

Investigating the Sheep Immune Response to Parasitic Worm Infection

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Why are parasitic worms important?

- The UK farming industry loses in excess of £100m per annum due to worm treatments and loss of production through worm infection
- Drug-resistance is now widespread across the globe
- Continued use of anthelmintics is not sustainable long-term.

Breeding for resistance

- Sheep naturally acquire resistant immunity with exposure to the parasites
- Resistant immunity is heritable
- Parasite-resistant sheep produce high levels of antibodies that clear worm infection
- Antibodies (IgA) are produced by a pathway activated by T helper type 2 (Th2) cells
- Susceptible sheep have an inflammatory reaction to the worms characterised by Th1 and Th17 cells
- The genes involved in these pathways could be key to genetic selection of resistant sheep.

Genes of interest

- Below are the genes under investigation in this study
- These genes have been sequenced for the first time in parasite-infected sheep
- Mutations within these genes have been identified.

Phenotype	Responding cell type	Associated Gene(s)	Variant(s)
Resistant	Th2	GATA3	GATA3v1
Susceptible	Th1	TBX21	
Susceptible	Th17	RORC2	RORC2v1
Susceptible	Th17	RORA	v1-5

Experimental lambs

- 45 Blackface ewe lambs were born and raised parasite-free until 12wks old when artificial trickle infection (2000 L3 3x per wk) began for 13 wks
- Resistant lambs cleared the infection after 6 wks; Susceptible lambs did not clear infection.

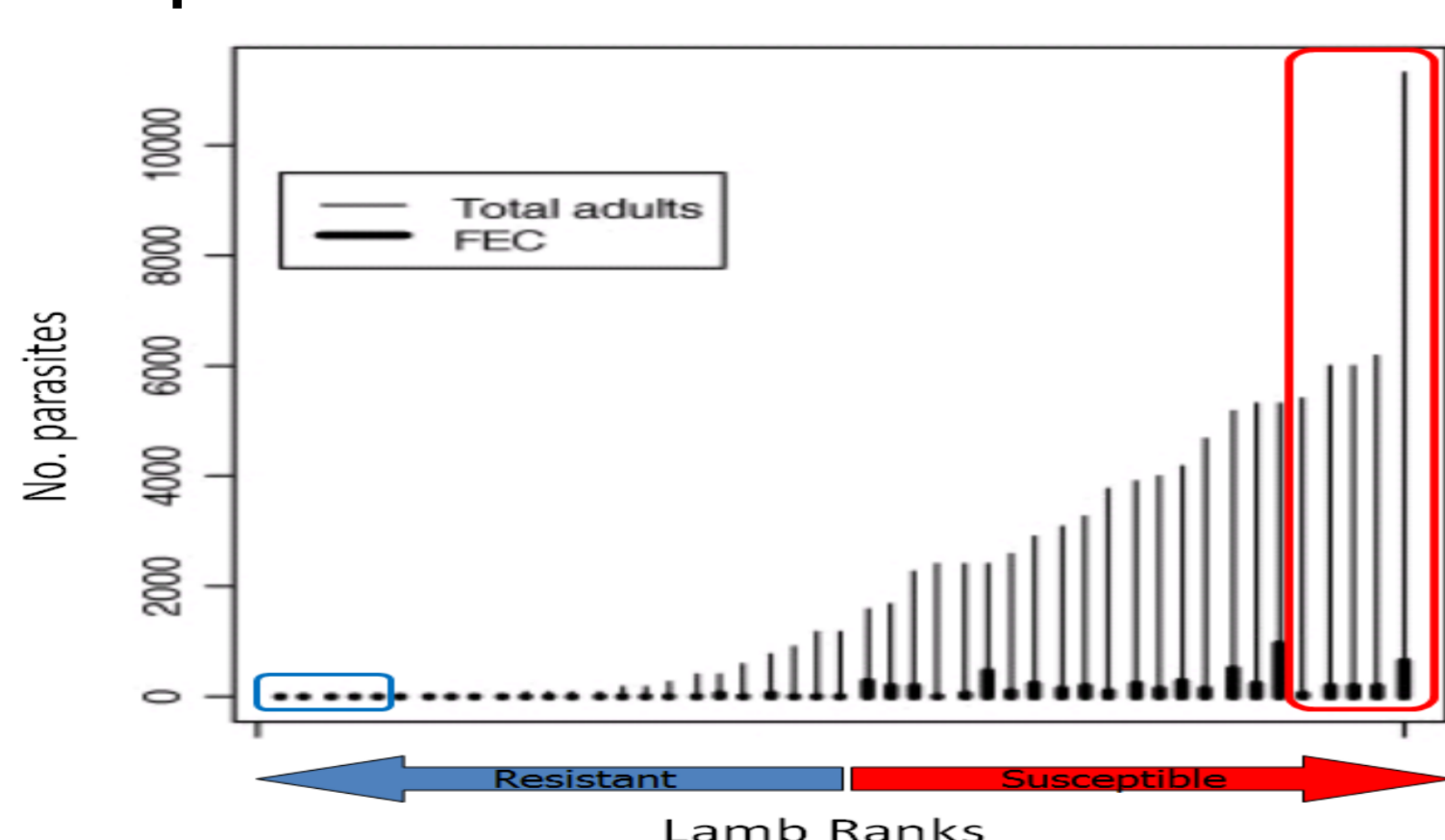


Figure 1. Infected lambs ranked by adult worm count and faecal egg count post mortem (Pemberton et al. 2011. Molecular Ecology 20:910-919)

Quantifying genes of interest

Using RT-qPCR, the genes of interest and their splice variants were quantified in the 9 most Resistant and 9 most Susceptible lambs.

Once the resistant/susceptible phenotype is well established, there is little difference in overall expression of the transcription factors within the lymph node.

However, one variant of RORA (v5) had fold change difference of 1.57 between groups (p-value 0.08). So this variant was analysed further in all infected lambs.

Absolute quantification of gene variants

Using Absolute RT-qPCR, RORAv2 and RORAv5 were quantified in all 45 infected lambs.

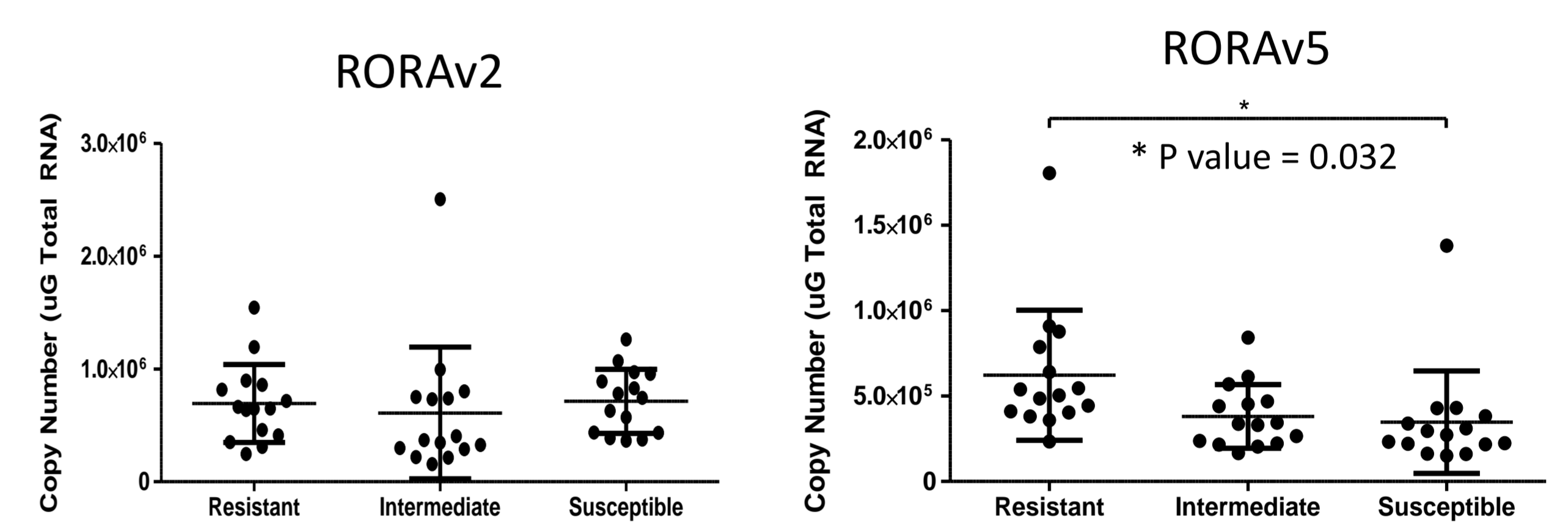


Figure 2. Levels of gene expression in Resistant, Intermediate and Susceptible lambs.

RORAv5 has significantly increased expression in Resistant compared to Susceptible lambs.

Correlation of gene variants to phenotypes

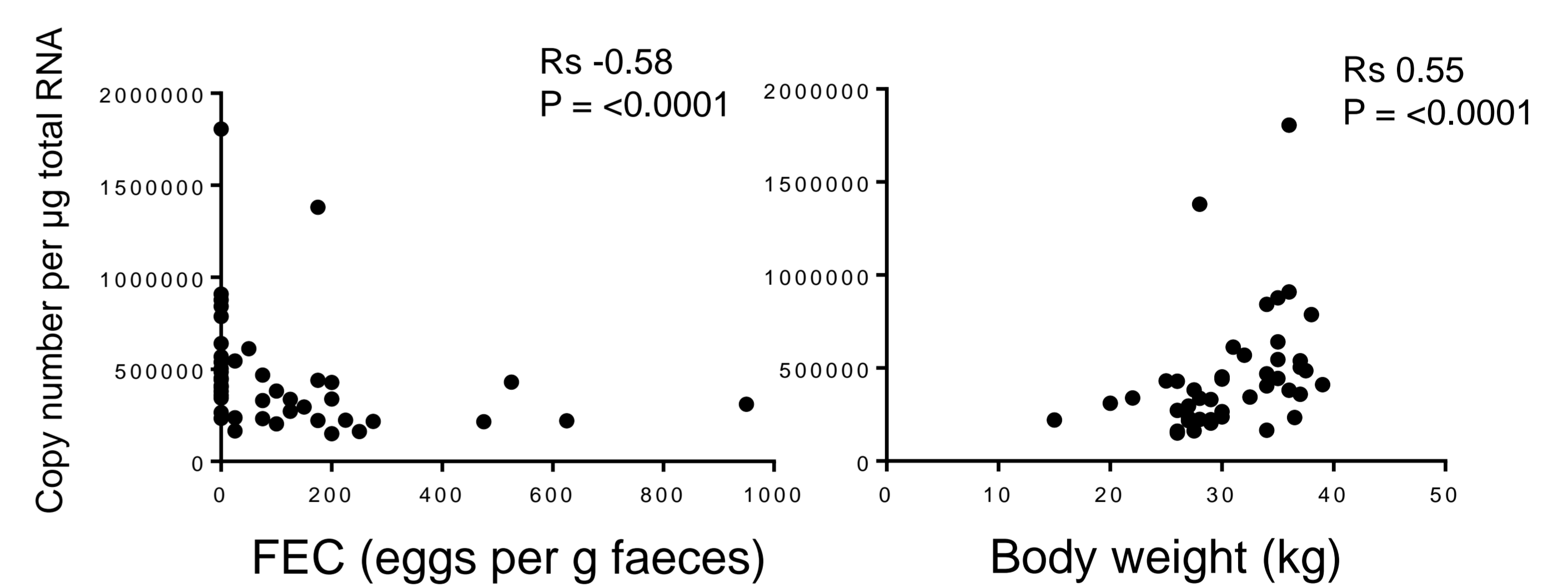


Figure 3. RORAv5 expression level correlated to faecal egg count and body weight

Gene	Phenotype	Correlation	P value
RORAv5	Adult worm count	-0.55	<0.0001
	Faecal egg count	-0.58	<0.0001
	Body weight	0.55	<0.0001
RORAv2	IgA	-0.29	0.025

CONCLUSIONS

- RORAv5 is associated with Resistance through significant increased expression in R lambs, significant negative correlation with AWC and FEC and significant positive correlation with BW
- RORAv2 is associated with Susceptibility through significant negative correlation with IgA levels.