultures during a co-grazing trial 12
  4.2 PCR assays 13
  4.3 Body and Fleece weights 14
5. Discussion 15
6. Conclusion 22
7. Acknowledgements 23
8. References 24
Appendix 31

45 **1. Abstract**

46

47 The Camden Park Estate (CPE) sheep are an inbred line of sheep that are reported to
48 originate from the colonial line of Australian Merino. The CPE merinos are
49 maintained as a closed flock for many years and have long been suspected to be
50 more resistant to gastrointestinal nematodes when compared to contemporary
51 sheep. However, this anecdotal evidence has not been tested or the possible
52 resistance investigated. This study investigated the seasonal egg counts of the CPE
53 merino when co-grazed with the Australian Meat Merino (AMM). Wether lambs
54 from the CPE and AMM lines were co-grazed for 10 months on *Haemonchus*
55 *contortus* prone pastures. Faecal egg counts (FEC), Multiplex Tandem PCR (MT-PCR)
56 for eggs speciation and bulk larval cultures (LC) were completed and compared
57 between the sheep lines. FEC were significantly lower (P=0.03) in the CPE (mean
58 8746 ± 1289.0 S.E, n=40) compared to AMM sheep (mean 12541 ± 1187.8 S.E, n=49)
59 during acute haemonchosis at the initial sampling during the Australian summer
60 (February). Subsequent sample collections (April, July, September) occurred under
61 medicated management. These subsequent sample collections did not reveal any
62 significant FEC differences between the sheep lines. The MT-PCR and LC confirmed a
63 >95% prevalence of *H. contortus* in February. This reduced to 71% and 93% in the
64 CPE and AMM lines respectively during the Australian Winter with other nematodes
65 present including *Trichostrongylus colubriformis*, *Oesophagostomum* spp and
66 *Cooperia curteci*. This is the first study to assess the anecdotal evidence of resistance
67 of the CPE line of merino during *H. contortus* infection. Current management
68 practices and ethical considerations limited interpretation of the subsequent

69 sampling time points. The prevailing weather conditions during summer-autumn and
70 an increasing *Haemonchus* challenge resulted in the use of an anthelminthic
71 treatment with long residual effects. An extension of this study under controlled
72 experimental conditions, such as a pen trial, would be required to further investigate
73 the significant difference observed in the summer result which suggested the CPE
74 line was more innately resistant to gastrointestinal nematodes.
75
76 Key Words: Faecal Egg Count, host resistance, Larval Culture, Merino, MT-PCR,
77 nematode
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2. Introduction

Gastrointestinal nematodes (GIN) act as the greatest constraint to the Australian sheep industry, nationally costing over $435 million dollars a year due to loss of production, loss of stock and costs of control (Lane et al., 2015). Current strategies to control nematodes is based on Integrated Parasite Management (IPM), which incorporates nutrition, genetic selection (Besier and Love, 2003), grazing management and the effective use of drenches (Kelly et al., 2010). With the increasing incidence of anthelmintic resistance, reliance on IPM has never been more crucial (Woodgate and Besier, 2010).

Resistance to nematodes dependent on the ability of the host to mount an effective and increased immune response in the presence of parasite infestation. Several breeds of sheep have been identified to have natural resistance to GIN such as the Barbados Blackbelly. This breed when challenged has significantly lower faecal egg output and approximately 1000 less *Haemonchus contortus* larvae at post-mortem compared to susceptible controls (Gruner et al., 2003; Terefe et al., 2007).

Genetic differences within breeds have also been exploited to produce resistance to nematodes. The Rylington Merino bred by the Department of Agriculture Western Australia, averaged 100 eggs per gram (EPG) of faeces compared 700 EPG in the control sheep when trickle infected with Trichostrongylus colubriformis (Liu et al., 2007). Genetic improvement has been made by exploiting factors such as lower FECs, the Carbohydrate Larval Antigen (CarLA), famacha and the use of biomarkers

117 to measure for resistance. Ultimately these nematode resistant individuals will have
118 fewer parasites or lowered nematode egg production (Douch et al., 1996).
119
120 Resistance to GIN parasites is associated with the development of allergic Th2-type
121 immune responses over approximately 6 months after first infection (Emery, 1996),
122 with a heritability of around 0.3 (Dominik, 2005). Resistance is most pragmatically
123 measured by reduced Faecal Egg Counts (FEC) (Douch et al., 1996).and is also used
124 for the diagnosis of worm infections, with levels >500 EPG recommended as
125 decisions to treat. Therefore, although relatively insensitive, FEC is an essential,
126 cheap assay, widely used as a selection tool for genetic progress towards breeding
127 for parasite resistance (Douch et al., 1996). Larval Cultures (LC) are an important
128 assay to determine the species of nematodes present in infections, often utilised by
129 researchers and commercial businesses to determine levels of resistance in
130 nematode species to specific chemical treatments. As a number of strongyle worms
131 have similar eggs, cultures are incubated for 5-7 days to permit development L3
132 stage larvae, allowing species differentiation in mixed infections (Whitlock, 1956).
133
134 Recent molecular developments have enabled a faster and more reliable method for
135 differentiating species of nematodes, compared to traditional techniques. It involves
136 Multiplex Tandem PCR (MT-PCR) of nematode egg DNA which is extracted directly
137 from faeces (Roeber et al., 2012). PCR assays have been able to identify nematode
138 species with a sensitivity up to 10 eggs per gram, (McNally et al., 2013) and r_s=0.77
139 with LC (Roeber et al., 2012).

140

141 Anecdotal evidence from several managers and researchers has suggested that the
142 randomly bred CPE line of merino had fewer parasites compared to modern
143 merinos. This anecdotal evidence provided an ideal opportunity to investigate the
144 seasonal egg counts of the CPE and AMM when co- grazed. In addition to
145 measurements of body weight, fleece weight and the regular collection of faeces for
146 FEC and LC, this study also applied the semi-automated MT-PCR methodology for
147 comparison and to definitively estimate the strongyle population structure in the
148 field. This study aims to confirm whether the anecdotal evidence that the Camden
149 Park Estate (CPE) heritage line of merino is more resistant to GIN when compared to
150 contemporary lines such as the Australian Meat Merino (AMM), during co grazing on
151 parasite-prone pastures through the season from early Summer to the next Spring.

152

153 **3. Materials and Methods**

154

155 **3.1 Animals, experimental design and parasite management**

156

157 This study was conducted at the Elizabeth Macarthur Agricultural Institute in
158 Menangle, New South Wales, Australia where the CPE are maintained separately on
159 a dedicated section of the property. A flock made up of 80 CPE Merinos and 80 AMM
160 wethers were co-grazed throughout the year. Menangle is a summer rainfall area
161 and *Haemonchus contortus* was the nematode of concern from the start of the trial

162 in December (5 months of age). The mob was rotated to new pastures as required.
163 The study was approved by EMAI Animal Ethics Committee (AEC Reference No:
164 M14/08).
165
166 The CPE flock is maintained as a discreet line without selection for genetic
167 improvement, however, culling of genetic defects and individuals that receive
168 veterinary interventions is undertaken (Aggs, 2012). While the CPE breeding stock
169 are not available for research purposes the wether portion is available. For this
170 study, wether lambs from the CPE and AMM were weaned and weighed in mid-
171 December 2014 and were co-grazed thereafter. They were each drenched orally with
172 Noromectin® (Ivermectin) on 8/1/15 to body weight. The mob was predominantly
173 grazed on native pastures, however chicory and oaten pastures were also utilised
174 when required. They were mustered and walked into the yards for sampling.
175
176 Faecal samples were collected from a subset of the flock (n=40 and n=49 for CPE and
177 AMM respectively) and the sheep drenched on four occasions throughout the year
178 (Figure 1). Due to drench efficacy concerns, a number of randomly selected
179 individuals were sampled two weeks after D1 and the mob redrenched with Q-
180 Drench. Local weather data was retrieved from http://www.bom.gov.au
181

182 **3.2 Faecal sample collection and egg counts**

183

184 Faecal sample collections occurred four times throughout the year, S1-S4 (Figure 1).
185 At each sample collection, the sheep were yarded and fresh faecal samples were
186 collected directly from the rectum (2-15g). Samples were placed into plastic jars and
187 labelled with sheep identification number, before being placed into a portable
188 refrigerator, transported and stored at 4°C. The faecal samples were manually
189 homogenised and subsampled to perform Faecal Egg Counts (FEC), Polymerase
190 Chain Reaction (PCR) assays and Larval cultures (LC).
191
192 FEC were conducted at EMAI using standard protocols of the modified McMaster
193 method (Whitlock, 1948). Individual samples were loaded onto two chambers of a
194 Whitlock slide (sensitivity 20 eggs/gram of faeces). Nematode eggs were counted
195 under a microscope using the 10X objective. Strongyle, *Nematodirus* and *Monezia*
196 eggs were observed, counted and recorded for each individual sample.
197

198 **3.3 Larval Culture**

199
200 Pooled LC's were established for the CPE and AMM lines following FEC according to
201 the sheep identification tags following FEC. LC were conducted as described by
202 Whitlock (1956).
203
204 After incubation for 7-10 days, the CPE and AMM cultures were removed from the
205 incubator and larvae harvested for 48 hours. The larvae collected over this period
206 were pooled from each culture and trained staff of the State Veterinary Diagnostic

207 Laboratory (SVDL) at EMAI performed larval differentiation counts. One hundred
208 larvae from each culture were observed and differentiated based on morphological
209 features such as the length of the sheath tail and the shape of the head.
210

211 **3.4 PCR for worm identification**

212

213 **3.4.1 Isolation of DNA**

214

215 Nematode genomic DNA was isolated from individual sheep faecal samples (n=162),
216 which had been stored in Eppendorf tubes at -20°C post sampling.
217

218 The ISOLATE Faecal DNA Kit (Bioline, Sydney, Australia) procedure was followed. In
219 brief, approximately 150mg of faeces were processed on the Fastprep24 machine
220 using lysis buffer and beads. The lysate was then filtered and 100μl of DNA eluted.
221 This was divided into 50μl aliquots in separate Eppendorf tubes labelled with sample
222 number, dated and stored at -20°C. These were stored in two separate locations
223 until further analysis. A negative control of 100 μl of PBS was also processed each
224 day DNA extractions were performed. DNA extractions were performed after each
225 sampling period.
226

227 **3.4.2 PCR Assay**

228

229 The Easy-Plex kit system developed by AusDiagnostics Pty. Ltd (Australia) was
230 utilised to perform a Multiplex Tandem PCR (MT-PCR). This kit contained specific
231 primers for *Teladorsagia circumcincta, Haemonchus contortus,* all members of the
232 *Trichostrongylus* genus*, Chabertia ovina, Oesophagostomum venulosum,*
233 *Oesophagostomum columbianum* and *Cooperia curteci* (AusDiagnostics Pty. Ltd,
234 Australia, Catalogue number: 38091). The tubes containing the primers had 5 µl of
235 the isolated DNA from faecal samples (n=9) and extraction control or PCR water
236 added. The tubes were then loaded onto the robotic platform with oil, water and
237 mastermixes along with assay and dilution rings (AusDiagnostics Pty. Ltd, Australia).
238
239 The Easy Plex Assay utilised a semi-automatic robotic system to run 10 initial
240 amplification cycles after which the assay rings were heat sealed and placed in the
241 Rotor-Gene thermal cycler (QIAGEN) for a further 30 cycles.
242
243 The results of the PCR were detailed in the Easy Plex Results software program
244 where information on the melt curves, take off values and template concentration
245 were located. The assay rings were labelled with the run number and date and the
246 amplified products were stored at -20°C for future sequencing analysis if required.
247

248 **3.5 Body and Fleece Weights**

249
250 Body weights were initially measured when the mob was formed after weaning
251 (22/12/14) and again after sampling to determine drenching rate on the 29/7/2015.
252 The weight of fleece cut by each sheep was measured at shearing (20/7/15)

253

254 **3.6 Statistical Analysis**

255

256 Data was analysed using Microsoft Excel. Outliers (± the Standard Error) were
257 removed to eliminate noise of FEC and PCR output. Significant differences were
258 calculated using the "tTest" function.

259

260 **4. Results**

261

262 **4. 1 Comparison of faecal egg counts and larval cultures during a co-grazing trial**

263

264 Wether sheep of CPE and AMM lines were co-grazed one month after weaning
265 (Figure 1). During the month of January the area received a total of 126.6mm of rain
266 over 15 days and an a total of 219.8 mm of rain over 23 days in April. In late January
267 and early February, it was noted that sheep suffered from suspected haemonchosis.
268 During the trial, haemonchosis accounted for the deaths of 3 CPE.

269

270 During S1 (February), the CPE had significantly lower mean FEC of 8,746 egg per
271 gram of faeces (S.E. 1289.0, n=40) compared to AMM mean of 12,541 egg per gram
272 of faeces (P=0.03) (Table 1). Subsequently at sampling S2, S3, S4, the FEC were lower
273 for both flocks (range 0-1525, 75-40725, 0-5375 respectively) and no significant
274 differences between the egg counts was observed (Table 1).

275

276 The larval differentiation counts performed following each sampling, are shown in

277 Table 2. The predominant species of nematode throughout the sampling period

278 (Summer to Spring) was *H. contortus.* The prevalence of *H. contortus* ranged from

279 71-98% in the CPE sheep and 80-98% in the AMM sheep (Table 2).

280

281 **4.2 PCR assays**

282

283 The EasyPlex system was used to perform MT-PCR on the DNA extracted from

284 samples taken at S1-S3. All but one sample from S3 contained *H. contortus*. The

285 prevalence of *Trichostrongylus colubriformis*, *Oesophagostomum venulosum,*

286 *Cooperia curteci* and *Teladorsagia circumcincta* varied throughout each sampling

287 period. *H. contortus* was most prevalent in Summer, whilst *T. colubriformis* and *O.*

288 *venulosum* became more abundant with the seasonal change, during Autumn and

289 Winter.

290

291 Overall, there was a low correlation between the FEC and the concentration of DNA

292 copies for each species. (Figure 2). The highest correlation of R^2=0.6 was observed

293 for *H. contortus* in the final PCR run where the least number of samples were

294 involved (n= 13). The concentration of the estimated copy number for each

295 nematode species detected, varied across samples and no clear or significant

296 relationship was observed with FEC's.

297

298 Comparison of the larval differentiation counts performed on larvae from faecal
299 cultures and the identification of DNA extracted from nematode eggs by MT-PCR
300 indicated PCR was a good indicator of *H. contortus* prevalence. Significant
301 differences only occurred between the two techniques at S2 for both flocks (Table
302 3). The closest relationships between LC and PCR was observed at S1. There was a
303 greater variation and no clear relationship between the two assays for *T.*
304 *colubriformis* and *Oesophagostomum* spp. (Figure 3). PCR revealed a greater mix of
305 infection compared to the LC, with the PCR able to discriminate to a higher degree
306 species other than *H. contortus*.
307

308 **4.3 Body and Fleece weights**

309

310 The average body weights calculated for the two flocks at S1 and S3 are presented in
311 Table 4. Average weight gains over the 7-month period for the CPE and AMM sheep
312 were 24% and 25% respectively, which did not differ significantly (P>0.05). The
313 maximum weight gains were 8.3 kg for the CPE and 17.3 kg for the AMM sheep.
314 During the study period around 10% of the CPE sheep lost weight compared to only
315 2% of the AMM sheep. There was a significant difference (P<0.05) between the two
316 flocks at both the first and second weighing with the AMM being significantly
317 heavier.
318

319 The AMM sheep had significantly higher fleece weights compared to the CPE sheep
320 when fleeces were weighed at shearing (20/7/15). Fleece weights for the CPE and
321 AMM ranged from 609-1458 g and 1218-2474 g respectively (Table 5).
322

323 **5. Discussion**

324

325 To our knowledge, this is the first study to investigate the potential resistance status
326 of Camden Park Estate (CPE) sheep to gastrointestinal nematodes. While these
327 sheep were grazed with Australian Meat Merinos (AMM), they displayed displayed a
328 significantly lower worm burden status, assessed by FEC, during the first sample
329 period (Table 1). This could have been the result of stronger innate immunity
330 presented in the naïve CPE sheep after the peak worm burden threshold was
331 reached at 6-8 weeks (Barger and Jambre, 1988). At this time increased immune
332 response is known to prevent new infections from establishing. This is achieved by
333 mucosal inflammatory reactions (Emery, 1996; Williams et al., 2010), preventing L3
334 establishment. Immunity also retards worm development and reduces worm
335 fecundity (Emery, 1996). This decreases worm egg output, and hence pasture
336 contamination and transmission. Development of immunity is therefore important,
337 occurring when the threshold is reached and the animal is continually challenged
338 (Emery, 1996).
339

340 This trial experienced an extreme worm challenge in late summer due to the
341 prevailing weather conditions, requiring the repeated use of alternative

342 anthlemintics, which may have influenced the development of immunity during
343 Autumn and the outcomes of the project. Al-Saadi et al. (1984) revealed that
344 anthelmintic treatments have a negative influence on the development of resistance
345 to lung worms through preventing the development of immunity. This could also
346 occur for gastrointestinal nematodes where anthelmintics interfere with immune
347 responses through the removal of nematodes from the gastrointestinal tract.
348 Statistical analysis of FEC of the subsequent sample periods did not find a significant
349 difference between these two lines of sheep. Additionally, FECs at the second
350 sample period were significantly lower than FECs during the first sample period. This
351 sample period fell just after the Egg Reappearance Period (ERP) of Q Drench and
352 therefore is reflected by the lower FECs, which may not provide adequate challenge
353 for the development of immunity and the production of high egg counts. Residual
354 activity of anthelmintics occurred at the final sample period where the sheep were
355 still under medication from D4 with Startect®, which had been given X weeks later.
356 This resulted in an inaccurate representation of worm burden status because
357 sampling fell within the ERP. However, there was a 22-week period between D3 and
358 D4, exceeding the 7-week ERP for abamectin, the longest acting chemical in Q-
359 Drench. This winter sample period in July (S3) revealed EPG averages of 4207 and
360 3852 for the CPE and AMM sheep, respectively, which is half the output from the
361 naïve sheep at the first sampling in Summer (S1). Though these numbers are
362 sufficient to require a drenching decision, the intake over time or delayed
363 establishment caused by the previous drench may not provide enough challenge to
364 produce a significant difference as in S1. However, some individuals expressed egg
365 counts greater than 20000 EPG for both flocks which resulted in the deaths of three

366 CPE sheep. As the sheep have become older, both the AMM and CPE sheep appear
367 to have developed a stronger acquired immunity from exposure to GIN. It may mean
368 that the CPE sheep exhibited a stronger innate resistance to the first infection
369 compared to the AMM sheep that developed a more effective acquired resistance
370 on subsequent challenges, and were simply more vulnerable with the lack of
371 acquired immunity in summer. This has been demonstrated for the progeny of the
372 "Golden Ram" which had high levels of primary *H. contortus* infections but were
373 highly resistant thereafter (Gill, 1991).
374
375 Studies have shown that anthelmintic treatments can negatively influence the
376 development of immunity to nematodes as resistance to infection is not observed
377 (Barger, 1988). Additionally, Barger (1998) demonstrated that this immunity does
378 not redevelop again until 8 weeks after reinfection revealing how constant
379 drenching, especially in younger animals may lead to loss of immunity. The negative
380 effects of anthelmintic treatment have also been demonstrated by Donald et al.,
381 (1969) and Whitlock and Georgi (1976), where anthelmintic treatments are observed
382 to interfere with development and maintenance of resistance, and potentially
383 making the effects of GIN more severe (Donald et al., 1969; Whitlock and Georgi,
384 1976). No significant difference in FEC was found after the initial S1 sampling. This
385 may be due to the high level of *Haemonchus* challenge, a result of the favourable
386 temperatures and rainfall in summer. The weather conditions allowed a rapid and
387 continual lifecycle from when the eggs are passed out in the faeces, developing into
388 the infective L3 larval stage, ingested by the grazing animal and growing into an adult
389 over 2-3 weeks before producing eggs. In these optimal environmental conditions of

390 adequate moisture and temperature, the nematode lifecycle development of L3
391 from eggs can be completed in as little as 4 days (Cheah and Rajamanickam, 1997)
392 compared to the average prepatent period of 18-21days. As a limiting factor, drench
393 management of the co-grazing trial may have therefore influenced the outcomes of
394 the project, and allowing for the development of immunity. Conversely, these
395 animals were under constant challenge from *Haemonchus contortus,* and in addition
396 to the 22-week period to allow the development of immunity, this provides insight
397 towards the lack of inherent resistance to nematodes in the CPE lines. Future trials
398 could be conducted where artificial infections are produced in both lines and the
399 animal's responses monitored without risk of supplementary infection from pasture.
400 The immune systems of the sheep could then be primed without risk of death.
401
402 Fleece and body weights of the CPE are significantly lower than the AMM as
403 expected due to the differences in body size. However, the CPE gained a similar
404 percentage in body weight of ~24% in that period as the AMM (Table 4). This is does
405 not support findings by Brusdon (1964) who found depressions in weight gains of up
406 to 52%, and Riffkin and Dobson (1979) who also found resistance traits were
407 detrimental towards production (Rifkin and Dobson, 1979). Increased immunity and
408 hence resistance to nematodes can be costly in terms of increased nutrition (Liu et
409 al., 2005a). However, studies of resistant strains of sheep, such as the Rylington
410 Merino, have demonstrated that decreased effects of parasitism and faecal egg
411 output due to resistance is possible without detriment to production (Liu et al,
412 2005b). Controlled infection studies of the CPE line in the future could allow a

413 greater insight into the mechanisms of immunity and resistance and its effects on
414 production
415

416 Previous studies have reveal that grazing of susceptible and resistant lines needs to
417 occur separately to reveal the full potential for breeding for resistance (Greeff and
418 Karlsson, 2006). Grazing separately of resistant and control sheep has revealed close
419 to a 10-fold decrease in pasture larvae contamination (Bisset et al., 1997). It would
420 therefore be beneficial for another study to be conducted on the CPE sheep where
421 select individuals with lower EPGs were grazed separately from a control flock given
422 a similar initial worm burden status. It is also known that immunity to one species
423 rarely confers protection to another species (Barnes and Dobson, 1993). Different
424 worm species produce different levels of persistence of immunity, with
425 *Trichostrongylus colubriformis* immunity being more persistent compared to *H.*
426 *contortus* (Barnes and Dobson, 1993). Future direction for the study could involve
427 comparisons of not only mixed infections in the field and observing reduction in egg
428 counts but also artificial infections of both mixed and single species nematode
429 infections.

430

431 This study also applies the semi-automated MT-PCR methodology for comparison
432 and estimation of the nematode population structure in these naturally infected
433 sheep. There was no high correlation with PCR output when compared to the FEC,
434 confirming that MT-PCR is currently unable to replace FEC due to issues with gene

435 copy numbers (Harmon et al., 2007). However, when presented against the LCs, the
436 PCR was able to produce similar results to Rober et al (2012), with a good
437 relationship between the MT-PCR species prevalence compared to the LC species
438 prevalence for *H. contortus* only. MT-PCR approach improved sensitivity to detect
439 the genus *Oesophagostomum* compared to bulk LC. It is, however, not known if the
440 bulk faecal samples contained *Oesophagostomum* eggs that either did not hatch or
441 hatched larvae were not accounted for in the 100 larvae counted, due to MT-PCR
442 not being performed on an aliquot of the bulk samples of LC.
443
444 One of the caveats with PCR is the extraction of DNA from the sample, in this case
445 DNA out of nematode eggs deposited into the sheep's faeces Compounds inhibiting
446 PCR in faeces, the lack of homogeneity of the sample, low number of eggs, small
447 sample size of 100-200mg of faeces and the difficulty encountered in releasing DNA
448 from hard walled eggs, all act as limitations to this technique (Harmon et al., 2007;
449 Verweij et al., 2007). Roeber et al. (2012) compared two methodologies for
450 nematode DNA isolation, DNA isolation directly from faeces using the Powersoil DNA
451 purification kit (MoBio, USA) and combined egg flotation and column purification.
452 They found that the egg flotation and column purification technique was able to
453 produce superior worm species determination when sensitivity was analysed and
454 reported this method to be superior for sensitivity in low FEC situations (Roeber et
455 al., 2012). Due to the high number of eggs present in most of the samples from this
456 trial, (approximately 1000 EPG), and for uniformity across samples, DNA was isolated
457 directly from the faeces using a kit which simplified the application for diagnosis. The
458 small amount of faeces utilised was of further concern and may have made the assay

459 less sensitive. In fact Roeber et al. (2012) found that sensitivity dropped by up to
460 13% when sample size decreased (Roeber et al., 2012). This would become more
461 important when monitoring individuals with low burdens of 200 EPG or less. In
462 addition, the release of eggs by adult nematodes into the faeces is not uniform,
463 which may produce samples that are not a true representation of the worm burden
464 in individual carries (Villanúa et al., 2006). One of the benefits of the PCR assay is its
465 superior sensitivity compared to FECs. For example, PCR analysis of an individual
466 sample where FEC was recorded as 0 EPG (FEC sensitivity 20 EPG), revealed a mixed
467 infection with >60% of DNA gene copies present for *H. contortus.* The method of
468 DNA isolation may not be of great importance given the degree of sensitivity of the
469 assay, which is contrary to the hypothesised need for large scale DNA isolations from
470 several grams of faeces (Demeler et al., 2013).
471
472 There are clear advantages of utilising MT-PCR over the traditional LC, where LCs can
473 be laborious, time consuming, require skill and cultures being fastidious and leading
474 to bias (Dobson et al., 1992). Though the assay can be costly, species identification
475 using DNA based diagnostics is likely to be more simplified when considering mixed
476 infections and understanding host and parasite interactions. Utilising DNA extracted
477 from the faeces also allows diagnosis without the need for culling individuals for
478 total worm counts (Wimmer et al., 2004). To achieve uniform results across multiple
479 flocks of naturally infected sheep, further trials and replicates need to be conducted
480 for this methodology to become an efficient and reliable diagnostic tool. Additional
481 species may also need to be included in the assay such as *Nematodirus* spp and
482 *Moniezia* spp which were observed during this trial. Results from this study provide

483 new information and extend previous research utilising similar methodology of MT-
484 PCR in assessing sheep naturally infected with GIN.
485

486 **6. Conclusion**
487

488 This study has provided scientific evidence that the CPE line of sheep displayed
489 greater potential of innate resistance to gastrointestinal nematodes during the first
490 large *Haemonchus* challenge in summer based on an initial reduced FEC. This
491 advantage was not apparent throughout the remainder of the trial. This inherent
492 host resistance to nematodes may be the ability of the host to mount an increased
493 immune response in the presence of extreme parasite infestation. This study
494 provides an opportunity to further examine this through conducting trials with
495 controlled environmental conditions in a pen where they are artificially infected with
496 nematode larvae to observe any dose relationships and reductions in FEC. Due to the
497 similarities in the weight gains of both the CPE and AMM sheep in the presence of
498 worm burdens, resilience determinants such as regular changes in body weights and
499 fleece characteristics could also be observed where the host is able to counter the
500 deleterious effects of parasitism, cope with the parasite load and still be productive.
501 The semi-automated MT-PCR assay could also be a future tool for rapid diagnosis of
502 nematode species in a rapid way with results from the MT-PCR available in 2 hours
503 compared to the 7 days of the LC. The ability of this assay to be potentially more
504 specific than LC also allows for an increased accuracy in diagnosis for a more
505 informed management strategy. Future studies into increasing the specificity for

506 species other than *Haemonchus contortus* could be more beneficial in mixed

507 infection situations.

508

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510

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517

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694 **Appendix**

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696 Tables

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698 Table 1. Comparison of Faecal Egg Counts (Eggs Per Gram of faeces) from the

699 Camden Park Estate (CPE) and Australian Meat Merino (AMM) sheep at each

700 sampling (S) expressed as Mean± Standard Error (SE), significance level (p-value), the

701 range (minimum-maximum) and number of individuals sampled (n).

S	Flock	Mean ± S.E	n	Range	Significance (p-value)
1	CPE	8746 ± 1289.0	40	1175-48500	0.03
	AMM	12541 ± 1187.8	49	525-43925	
2	CPE	245 ± 42.0	38	0-1300	0.9
	AMM	241 ± 42.0	46	0-1525	
3	CPE	4207 ± 719.6	40	75-20400	0.8
	AMM	3852 ± 962.5	49	150-40725	
4	CPE	722 ± 140.4	38	0-3575	0.8
	AMM	676 ± 174.4	47	0-5375	

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707 Table 2. Larval Differentiation counts performed on larvae harvested from faecal
708 cultures of the Camden Park Estate (CPE) and Australian Meat Merino (AMM) at
709 each sampling period (S) for *Haemonchus contortus, Trichostrongylus colubriformis,*
710 *Oesophagostomum* spp*, Cooperia* spp and *Teladorsagia circumcincta*.

S	CPE	AMM
1	98% *H. contortus,* 2% *T. colubriformis*	
2	93% *H. contortus,* *7% Oesophagostomum*	95% *H. contortus,* *3% Oesophagostomum spp*, *1% T. colubriformis,* *1% Cooperia spp.*
3	71% *H. contortus,* 29% *T. colubriformis*	93% *H. contortus,* 7% *T. colubriformis*
4	90% *H. contortus,* 10% *T. colubriformis*	80% *H. contortus,* *19% T. colubriformis,* *1% Cooperia spp.*

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718 Table 3. Comparison of the percentage results from Larval Cultures (LC) and PCR
719 assays of the two flocks Camden Park Estate (CPE) and Australian Meat Merino
720 (AMM) from S1-S3 for *Haemonchus contortus, Trichostrongylus colubriformis,*
721 *Oesophagostomum* spp, *Cooperia* spp and *Teladorsagia circumcincta.* Any significant
722 differences (P<0.05) between the two assays for each flock is indicated with *.

S	**Flock**	**Assay**	**Mean Species %**				
			H. contortus	***T. colubriformis***	***Oesophagostomum spp***	***Cooperia spp***	***T. circumcincta***
1	CPE	LC	98	2			
		PCR	100				
	AMM	LC	98	2			
		PCR	96	4			
2	CPE	LC	93	7			
		PCR	74 *	9	20 *		4
	AMM	LC	95	3	1	1	
		PCR	67 *	10 *	15 *	1	
3	CPE	LC	71	29			
		PCR	74	3 *	23 *		
	AMM	LC	93	7			
		PCR	30 *	10	60 *		

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726 Table 4. Comparison of body weights (kg) from the Camden Park Estate (CPE) and
727 Australian Meat Merino (AMM) sheep at S1 and S3 expressed as Mean ± Standard
728 Deviation (SD) and the associated percentage weight gain.

S	**CPE**	**AMM**
1	15.5 kg ± 2.8	24.4 kg ± 4.0
3	18.8 kg ± 2.2	32.0 kg ± 3.8
Weight gain (%)	24	25

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745 Table 5. The average unskirted fleece weight Mean(kg) ± Standard Deviation (SD) of

746 the Camden Park Estate (CPE) and Australian Meat Merino (AMM) sheep, measured

747 at shearing 20/7/15 (S3).

	Average fleece weight (kg)
CPE	1.14 ± 0.19
AMM	1.92 ± 0.30

748 Figures

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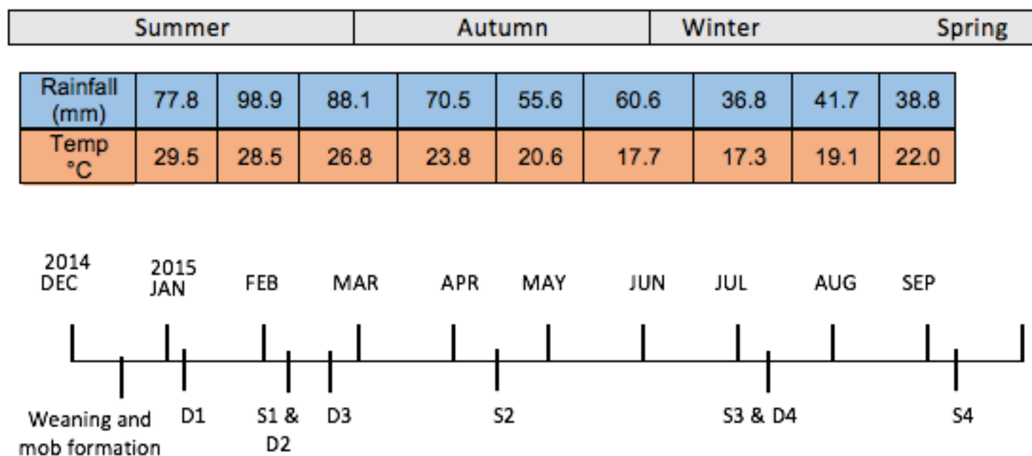

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751 Figure 1. Timeline of management and sampling periods of the Camden Park Flock
752 (CPF) and Australian Meat Merino (AMM). The mob of 80 CPF and 80 AMM wethers
753 was formed on the 22nd December 2014. Drenching occurred 4 times throughout the
754 trail; D1 8th January with Noromectin (Ivermectin), D2 10th February with
755 Zolvix®(Monepantel), D3 26th February with Q-Drench (Abamectin, Abendazole,
756 Closantel, Levamisole) and D4 29th July with Startect® (Derquantel, Abamectin). Four
757 Samplings were also made; S1 on 9th February, S2 on 20th April, S3 on 29th July and S4
758 on 10th September. Mean rainfall and mean maximum temperature from January to
759 September is displayed (BOM).

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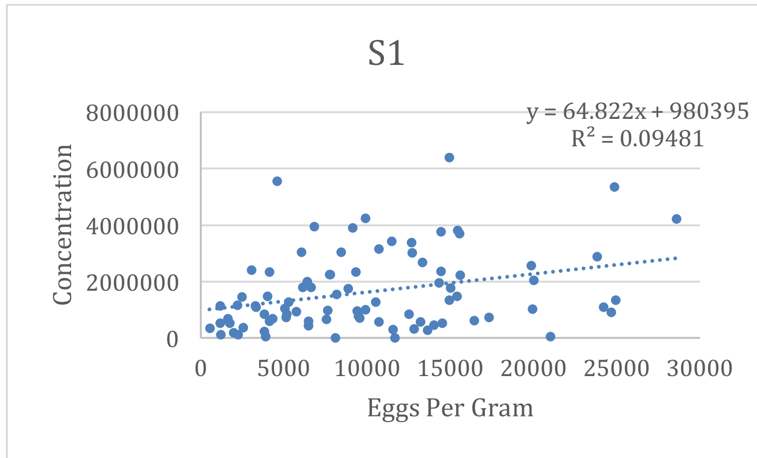

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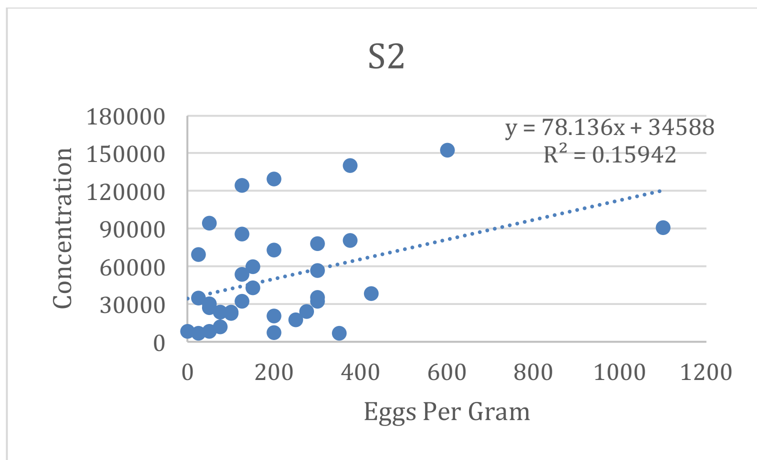

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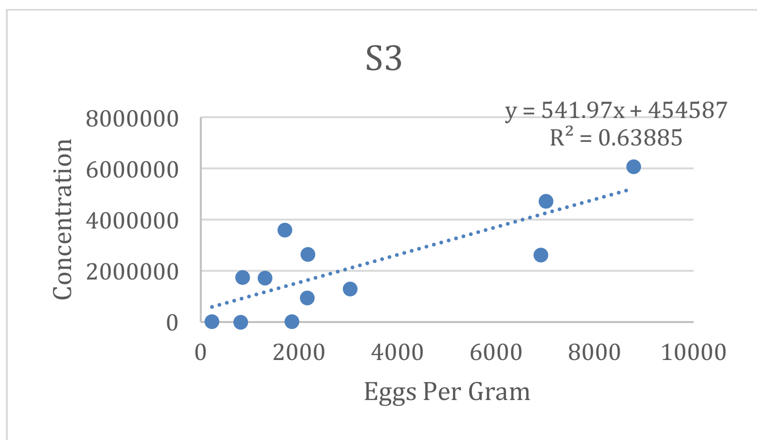

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766 Figure 2. The relationship between Faecal Egg Counts (Eggs Per Gram) and MT-PCR

767 copy number concentration for *Haemonchus contortus* at sampling times S1-S3 (n=

768 89, 38 and 13 respectively).

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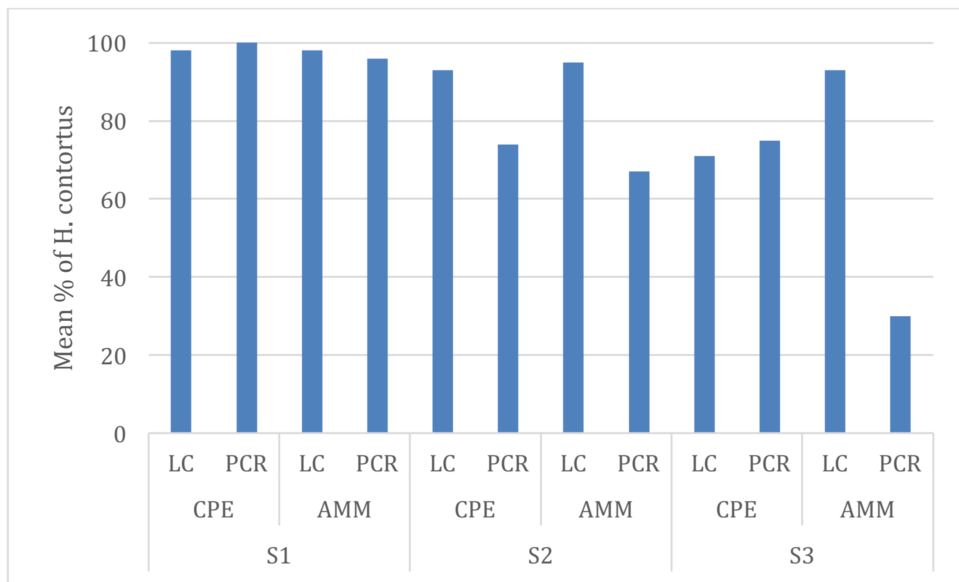
771 **A:**



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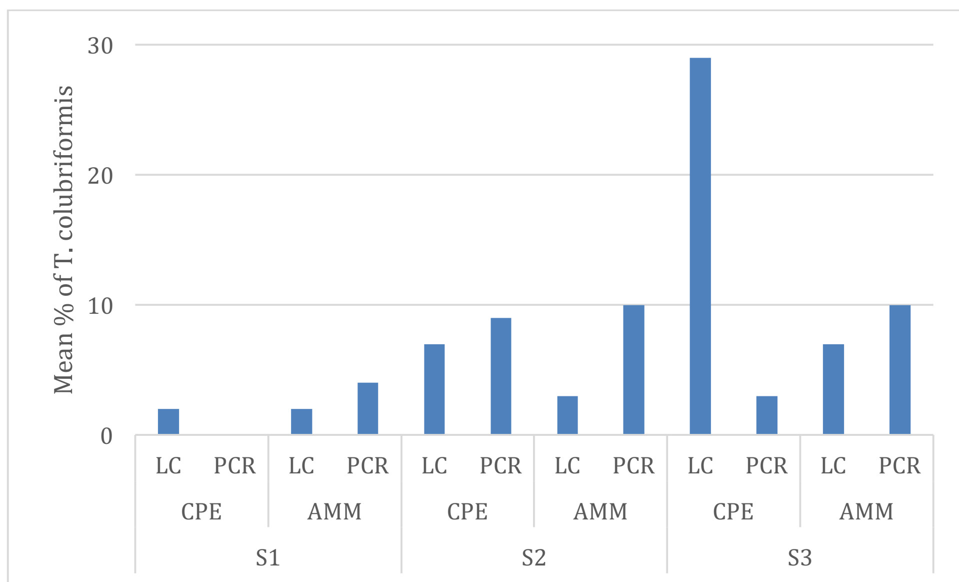
773 **B:**



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775 **C:**

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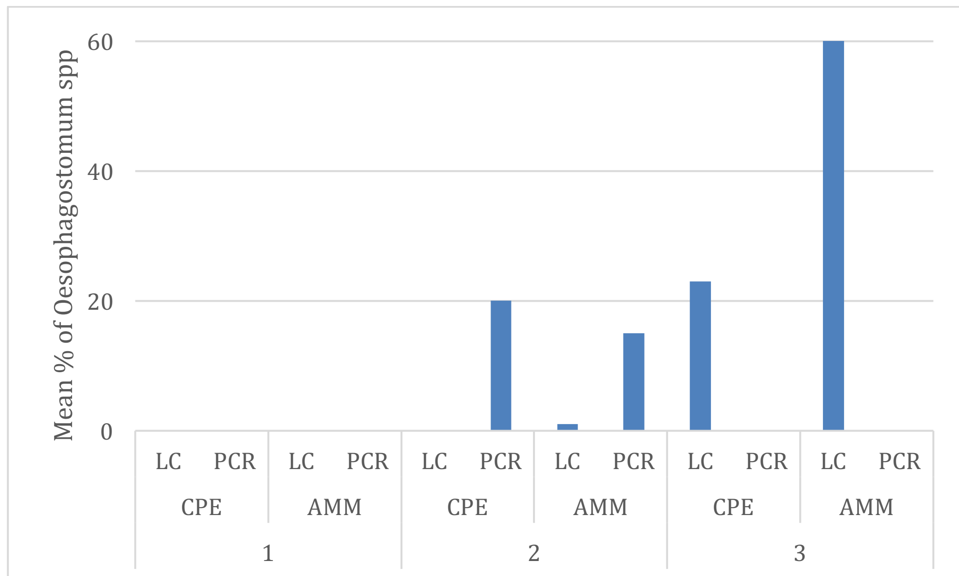

777 Figure 3. Comparison of the percentage results from Larval Cultures (LC) and PCR

778 assays of the two flocks Camden Park Estate (CPE) and Australian Meat Merino

779 (AMM) from S1-S3 for A: *Haemonchus contortus,* B: *Trichostrongylus colubriformis*

780 *and* C: *Oesophagostomum spp.*

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