RNA interference for selective gene knockdown and vaccine candidate identification in the ectoparasitic mite *Psoroptes ovis*.

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Introduction to Sheep Scab

• Sheep scab is a highly contagious ectoparasitic disease of sheep, endemic in the UK, caused by the Astigmatid mite *Psoroptes ovis*, causing a serious welfare issue due to intense pruritis and severe exudative dermatitis (**Figure 1**)

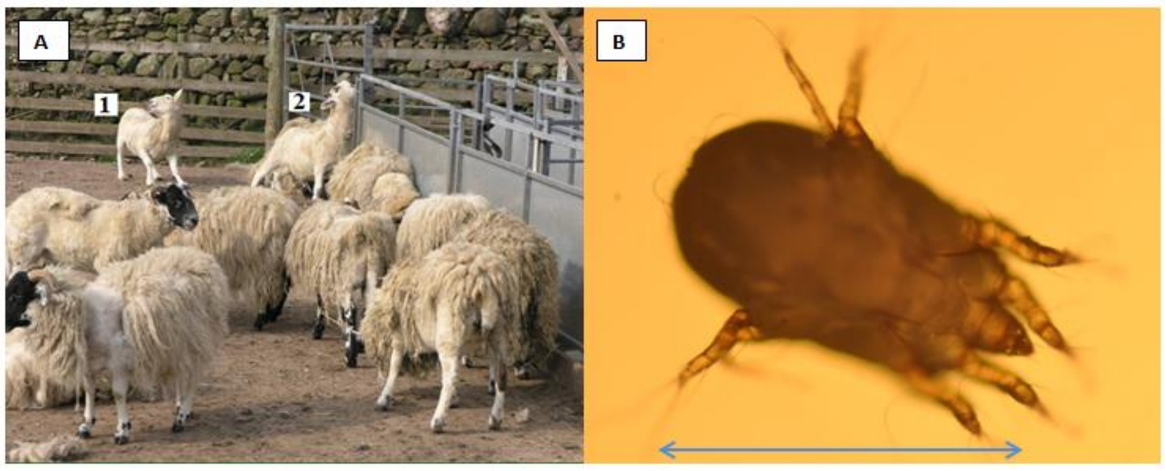


Figure 1 - Overview of sheep scab disease: [A] Stereotypies associated with *P. ovis* infestation, sheep 1 is displaying typical hyper-flexion, sheep 2 is displaying excoriation behaviour (Photo, N. Sargison) [B] Adult female *P. ovis*, scale bar = 500µm.

• Currently controlled by chemotherapeutics, at an annual cost of £8-14 million [1,2]. However, concerns over their sustainablity is driving the search for new methods of control

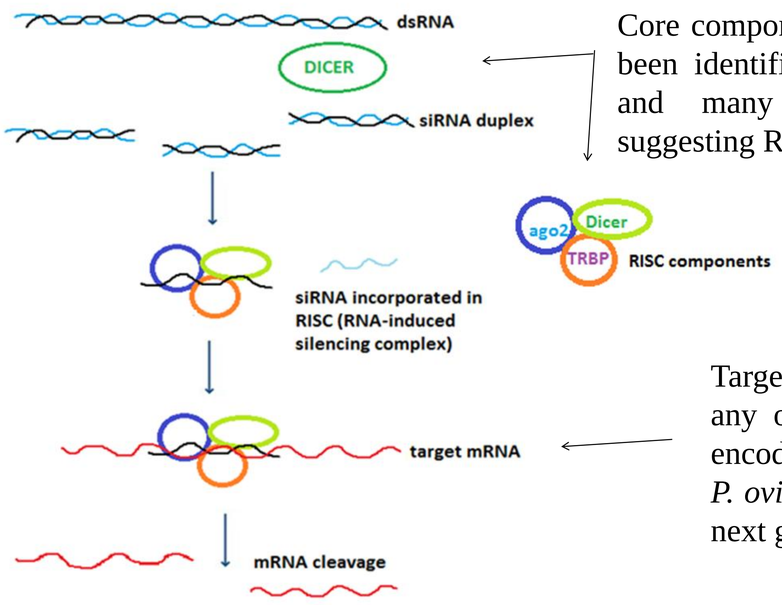
• Next generation sequencing of the *P. ovis* transcriptome has generated 12,160 expressed sequence tags, which now require filtering and screening for target identification

• A novel, high-throughput method is required to screen this large dataset to identify new vaccine candidates

RNA Interference (RNAi)

• Widely used method of gene silencing (**Figure 2**), could be used to screen potential vaccine candidates, an approach widely applied in tick research

• Significant prior RNAi research in ectoparasites eg. *Varroa destructor* [3]



Core components of the RNAi pathway have been identified in the *P. ovis* transcriptome and many systemic components also, suggesting RNAi is feasible in *P. ovis*.

Target mRNA; this could encode any of the 12,160 putative gene-encoding isotigs from the MRI *P. ovis* transcriptome, generated by next generation sequencing.

Figure 2 - Artificial RNAi pathway summary. Small interfering RNA and double stranded RNA molecules can be designed using the MRI in-house *P. ovis* transcriptome.

RNAi pathway in *Psoroptes ovis*

Table 1 - List of RNAi pathway genes, adapted from Grbic *et al*. 2011, [4] detected in *P. ovis, T. urticae, S. scabiei, R. microplus* and a selection of organisms for which an annotated genome exists.

Gene name	RNAi pathway gene detected							
	Psoroptes ovis	*Tetranychus urticae*	*Sarcoptes scabiei*	*Rhipicephalus microplus*	*Ixodes scapularis*	*Caenorhabditis elegans*	*Tribolium castaneum*	*Drosophila melanogaster*
Dicer	+	+	+	+	+	+	+	+
Argonaute	+	+	+	+	+	+	+	+
Exportin	+	+	+	- *	+	+	+	+
Loquacious/TRBP	-	+	-	-	+	+	+	+
Pasha	+	+	+	-	-	+	+	+
Drosha	-	+	-	-	-	+	+	+
RdRP	-	+	-	+	+	+	-	-
R2D2	-	-	-	-	-	+	+	+
C3PO	-	-	-	-	-	+	+	-
VIG	-	+	-	-	+	+	-	+
GW182	-	+	-	-	-	+	-	+
Piwi/ago-3/ Aubergine	+	+	-	+	-	+	+	+

+ denotes genes that were identified through literature or database searches.
- denotes genes that were not detected in the author's search and does not necessarily imply the gene is absent in the species listed.
* homologue of putative *I. scapularis* exportin, detected in *Rhipicephalus pulchellus* (Blast E-value = 0.0).

Promising Initial Results

- Optimised RNA extraction technique for use with low numbers of mites
- Interrogated the *P. ovis* transcriptome to identify components of the RNAi pathway and to identify potential targets for RNAi (**Table 1**)
- Established a non-invasive overnight immersion technique for demonstrating RNA uptake in *P. ovis*, using fluorescently-labelled siRNA (**Figure 3)**

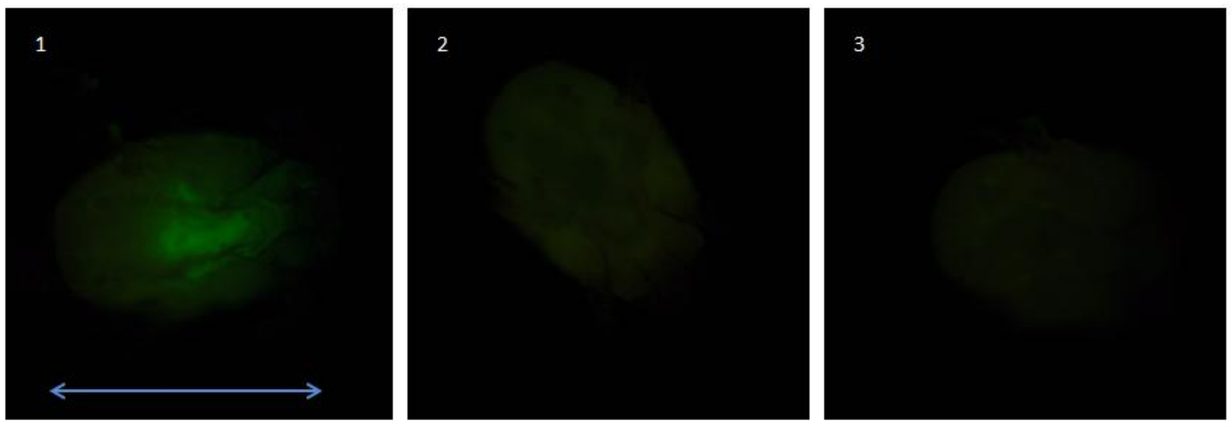


Figure 3 - Female *P. ovis* fluoro-siRNA uptake assessment results are presented: adult females following immersion overnight in fluoro-siRNA solution (1), 0.9% NaCl buffer (2) and untreated control female exposed to air (3). Scale bar = 500 µm.

• Following dsRNA immersion experiments, viable cDNA from extracted RNA was produced and qPCR performed to determine mRNA transcript levels (**Figure 4**)

Pso o 2 dsRNAi trial qPCR results

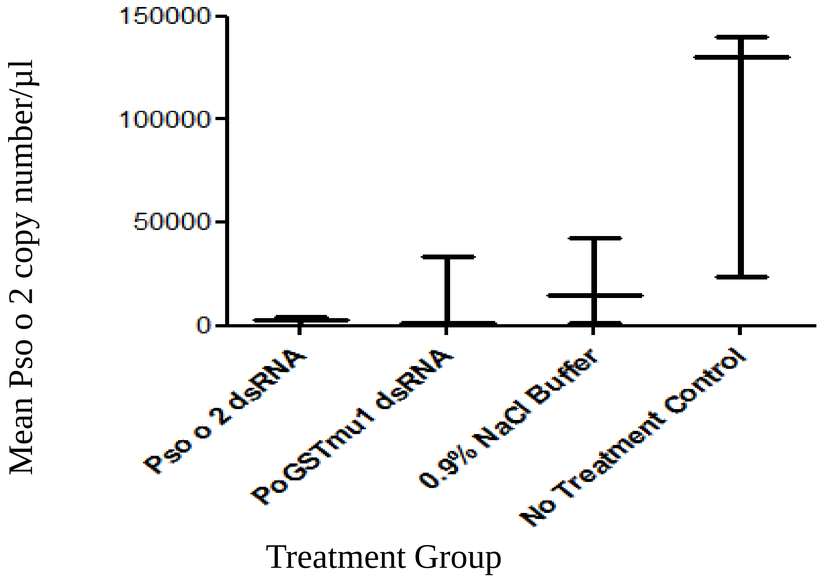


Figure 4 - Pso o 2 mRNA expression for each treatment group normalised to the expression of the reference gene β-actin. Mites were immersed in dsRNA encoding Pso o 2, dsRNA encoding *Po*GST-mu1 (control to determine specificity of RNAi), 0.9% NaCl buffer alone or exposed to air. A one way ANOVA with *post-hoc* Tukey's Multiple Comparison Test was carried out - Pso o 2 copy number was significantly different between the 'Pso o 2 dsRNA' and 'No Treatment Control' treatment groups (p=0.0361).

Future Research Directions

• Assess further *P. ovis* genes with RNAi – develop positive control candidate

• Assess translational level effect of dsRNAi, focussed on *Po*GST-mu1 initially

• Develop *in vivo P. ovis* dsRNAi model to look at impact of RNAi on early infestation

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References

1. Bates, P. (2006) Presentation to the Annual Meeting of the Irish Society for Parasitology, Celbridge, 1st December 2006.
2. Nisbet *et al*. (2008) *Experimental Parasitology*, 120: 194-9
3. Campbell *et al*. (2010) *Parasites & Vectors*, 3: 73
4. Grbic *et al*. (2011) *Nature*, 479: 487-92

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