

Scotland's Rural College

## Salivary IgA: A biomarker for resistance to *Teladorsagia circumcincta* and a new estimated breeding value

Fairlie-Clarke, Karen; Kaseja, K; Sotomaior, Cristina; Brady, Nicola; Moore, Kirsty; Stear, Michael

*Published in:*  
Veterinary Parasitology

*DOI:*  
[10.1016/j.vetpar.2019.04.005](https://doi.org/10.1016/j.vetpar.2019.04.005)

Print publication: 01/05/2019

*Document Version*  
Peer reviewed version

[Link to publication](#)

### *Citation for published version (APA):*

Fairlie-Clarke, K., Kaseja, K., Sotomaior, C., Brady, N., Moore, K., & Stear, M. (2019). Salivary IgA: A biomarker for resistance to *Teladorsagia circumcincta* and a new estimated breeding value. *Veterinary Parasitology*, 269, 16-20. <https://doi.org/10.1016/j.vetpar.2019.04.005>

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 Salivary IgA: a biomarker for resistance to *Teladorsagia*  
2 *circumcincta* and a new Estimated Breeding Value

3

4 Karen Fairlie-Clarke<sup>a\*</sup>, Karolina Kaseja<sup>c</sup>, Cristina  
5 Sotomaior<sup>b</sup>, Nicola Brady<sup>a</sup>, Kirsty Moore<sup>c</sup>, Michael Stear<sup>d</sup>

6

7 <sup>a</sup>Institute of Biodiversity, Animal Health and Comparative  
8 Medicine, University of Glasgow, Garscube Campus,  
9 Bearsden Road, Glasgow, Scotland, G61 1QH, UK.

10

11 <sup>b</sup>Graduate Program in Animal Science, School of Life  
12 Sciences, Pontifícia Universidade Católica do Paraná  
13 (PUCPR), Curitiba, Paraná 80215-901, Brazil.

14

15 <sup>c</sup>Animal and Veterinary Sciences, Scotland's Rural College  
16 (SRUC), Peter Wilson Building, Kings Buildings, West Mains  
17 Road, Edinburgh EH9 3JG, UK

18

19 <sup>d</sup>Department of Animal, Plant and Soil Science, Agribio, La  
20 Trobe University, Bundoora, VIC, 3086, Australia

21

22 \*corresponding author

23 [karen.fairlie-clarke@moredun.ac.uk](mailto:karen.fairlie-clarke@moredun.ac.uk)

24 Tel: +44 141 330 2886

25 Moredun Research Institute

26 Pentlands Science Park,

27 Bush Loan,

28 Penicuik,

29 Midlothian,

30 EH26 0PZ

31 Scotland, UK.

32

33 Keywords: IgA, nematode resistance, saliva test,  
34 *Teladorsagia circumcincta*, selective breeding

35

36 Abstract

37 *Teladorsagia circumcincta* is the dominant nematode of  
38 sheep in cool, temperate climates. Faecal nematode egg  
39 counts (FEC) are widely used to identify the intensity of  
40 infection and as a measure of host resistance to nematodes.  
41 However due to density-dependent effects on worm  
42 fecundity the relationship between FEC and worm burden  
43 is not linear. In addition collecting FEC data is challenging  
44 on a practical level and there is a need for more reliable  
45 markers of resistance. There are two major known  
46 mechanisms of immunity to *T. circumcincta*: IgE against  
47 third stage larvae (L3), which inhibits larval establishment  
48 and IgA against fourth stage larvae (L4), which inhibits  
49 parasite growth. We measured salivary IgA responses in  
50 over 5000 animals against L3 antigen by Enzyme Linked  
51 Immunosorbent Assay (ELISA). Antigen-specific IgA levels  
52 were negatively correlated with FEC ( $r=-0.26$ ,  $SE=0.02$ ) and  
53 were heritable ( $h^2 = 0.16$ ,  $SE=0.04$ ) indicating that they can  
54 be used to identify resistant animals suitable for inclusion  
55 in selective breeding programs. Ecological theory predicts  
56 that a trade-off between immunity and host-growth will  
57 exist due to competing energetic needs. Antigen-specific IgA

58 responses were not negatively correlated with muscle  
59 deposition such that the expected trade-off between growth  
60 and immunity was not apparent. Our analyses indicate that  
61 selection for antigen-specific IgA is possible without  
62 impacting on the production traits for the Lleyn breed.

63

#### 64 1. Introduction

65 Infection with gastro-intestinal strongyles such as  
66 *Teladorsagia circumcincta* presents a significant problem to  
67 the sheep breeding industry, impacting both the welfare  
68 and productivity of animals. Anthelmintics are commonly  
69 used to control helminth infection but reliance on this  
70 method alone is ill-considered in light of growing  
71 anthelmintic resistance (Traversa and von Samson-  
72 Himmelstjerna, 2016). An integrated approach to ensure  
73 sustainable control strategies for the future is the main  
74 objective of research in this area and requires improved  
75 phenotypic and genetic markers of resistance and resilience  
76 to inform selective breeding programs (Jackson et al.,  
77 2009).

78

79 Whilst both resistance and resilience contribute to control  
80 of helminth infection it may be more preferable when  
81 considering productivity to select for resilient sheep that  
82 can better tolerate the infection and grow in spite of worm

83 burden. Previous research has demonstrated that there are  
84 two main mechanisms of immunity to *T. circumcincta*  
85 infection. Immunoglobulin E (IgE) against third stage larvae  
86 (L3), which inhibits larval establishment and  
87 Immunoglobulin A (IgA) against fourth stage larvae (L4),  
88 which inhibits parasite growth (Lee et al., 2011; Martinez-  
89 Valladares et al., 2005; Murphy et al., 2010; Strain et al.,  
90 2002). The IgE response, although protective is associated  
91 with pathology that leads to a relative protein deficiency in  
92 the animal (Stear et al., 2003). Thus resistance to the  
93 infection is costly both in terms of welfare and productivity.  
94 The IgA response impairs the growth of the worms  
95 resulting in shorter and crucially less fecund worms that  
96 contribute less to pasture contamination (Stear et al., 1995;  
97 Strain et al., 2002). IgA without the pathology and negative  
98 impact on animal growth associated with IgE has potential  
99 as a marker of resilience.

100

101 Traditionally low faecal nematode-egg count (FEC) is used  
102 as a marker of resistance in selective breeding programmes.  
103 However, mathematical modelling indicates that IgA has  
104 advantages over FEC as a selection tool; after 7 generations  
105 of selection based on plasma IgA a drop in FEC of 85% was  
106 achieved whereas selection on FEC alone gave only a 50%  
107 reduction (Prada Jimenez de Cisneros et al., 2014). There

108 are also other disadvantages of using FEC as a measure of  
109 infection intensity; worm density-dependent effects on egg  
110 production mean that high worm burdens do not always  
111 tally with high FEC (Bishop and Stear, 2000; Romeo et al.,  
112 2014; Smith et al., 1987), sampling per rectum is invasive  
113 and often samples can't be obtained. Measuring IgA in  
114 plasma is also invasive and requires specialist training.

115

116 Salivary IgA to a carbohydrate larval antigen (CARLA) has  
117 been used successfully to measure immunity to  
118 gastrointestinal nematodes (Shaw et al., 2012). Here we  
119 have developed an Enzyme Linked Immunosorbent Assay  
120 (ELISA) to measure *T. circumcincta*-specific IgA in saliva.  
121 The test measures the IgA response to L3 larvae with the  
122 rationale that resistance results from the recognition of  
123 multiple antigens (Ellis et al., 2014). This test is deliberately  
124 targeted to the predominant nematode infection in the UK  
125 and other temperate climates. Breeders and veterinarians  
126 have readily adopted this test and results are reported as  
127 an Estimated Breeding Value (EBV) for inclusion in the  
128 selective breeding programmes of the Lley Performance  
129 Recording group.

130

## 131 2. Material & Methods

### 132 2.1 *Animals and sample collection*

133 Eighteen farms owned by Performance Recorded Lleyn  
134 Breed Society members throughout the UK were involved  
135 in the study and bred all the Lleyn animals that were tested  
136 (n=5201). Sampling was undertaken between the months  
137 of July-September to coincide with exposure to  
138 *Teladorsagia circumcincta* through grazing. For lambs  
139 sampled between 2014 and 2017 the average age at  
140 sampling was 193days of age. The age distribution of  
141 animals in the study is shown in Figure 1.

142

143 Saliva samples were collected by insertion of a dental swab  
144 (Robinson Healthcare, 12mm), secured by forceps, into the  
145 cheek pouch of the animal. Swabs were sealed in 15ml  
146 falcon tubes and saliva recovered by centrifugation at 449  
147 rcf for 5 minutes. Saliva was then frozen at -20°C before use  
148 in ELISA.

149

## 150 2.2 Antigen and ELISA

151 *T. circumcincta* antigen was prepared by homogenisation of  
152 1 million L3 in 10mM Tris-HCL with addition of protease  
153 inhibitors (0.5M EDTA, 0.5M EGTA, 1M NEM, 0.33M PMSF,  
154 0.1M TPCK, 1mM pepstatin) and 2% sodium deoxycholate.  
155 Homogenisation was performed at 30Hz for 6 mins in a  
156 Retsch MM400 mixer mill. Antigen was passed through a  
157 0.2µm syringe filter and frozen before use in ELISA.

158  
159 Antigen-specific salivary IgA ELISA: 96 well BD Falcon  
160 ELISA plates were coated with 5µg/ml L3 antigen diluted in  
161 0.06M carbonate buffer (0.04 M NaHCO<sub>3</sub>, 0.02 M NaCO<sub>3</sub>,  
162 pH9.6) and incubated overnight at 4°C. Non-specific  
163 binding was blocked with 4% Marvel milk powder in  
164 Phosphate-buffered saline with 0.1% Tween 20 (PBST) for  
165 2 h at 37 °C. Wells were washed three times in PBST after  
166 each subsequent step. Saliva samples were added at 1:4  
167 dilution using PBST as a diluent and incubated for 30  
168 minutes at 37 °C. Isotype-specific detection antibody  
169 horseradish peroxidase conjugated rabbit anti-ovine IgA  
170 (AbD serotec AHP949P) was added at 1:15 000. Plates were  
171 incubated for 30 minutes at 37 °C. An additional wash in  
172 distilled water was carried out before developing with 3,3',  
173 5,5'-tetramethylbenzidine (TMB) peroxide substrate  
174 (Pierce <sup>TM</sup>), at room temperature for 5 min. Optical density  
175 (O.D.) was read at 450nm using a spectrophotometer.  
176 Results were expressed as an O.D. Index calculated as  
177 (sample O.D. – control O.D.) / (high-responder O.D. – control  
178 O.D.). This O.D. Index and animal pedigree were used to  
179 generate an estimated breeding value for antigen-specific  
180 IgA (IgA EBV).  
181



182 To ensure standardisation of IgA responses from year to  
183 year the high responder pool was created from 6-10 sheep  
184 and this provides enough material to test thousands of  
185 animals. When a new batch of high responder pool is  
186 created the reactivity of the previous pool and the new pool  
187 are carefully compared by testing multiple samples with  
188 both batches.

189

### 190 *2.3 Calculation of IgA Estimated Breeding Value (EBV)*

191 At the time of producing IgA EBVs, raw antigen-specific IgA  
192 data were available for 5,201 Lleyn animals, measured  
193 between 2014 and 2016 from 15 flocks. Basic data edits  
194 were undertaken to remove data for animals that were  
195 sampled multiple times or outside of 100 to 350 days of  
196 age. To normalise the IgA phenotype distribution a Box-Cox  
197 procedure was applied using a square root transformation  
198 and extreme outliers removed. To allow genetic parameter  
199 estimation with the other traits routinely evaluated in the  
200 national Lleyn genetic evaluation, Faecal Egg Count (FEC)  
201 (log transformed), birth weight (kg), 8 week weight (kg), 21  
202 week weight (kg), ultrasound muscle depth (mm),  
203 ultrasound fat depth (mm) and ewe weight at tuppung (kg)  
204 were extracted from the genetic evaluation database for the  
205 flocks that recorded antigen-specific IgA. For genetic  
206 parameter estimation, animals that were fostered, the

207 result of embryo transfer, not purebred or from a litter size  
208 greater than 3 were removed from the dataset. Any record  
209 which was part of a small (< 5 animals) or single sire  
210 contemporary group were set to missing, as were records  
211 outside 3 standard deviations from the contemporary  
212 group mean.

213

214 Contemporary groups (CG) were as defined in the routine  
215 national genetic evaluations (Ceyhan, 2015). For birth- and  
216 8 week- weight the CG was defined as flock of birth, season  
217 and sex (Flock-Season-Sex CG). The same definition, but  
218 with the inclusion of a user defined management group  
219 (Flock-Season-Sex-Managementgroup CG) was used for 21  
220 week weight, ultrasound muscle and fat depth and Faecal  
221 Egg Count. In all cases, season was based on date of birth  
222 and within year (defined from August to July) was sliced so  
223 that consecutive animals were not more than 28 days apart  
224 in age and the total time span of a CG was not more than  
225 155 days (5.5 months). The CG for ewe weight at tugging  
226 was the herd and year of tugging (Tugging herd-year CG).  
227 For IgA it was decided to use a similar CG as for 21 week  
228 weight, ultrasound scans and FEC, however including the  
229 IgA management group. A 5 generation pedigree was built  
230 for animals with phenotype information.

231

232 For both parameter estimation and the calculation of EBVs  
233 the statistical models used for routine genetic evaluation  
234 were used for all traits other than IgA. The statistical  
235 models fitted were as follows;

236

237 Birth weight = Flock-Season-Sex CG + litter size born + dam  
238 age + animal genetic effect + dam genetic effect +  
239 permanent environment effect

240 8 week weight = Flock-Season-Sex CG + litter size reared +  
241 dam age + animal genetic effect + dam genetic effect +  
242 permanent environment effect

243 21 week weight / ultrasound muscle depth /ultrasound fat  
244 depth / FEC = Flock-Season-Sex-Managementgroup CG +  
245 litter size reared + dam age + scanning age + Flock-Season-  
246 Sex-Managementgroup CG \* scanning age + animal genetic  
247 effect

248 Ewe weight at tugging = Tugging herd-year CG + animal  
249 genetic effect

250

251 The CG, litter size and dam age terms were fitted as fixed  
252 class effects. CG was as defined above, litter size born was  
253 the number of lambs born in the same litter, litter size  
254 reared was the number of lambs born and raised in the  
255 same litter. Dam age was the age of the dam in years, with  
256 dams over 5 years of age being recorded as 5 yrs.

257

258 Scanning age was the age of the animal at the time of the 21  
259 week weight and ultrasound scans were measured. It is  
260 recorded in days and was fitted in the model as a fixed  
261 covariate.

262

263 Fitted as random effects were the additive, dam and  
264 permanent environment genetic effects.

265

266 The final statistical model for IgA was as follows;

267  $IgA = \text{Flock-Season-Sex-ManagementGroup CG} + \text{litter size}$   
268  $\text{born} + \text{age when IgA sample taken} + \text{animal genetic effect}$

269

270 Model terms were fitted in the same manner as above, with  
271 age at IgA sample being recorded in days and fitted as a  
272 fixed covariate.

273

274 ASReml (Gilmour, 2009) was used to estimate genetic  
275 parameters to produce EBVs. To estimate (co)-variance  
276 components, a series of uni- and bi- variate animal models  
277 (described above) were fitted. The software MiX99  
278 (Lidauer, 2011) was used to produce EBVs based on the  
279 variance components estimated and the models described  
280 above.

281

282 3. Results

283 After basic data edits antigen-specific IgA records were  
284 available for 5,188 animals, of which 4,473 also had FEC  
285 records available in the national Lleyn genetic evaluation.  
286 Table 1 describes the raw data that was available for  
287 genetic parameter estimation. A small to moderate  
288 heritability of 0.16 (0.04) was estimated for antigen-specific  
289 IgA transformed to a normal scale (Table 2), this was  
290 double that of the heritability calculated for FEC in the same  
291 cohort (Table 2). In addition to being heritable, there was  
292 sufficient genetic variation to enable selection of animals  
293 for increased antigen-specific IgA activity.

294

295 Genetic relationships estimated from bi-variate analysis are  
296 reported in Table 2. Moderate genetic correlations  
297 between IgA and FEC were estimated with a negative  
298 correlation (-0.26 (0.02)) between IgA and FEC Strongyles.  
299 These correlations indicate that as IgA increases  
300 genetically, FEC Strongyles decreases improving host  
301 resistance for these types of worms. A positive correlation  
302 (0.27 (0.19)) between IgA and FEC Nematodirus was also  
303 observed, however this correlation estimate was not  
304 significantly different from 0.

305

306 Parameters were unable to be estimated for birth and eight  
307 week weight due to convergence issues. The correlations  
308 between antigen-specific IgA activity and production traits  
309 were not significantly different from 0. These results  
310 indicate that selection for antigen-specific IgA is possible  
311 without any adverse influence on production traits for the  
312 Lleyn breed.

313

314 Based on the genetic parameters estimate, EBVs were  
315 produced for IgA with the only correlation fitted for IgA  
316 being with FEC Strongyles (-0.26 (0.02)). The average IgA  
317 EBV was 0.00 and ranged between -0.17 and 0.18 with a  
318 standard deviation of 0.01. A simple correlation between  
319 sire EBV and progeny IgA was undertaken for sires with  
320 more than 10 progeny recorded for IgA. After adjusting the  
321 phenotypes for the fixed effects fitted in the statistical  
322 model, a correlation of 0.91 was observed (Figure 2). This  
323 relationship suggests that sires with higher EBVs for  
324 IgA will produce offspring with genetics to produce higher  
325 IgA responses.

326

#### 327 4. Discussion

328 This study has shown that antigen-specific salivary IgA  
329 responses are heritable and negatively correlated with FEC  
330 making them suitable to identify animals resistant to

331 nematodes for use in selective breeding programs. Antigen-  
332 specific IgA responses have twice the heritability of FEC  
333 (which is currently used to identify resistant animals)  
334 suggesting that selection based on IgA would be more  
335 efficient. Furthermore we predict there would be no  
336 adverse effect of selection on production traits  
337 (correlations between antigen-specific IgA and fat and  
338 muscle deposition were not significantly different from  
339 zero). This is in contrast to ecological theory which  
340 predicts that a trade-off between immunity and host-  
341 growth will exist due to competing energetic needs  
342 (Klasing, 2004). It is possible that this trade-off is not  
343 evident for the measures of immunity and growth used  
344 here because the IgA response to *T. circumcincta* is not  
345 known to cause pathology and a relative protein deficiency  
346 as is the case for the IgE response (Stear et al., 2003).

347

348 Previous research has focussed on plasma IgA responses to  
349 *T. circumcincta* L4 antigens (JP et al., 2014; Strain et al.,  
350 2002). Here we opted to use L3 antigens as they are easier  
351 and less costly to produce than L4 sourced antigens. There  
352 is good evidence that L3 antigens can be used as a proxy for  
353 L4 (McRae et al., 2014) and a strong correlation between  
354 plasma IgA responses to L3 and L4 antigens has previously  
355 been shown (Stear et al., 1995). Despite being easier to

356 produce it is likely that responses to L3 antigens will have  
357 weaker relationships to FEC and perhaps productivity than  
358 L4 and in the future the use of L4 antigens or a suite of L4  
359 recombinant antigens in ELISA may be advantageous.  
360 Indeed the heritability of IgA activity to L4 antigens in  
361 plasma of Scottish Blackface sheep ( $0.56 \pm 0.11$ ) (Strain et  
362 al., 2002) was higher than that measured here to L3  
363 antigens in saliva of Lleyn sheep. It should be noted  
364 however that these two heritabilities are not directly  
365 comparable as different data transformations and statistical  
366 models were used. Importantly in each case the heritability  
367 of IgA responses was approximately twice that of FEC  
368 (Bishop, 1996).

369

370 The strength of the relationship between salivary IgA and  
371 plasma IgA or indeed mucosal IgA at the site of infection  
372 (abomasum) remains to be determined. However, the  
373 majority of IgA present in saliva is derived from B-  
374 lymphocytes that migrate from the gut associated lymphoid  
375 tissue (Brandtzaeg, 2007a, b). This underlying biology and  
376 previous use of L3 antigens in place of L4 (McRae et al.,  
377 2014) supports our use of salivary IgA responses to L3  
378 antigens as an indicator of the protective mucosal IgA  
379 response to L4 antigens. This rationale was upheld by the  
380 favourable genetic correlation with FEC that we observed.



381

382 We advocate using salivary IgA as an indicator of resistance  
383 to *T. circumcincta* as on practical level saliva is easy to  
384 collect for farmers or veterinarians and there is no  
385 possibility of animals not providing a sample. Samples are  
386 also easy to process in large numbers and, as salivary IgA is  
387 quite stable, can be shipped at room temperature.  
388 Alternatively the saliva (or saliva soaked swab) can be  
389 stored at -20°C prior to analysis without impacting the  
390 result.

391

392 Selection for resistance to nematodes based on FEC in the  
393 UK requires an average flock level of 100 eggs per gram  
394 (epg) or higher (Signet Breeding Services, 2014). For FEC it  
395 is also crucial that lambs have not been treated with  
396 anthelmintics for at least 4 weeks. IgA activity is less  
397 affected by anthelmintic use and (as with FEC) flock EBV  
398 will be useful for animals that have been exposed to similar  
399 levels of infection by grazing the same pasture. Currently  
400 the Lleyn breeders are utilising both FEC and IgA EBVs, as  
401 the required flock responses in IgA activity are still to be  
402 determined.

403

404 In this study we measured antigen-specific responses to *T.*  
405 *circumcincta* larval antigens. Of course the animals were

406 grazed on pasture and so would be exposed to and infected  
407 with other parasitic nematodes (e.g. *Nematodirus battus*,  
408 *Cooperia curticei*, *Oesophagostomum* and *Trichostrongylus*  
409 species). We would anticipate some cross-reactivity in the  
410 antibody response to *T. circumcincta* and these other  
411 species and this may be beneficial to the host. However,  
412 larval culture for speciation performed on a subset of faecal  
413 samples revealed these lambs to be predominantly infected  
414 with *T. circumcincta* (unpublished). In addition to the  
415 favourable negative correlation between IgA and FEC  
416 Strongyles, a positive correlation between IgA and FEC  
417 *Nematodirus* was observed (Table 2). However, this  
418 correlation estimate was not significantly different from 0.  
419 The difference in direction was unexpected, given that a  
420 strong positive genetic correlation (0.61 (0.16)) between  
421 the two FEC traits was estimated. It is worth noting that  
422 *Nematodirus* counts are zero-inflated (Denwood et al.,  
423 2008) and so estimates using faecal egg counts for this  
424 species need to be treated cautiously.

425

## 426 5. Conclusions

427 Salivary IgA responses to *T. circumcincta* larval antigens  
428 can be used to identify animals resistant to nematodes for  
429 inclusion in selective breeding programs. Antigen-specific  
430 IgA responses have twice the heritability of FEC (which is

431 currently used to identify resistant animals) suggesting that  
432 selection based on IgA would be more efficient. On a  
433 practical level saliva is easy to collect for farmers or  
434 veterinarians and there is no possibility of animals not  
435 providing a sample. The research reported here was  
436 conducted on Lleyn sheep, however the IgA test is  
437 appropriate for use throughout the sheep breeding industry  
438 as a tool for breeders to identify animals resistant to  
439 nematode infection. Selection of animals based on the IgA  
440 test could play an important role in the integrated  
441 management of *T. circumcincta* and reduce the need for  
442 treatment with anthelmintics.

443

#### 444 Funding

445 This work was supported by BBSRC Animal Health  
446 Research Grant BB/L004070/1 and the University of  
447 Glasgow.

448

#### 449 Acknowledgements

450 We would like to thank the Performance Recorded Lleyn  
451 Breed Society and Agriculture and Horticulture  
452 Development Board (AHDB) and Signet Breeding services  
453 for their involvement and enthusiasm. Catherine Nakielny  
454 (KN consulting) performed faecal egg counts. Thanks also  
455 to Dr Dave Bartley and Alison Morrison (Moredun Research

456 Institute) for the supply of *T. circumcincta* larvae. For  
457 technical assistance we would like to thank C. McComb, K.  
458 O'Neill, M. Robinson and J. McGoldrick.

459

#### 460 References

- 461 Bishop, S.C., Stear, M.J., 2000. The use of a gamma-type  
462 function to assess the relationship between the  
463 number of adult *Teladorsagia circumcincta* and total  
464 egg output. *Parasitology* 121, 435-440.
- 465 Bishop, S.C.B., K.; McKellar, Q.; Park, M.; Stear, M., 1996.  
466 Genetic parameters for faecal egg count following  
467 mixed, natural, predominantly *Ostertagia*  
468 *circumcincta* infection and relationships with live  
469 weight in young lambs. *Anim Sci* 63, 423-428.
- 470 Brandtzaeg, P., 2007a. Do salivary antibodies reliably  
471 reflect both mucosal and systemic immunity? *Ann N*  
472 *Y Acad Sci* 1098, 288-311.
- 473 Brandtzaeg, P., 2007b. Induction of secretory immunity and  
474 memory at mucosal surfaces. *Vaccine* 25, 5467-  
475 5484.
- 476 Ceyhan, A.M., K; Mrode, R, 2015. The Estimation of  
477 (co)variance components growth, reproduction,  
478 carcass, FECS and FECN traits in Lleyn sheep. *Small*  
479 *Ruminant Res* 131, 29-34.
- 480 Denwood, M.J., Stear, M.J., Matthews, L., Reid, S.W., Toft, N.,  
481 Innocent, G.T., 2008. The distribution of the  
482 pathogenic nematode *Nematodirus battus* in lambs  
483 is zero-inflated. *Parasitology* 135, 1225-1235.
- 484 Ellis, S., Matthews, J.B., Shaw, D.J., Paterson, S., McWilliam,  
485 H.E., Inglis, N.F., Nisbet, A.J., 2014. Ovine IgA-reactive  
486 proteins from *Teladorsagia circumcincta* infective  
487 larvae. *International journal for parasitology* 44,  
488 743-750.
- 489 Gilmour, A.R., Gogel, B.J., Cullis, B.R. and Thompson, 2009.  
490 ASReml User Guide Release 3. VSN Interntional Ltd,  
491 Hemel Hempstead, HP1 1ES, UK.
- 492 Jackson, F., Bartley, D., Bartley, Y., Kenyon, F., 2009. Worm  
493 control in sheep in the future. *Small Ruminant Res*  
494 86, 40-45.
- 495 JP, D.E.C., Matthews, L., Mair, C., Stefan, T., Stear, M.J., 2014.  
496 The transfer of IgA from mucus to plasma and the  
497 implications for diagnosis and control of nematode  
498 infections. *Parasitology* 141, 875-879.
- 499 Klasing, K.C., 2004. The costs of immunity. *Acta Zoologica*  
500 *Sinica* 50, 961-969.

- 501 Lee, C.Y., Munyard, K.A., Gregg, K., Wetherall, J.D., Stear, M.J.,  
502 Groth, D.M., 2011. The influence of MHC and  
503 immunoglobulins a and e on host resistance to  
504 gastrointestinal nematodes in sheep. *J Parasitol Res*  
505 2011, 101848.
- 506 Lidauer, M.H., Matilainen, K., Mantysaari, E.A. and Strandén,  
507 I., 2011. Technical reference guide for MiX99 release  
508 IV/2011. MTT Agrifood Research Finland, Jokioinen.
- 509 Martínez-Valladares, M., Vara-Del Rio, M.P., Cruz-Rojo, M.A.,  
510 Rojo-Vazquez, F.A., 2005. Genetic resistance to  
511 *Teladorsagia circumcincta*: IgA and parameters at  
512 slaughter in Churra sheep. *Parasite immunology* 27,  
513 213-218.
- 514 McRae, K.M., Good, B., Hanrahan, J.P., Glynn, A., O'Connell,  
515 M.J., Keane, O.M., 2014. Response to *Teladorsagia*  
516 *circumcincta* infection in Scottish Blackface lambs  
517 with divergent phenotypes for nematode resistance.  
518 *Veterinary parasitology* 206, 200-207.
- 519 Murphy, L., Eckersall, P.D., Bishop, S.C., Pettit, J.J., Huntley,  
520 J.F., Burchmore, R., Stear, M.J., 2010. Genetic  
521 variation among lambs in peripheral IgE activity  
522 against the larval stages of *Teladorsagia*  
523 *circumcincta*. *Parasitology* 137, 1249-1260.
- 524 Prada Jimenez de Cisneros, J., Stear, M.J., Mair, C., Singleton,  
525 D., Stefan, T., Stear, A., Marion, G., Matthews, L., 2014.  
526 An explicit immunogenetic model of gastrointestinal  
527 nematode infection in sheep. *Journal of the Royal*  
528 *Society, Interface / the Royal Society* 11.
- 529 Romeo, C., Wauters, L.A., Cauchie, S., Martinoli, A.,  
530 Matthysen, E., Saino, N., Ferrari, N., 2014. Faecal egg  
531 counts from field experiment reveal density  
532 dependence in helminth fecundity: *Strongyloides*  
533 *robustus* infecting grey squirrels (*Sciurus*  
534 *carolinensis*). *Parasitol Res* 113, 3403-3408.
- 535 Shaw, R.J., Morris, C.A., Wheeler, M., Tate, M., Sutherland,  
536 I.A., 2012. Salivary IgA: a suitable measure of  
537 immunity to gastrointestinal nematodes in sheep.  
538 *Veterinary parasitology* 186, 109-117.
- 539 Signet Breeding Services 2014. The Signet guide to  
540 breeding for worm resistance.
- 541 Smith, G., Grenfell, B.T., Anderson, R.M., 1987. The  
542 regulation of *Ostertagia ostertagi* populations in  
543 calves: density-dependent control of fecundity.  
544 *Parasitology* 95 ( Pt 2), 373-388.
- 545 Stear, M.J., Bishop, S.C., Doligalska, M., Duncan, J.L., Holmes,  
546 P.H., Irvine, J., McCririe, L., McKellar, Q.A., Sinski, E.,  
547 Murray, M., 1995. Regulation of egg production,  
548 worm burden, worm length and worm fecundity by

549 host responses in sheep infected with *Ostertagia*  
550 *circumcincta*. *Parasite immunology* 17, 643-652.  
551 Stear, M.J., Bishop, S.C., Henderson, N.G., Scott, I., 2003. A  
552 key mechanism of pathogenesis in sheep infected  
553 with the nematode *Teladorsagia circumcincta*. *Anim*  
554 *Health Res Rev* 4, 45-52.  
555 Strain, S.A., Bishop, S.C., Henderson, N.G., Kerr, A., McKellar,  
556 Q.A., Mitchell, S., Stear, M.J., 2002. The genetic  
557 control of IgA activity against *Teladorsagia*  
558 *circumcincta* and its association with parasite  
559 resistance in naturally infected sheep. *Parasitology*  
560 124, 545-552.  
561 Traversa, D., von Samson-Himmelstjerna, G., 2016.  
562 Anthelmintic resistance in sheep gastro-intestinal  
563 strongyles in Europe. *Small Ruminant Res* 135, 75-  
564 80.  
565

566

567 Table 1: A summary of the edited Lleyn data used to

568 estimate genetic parameters

<b>Trait</b>	<b>n</b>	<b>average</b>	<b>min</b>	<b>max</b>	<b>Std. dev</b>
birth weight (kg)	66,626	4.14	2.0	7.0	0.96
8 week weight (kg)	268,594	19.17	5.7	32.8	4.36
21 week weight (kg)	102,035	35.76	13.0	58.6	7.44
Ultrasound muscle depth (mm)	63,499	24.08	13.7	34.5	3.41
Ultrasound fat depth (mm)	62,825	2.60	0.1	7.13	1.36
ewe weight at tuppung (kg)	13,398	54.71	20	90	12.12
Faecal egg count – Strongyles (log transformed)	5,840	5.12	0.0001	9.00	2.08
Faecal egg count – Nematodirus (log transformed)	5,840	1.89	0.0001	7.62	2.36
transformed IgA	4,622	0.71	0.03	1.99	0.35

569

570

571 Table 2: Estimates of phenotypic variances, heritability and  
 572 phenotypic and genetic correlations between transformed  
 573 IgA and production traits of UK Lleyn sheep

<b>Trait</b>	<b>Phenotypic Variance</b>	<b>Heritability</b>	<b>Phenotypic correlation with IgA</b>	<b>Genetic correlation with IgA</b>
transformed IgA	0.08 (0.002)	0.16 (0.04)	-	-
Faecal egg count - Strongyles (log transformed)	2.68 (0.06)	0.08 (0.03)	-0.002 (0.02)	-0.26 (0.02)
Faecal egg count - Nematodirus (log transformed)	4.55 (0.11)	0.15 (0.04)	0.007 (0.02)	0.27 (0.19)
Ultrasound muscle depth (mm)	4.85 (0.09)	0.37 (0.03)	0.05 (0.02)	0.08 (0.12)
Ultrasound fat depth (mm)	1.04 (0.02)	0.41 (0.03)	0.04 (0.02)	0.04 (0.12)
21 week weight (kg)	20.54 (0.25)	0.45 (0.02)	0.02 (0.02)	-0.02 (0.11)
ewe weight at tugging (kg)	37.81 (0.85)	0.37 (0.03)	0.04 (0.04)	0.25 (0.15)

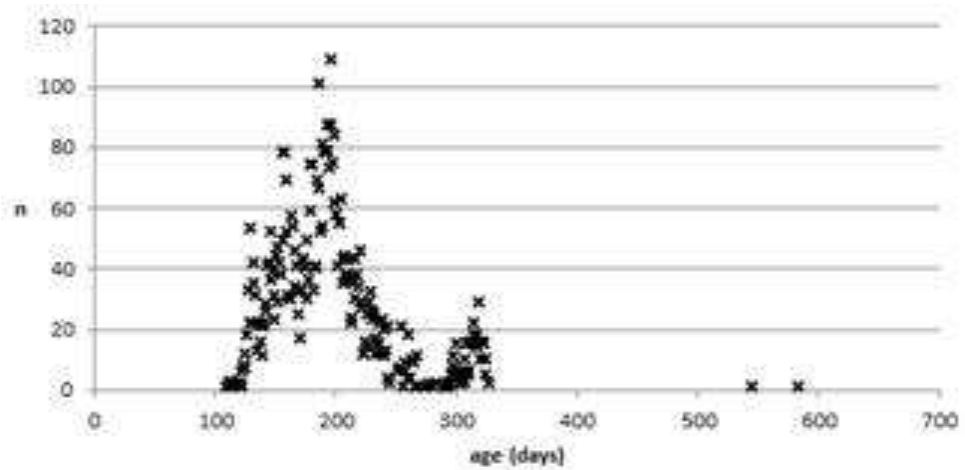
574

575



576 Figure 1: Age distribution of animals sampled between 2014  
577 and 2017. Animals greater than 350 days old were excluded  
578 from final analysis. The mean age at sampling for all animals  
579 was 193 days. For males the mean age was 182 days and for  
580 females 196.

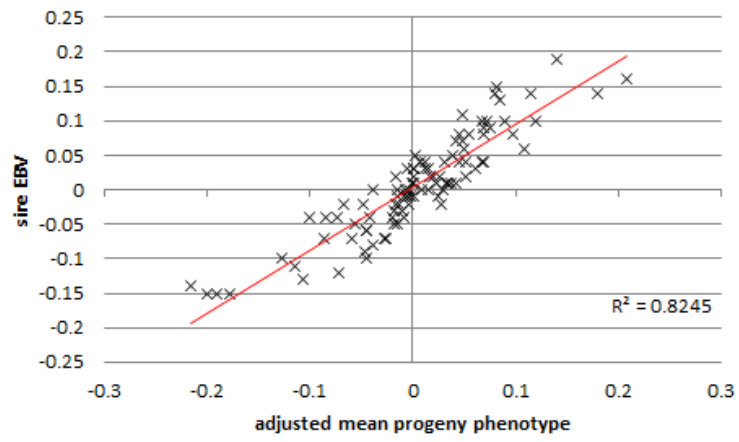
581



582

583

584 Figure 2: Sire IgA EBV and the mean progeny IgA phenotype  
585 adjusted for fixed effects



586