African Journal of Biotechnology Vol. 12(12), pp. 1350-1356, 20 March, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2693
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Development of an RNA-interference procedure for gene knockdown in the poultry red mite, *Dermanysus gallinae*: Studies on histamine releasing factor and Cathepsin-D

Lucy M. Kamau[1,2]*, Harry W. Wright[3], Alasdair J. Nisbet[3] and Alan S. Bowman[1]

[1]University of Aberdeen, School of Biological Sciences, Aberdeen, AB24 2TZ, Scotland, UK.
[2]Department of Zoological Sciences, Kenyatta University, P. O Box 43844, 00100, Nairobi, Kenya.
[3]Parasitology Division, Moredun Research Institute, Midlothian EH26 OPZ, Scotland, UK.

Accepted 16 November, 2012

The poultry mite, *Dermanyssus gallinae*, is a parasite of fowl and birds worldwide, causing huge economic losses in production systems. We investigated gene-knockdown approach in identifying potential vaccine antigens, targeting Histamine Releasing Factor (HRF) and Cathepsin D (C), through immersion in dsRNA derived from the two genes, for 7 and 16 h respectively, alongside LacZ control. Quantitative reverse transcriptase (RT)-PCR was used to estimate the extent of gene knockdown. The results show successful knockdown of HRF target after 16 h of immersion in dsRNA. It is suggested that optimization of delivery methods for dsRNA and targeting more genes could enhance knockdown.

Key words: *Dermanyssus gallinae* (Acari: Astigmata), RNA interference (RNAi), gene silencing, histamine releasing factor, Cathepsin D.

INTRODUCTION

The poultry red mite, *Dermanyssus gallinae* (Acari: Dermanyssidae) is a cosmopolitan parasite of fowl, caged and wild birds. The mite dwells in crevices of nests of birds and cages and nests of small mammals. It is reported in all types of egg production systems with greater abundance in free-range and barn systems compared to battery units (Hoglund et al., 1995; Fiddes et al., 2005). Infestations cause serious health and welfare concerns resulting in reduced productivity, anaemia, aggression, cannibalism, increased death risks and food demand, reduced time for bird resting, anaemia, decreased disease resistance, egg-laying and reduced egg quality. Poultry red mites are also implicated in transmission of poultry diseases, such as salmonellosis (Afrim et al., 2011; Moro et al., 2009; Lesna et al., 2009) and in causation of pruritic dermatitis in poultry workers and veterinarians through contact in poultry houses.

The mite occurs worldwide in many European countries. It is the most economically deleterious parasite of laying hens (Claude, 1998) where control and production losses have been estimated at € 130 million per annum (Van Emous et al., 2006). Massive infestations are reported in France (Beugnet et al., 1997), Sweden (Maurer and Baumgartner, 1992, 1994; Maurer et al., 1993), and the Netherlands (van Emous et al., 2006) occurring throughout the year and virtually in all laying hen, broiler breeder, rearing hen and parental stock houses. In Kenya, and many African countries, the extent of damage caused by this mite is largely un-documented as it occurs mostly in free-range egg laying hens which are mainly reared for subsistence con-

*Corresponding author. E-mail: kamaulucy05@gmail.com or lmuthoni25@yahoo.com. Tel: +254722356568.

Abbreviations: HRF, Histamine releasing factor; **Dg-CatD,** Dermanyssus gallinae cathepsin D; **C,** cathepsin D; **RNAi,** RNA interference; **dsRNA,** double stranded RNA; **FEC,** faecal egg counts; **siRNAs,** small interfering RNA molecules.

sumption. Currently, there is a growing local and regional market for the indigenous chicken in Kenya, both for meat and eggs and the losses caused by the mite infestation will be of much greater significance. It is apparent that the mite problem will increase in several countries in future and therefore control methods must be reviewed in an effort to improve them (Claude, 1998).

Control of *D. gallinae* is typically by use of synthetic acaricides such as carbaryl, diazinon, dichlorvos and permethrine. The continued use of these chemicals in control is hampered by the development of inheritable resistance, residues in meat and eggs and other environmental effects (Claude, 1998), while other chemicals are toxic or harm birds welfare. Effective administration and effectiveness is also limited by the fact that mites hide in crevices and other narrow places in the poultry farm where they are not easily reached by pesticide sprays (Lesna et al., 2009). There is therefore a significant and growing need for development of alternative control methods, such as development of vaccines against this ectoparasite. The advantage of vaccination against vectors is that it eliminates both the vector and the diseases it transmits (de la Fuente et al., 1998; 2006; 2007).

Histamine-releasing factor (HRF) is a cytokine produced by many cell types, in many animal species including humans where it causes IgE-mediated histamine release from basophils. In ticks, a histamine release factor (HRF) has been identified in several species and shown to be present in a variety of tissues including salivary glands, mid-gut, ovary and haemocytes and in all developmental stages and is shown to have potential as a vaccine using rat basophils (Mulenga et al., 2003a, b; Mulenga and Azad 2005). In *D. gallinae*, an orthologue of the tick HRF protein has been characterized and the recombinant form was shown to have potential for use as a vaccine against poultry mite infestation in hens (Bartley et al., 2009).

Cathepsin D and other aspartic-like proteinase enzymes have been demonstrated in extracts of *Psoroptes ovis* and *Psoroptes cuniculi* mites and associated with digestive role. In *D. gallinae*, an aspatyl protease (Dg-CatD) and a cysteine protease (Dg-CatL) have been isolated and shown to exhibit similarity with a tick lysosomal cathepsin D- like protease and also cathepsin-like proteins from various parasitic invertebrates. These proteins are associated with blood digestion in the parasitic invertebrates and have demonstrated high potential as vaccine candidates (Bartley et al., 2012). Cathepsin B and L secreted by *Fasciola hepatica* in all life cycle stages is shown to play roles in migration, feeding survival and virulence of the parasite and are proposed targets for vaccination (Jayaraj et al., 2010).

RNA intereference (RNAi) gene silencing technique is becoming a powerful tool for investigating the functional role of specific genes that may have potential as vaccines or chemotherapeutic targets. The mechanism of RNAi involves *in vivo* enzymatic production of small interfering RNA molecules (siRNAs) from larger introduced dsRNA. The siRNA destroy target mRNA, thereby silencing the target gene at post-transcriptional stage. RNAi has been used to investigate therapeutic intervention targets in several invertebrates including gut proteins in tsetse flies (Walshe et al., 2009), moulting genes in *Tribolium castaneum* (Minakuchi et al., 2009) growth genes in shrimps (Hui et al., 2008), aquaporins in aphids (Shakesby et al., 2009) and in ticks and mosquitoes.

Different methods of introducing dsRNA have been tested in different species including intra-haemocoelic injection in arthropods, immersion in solutions containing dsRNA employed for nematodes, planarians and mosquito larvae. The results of gene knockdown are varied between individual genes and even different species of animals. RNAi has been applied successfully in a range of genes in *Caenorhabditis elegans* nematodes to study gene function but in parasitic nematodes it was less effective. In the sheep nematode, *Haemonchus contortus,* some genes seem to be more susceptible to RNAi than other genes. Silencing was achieved in *H. Contortus* for a candidate vaccine designated as HII, through soaking L3 infective larvae in dsRNA for 24 h and larvae remained viable after this treatment. Silencing HII gene reduced faecal egg counts (FEC) and worm burden in sheep infected with HII dsRNA treated larvae compared to controls, by at least 50% (Samarasinghe et al., 2010).

So far there are no RNAi studies reported in poultry mites and therefore this study aimed at investigating RNAi gene knockdown by immersion as a method of introducing dsRNA into the mites targeting the orthologues of histamine releasing factor (HRF) and Cathepsin D proteins which have shown potential for use as vaccine candidates. The small size of the mite prevented micro-injection of dsRNA.

MATERIALS AND METHODS

Mites

D. gallinae mites were collected from commercial egg production unit and stored in 75 cm^2 culture flask (Corning), with a vented cap. They were conditioned overnight at room temperature (~21±2°C) at the parasitology division of Morem Research Institute before being sent to the School of Biological Sciences at Aberdeen University, in Scotland for use in the experiments.

To evaluate the minimum number of mites for analysis, total RNA was extracted separately, from a single mite, two mites, and six mites using RNA isolation kit (Zymo Research). The RNA was dissolved in 10 µl of sterile nuclease-free water (Sigma) and DNase treated using 1 µl (1 U) of RQ1 DNase, was quantified (Nanodrop Spectrophotometer), used in a 25 µl reaction to make 1^{st} strand cDNA using Oligo dT primer (Promega, Southampton, UK) and 4 µl of the cDNA was then used for PCR using target genes (HRF and C) and also 'house' keeping gene (Actin) primers.

Table 1. Total RNA extracted from varying number of mites.

Number of mite	Concentration of RNA (ng/µl	Total RNA (ng)
1	14.6	146
1	16.5	165
2	29.9	299
6	47.4	474

Generation of dsRNA from *D. gallinae* target genes

Generation of dsRNA from target genes was done in three steps: 1) total RNA extraction; 2) RT-PCR to generate cDNA and 3) generation of sense and anti-sense single stranded RNA strands and annealing to form dsRNA.

Total RNA was extracted from adult mites using mini RNA isolation kit (Zymo Research), quantified (Nanodrop spectrophometer) and DNase (Promega) treated as above, prior to using it to make cDNA using 0.5 µg of oligo d(T) 15 (Promega, Southampton, UK) using Bioscript-reverse transcriptase system (Bioline) by incubation at 42°C for 60 min and the reaction was stopped by heating to 70°C for 5 min according to the manufacturer's instructions.

The cDNA from the mites was used as template for PCR with specific primers derived from histamine releasing factor (HRF), cathepsin D and Actin as a house- keeping gene. Primers to amplify *D. gallinae* HRF were based on HRF sequence Genbank Acc. No FM179713 (HRF forwards 5-'ggcaggctcaaaccctgctgaggg-3'and reverse 5'-gcgtagtttaggatagcgacaca-3') and cathepsin D-1 Genbank Acc. HE565350, (forwards 5'-cgtccaagtgtcccagctctaac-3' and reverse 5'-gcccatgccaagaataccgtcat-3') to amplify fragments of 322 bp and 254 bp respectively. The PCR reactions to amplify Cathepsin D fragments consisted of 1 µl of cDNA template (containing 10-50 µg cDNA), 5 µl of 10X reaction buffer, 2 µl of 50 mM $MgCl_2$, 1 µl of dNTPs (25 mM each), 1 µl primer (10 mM each), 0.5 µl (1.25 U) of Taq Polymerase (Bioline, London, UK) and DEPC –treated water to give a 50 µl total volume. PCR cycling conditions were 1 cycle of 5 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 50°C, 30 s at 72°C and final extension at 72°C for 7 min. PCR reactions for HRF and actin amplification used 50 ng template DNA in 50 µl reaction and cycling conditions; 94°C for 2 min, followed by 32 cycles of 94°C 30 s, 60°C 30 s, 72°C 1 min, and 72°C for 7 min final extension. At the end of the PCR, 8 µl of each product was analyzed on a 1.5% agarose gel and stained with ethidium bromide (1.0 µl of 10 mg/ml stock solution for a 50 ml minigel).

The remaining PCR products from the two genes were used to set up T7 TOPO^R linking reactions (BLOCK iT RNAi TOPO^R transcription Kit, Invitrogen) before being used in separate secondary amplification reactions of sense and antisense DNA which was conducted using either gene specific reverse or forward primer with the BLOCK iT^{TM} T7 primer. The sense and anti-sense DNA strands, including the T7 TOPO^R linker, were used in reverse transcriptase reactions to generate single stranded sense RNA (from dsDNA with 5' T7) and antisense RNA strands (from dsDNA with 3' T7). Finally, equal amounts of sense and anti-sense ssRNA for each target were combined and incubated in boiling water and the water was allowed to cool at room temperature for 1½ h. This allowed annealing to make double stranded RNA for each fragment. At the end of the transcription, 5 µ aliquots of sense and anti-sense as well as dsRNA were analysed for quality on a 1.2% agarose gel, stained with ethidium bromide and visualized under UV transilluminator. A control lacZ plasmid provided in the TOPO^R BLOCK iT Kit was PCR amplified alongside the target genes and dsRNA generated from the plasmid was used in control experiments.

RNAi experiments and validation of knockdown in HRF treated mites

An average of three individual mites clean from poultry house detritus were put in separate sterile Eppendorf tubes and 5 to 10 µl of double stranded RNA (dsRNA) from target genes C or HRF or control LacZ was added. The tubes were tapped gently to ensure mites were in contact with the liquid. The tubes were spun briefly in microfuge to ensure mites were completely immersed or well washed in the dsRNA solution. The tubes were wrapped with kitchen foil to exclude light and then kept in the incubator at 25±2°C. The experiment was set up in 3 replicates for each gene fragment and control.

The tubes were incubated for 7, 16 and 24 h. At the end of each incubation period, the tubes were removed from the incubator and 20 µl of medium M199 (supplemented with 20 mM MOPS and 50 mg/ml ampicillin) was added to each tube before storing the mites at -80°C until the time for total RNA extraction. Total RNA was extracted from the treated mites using Mini RNA Kit (Zymo Research) according to the manufacturers' instructions, was quantified, DNase treated and used to generate cDNA by reverse transcriptase PCR using Oligo dT primer and incubation at 42°C for 1 h, and a final extension at 70°C for 5 min according to the manufacturer's instructions. The cDNA samples were used in quantitative PCR using Cathepsin D, Histamine Releasing Factor and actin (for normalisation). Products were analysed by agarose gel electrophoresis and visualized with ethidium bromide staining. The relative quantity of PCR product was estimated using densitometer.

RESULTS AND DISCUSSION

Evaluation of minimum number of mites for use in treatments and PCR analysis

Table 1 shows representative amounts of total RNA isolated from single, two mites and six mites. It was shown that PCR amplified the target genes from only a fraction of cDNA obtained from a single mite (calculated to be approximately 1/8 of a mite). Figure 1 shows the sample PCR using cDNA generated from single mite and 2 mites for HRF target gene.

It was shown that the relative expression of HRF gene in dsRNA treated mites was reduced compared to the control mites (reduced intensities and size of bands shown in Figure 2A). When the gels were subjected to densitometer analysis to estimate relative amounts of

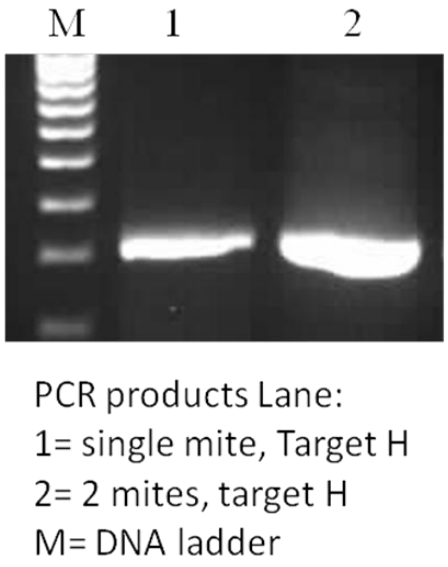

Figure 1. Evaluation of minimum number of mites for the use in PCR. Total RNA was extracted from single mite and two mites (Lane 1 and 2 respectively), DNase treated and used in a 25 µl 1st strand cDNA synthesis reaction. 4 µl of the cDNA was then used for PCR using primers derived from HRF (Genbank Acc. No FM179713). 8 µl of the PCR product was loaded in a 1.6% agarose gel, stained with eithidium bromide and visualized under UV transilluminator.

DNA in each band, and compared with lacZ control, 2/3 of HRF treated mites had HRF: actin ratio well below the range obtained for LacZ control treated mites (Figure 2B). All 3 HRF treated tubes had Cathepsin:actin ratio similar to lacZ treated controls (Figure 2C). This shows that the expression levels of the non-target genes actin and cathepsin in this experiment were relatively similar and that the reduction in HRF amplification was possibly due to knockdown in expression of the targeted gene. HRF dsRNA immersions for 7 h did not yield detectable knockdown results (data not shown), possibly due to short exposure time. Validation of knockdown for Cathepsin D after 16 and 24 h did not yield significant results (data not shown).

The present study demonstrates the successful gene knockdown for HRF in *D. gallinae* by immersing the mites in a small volume of dsRNA solution in water. The knockdown was apparent at the end of 16 h immersion period. Though it was not possible to analyse post treatment effects, the present studies have demonstrated that it is possible to induce RNAi gene knockdown in poultry mites. Application of dsRNAi in *D. gallinae* would allow investigators to determine the effects of knockdown of certain genes on survival, reproduction and infectivity of mites. This could be used to study functions of certain genes or even characterize genes associated with specific functions which could be targeted for vaccines or even drugs treatment. Specific genes associated with host inflammation could be knocked down to reduce scaling pathology of infected chicken.

In the present studies, immersion of mites in dsRNA solution was used to demonstrate gene knockdown for HRF target. The small size of the mite (0.75 to 1.5 mm) (Nisbet and Billingsley, 2000) prevents micro-injection of dsRNA from being practical. A similar method has been applied in *D. gallinae* for introduction of Salmonella infection via cuticular contact and also through blood meal. In the present study, there was a possibility that the mites fed on dsRNA for sometime after they were immersed in the solution or the RNA entered the mites via the cuticle. Further studies could be done possibly by utilization of *in-vitro* feeding device (Bruneau et al., 2001; Wright et al., 2009;).

Attempts in gene knockdown of cathepsin D target did not yield significant results. This is not surprising since RNAi is reported to produce different silencing effects in different genes even within the same organism. Based on PCR amplification efficacies, we speculate that Cathepsin D in *D. gallinae* may be a high copy gene and possibly the degree of knockdown was not detectable using the current experimental protocol. Further studies are required in future to validate these results.

Conclusions

The study demonstrates the dsRNA knockdown in economically and medically important *D. gallinae*, using Histamine releasing factor (HRF) as target gene after 16 h immersion of mites in double stranded RNA (dsRNA) solution. Knockdown was specific for the target (HRF) whereas the non-target (Cathepsin D) was not affected by HRF dsRNA treatment. These results suggest that it is possible to induce RNAi gene knockdown in poultry mites just like in many other arthropods that have been tested to date. An efficient method of introducing dsRNA into the mite needs to be developed since immersions conducted in this study did not allow the mites to live after treatment. Hence, it was not possible to evaluate knockdown effects on mites post-treatment. This is the first time dsRNAi has been demonstrated in *D. gallinae*. Further work on other relevant genes needs to be done to verify these results.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the Commonwealth Scholarship Commission in the United Kingdom who awarded L. Kamau post-doctoral fellowship to carry out the work at the University of Aberdeen, In Scotland, UK. We are grateful to Moredun Research Institute for providing the mites.

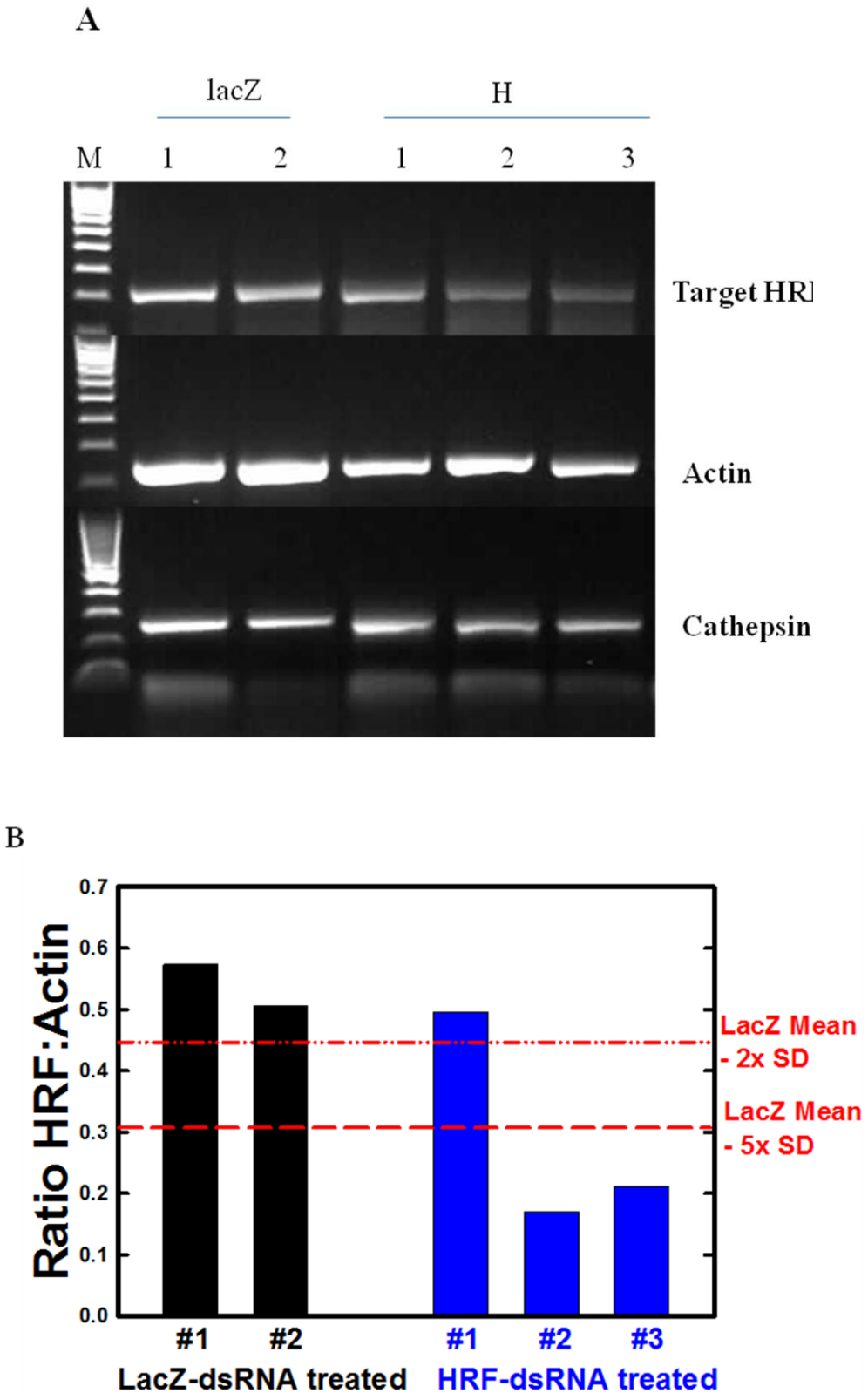

Figure 2. A and B. Validation of knockdown by PCR and relative densities of PCR products in dsRNA treated mites compared to controls. **A.** PCR showing relative expression of target gene histamine releasing factor (HRF) in dsRNA treated mites (H lanes 1, 2, and 3) relative to mites treated with dsRNA derived from LacZ control (LacZ lanes 1 and 2). **B.** Ratio of HRF: actin amplification in HRF treated mites compared to lacZ controls.

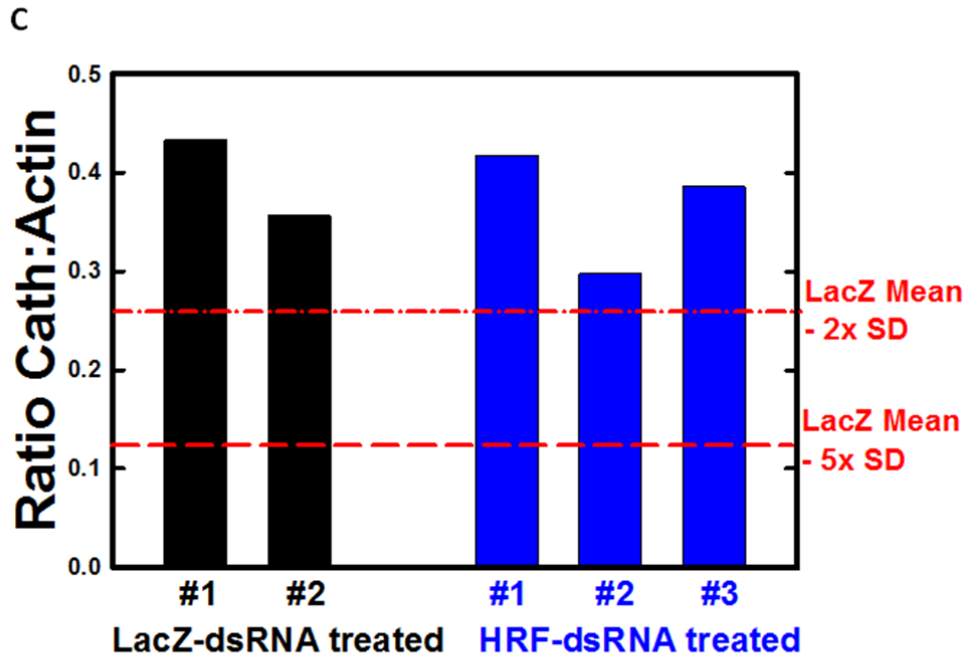

Figure 2. C. Validation of knockdown by PCR and relative densities of PCR products in dsRNA treated mites compared to controls. **C.** Ratio of Cathepsin: Actin in HRF treated mites. On average 3 to 5 mites were immersed in 10 to 12 µl of dsRNA solution (containing 1 mg dsRNA) and incubated for 16 hours at 25+2°C in the dark. After incubation, RNA was extracted from mites, DNase treated and used for PCR using HRF, Actin and cathepsin specific primers. Both Actin and cathepsin (non-treatment genes) were used to normalize the gel.

REFERENCES

Afrim H, Kurtesh S, Skender M, Behlul B, Fatgzim L, Avni R, Rezart P, Claudia H, Michael H, Olivier S (2011). Dermanyssus gallinae in layer farms in Kosovo: a high risk for salmonella prevalence. Parasit. Vectors 4:136.

Bartley K, Huntley JF, Wright HW, Nath M, Nisbet AJ (2012). Assessment of cathepsin D and L-like proteinases of poultry red mite, Dermanyssus gallinae (De Geer), as potential vaccine antigens. Parasitology 139(6):755-765.

Bartley K, Nisbet AJ, Offer JE, Sparks NH, Wright HW, Huntley JF (2009). Histamine Release Factor from Dermanysus gallinae (De Geer): Characterization and in vitro assessment as a protective antigen. Int. J. Parasitol. 39:447-456.

Beugnet F, Chauve C, Gauthey M, Beert L (1997). Resistance of the red poultry mite to pyrethroids in France. Vet. Rec. 140:577-579.

Bruneau A, Dernburg A, Chauve C, Zenner L (2001). First in vitro cycle of the chicken mite, Dermanyssus gallinae (DeGeer 1778), utilizing an artificial feeding device. Parasitology 123(Pt 6):583-589.

Claude Chauve (1998). The poultry red mite Dermanysus gallinae (De Geer, 1778): current situation and future prospects for control. Vet. Parasitol. 97:239-245.

de la Fuente J, Almazan C, Blouin EF, Naranjo V, Kocan KM (2006). Reduction of tick infestations with Anaplasma marginale and A. Phagocytophilum by targeting the tick protective antigen subolesin. Parasitol. Res. 100:85-91.

de la Fuente J, Kocan KM, Almazan C, Blouin EF (2007). RNA interference for the study and genetic manipulation of ticks. Trends Parasitol. 23:427-433.

de la Fuente J, Rodriguez M, Redondo M, Montero C, Garcia-Garcia JC, Mendez L, Serrano E, Valdes M, Enriquez A, Canales M, Ramos E, Boue O, Machado H, Lleonart R, de Armas C.A, Rey S, Rodriquez JL, Artiles M, Garcia L (1998). Field studies and cost effectiveness analysis of vaccination with Gavac against cattle tick *Boophilus microplus*. Vaccine 16:366-373.

Fiddes MD, Le Gresley S, Parsons DG, Epe C, Coles GC, Stafford KA (2005). Prevalence of the poultry red mite Dermanysus gallinae in England. Vet. Rec. 157:233-235.

Höglund J, Nordenfors H, Uggla A (1995). Prevalence of the poultry red mite, Dermanysus gallinae, in different types of production systems for egg layers in Sweden. Poult. Sci. 74:1793-1798.

Hui JHL, Tobe SS, Chan SM (2008). Characterization of putative farnesoic acid O- methyltransferase (LvFAMeT) cDNA from white shrimp, *Litopenaeus vannamei*: Evidence for its role in molting. Peptides 29:252-260.

Jayaraj R, Piedrafita D, Dynon K, Grams R, Spithill TW Smooker PM (2010). Vaccination of rats against fascioliasis by a multivalent vaccine of stage-specific cathepsin proteases induces significant protection. IProceedings of British Society for Parasitology Spring Meeting and Leishmaniasis seminar, Cardiff University Wales, UK. March 29th – April 1st 2010.

Lesna I, Wolfs P, Faraji F, Roy L, Komdeur J, Sabelis MW (2009). Candidate predators for biological control of the poultry red mite Dermanyssus gallinae. Exp. Appl. Acarol. 48(1-2):63-80.

Maurer V, Baumgartner J (1992). Temperature influence of life table statistics of the chicken mite Dermanysus gallinae (Acari: Dermanyssidae). Exp. Appl. Acarol. 15:27-40.

Maurer V, Baumgartner J (1994). A population model for Dermanysus gallinae (Acari: Dermanyssidae). Exp. Applic. Acarol. 18:409-422.

Maurer V, Baumgartner J, Bieri M, Folsch DW (1993). The occurrence of the chicken mite Dermanysus gallinae (Acari: Dermanyssidae) in Swiss poultry houses. Mitteilungen der schweizerischen Entomologischen Gelleschaft 66:87-97.

Minakuchi C, Namiki T, Shinoda T (2009). Kruppel homolog 1, an early juvenile hormone-response gene downstream of Methoprene-tolerant, mediates its anti-metamorphic action in red flour beetle

Tribolium castaneum. Dev. Biol. 325:341-350.
Moro CV, De Luna, CJ, Tod Alexander, Guy JH, Sparagano OAE, Zenner L (2009). The poultry red mite (Dermanyssus gallinae): a potential vector of pathogenic agents. Exp. Appli. Acarol. 48:93-104.
Mulenga A, Azad AF (2005). The molecular and biological analysis of ixodid ticks histamine release factors. Exp. Appl. Acarol. 37:215-229.
Mulenga A, Macaluso KR, Simser JA, Azad AF (2003a). Dynamics of Rickettsia-tick interactions: identification and characterization of differentially expressed mRNAs in uninfected and infected *Dermacenter variabilis*. Insect Mol. Biol. 12:185-193.
Mulenga A, Macaluso KR, Simser JA, Azad AF (2003b). The American dog tick, *Dermacentor variabilis*, encodes a functional histamine release factor homolog. Insect Biochem. Mol. Biol. 33:911-919.
Nisbet AJ, Billingsley PF (2000). A comparative survey of the hydrolytic enzymes of ectoparasitic and free-living mites. Int. J. Parasitol. 30(1):19-27.
Samarasinghe B, Knox D, Britton C (2010). In vitro and in vivo RNAi of Haemonchus contortus H11. Proceedings of British Society for Parasitology Spring Meeting and Leishmaniasis seminar, Cardiff University Wales, UK. March 29th – April 1st 2010.
Shakesby AJ, Wallace IS, Issacs HV, Pritchard J, Roberts DM, Douglas AE (2009). A water-specific aquaporin involved in aphid osmoregulation. Insect Biochem. Mol. Biol. 39:1-10.
Van Emous RA, Fiks-Van Niekerk TGCM, Mul MF (2006). € 11 million damage for the sector: enquiry into the cost of mites to the poultry industry. De pluimveehouderij 35:8-9.
Walshe DP, Lehane SM, Lehane MJ, Haines LR (2009). Prolonged gene knockdown in the tsetse Glossina by feeding double stranded RNA. Insect Mol. Biol. 18:11-19.
Wright HW, Bartley K, Nisbet AJ, McDevitt RM, Sparks NH, Brocklehurst S, Huntley JF (2009). The testing of antibodies raised against poultry red mite antigens in an *in vitro* feeding assay; preliminary screen for vaccine candidates. Exp. Appl. Acarol. 48(1-2):81-91.