



MICROARRAY EVIDENCE FOR OFF TARGET EFFECTS IN TICK RNA INTERFERENCE EXPERIMENTS, AND THE LACK OF STRONG CORRELATION BETWEEN DSRNA AND ANTIBODY PHENOTYPES IN TICK IN VITRO TREATMENTS FOR VACCINE CANDIDATE SCREENING.

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Knowledge of cattle tick (*Rhipicephalus (Boophilus) microplus*; Acari:Ixodidae) molecular pathways has been hampered by the lack of an annotated genome. In addition, most of the tick expressed sequence tags available to date consist of ~50% unassigned sequences without predicted functions. The most common approach is the application of RNA interference (RNAi) methods to investigate gene function and to confirm potential of targeted genes as vaccine antigens. This has been widely adopted despite lack of knowledge of the tick RNAi pathway, double-stranded RNA (dsRNA) uptake mechanisms, or host immune recognition of the targeted genes. A strong knockdown phenotype of adult female ticks had been previously observed using a 594 bp dsRNA targeting the cattle tick homologue for the *Drosophila Ubiquitin-63E* gene leading to death or nil and deformed eggs if the female tick survived (Kurscheid et al. 2009). A NimbleGen cattle tick custom microarray based on the BmiGI.V2 database of *R. microplus* ESTs (14 x 50-mer probes for 13,601 targets of the total 13,643 ESTs) was used to evaluate the expression of mRNAs harvested from ticks treated with the tick *Ubiquitin-63E* 594 bp dsRNA. A total of 144 ESTs including TC6372 (*Ubiquitin-63E*) were down-regulated. The results substantiated the knockdown phenotype with ESTs associated with ubiquitin proteolysis as well as oogenesis, embryogenesis, fatty acid synthesis and stress responses. A bioinformatics analysis was undertaken to predict off target effects (OTE) resulting from the in silico dicing of the 594 bp *Ubiquitin-63E* dsRNA which identified 10 down-regulated ESTs (including TC6372) within the list of differentially expressed ESTs on the microarrays. Subsequent knockdown experiments utilising 196 bp and 109 bp dsRNAs, and a cocktail of short hairpin RNAs (shRNA) targeting *Ubiquitin-63E* demonstrated similar phenotypes for the dsRNAs but nil effect following shRNA treatment. Quantitative real time PCR analysis confirmed differential expression of TC6372 and selected ESTs including OTE targets. We also compared female tick in vitro treatments using corresponding dsRNAs and antibodies targeting 11 different ESTs known to be recognised by the bovine immune system. Only two ESTs demonstrated a correlation between gene knockdown and antibody treatment resulting in death and poor egg production, while 7 demonstrated antibody effects on survival but no effect following dsRNA treatment and 2 showed the converse with dsRNA effects on survival only. This is the first study addressing tick dsRNA delivery methods demonstrating the minimisation of predicted OTEs in shorter dsRNA treatments (~100-200 bp) and also illustrating the need for caution when interpreting knockdown phenotypes. We also demonstrate that dsRNA knockdown phenotypes will not always correlate to antibody or immune responses for the identification of putative vaccine antigens.

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