A COMPARISON BETWEEN SHEEP BRED FOR WORM RESISTANCE AND UNSELECTED CONTROLS WHEN EXPOSED TO LOW LARVAL CHALLENGE DURING SUMMER

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SUMMARY

Sheep in winter-rainfall regions typically show a rise in faecal worm egg count (WEC) over the summer-autumn period, when pasture larval challenge is low or near zero. This rise is important epidemiologically for worm control. Our study found that the peak WEC of sheep bred for worm-resistance was reduced by 43% compared to unselected control sheep during the summer-autumn period. Total worm counts confirm that the rise in WEC was not due to an increased worm burden. Explanations for the summer-autumn rise in WEC include an increased fecundity of existing adult worms or maturation of existing immature worms. However, at this stage the precise mechanisms behind the rise in WEC remain unclear. A marked increase in the WEC for both genotypes of sheep occurred when they were removed from pasture and housed for 3-4 weeks.

INTRODUCTION

Reducing the summer-autumn rise in WEC may be important to capture epidemiological benefits from sheep bred for low WEC in winter-rainfall environments. This is because eggs deposited during this period largely determine the peak larval challenge during winter (Anderson 1972), and hence the degree of parasitism and production losses (Niven *et al.* 2002). The summer-autumn period generally has low larval challenge and there is concern that sheep bred for low WEC, under high larval challenge conditions, may not suppress WEC during the summer-autumn period. This concern is based on the hypothesis that immunity is dependent on a threshold of ingested larval (Dineen 1963). The aim of this study was to investigate two hypotheses; (i) that sheep bred for low WEC (worm-resistant, R) will maintain lower WEC than unselected control (C) sheep throughout the summer-autumn period, and (ii) that the summer-autumn rise in WEC is caused by maturation of arrested *Teledorsagia* spp. rather than increased total worm burden. Hypothesis 2 is primarily concerned with the biological basis for the summer-autumn rise in WEC, rather than a comparison of the genotypes contained within this study.

MATERIALS AND METHODS

Animals. Animals were sourced from three epidemiologically independent replicates of Rylington Merino worm-resistant (R1, R2, R3) and unselected control (C1, C2, C3) mature ewes (n = 50/replicate) at the Mt Barker research station, Western Australia (Greeff & Karlsson 2006; Greeff *et al.* 2006). To test the first hypothesis we monitored a subset of 3-4 year old ewes from each replicate for WEC at four times during the 2007/2008 season (18th September, 11th December, 20th February, 28th March). Individuals were repeatedly measured, where possible, and a total of 473 WEC measures were made on 164 animals. Most animals (69%) had 3–4 WEC measures. Lactation status was recorded in early spring (September). The second hypothesis was tested by the necropsy of two ewes per replicate (2–7 years of age), before and after the summer-autumn rise in WEC. Animals were chosen from 6–9 cull animals per replicate. Animals for necropsy

were selected from culls based on WEC measurements at the above dates, either to reflect the observed WEC range (early summer) or on the basis of a demonstrated rise in WEC (late summer). After selection, animals were removed from their pasture contemporaries (11^{th} December or 25^{th} – 26^{th} February) and housed for 3–4 weeks on a mixed chaff and lupin ration. Housing prevented the ingestion of larvae so that worms counted in the early L_4 stage were truly arrested and were not confounded with recently ingested larvae. Total worm counts were performed over 7 (2^{nd} – 9^{th} January) or 2 days (18^{th} – 19^{th} March) and measurements included the count of adult worms in each genus, the count of immature L_5, delayed and early L_4, and WEC. Total worm burden was the sum of all counted worms.

Parasitology. WEC and necropsy measurements were made according to standard procedures at the Agriculture Western Australia laboratories, Albany. WEC was assessed using a modified McMaster method with 25 eggs per gram (epg) sensitivity. Larval cultures were conducted for each plot at each WEC measurement, or individually for necropsy animals; and a proportion of *H. contortus* was detected in early spring (C1, 0.28; R2, 0.12 & R3, 0.01), early summer (C2, 0.04; C3, 0.13) and late summer (C2, 0.22; R3, 0.70). To prevent potential bias, all WEC were adjusted for either the replicate or individual proportion of *H. contortus*. Necropsy procedures included the examination of the washings and digests from the abomasum and small intestine.

Statistical Analysis. WECs showed a skewed distribution and were transformed [$\log_{10}(x+10)$] for normality. Replicate geometric means and t-tests for each time-point are presented. To account for multiple measures over time a mixed, repeated measures linear model was fitted to log-transformed data. The model had the form:

$$\log_{10}(WEC_i+10) = \mu + G_j + T_k + G.T_{j,k} + L_l + R_{j,k,m} + ID_n + \varepsilon_i \quad (1)$$

where the fitted effects were the overall mean (μ) and fixed effects of genotype (G_j, j = R, C), time of measurement (T_k, k = 1…4), the interaction between genotype and time ($G.T_{j,k}$) and lactation status (L_l, l = wet, dry; k = 1 only); and random terms were replicate variance [replicate (m) x genotype x time interaction, $R_{j,k,m}$ where m = 1..3], between-animal variance (ID_n, for n^{th} animal) and within-animal variance (or residual error, ε_i). The linear model for necropsy data had the form:

$$\log_{10}(x_i+1) = \mu + G_j + T_k + G.T_{j,k} + \varepsilon_i \quad (2)$$

where i^{th} worm count was fitted to the overall mean (μ) and fixed effects of genotype (G_j), necropsy group (T_k, k = 1,2) and their interaction ($G.T_{j,k}$). Replicate structure could not be included due to small sample size per replicate. Necropsy WEC was $\log_{10}(x_i+10)$ transformed.

RESULTS AND DISCUSSION

Faecal worm egg count. WECs were low throughout the 07/08 season. However, temporal trends in mean WEC show a clear summer-autumn rise for both C and R genotype sheep (Table 1) and peak mean WEC was 43% lower in R animals. Approximately 50% of C and 30% of R genotype sheep had WEC > 200 epg in late summer (February) and early autumn (March). Unexpectedly, WECs for the necropsy animals were much higher than their genotype contemporaries on pasture at the time of slaughter. WECs increased markedly during 3–4 weeks of housed conditions. This rise makes inferences about the structure of parasite populations in pasture contemporaries impossible. The higher late summer WEC for R ewes in the late summer necropsy group was expected, as this group was chosen on the basis of a demonstrated rise in WEC (rather than to be representative of the genotype).

Geometric means show the variation between genotype replicates, as well as the high variation within each replicate (Table 2). The mixed model showed R animals to have significantly lower WEC than C animals when all measures are considered together (Table 3, $P < 0.05$), however the

time-point specific differences were never significant. The repeatability of WEC over the period was high (0.42), providing an upper-bound estimate of heritability for WEC over summer. Significant replicate and between-animal variance components show that (i) the variation between replicates was not simply due to the variation between animals and (ii) the variation between animals was not simply due to the variation between replicates ($P < 0.01$).

Table 1. Arithmetic mean faecal worm egg count (WEC; eggs per gram) for control (C) and resistant (R) genotype sheep from September 07 – March 08. Shown is mean for animals continuously grazed on pasture (mean of replicates) and the mean for the two necropsy groups removed from pasture in early (ES) or late (LS) summer. The mean for necropsy groups after removal from pasture is shown in italics

Animal group & genotype		E. Spring (September)	E. Summer (December)	*E. Summer necropsy*	L. Summer (February)	*L. Summer necropsy*	E. Autumn (March)
mean of replicates	C	142	56		346		324
"	R	53	31		198		206
ES necropsy gp	C	77	94	*2350*			
"	R	79	124	*845*			
LS necropsy gp	C	64	24		343	*1550*	
"	R	14	25		445	*1392*	

Table 2. Geometric mean faecal worm egg count (eggs per gram) for replicates 1-3 in control (C) and resistant (R) genotype sheep. Genotype geometric means are shown in bold. The range observed in each replicate is shown in parentheses

Replicate	Early spring (September)		Early summer (December)		Late summer (February)		Early autumn (March)	
C1	40	(0 – 485)	35	(0 – 300)	160	(0 – 950)	120	(0 – 975)
C2	65	(0 – 1875)	40	(0 – 360)	140	(0 – 1345)	140	(0 – 950)
C3	75	(0 – 600)	30	(0 – 740)	400	(75 – 1550)	400	(25 – 1325)
	60		**35**		**210**		**200**	
R1	60	(0 – 225)	35	(0 – 200)	140	(25 – 1600)	170	(25 – 1425)
R2	20	(0 – 25)	30	(0 – 150)	85	(0 – 475)	65	(0 – 180)
R3	50	(0 – 265)	30	(0 – 365)	130	(0 – 765)	210	(0 – 1050)
	40		**30**		**120**		**130**	
P- value^		*0.18*		*0.09*		*0.09*		*0.20*

^ one-sided t-test

Table 3. F-ratios and variance components for the mixed model of WEC

Effect	Df	F-ratio	Component	df	Variance
Lactation	1	10.1[a]	Replicate	24	2.07×10^{-2} [b]
Genotype	1	5.35 [a]	Between-animal	164	1.15×10^{-1} [b]
Time	1	37.9 [b]	Within-animal	463	1.60×10^{-1}
Genotype x Time	3	0.36			

[a] $P < 0.05$, [b] $P < 0.01$

Necropsy observations. Mean worm burdens were low and typical of what might be expected during summer (Table 4). A large range of worm burdens was observed, particularly in the early summer group. R animals tended to have lower total worm burden and WEC at necropsy. Fitted models generally had difficulty to predict the observed geometric means (not shown), indicating poor fit to the data, potentially due to the small sample sizes and high variability. WECs at necropsy were much higher than those observed on pasture, implying a change in the resident worm population under housed conditions or changes to faecal composition. Higher WEC may

have occurred due to increased egg production per worm or maturation of immature worms. Changes in WEC and a poor data fit by the models does not allow for valid testing of the second hypothesis. For future studies we recommend a shorter interval period of housing prior to necropsy (4 – 7 days) and the selection of animals for necropsy to be more representative of pasture contemporaries. These data suggest maturation of immature stages over summer, however increased worm fecundity cannot be excluded and these results would need to be reproduced. Worm burdens were stable over summer, potentially indicating low larval challenge and that an increased worm burden was not the cause of the summer rise in WEC for pasture contemporaries.

Table 4. Geometric mean worm counts from control (C) and resistant (R) genotype animals in early and late summer necropsy groups. The range of observed values is shown in parentheses.

	n	Total	*Teledorsagia*‡	*Trichostrongylus*‡	immature†,§	early L_4§	WEC
Early summer necropsy							
C	6	2950	70	120	80	1250	1750 α
		(450 – 11150)	(0 – 2450)	(0 – 6250)	(0 – 1100)	(150 – 3250)	(650 – 4650)
R	6	1950	470	90	130	180	610 β
		(400 – 5950)	(200 – 1300)	(0 – 1250)	(50 – 350)	(0 – 5200)	(100 – 1230)
Late summer necropsy							
C	6	3750	1120	1430	230	420	1250
		(1500 – 9100)	(350 – 3450)	(600 – 6750)	(50 – 1500)	(50 – 2000)	(600 – 3800)
R	6	1950	780	640	20	210	1070
		(600 – 3850)	(250 – 2450)	(250 – 2550)	(0 – 150)	(50 – 550)	(250 – 2800)
G x T		*Ns*	*ns*	*ns*	*0.06*	*ns*	*ns*
G.type		*0.19*	*0.33*	*0.61*	-	*0.17*	*0.25*
Time		*0.78*	*0.05*	*0.04*	-	*0.63*	*0.72*

α, β missing data; n = 3, 4

† contains immature L_5 and developing L_4; § all species; ‡ mostly adults, but some immatures were detected

CONCLUSIONS

Sheep selected for low WEC had a reduced mean WEC during the summer-autumn period compared to unselected sheep. This may partially explain reduced winter pasture contamination and epidemiologically-derived production benefits observed for R sheep in this environment (Greeff *et al.* 2006; Williams *et al.* 2006). The 43% reduction in peak WEC was less than reported under high larval challenge (89%, Greeff & Karlsson 2006). It seems sheep selected for low WEC may respond to larval challenge more readily than unselected controls. We could not test our second hypothesis as necropsy WECs were much higher than those on pasture. The increased WEC during housing maybe due to changes to faecal composition or indicate altered immunity; potentially due to stress, an absence of larval challenge or nutritional changes.

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