



Histamine Release Factor from *Dermanyssus gallinae* (De Geer): Characterization and in vitro assessment as a protective antigen [☆]

Kathryn Bartley ^{a,*}, Alasdair J. Nisbet ^a, Jill E. Offer ^b, Nicholas H.C. Sparks ^b, Harry W. Wright ^a, John F. Huntley ^a

^a Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ, Scotland, UK

^b Avian Science Research Centre, Animal Health Group, SAC Ayr, Auchincruive KA6 5HW, Scotland, UK

ARTICLE INFO

Article history:

Received 22 July 2008

Received in revised form 11 September 2008

Accepted 15 September 2008

Keywords:

Dermanyssus gallinae

Histamine-releasing factor

TCTP

Vaccine antigen

ABSTRACT

A cDNA encoding a 174-amino-acid orthologue of a tick histamine release factor (HRF) was identified from the haematophagous poultry red mite *Dermanyssus gallinae*. The predicted *D. gallinae* HRF protein (Dg-HRF-1) sequence is highly conserved with the tick HRFs (identity 52–54%) and to a lesser degree with translationally controlled tumour proteins (TCTP) from mammals and other invertebrates (range 38–47%). Phylogenetically, Dg-HRF-1 partitions with the tick HRF clade suggesting a shared lineage and potentially similar function(s). A recombinant Dg-HRF-1 protein (rDg-HRF-1) was produced and shown to induce degranulation of rat peritoneal mast cells in vitro, confirming conservation of the histamine-releasing function in *D. gallinae*. Polyclonal antibodies were generated in rabbits and hens to rDg-HRF-1. Western blotting demonstrated that native Dg-HRF is a soluble protein and immunohistochemical staining of mite sections revealed that the distribution of Dg-HRF, although ubiquitous, is more common in mite reproductive, digestive and synganglion tissues. A survey of hens housed continuously in a mite-infested commercial poultry unit failed to identify IgY specific for recombinant or native Dg-HRF, indicating that Dg-HRF is not exposed to the host during infestation/feeding and may therefore have potential as a vaccine using the concealed antigen approach. To test the protective capability of rDg-HRF-1, fresh heparinised chicken blood was enriched with yolk-derived anti-Dg-HRF IgY antibodies and fed to semi-starved mites using an in vitro feeding system. A statistically significant increase in mortality was shown ($P = 0.004$) in mites fed with anti-Dg-HRF IgY after just one blood meal. The work presented here demonstrates, to our knowledge for the first time, the feasibility of vaccinating hens with recombinant *D. gallinae* antigens to control mite infestation and the potential of rDg-HRF-1 as a vaccine antigen.

© 2008 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The blood-feeding poultry red mite *Dermanyssus gallinae* is the most economically important and widespread ectoparasite affecting commercial egg farming in Europe and causes considerable economic loss as a result of reduced production and mite control costs. The mite resides “off-host” in the cracks and crevices of the poultry house, becoming active and feeding at night. *Dermanyssus gallinae* is found in all types of egg production systems, but is present in greater numbers in free-range and barn systems than in battery units (Hoglund et al., 1995; Fiddes et al., 2005). Under ideal conditions *D. gallinae* can complete its life cycle (egg to egg) within 7 days and the mite population can double within

1 week (Maurer and Baumgartner, 1992). Mite numbers can reach in excess of 150,000 per hen, resulting in serious health and welfare issues for laying hens and potentially for poultry workers (Auger et al., 1979; Chauve, 1998; Kilpinen et al., 2005). In addition, *D. gallinae* has been implicated as a vector for several commercially important diseases such as Erysipelas and Salmonella (Chirico et al., 2003; Moro et al., 2005, 2007a,b). Chemical acaricide spraying is the current method generally employed for control; however the legislated withdrawal of toxic acaricides and the development of acaricide resistance (Beugnet et al., 1997; Nordenfors et al., 2001; Fiddes et al., 2005; Thind and Ford, 2007) has made alternative mite control strategies such as vaccination an attractive prospect.

Studies with Ixodid ticks have demonstrated the feasibility of using vaccination with “exposed” salivary antigens to reduce infestation (e.g. Iris protein; Prevot et al., 2007), however a protective natural immunity in hens based on exposed antigens of *D. gallinae* appears to be either absent or ineffective. “Concealed” antigens of

[☆] Note: Nucleotide sequence data reported in this paper are available in the EMBL, GenBank and DDJB databases under the Accession No. FM179713.

* Corresponding author. Tel.: +44 0 131 445 5111; fax: +44 0 131 445 6235.

E-mail address: Kathryn.bartley@moredun.ac.uk (K. Bartley).

ectoparasites have shown most promise as exemplified by the commercially available TickGARD plus™/Gavac™ vaccines which are based on the concealed Bm86 midgut antigen from the cattle-tick *Rhipicephalus (Boophilus) microplus*. The TickGARD™ vaccine operates by generating antibodies to Bm86, which are ingested with a blood meal and bind to the antigen in tick gut epithelia inducing damage (Willadsen et al., 1989). An additional benefit of vaccination is the control of vector-borne diseases, such as Babesiosis and Anaplasmosis, by eliminating the tick vector (de la Fuente et al., 1998) and in some cases impairing the ability of ticks to become infected, thus reducing transmission (de la Fuente et al., 2006).

A histamine release factor (HRF), able to induce histamine release in rat basophils, was identified in several species of ticks (Mulenga et al., 2003a,b; Mulenga and Azad, 2005). The tick HRF protein is expressed in a variety of tissues including salivary gland, mid-gut, ovary and haemocytes and in all developmental stages, making it an ideal tick vaccine antigen candidate (Mulenga and Azad, 2005). Although the function of HRF in ticks is unclear, a related protein found in mammals, nematodes and other invertebrates (translationally-controlled tumour protein, TCTP; synonymous with HRF) has several described functions including: microtubule binding, cell growth and cell cycle progression, inhibition of apoptosis, histamine release and other cytokine-like activities; suggesting diverse regulatory functions (reviewed in Bommer and Thiele, 2004). The anti-malarial drug artemisinin is effective against *Plasmodium* and Schistosome parasites, and although the mechanism of the drug has yet to be elucidated, it has been shown that the parasites' HRF/TCTP proteins directly interact with artemisinin (Bhisutthibhan et al., 1998). A study by Walker et al. (2000) demonstrated that increased expression of *Plasmodium* HRF/TCTP is associated with a decreased susceptibility to artemisinin. This has led to the proposal that HRF/TCTP is a valid target for drug or vaccine development for control of, or therapy for, these parasite species (Rao et al., 2002).

The aims of the work presented here were to characterize an orthologue of the tick HRF protein in *D. gallinae* and determine its potential as a vaccine antigen. We believe the work presented here is the first report demonstrating the potential of vaccination with recombinant *D. gallinae* antigens as a valid mite control strategy.

2. Materials and methods

2.1. Parasite material and eggs from infested hens

Dermanyssus gallinae mites and hen eggs were collected from a large mite-infested commercial battery unit which had been populated with the same Lohmann's Brown hens for 40 weeks. Large numbers (>5000) of mixed stage and gender mites were collected by scraping aggregated mites from the poultry cages and transferring into vented tissue culture flasks. The percentage of the mites held in the flask that had recently fed was immediately estimated by their appearance under light microscopy. Mites were only utilized if approximately 60% or more of the flask population had recently fed.

2.2. Cloning and sequencing of the *D. gallinae* HRF gene

Mites, free from poultry house detritus, were snap-frozen in liquid nitrogen and total RNA was immediately purified by homogenising mites in TRIzol® Reagent (Invitrogen) and enriched for mRNA using a poly(A)Purist™ Kit (Ambion) following the manufacturer's instructions. A cDNA expression library was constructed from 1 µg mRNA using the SMART™ cDNA Library Construction

Kit (Clontech), employing the primer extension method. DNA sequencing of randomly selected clones resulted in the identification of a truncated cDNA encoding an orthologue of the tick HRF.

The complete coding sequence of the *D. gallinae* HRF gene (*Dg-hrf-1*) was obtained by rapid amplification of cDNA ends (RACE) using RACE-ready cDNAs generated with the SMART™ RACE cDNA Amplification Kit (Clontech) and the gene-specific oligonucleotide primers HRF-1F (5'-GAGGGCGAGGTGTCTTGGCAGGC-3') and HRF-1R (5'-GCCATGCACTGGTTCTTGAGTTCGG-3') following the manufacturer's protocol. RACE PCR products were ligated into a pGEM T-Easy plasmid vector (Promega) and transformed into competent *Escherichia coli* cells (strain JM109, Promega). Individual positive clones were picked, based on blue/white selection, and grown overnight at 37 °C in Luria Bertani (LB) medium containing 100 µg ml⁻¹ of ampicillin. Plasmid DNA was purified using the QIAspin plasmid miniprep kit (Qiagen). The DNA sequence was obtained using automated sequencing with HRF-1F and HRF-1R primers (Moredun Functional Genomics Unit). The *Dg-hrf-1* DNA sequence was deposited in EMBL under Accession No. FM179713.

2.3. Phylogenetic analysis

The predicted amino acid sequence of the full-length *Dg*-HRF-1 sequence was aligned with putative orthologues from other species of invertebrate and vertebrate organisms using the Clustal X algorithm (Jeanmougin et al., 1998) and a neighbour-joining tree drawn according to relationships inferred by the multiple sequence alignment. The neighbour joining tree was bootstrapped 1000 times using Clustal X (Jeanmougin et al., 1998) and the resulting tree viewed with TreeView (Page, 1996).

2.4. Expression of recombinant *Dg*-HRF-1 protein

The complete *Dg*-HRF-1 coding region was amplified by PCR with the oligonucleotide primers HRF-for (5'-AATTCGGATCCG **ATG**CTCATCTACAGGGACATC-3') and HRF-rev (5'-CCGAAGCTTGA CCTTCTCTCCGCAAC-3') that contain the ATG start codon (bold) and the restriction sites HindIII and BamHI (italicised) which allowed ligation into the cloning site of the pET22b(+) expression plasmid (Novagen) in the correct direction and in-frame with the 5'-*pelB* leader sequence and 3'-polyhistidine tag flanking the cloning site. PCR was performed using the Advantage® 2 PCR Kit (Clontech) with the cycling conditions: 25 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 90 s, followed by 1 cycle of 72 °C/5 min. The 544-bp PCR product was digested with HindIII and BamHI (Roche), purified using the QIAprep PCR cleanup kit (Qiagen) and inserted into the pET22b(+) vector using the Rapid Ligation kit (Promega).

Expression was carried out in BL21-CodonPlus®(DE3)-RIPL *E. coli* cells (Stratagene). Following induction with 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG), the recombinant *Dg*-HRF-1 (rDg-HRF-1) protein was expressed at 30 °C for 3 h. Insoluble rDg-HRF-1 protein was recovered from cell lysates prepared from inclusion body fractions. rDg-HRF-1 was purified by binding to His-Trap™ HP columns (GE healthcare) using 8 M urea reducing conditions and eluted with increasing imidazole concentrations, according to the manufacturer's instructions. Solubilized rDg-HRF-1 was refolded by dialysis against PBS with a decreasing gradient of urea.

2.5. Fractionation of *D. gallinae* proteins

Mites were successively homogenized in PBS, 0.1% Tween 20/PBS, 1% Triton X-100/PBS and 8 M urea in order to obtain protein fractions comprised of soluble, membrane-associated, integral-membrane and insoluble *D. gallinae* proteins, following a method described previously (Smith et al., 2002).

2.6. Generation of immunoglobulins specific for Dg-HRF-1

Two New Zealand White rabbits and two Lohmann Brown hens were each injected with 75 µg rDg-HRF-1 protein in PBS plus 200 µg QuilA adjuvant. The animals were injected again 2 and 4 weeks later with the same rDg-HRF-1/adjuvant concentrations. Blood was collected from rabbits and eggs from the hens prior to injection (negative control antibodies) and again at 2 weeks after the third injection. Serum was prepared from rabbit blood and IgY purified from the egg yolks using the Eggstract kit (Promega) according to manufacturer's instructions. Care of animals and experimental procedures were carried out with institute ethics committee approval in accordance with United Kingdom Animals (Scientific Procedures) Act, 1986.

2.7. PAGE and Western blotting

Proteins were electrophoresed on NuPAGE® Novex Bis–Tris Gels 4–12% minigels (Invitrogen) under reducing conditions using the NuPAGE® reducing agent and 2-(N-Morpholino)ethanesulphonic acid (MES)–SDS running buffer according to the manufacturer's guidelines. Protein bands were visualized by staining with SimplyBlue™ SafeStain (Invitrogen).

Proteins were transferred from unstained gels to nitrocellulose membranes using the iBlot™ Gel Transfer Device (Invitrogen) according to the manufacturer's instructions. Blots were washed twice in PBS–Tween 20 (PBS, 0.05% Tween 20 (Sigma); PBST) before incubating in blocking buffer (5% (w/v) Marvel skimmed milk powder in PBST) for 30 min to block non-specific binding sites. Blots were washed and then incubated in rabbit or chicken antibodies (1:100 in blocking buffer) for 1 h at room temperature. Blots were washed and specifically-bound antibody detected with anti-rabbit-horse radish peroxidase (HRP) (DAKO) or anti-IgY-HRP (Sigma) conjugate antibody (1:2000, 1:30,000 in blocking buffer, respectively) and visualized with SIGMAFAST™ 3,3'-Diaminobenzidine (DAB; Sigma).

2.8. Immunohistochemical staining of *D. gallinae* sections

Freshly collected mites were immersed in Carnoy's fixative (60% ethanol, 30% chloroform, 10% acetic acid) and stored at 4 °C until required. Fixed mites were immobilised in 1% molten agarose and, once solidified, set in paraffin wax. Five-micrometre sections were cut and dried onto glass slides at 37 °C for 16 h. The sections were dewaxed in xylene, rehydrated and washed twice for 5 min each with Tris-buffered saline (25 mM Tris, 137 mM NaCl, 2.7 mM KCl; TBS). Endogenous peroxidase was inactivated by immersing slides in 1% hydrogen peroxide in methanol for 30 min before sections were blocked with normal goat serum (1:4 in TBS). Consecutive sections were labeled with the primary antibodies: rabbit anti-Dg-HRF serum and rabbit negative control serum (1:100 in TBS). Unbound antibody was removed by washing three times in TBS for 5 min each. Specifically bound antibody was detected with the secondary anti-rabbit-HRP conjugate antibody (1:200 in TBS), visualized with SIGMAFAST™ DAB and counterstained with haematoxylin for 1 min. Sections were washed in water, dehydrated and mounted in DPX (Sigma). Consecutive control sections were performed in parallel to assess levels of non-specific binding of the secondary antibody (conjugate control) and residual endogenous peroxidase activity (DAB control). Control sections were performed following an identical procedure except that the primary antibody was substituted for PBS (conjugate control), and both the primary and secondary antibodies were substituted for PBS (DAB control).

2.9. In vitro feeding assay

Mites were conditioned in vented tissue culture flasks for use in the in vitro feeding assay by maintaining them at room temperature (22 ± 3 °C) for 7 days followed by 21 days at 4 ± 2 °C. Pre-conditioned mites were fed fresh chicken blood enriched with antibodies using the in vitro feeding device described by McDevitt et al. (2006). One group of mites received blood supplemented with 36 USP-units of heparin sodium salt (Sigma) ml⁻¹ blood and 3.5 mg ml⁻¹ purified chicken anti-Dg-HRF IgY. The control group was fed in parallel and received the same heparinised blood but enriched with 3.5 mg ml⁻¹ purified negative control chicken IgY. Fed mites were isolated and observed daily for 5 days. The experiment was repeated three times and the mean mortality rates of the two groups compared. Statistical analysis of mite mortality 5 days after feeding was performed using a generalized linear mixed model. "Group" (positive or negative) was fitted in the model as a fixed effect and "experiment" as a random effect. The analysis was carried out using Genstat 10th edition and a *P* value <0.05 was deemed statistically significant.

2.10. Biological activity of rDg-HRF-1

Rat peritoneal cells (RPCs) were obtained by peritoneal lavage. Briefly, four Sprague–Dawley rats were euthanized and the peritoneal cavity washed with 20 ml wash medium (Hanks balanced salt solution (Sigma), 2% FBS, 100 unit ml⁻¹ penicillin and 100 mg ml⁻¹ streptomycin (Northumbria Biologicals), 20 USP units ml⁻¹ heparin sodium salt (Sigma)). Cells were recovered by centrifuging at 200g for 5 min, pooled and washed once in wash medium. Cells were suspended in Earle's medium (Earle's balance salts (without phenol red and sodium bicarbonate, supplemented with 1.8 mM CaCl₂, 0.8 mM MgSO₄ and 5.5 mM D-Glucose), 2% FBS) to a final concentration of 1×10^7 cells ml⁻¹. Approximately 5% of the RPC population were rat peritoneal mast cells (RPMCs) as determined by morphological examination of cytosmeas stained with Leishman's stain.

RPMC degranulation in response to stimulation with rDg-HRF-1 and compound 48/80 (Sigma) was estimated by measuring the activity of the released granule-associated acid hydrolase, β-hexosaminidase using a method described by Schwartz et al. (1979). Briefly, 3×10^6 RPCs were stimulated with equal volumes of rDg-HRF-1, compound 48/80 (positive control), heat-denatured rDg-HRF-1 (bacterial endotoxin control, heat denatured for 20 min prior to use) or media (negative control) and the intracellular and released β-hexosaminidase quantified by the colourmetric change (OD_{405nm}) of the substrate *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Sigma). The assay concentrations of rDg-HRF-1 and compound 48/80 were set at 0.5 µg ml⁻¹ based on the results of histamine release in response to recombinant tick HRF (Mulenga et al., 2003b). The assay was performed in triplicate and the mean β-hexosaminidase release was expressed as a percentage of the total β-hexosaminidase content and calculated by the formula:

$$\% \beta\text{-hexosaminidase release} = (\text{OD supernatant} / \text{OD supernatant} + \text{OD pellet}) \times 100$$

3. Results

3.1. DNA sequence analysis of Dg-HRF-1 and phylogeny

A truncated cDNA isolated from the cDNA expression library exhibited sequence homology to several tick HRF proteins. The sequence of the full-length 525 bp open-reading frame (ORF)

predicted to encode Dg-HRF-1 was obtained using 3' and 5' RACE analysis (Accession No. FM179713). The predicted protein sequence of Dg-HRF-1 was 174 amino acids in length and exhibited greatest identity to known tick HRF orthologues (ranging from 52% to 54%) and a lesser identity with the HRF/TCTP derived from mammals and other invertebrates (range 38–47%). The similarity of the Dg-HRF-1 with the tick HRF proteins was also inferred by the construction of a neighbour-joining tree (Fig. 1) and was supported by bootstrap analysis based on 1000 resampled datasets; with the Dg-HRF-1 partitioning closely with sequences derived from ticks. Other invertebrates formed discrete groupings corresponding to their defined taxonomic groups. The mammalian group clearly diverged from all of the invertebrates and this division was supported by 100% of the bootstrap predictions.

The Dg-HRF-1 protein was predicted to have a molecular mass of 19.4 kDa and a pI 4.48. No signal peptides were evident and Dg-HRF-1 was predicted to be cytoplasmic ($k = 23$) with two potential endoplasmic reticulum retention sequences (XXRR and KKXX) by pSORT II analysis (Nakai and Horton, 1999).

3.2. Expression of rDg-HRF-1 and identification of native HRF in mite protein extracts

rDg-HRF-1 was expressed in *E. coli* using the pET22b(+) vector system to produce a protein with an approximate molecular mass of 23 kDa with a C-terminal histidine tag to aid purification and N-terminal cleavable pelB leader sequence to enhance solubility. Despite the presence of the pelB leader sequence and adjustment

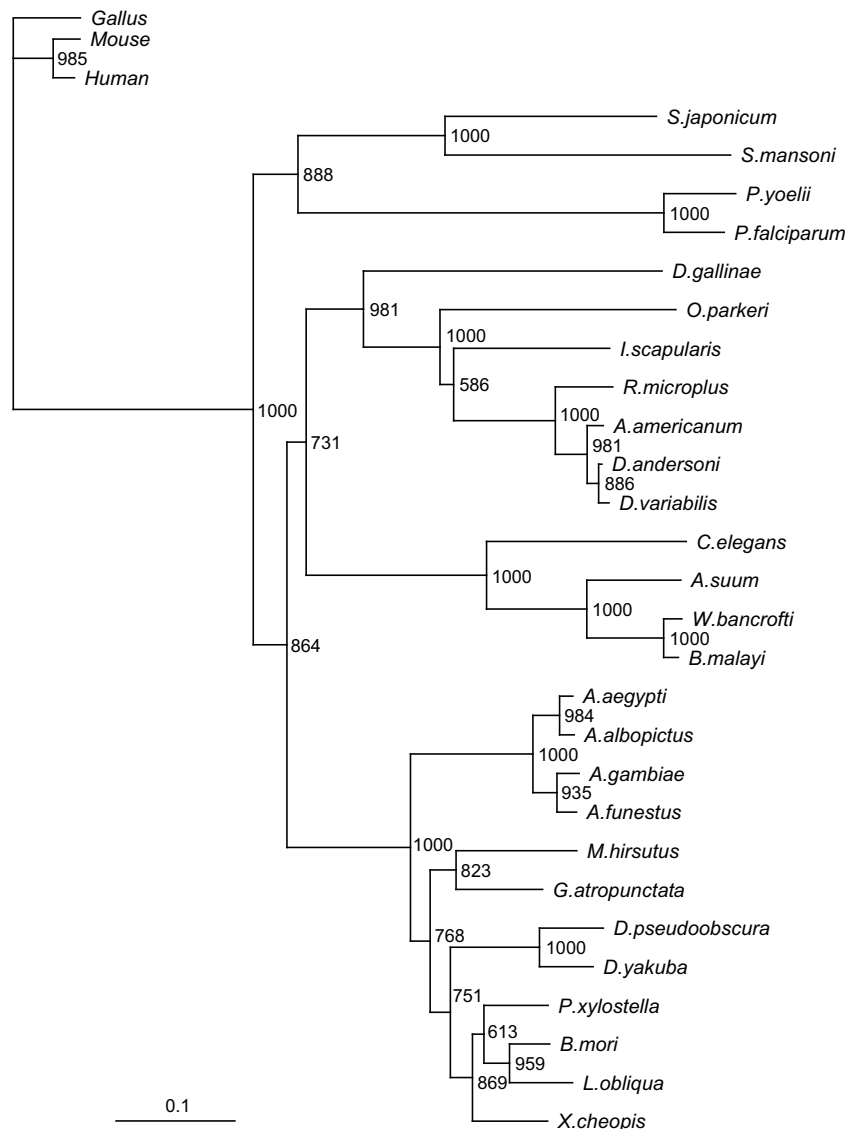


Fig. 1. Phylogenetic relationship between the predicted amino-acid sequences of selected mammalian, arthropod and other invertebrate histamine release factor (HRF)/translationally-control tumour proteins (TCTP). A neighbour-joining tree was constructed using the ClustalX algorithm, resampled using a 1,000 bootstrap analysis (Jeanmougin et al., 1998) and the resulting tree viewed with TreeView (Page, 1996) with *Gallus gallus* as the out-group. The degree of bootstrap support for each partition is annotated at the nodes. HRF/TCTP sequences included in the analysis: Mammalian species: *G. gallus* (P43347), Mouse (P63028) and Human (Q5W0H4). Nematode species: *Caenorhabditis elegans* (Q93573), *Ascaris suum* (A1BQ59), *Wuchereria bancrofti* (AAK71499), *Brugia malayi* (AAC47622), Protozoan species: *Plasmodium yoelii* (XP_725272), *Plasmodium falciparum* (Q8I3Z5). Trematode species: *Schistosoma japonicum* (AAB42079), *Schistosoma mansoni* (AAL11633). Ixodida species: *Ornithodoros parkeri* (ABR23352), *Ixodes scapularis* (Q4PLZ3), *Rhipicephalus microplus* (AAY67698), *Amblyomma americanum* (AAY67700), *Dermacentor andersoni* (AA67699), *Dermacentor variabilis* (AAL75585). Other arthropod species: *Aedes aegypti* (Q1HR79), *Aedes albopictus* (Q5MIP6), *Anopheles gambiae* (Q7QCK2), *Anopheles funestus* (Q06DG2), *Maconellicoccus hirsutus* (A2I3U6), *Graphocephala atropunctata* (Q1W2B7), *Drosophila pseudoobscura* (Q293Y0), *Drosophila yakuba* (Q6XIN1), *Plutella xylostella* (Q60F51), *Bombyx mori* (A2TK65), *Lonomia oblique* (Q5MGM6), *Xenopsylla cheopis* (A2IAB5).

of expression conditions, the majority of rDg-HRF-1 was directed to bacterial inclusion bodies, from which it was purified using a nickel affinity column under reducing conditions and then refolded by dialysis against PBS (Fig. 2). The identity of the purified recombinant protein was confirmed by MALDI-TOF-MS.

Antibodies specific for rDg-HRF-1 were generated in rabbits and hens and the specificity to rDg-HRF-1 confirmed by Western blot (Figs. 3 and 4). A double-band corresponding to the purified rDg-HRF-1 protein was visualised with the rabbit and chicken anti-rDg-HRF-1 antibodies. The double-band was probably the result of incomplete cleavage of the 22-amino-acid pelB leader signal peptide during expression under conditions imperfect for soluble protein formation.

Soluble, membrane-associated, integral membrane and insoluble fractions of *D. gallinae* proteins were immuno-screened with rabbit anti-rDg-HRF-1 antibodies (Fig. 3) and a protein was detected in the soluble mite fraction with an approximate molecular mass corresponding to that predicted for Dg-HRF (19.4 kDa).

PAGE was carried out to further fractionate the soluble *D. gallinae* proteins and LC/MS-MS performed with proteins excised from the gel with a M_r range of 17–21 kDa. Peptides with homology to the predicted rDg-HRF-1 protein sequence were identified, confirming the existence of soluble native HRF in *D. gallinae* in the M_r range of 17–21 kDa, which is also comparable to the M_r the protein detected by Western blot.

3.3. Natural immunity to Dg-HRF in layers

rDg-HRF-1 and the soluble mite protein fraction were immuno-screened with IgY purified from eggs randomly sampled from a battery system at a commercial egg farm. The donor hens were 60 weeks old and had been housed in the battery system for

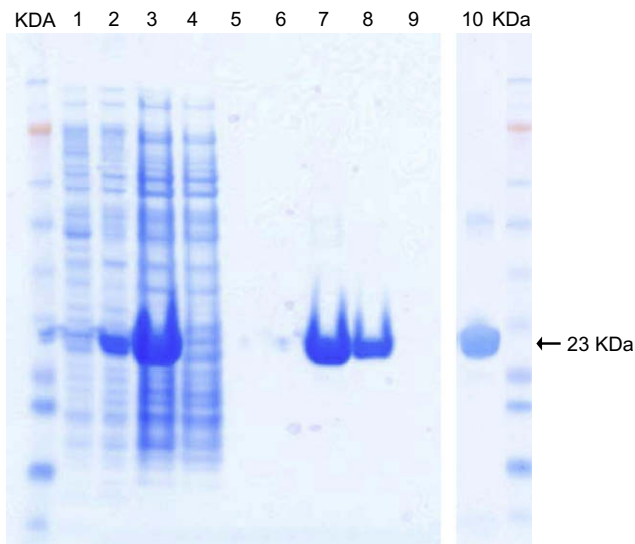


Fig. 2. Expression and purification of recombinant *Dermanyssus gallinae* histamine release factor (rDg-HRF). rDg-HRF-1 protein was expressed as a histidine and pelB fusion in *Escherichia coli* and insoluble protein recovered from bacterial cell lysates prior to induction with 1 mM Isopropyl β -D-1-thiogalactopyranoside (IPTG) (lane 1) and after induction with IPTG (lane 2). rDg-HRF-1 protein was purified from induced bacterial cell lysates by binding to a nickel column using 8 M urea under reducing conditions. Unbound rDg-HRF-1 and *E. coli* proteins were removed in column flow-through (fraction A, lane 3) and rDg-HRF-1 eluted with the following increasing gradient of imidazole: 50 mM (fraction B, lane 4), 200 mM (fraction C, lane 5), 350 mM (fraction D, lane 6) and 500 mM (fractions E–G, lanes 7–9). Fractions E and F were pooled and the protein refolded by dialysis against a decreasing gradient of urea to obtain soluble rDg-HRF-1 in PBS (lane 10). Proteins were electrophoresed on NuPAGE® Novex 4–12% Bis-Tris minigels (Invitrogen) and protein bands were visualized with SimplyBlue™ SafeStain.

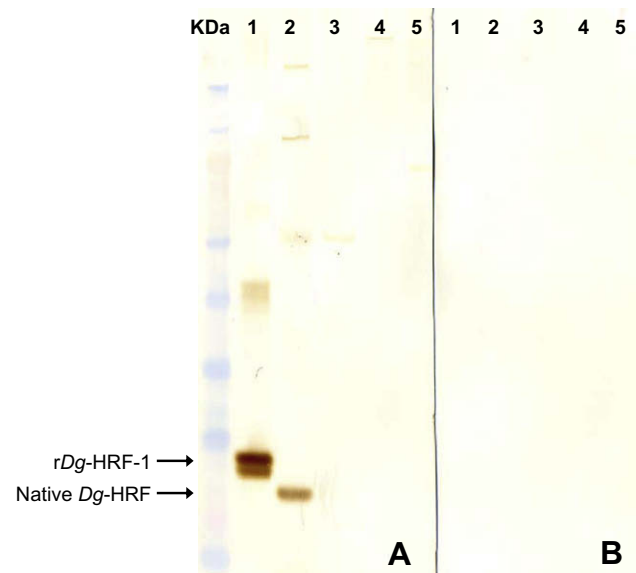


Fig. 3. Western blot analysis of recombinant *Dermanyssus gallinae* histamine release factor (rDg-HRF) and fractionated *D. gallinae* mite proteins. Twenty-five nanograms rDg-HRF-1 and 25 μ g of fractionated *D. gallinae* proteins were electrophoresed on a 4–12% Bis-Tris gel and transferred to nitrocellulose. Blots were incubated with positive rabbit polyclonal serum raised against rDg-HRF-1 (A) and negative rabbit serum (B), then specifically bound antibodies detected with anti-rabbit-horse radish peroxidase (HRP) conjugated antibody (DAKO) and visualized with SIGMAFAST™ 3,3'-Diaminobenzidine. Positive serum antibodies (A) recognised the recombinant rDg-HRF-1 (lane 1) and the native Dg-HRF present in the soluble mite fraction (lane 2), but not in the membrane-associated (lane 3), integral-membrane (lane 4) or insoluble (lane 5) mite extracts. Negative serum antibodies (B) did not recognise recombinant or native Dg-HRF proteins.

approximately 40 weeks, during which they had been exposed to heavy infestations of mites. The recombinant and native Dg-HRF were not detected with IgY from the commercially-sourced eggs or with a negative control IgY. In contrast the rDg-HRF-1 and native Dg-HRF proteins were both clearly detected by positive control IgY (Fig. 4). These data suggest that antibodies specific to Dg-HRF are not generated in response to natural infestation.

3.4. Immunolocalization of Dg-HRF in mite tissues

Immunohistochemical staining of longitudinal sections through an adult female *D. gallinae* mite were carried out to visualize the anatomical distribution of the Dg-HRF protein. Consecutive 5- μ m sections were screened with rabbit anti-rDg-HRF-1 serum and a negative control rabbit serum for direct comparison (Fig. 5). The staining and hence distribution of Dg-HRF appears to be widespread but specific to certain tissues, notably the synganglion nervous tissue, ventriculum and other digestive tract tissue, fat body and the reproductive tissues including the ovary, oviduct, cornus sacculus and the vaginal gland chamber, suggesting a greater concentration of HRF in these tissues. No evidence was seen of non-specific binding of the conjugate to the control sections (results not shown).

3.5. Effect of ingested anti-Dg-HRF IgY on the mite survival

The ability of ingested IgY specific for Dg-HRF-1 to induce mortality in mites was investigated using the in vitro feeding device. Mites were fed fresh heparinised chicken blood that was enriched with purified anti-Dg-HRF IgY or with IgY from a negative control hen and mortality compared between the two groups. The experiment was repeated on three separate occasions using different

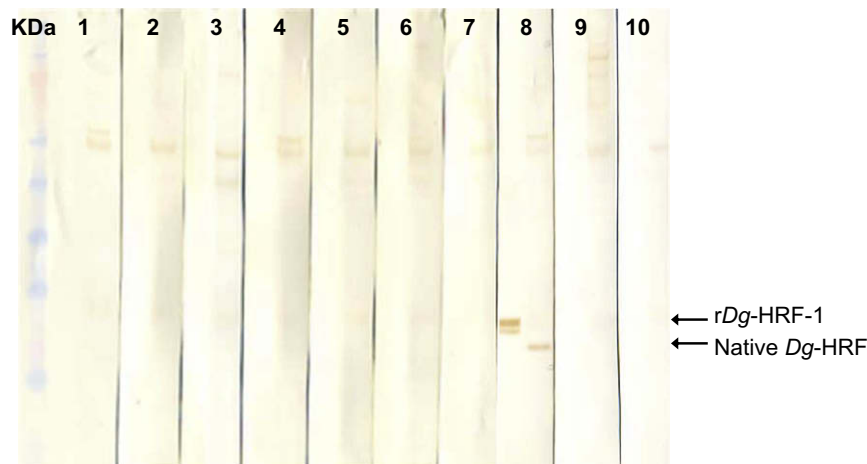


Fig. 4. Immunoreactivity of yolk IgY immunoglobulins with recombinant *Dermanyssus gallinae* histamine release factor (rDg-HRF) and soluble *D. gallinae* mite proteins. Twenty-five ng rDg-HRF-1 and 25 µg soluble mite extract were localised onto each Western blot strip and each strip probed with 75 µg ml⁻¹ yolk-derived IgY from: commercial poultry unit hens (strips 1–7), positive control hen IgY generated by injection with rDg-HRF-1 (strip 8) and negative-control (mite na) hen (strip 9). Specifically bound IgY was detected with anti-IgY-horse radish peroxidase (HRP) conjugated antibody (Sigma) and visualized with SIGMAFAST™ 3,3'-Diaminobenzidine. Positive hen IgY recognised the recombinant and native Dg-HRF proteins, the IgY from negative control and commercial hens did not. Non-specific binding of the conjugated antibody with the recombinant or soluble mite extract was not observed (strip 10).

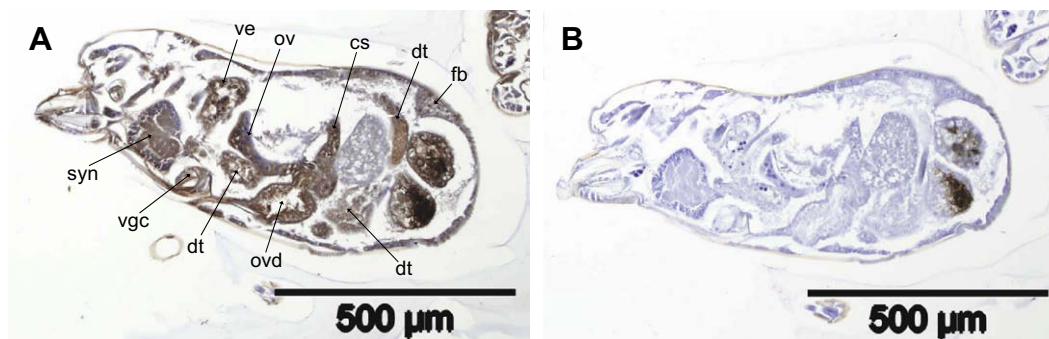


Fig. 5. Immunohistochemical staining of a longitudinal section through an adult female *Dermanyssus gallinae* mite showing the anatomical distribution of the *D. gallinae* histamine release factor (Dg-HRF) protein. Consecutive 5-µm mite sections were screened with positive rabbit polyclonal serum raised against recombinant Dg-HRF-1 (A) and negative-control rabbit serum (B). Specifically bound antibody was detected with an anti-rabbit-horse radish peroxidase (HRP) conjugated antibody (DAKO), visualized with SIGMAFAST™ 3,3'-Diaminobenzidine (DAB) and counterstained with haematoxylin. Consecutive control sections were also screened with anti-rabbit-HRP conjugate and DAB only, omitting the primary antibody (rabbit anti-Dg-HRF) binding step, and no evidence was seen of non-specific binding of the conjugate to the sections (results not shown). The distribution of Dg-HRF appears to be ubiquitous, however several tissues, notably the synganglion nervous tissue (syn), ventriculus (ve) and other digestive tract (dt), fat body (fb) and the reproductive tissues including the ovary (ov), oviduct (ovd), cornus sacculus (cs) and the vaginal gland chamber (vgc), all appear to be very intensely stained suggesting a greater concentration of HRF in these tissues.

batches of mites and chicken skin feeding membranes and this may have influenced feeding rates. There was little variation in the numbers of fed mites recovered from the feeding chambers between groups within individual experiments, however a greater variability in the number of fed mites recovered occurred between the three experiments (mite numbers: experiment 1 = 114 and 119; experiment 2 = 41 and 62; experiment 3 = 190 and 191). To allow comparison of mortality between the three experiments, results were expressed as a cumulative percentage of mortality.

Mortality occurred in the 4 days post-feeding in the group that received the anti-Dg-HRF IgY and reached mean cumulative percentage mortality of 9.24% (SD ± 1.63). In comparison, the mortality that occurred in the negative control group was limited to the first 3 days post-feeding and was less with a mean cumulative percentage mortality of 2.74% (SD ± 3.31). Overall, the effect of anti-Dg-HRF IgY on the survival of mites occurred mostly within 48 h of IgY ingestion (Fig. 6) and there is strong evidence ($P=0.004$) of a difference in the mortality rate between the two groups at day 5 post feeding.

3.6. Biological activity of rDg-HRF-1

The ability of rDg-HRF-1 to induce degranulation of RPMCs was evaluated by measuring the percentage of cellular β-hexosaminidase released in response to stimulation with rDg-HRF-1 and comparing this with the spontaneous release induced by media (negative control) and the calcium ionophore compound 48/80 (positive control). The mean % of β-hexosaminidase released from RPMCs stimulated with rDg-HRF-1 was 29.37% (SD ± 3.79) and was similar to the 30.63% release (SD ± 0.58) in response to the positive control compound 48/80 (Fig. 7). The % of β-hexosaminidase spontaneously released by RPMCs (media control) was 21.88% (SD ± 4.16), which is approximately 8% less than that measured with rDg-HRF-1. Stimulation with heat-denatured rDg-HRF-1 resulted in 22.51% release (SD ± 4.62), which is similar to the spontaneous release in the negative control. Hence degranulation stimulated by rDg-HRF-1 is unlikely to be the result of contaminating bacterial endotoxins.

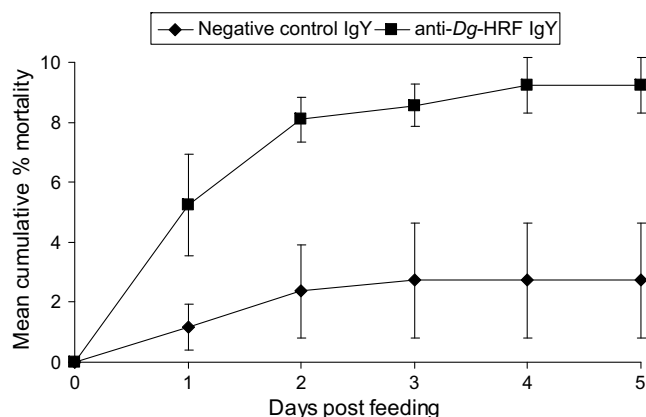


Fig. 6. The effect of ingested anti-recombinant *Dermanyssus gallinae* histamine release factor (rDg-HRF) IgY on mite survival. Starved *D. gallinae* mites were fed with fresh heparinised chicken blood enriched with either 3.5 mg ml⁻¹ yolk-derived anti-rDg-HRF polyclonal IgY or 3.5 mg ml⁻¹ purified yolk-IgY from a negative control hen. Fed mites were monitored daily for 5 days and scored for mortality. The experiment was repeated three times and the cumulative mean mortality rates of the two groups (y-axis) are plotted against time (x-axis), the SEM is represented by the vertical error bars. The number of mites that were dead by day 5 were analysed using a generalized linear mixed model and there is strong evidence ($P = 0.004$) of a difference in the mortality rate between the two groups.

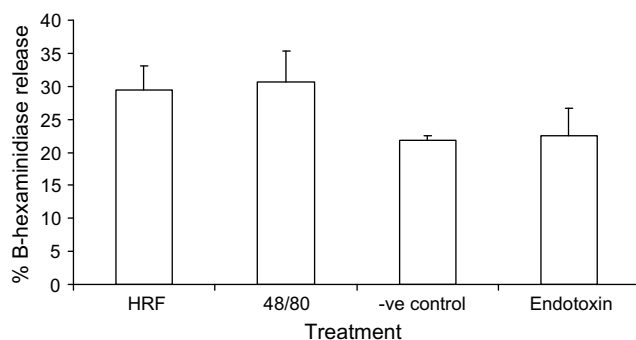


Fig. 7. Biological activity of recombinant *Dermanyssus gallinae* histamine release factor (rDg-HRF-1). Degranulation of rat peritoneal mast cells (RPMCs) in response to stimulus was evaluated by measuring the percentage of cellular β -hexosaminidase released from cells. The percentage of β -hexosaminidase released from RPMCs (y-axis) stimulated with rDg-HRF-1 was compared with the percentage released spontaneously by stimulation with media only (negative control) to identify specific degranulation activity of rDg-HRF-1. Compound 48/80 was used at a concentration of 0.5 μ g ml⁻¹ to induce maximal degranulation (positive control) and 0.5 μ g ml⁻¹ heat denatured rDg-HRF-1 was used to assess the stimulatory effect of contaminating bacterial endotoxins present in purified rDg-HRF-1 (endotoxin control). The assay was performed in triplicate and error bars represent the SD.

4. Discussion

Here we describe the identification and characterization of an orthologue of the tick HRF protein in *D. gallinae*. To our knowledge this is the first description of this type of molecule in mites. We have also provided preliminary evidence demonstrating the potential of rDg-HRF as a vaccine antigen for *D. gallinae*.

Phylogenetic analysis of the *Dg*-HRF-1 protein sequence clearly demonstrated the close relationship of this molecule in the Mesostigmatid *D. gallinae* mite and Ixodid tick species. *Dermanyssus gallinae* and Ixodid ticks both belong in the Parasitiformes superorder of the Acari subclass (Evans, 1992; Wheeler et al., 2000), however as no representative HRF/TCTP sequences from mites belonging to the other acarine superorders (Acariformes and Opilioacariformes) were available, it was impossible to infer the relationship of *D. gal-*

linae within the wider Acari subclass from this data. However, a previous study examining the relationships between tropomyosin protein sequences also demonstrated that the *D. gallinae* sequence partitioned with Ixodid tick sequences, rather than with the Acariformes mite sequences (Nisbet et al., 2006a,b) underlining the close phylogenetic relationship between *D. gallinae* and tick species. The taxonomic relationship between *D. gallinae* and the Ixodid ticks is possibly reflected in similarities in physiology and lifestyle, such as off-host residence, host location strategies and feeding mechanisms.

The function of HRF in ticks is not yet clear. Mulenga et al. (2003b) demonstrated that a recombinant version of HRF from the dog tick *Dermacentor variabilis* induced the release of histamine from rat basophils, and in this respect shares function with HRF/TCTP homologues found in mammals and other invertebrates (miHRF/TCTP) (MacDonald et al., 1995, 2001). Preliminary data presented here indicates that modest histamine release also occurred when RPMCs were stimulated with rDg-HRF-1, indicating that the histamine-release function is also conserved in *D. gallinae*.

Similar to tick and other miHRF/TCTPs (Macdonald et al., 2001; Rao et al., 2002; Gnanasekar et al., 2002), *Dg*-HRF is soluble but lacks signal peptides associated with secretion. However, despite the absence of signal peptides, secretion of HRF/TCTP has been confirmed in several mammal and invertebrate species (Bommer and Thiele, 2004; Amzallag et al., 2004). Secretion of miHRF/TCTP may be crucial not only for histamine release, but also for other extracellular cytokine-like effects, for example: activation of basophils and eosinophils (Macdonald et al., 2001; Rao et al., 2002; Vonakis et al., 2008) and stimulation of B-cell proliferation (Kang et al., 2001). The extracellular cytokine-like activities of secreted HRF/TCTP may be particularly important in other invertebrate parasites such as *Schistostoma mansoni* and filarial nematodes, possibly modulating host immune signalling and influencing vasodilation in a manner potentially beneficial to the survival of the parasite (Macdonald et al., 2001; Gnanasekar et al., 2002; Rao et al., 2002).

Mulenga et al. (2003b) initially postulated that secreted tick HRF may also play a role in the multifaceted control of localized inflammatory response around the tick/host interface, and that HRF-mediated histamine response during the late phase of feeding might facilitate vascularisation and hence enhance rapid engorgement. *Dermanyssus gallinae* feeds very rapidly; attachment to host, engorgement and host release is generally completed within 30–90 min (Chauve, 1998). In comparison hard ticks feed for several days, but it is only in the late phase of feeding that significant volumes of blood are ingested (Sonenshine, 1991). Given the similarity of the rapid feeding mechanism of *D. gallinae* with the late rapid engorgement phase of ticks, it might be tempting to hypothesize that *Dg*-HRF may also be a secreted salivary antigen involved in rapid feeding in *D. gallinae*. However, as antibodies specific for *Dg*-HRF were not detected in hens from a heavily infested unit, it suggests that *Dg*-HRF is not exposed to the host or it is not naturally immunogenic. The naturally infested hens sampled for this study had been housed in a heavily infested commercial poultry unit for 40 weeks ensuring sufficient exposure to *D. gallinae* salivary antigens for any measurable IgY response to have occurred. The apparent absence of IgY specific for *Dg*-HRF in naturally infested hens is unlikely to be the result of poor immunogenicity of *Dg*-HRF as the hens exhibited a vigorous and specific IgY response when injected with rDg-HRF-1.

These data suggest that in *D. gallinae*, HRF is unlikely to be a secreted salivary antigen and is probably concealed, which appears to be in contrast to the situation in ticks. Native HRF has been detected in tick saliva by Western blot, suggesting that it is an exposed salivary antigen (Mulenga et al., 2003b); although it is unclear whether HRF is a genuine secreted tick salivary antigen

or was detected due to contamination of saliva with haemolymph. Furthermore it remains to be determined whether naturally infested animals possess antibodies specific for tick HRF, which would clarify the status of HRF as an exposed or concealed tick antigen. HRF expression is not up-regulated in response to blood meal uptake (Mulenga et al., 2003b), which may indicate that HRF is not directly involved in tick-feeding as was originally proposed (Mulenga and Azad, 2005). In addition, the existence of miHRF/TCTP identified in non-blood feeding invertebrates and mammals suggest another function(s) for HRF in haematophagous ectoparasites.

HRF/TCTP proteins are expressed in a wide range of invertebrate and mammalian tissues and many of the described functions are regulatory (Mulenga et al., 2003b; Bommer and Thiele, 2004). Immunolocalization data presented here demonstrated that Dg-HRF is also ubiquitously distributed in mite tissues, which suggests that, like miHRF/TCTPs, Dg-HRF is potentially a regulatory protein intrinsic to most mite cell types. miTCTP/HRFs have been shown to interact with a variety of cellular proteins (e.g. tubulin, protein kinase p1k, translation elongation factor) and have been implicated in the regulation of cellular processes such as cell growth, cell cycle progression, malignant transformation and protein synthesis (Cans et al., 2003; reviewed by Bommer and Thiele, 2004). More recently HRF/TCTP has also been described as an antioxidant in filarial parasites (Gnanasekar and Ramaswamy, 2007), however this function is unlikely to be conserved in *D. gallinae* as the three cysteine residues critical for the antioxidant property are not conserved in the Dg-HRF-1 protein.

HRF protein is expressed in all life stages in Ixodid ticks (Mulenga and Azad, 2005); if this is also true for *D. gallinae*, vaccination with rDg-HRF-1 may be effective against all blood-feeding life stages. The ubiquitous distribution and prevalence of Dg-HRF, and its putative role in multiple biological processes, also make Dg-HRF an attractive vaccine prospect. The binding of ingested antibodies to the mite tissues may interfere with digestion and regulatory processes, and ultimately may affect mite survival, development and fecundity. Ingested host antibodies apparently remain functional in the gut environment in tick and mite species previously investigated (Jasinskas et al., 2000; Pettit et al., 2000), which suggests that ingested IgY would be able to directly access the Dg-HRF located in the gut of *D. gallinae* and potentially cause gut damage in a manner similar to Bm86 tick vaccine.

The protective capability of HRF/TCTP against haematophagous ectoparasites had not previously been tested. We have used an in vitro mite-feeding system in order to assess the effect of ingesting anti-Dg-HRF IgY on mite survival. The in vitro assay system offers several advantages over a conventional in vivo vaccination and mite challenge study for the preliminary assessment of antigens as vaccine candidates. The in vitro system allows the mortality rates of mites fed with antigen-specific antibodies to be rapidly and accurately defined; whereas mortality cannot be accurately measured in an in vivo trial which relies on an overall assessment of changes in mite populations over several months using mite trapping techniques. Hence in vivo trials, although necessary at a later stage of vaccine testing, are time-consuming and expensive. Furthermore, an accurate estimate of a mite population is difficult as mite trapping can be affected by, for example, subtle environmental differences and hen/mite behaviours. In contrast, the in vitro system allows the recovery of all mites that have fed on vaccinated blood, enabling individual mites to be isolated and monitored in defined environmental conditions. *Dermanyssus gallinae* mites fed with fresh heparinised chicken blood enriched with anti-Dg-HRF IgY showed strong evidence ($P = 0.004$) of a significant increase in mortality compared with mites fed with chicken-blood enriched with yolk antibodies from a negative control hen. Observed mortality was highest within the first 24-h post-feeding and in this respect

was similar to the immediate mortality observed for *D. gallinae* mites fed blood enriched with antibodies specific for a complex mixture of soluble native *D. gallinae* proteins (Wright et al., unpublished data). Although the increase in mortality was modest at approximately 7%, in a natural environment mortality may be much higher. In the in vitro system *D. gallinae* only fed once; whereas in a natural infestation, *D. gallinae* mites will feed three times in order to complete their development and an adult female mite will feed several more times in order to reproduce (reviewed in Nordenfors, H., 2001. Epidemiology and Control of the Poultry Red Mite, *Dermanyssus gallinae*. Ph.D. Thesis, Uppsala University). If there is an additive effect on mite mortality as a result of successive blood meals containing anti-Dg-HRF IgY, the reduction in mite numbers in commercial poultry houses may be significant enough to have a beneficial effect on poultry welfare and improve egg production.

Early vaccination studies with recombinant Bm86 tick vaccine also showed a comparably modest (24%) and immediate mortality as a result of antibody and complement induced gut damage (Kemp et al., 1989). The vaccine potential of Bm86 lay in the high proportion of surviving ticks that exhibited damage, low engorgement weights and 77% reduction in reproductive capability (Rand et al., 1989). Given the regulatory role of miHRF/TCTP in cell cycle progression, it is not surprising that miHRF/TCTP has also been found to be intrinsic to gamete formation and embryogenesis (Chen et al., 2007a,b; Vitale et al., 2007; Kubiak et al., 2008). Although the impact of anti-Dg-HRF IgY on fecundity and development was not monitored in this study, it will be interesting to determine whether these functions could be disrupted by vaccination. For this to occur, ingested IgY must first traverse the gut/haemolymph barrier in order to gain access to the Dg-HRF located in the reproductive tissues. In *D. gallinae* IgY immunoreactivity is observed throughout the mite body following blood-feeding, suggesting that IgY is capable of crossing into the haemolymph of *D. gallinae* and accessing organ-specific antigens (Nisbet et al., 2006a). Likewise in ticks, ingested antibodies have been shown to cross the gut barrier into the haemolymph (Brossard and Rais, 1984; Ben-Yakir et al., 1987; Tracey-Patte et al., 1987; Ben-Yakir, 1989) where they remain functional (Minoura et al., 1985; Jasinskas et al., 2000) and able to bind to target antigens (Ackerman et al., 1981; Jasinskas et al., 2000). Vaccination with the *Ixodes scapularis* subolesin antigen provides an example of how vaccination can target reproductive organs and disrupt oviposition in ticks (Almazan et al., 2005). The level of mortality induced by a single blood meal enriched with anti-Dg-HRF IgY was relatively modest in the present study. However, if ingested anti-Dg-HRF IgY proves able to impact on the reproductive potential of *D. gallinae* and thus reduce population growth and/or if repeated blood-feeding increases mortality, the prospect of Dg-HRF as a vaccine antigen will be significantly enhanced. Further work is planned to assess whether vaccination of hens with rDg-HRF can control mite populations by employing an in vivo vaccination and challenge regime that mimics commercial egg farming conditions.

To summarize, we believe this is the first identification of HRF in a mite species and it is also the first recombinant *D. gallinae* antigen to be evaluated for vaccine potential. The preliminary results obtained using the in vitro feeding system are encouraging and demonstrate an effect on *D. gallinae* survival in response to IgY generated by recombinant protein vaccination. Importantly this study has demonstrated proof of principle: that vaccination with recombinant mite proteins is a promising strategy for the control of *D. gallinae* infestation.

Acknowledgements

The authors thank the Moredun Proteomics Unit for the MALDI-TOF and LC/MS-MS analysis, Jill Sales (Biomathematics and

Statistics, Scotland) for the statistical analysis and the clinical staff at Moredun and SAC for their expertise. We gratefully acknowledge the practical assistance and support provided by local poultry farmers and the British Egg Marketing Board Educational Trust for their enthusiasm for this work and sponsoring Harry Wright in his Ph.D. studies. This work was funded by The Department for Environment, Food and Rural Affairs.

References

- Ackerman, S., Clare, F.B., McGill, T.W., Sonenshine, D.E., 1981. Passage of host serum components, including antibody, across the digestive tract of *Dermacentor variabilis* (Say). *J. Parasitol.* 67, 737–740.
- Almazan, C., Kocan, K.M., Blouin, E.F., de la Fuente, J., 2005. Vaccination with recombinant tick antigens for the control of *Ixodes scapularis* adult infestations. *Vaccine* 23, 5294–5298.
- Amzallag, N., Passer, B.J., Allanic, D., Segura, E., Thery, C., Goud, B., Amson, R., Telerman, A., 2004. TSAP6 facilitates the secretion of translationally controlled tumor protein/histamine-releasing factor via a nonclassical pathway. *J. Biol. Chem.* 279, 46104–46112.
- Auger, P., Nantel, J., Meunier, N., Harrison, R.J., Loisele, R., Gyorkos, T.W., 1979. Skin acariasis caused by *Dermanyssus gallinae* (de Geer): an in-hospital outbreak. *Can. Med. Assoc. J.* 120, 700–703.
- Ben-Yakir, D., Fox, C.J., Homer, J.T., Barker, R.W., 1987. Quantification of host immunoglobulin in the hemolymph of ticks. *J. Parasitol.* 73, 669–671.
- Ben-Yakir, D., 1989. Quantitative studies of host immunoglobulin G in the hemolymph of ticks (Acari). *J. Med. Entomol.* 26, 243–246.
- Beugnet, F., Chauve, C., Gauthey, M., Beert, L., 1997. Resistance of the red poultry mite to pyrethroids in France. *Vet. Rec.* 140, 577–579.
- Bhisutthibhan, J., Pan, X.Q., Hossler, P.A., Walker, D.J., Yowell, C.A., Carlton, J., Dame, J.B., Meshnick, S.R., 1998. The *Plasmodium falciparum* translationally controlled tumor protein homolog and its reaction with the antimalarial drug artemisinin. *J. Biol. Chem.* 273, 16192–16198.
- Bommer, U.A., Thiele, B.J., 2004. The translationally controlled tumour protein (TCTP). *Int. J. Biochem. Cell Biol.* 36, 379–385.
- Brossard, M., Rais, O., 1984. Passage of hemolysins through the midgut epithelium of female *Ixodes ricinus* L. fed on rabbits infested or reinfested with ticks. *Experientia* 40, 561–563.
- Cans, C., Passer, B.J., Shalak, V., Nancy-Portebois, V., Crible, V., Amzallag, N., Allanic, D., Tufino, R., Argenti, M., Moras, D., Fiucci, G., Goud, B., Mirande, M., Amson, R., Telerman, A., 2003. Translationally controlled tumor protein acts as a guanine nucleotide dissociation inhibitor on the translation elongation factor eEF1A. *Proc. Natl. Acad. Sci. USA* 100, 13892–13897.
- Chauve, C., 1998. The poultry red mite *Dermanyssus gallinae* (De Geer, 1778): current situation and future prospects for control. *Vet. Parasitol.* 79, 239–245.
- Chen, S.H., Wu, P.S., Chou, C.H., Yan, Y.T., Liu, H., Weng, S.Y., Yang-Yen, H.F., 2007a. A knockout mouse approach reveals that TCTP functions as an essential factor for cell proliferation and survival in a tissue- or cell type-specific manner. *Mol. Biol. Cell.* 18, 2525–2532.
- Chen, Z., Zhang, H., Yang, H., Huang, X., Zhang, X., Zhang, P., 2007b. The expression of AmphitCTP, a TCTP orthologous gene in amphioxus related to the development of notochord and somites. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 147, 460–465.
- Chirico, J., Eriksson, H., Fossum, O., Jansson, D., 2003. The poultry red mite, *Dermanyssus gallinae*, a potential vector of *Erysipelothrix rhusiopathiae* causing erysipelas in hens. *Med. Vet. Entomol.* 17, 232–234.
- de la Fuente, J., Rodriguez, M., Redondo, M., Montero, C., Garcia-Garcia, J.C., Mendez, L., Serrano, E., Valdes, M., Enriquez, A., Canales, M., Ramos, E., Boue, O., Machado, H., Leonart, R., de Armas, C.A., Rey, S., Rodriguez, J.L., Artilles, M., Garcia, L., 1998. Field studies and cost-effectiveness analysis of vaccination with Gavac against the cattle tick *Boophilus microplus*. *Vaccine* 16, 366–373.
- de la Fuente, J., Almazan, C., Blouin, E.F., Naranjo, V., Kocan, K.M., 2006. Reduction of tick infections with *Anaplasma marginale* and *A. phagocytophilum* by targeting the tick protective antigen subolesin. *Parasitol. Res.* 100, 85–91.
- Evans, G.O., 1992. Principles of Acarology. C.A.B International, Wallingford, UK.
- Fiddes, M.D., Le Gresley, S., Parsons, D.G., Epe, C., Coles, G.C., Stafford, K.A., 2005. Prevalence of the poultry red mite (*Dermanyssus gallinae*) in England. *Vet. Rec.* 157, 233–235.
- Gnanasekar, M., Rao, K.V., Chen, L., Narayanan, R.B., Geetha, M., Scott, A.L., Ramaswamy, K., Kaliraj, P., 2002. Molecular characterization of a calcium binding translationally controlled tumor protein homologue from the filarial parasites *Brugia malayi* and *Wuchereria bancrofti*. *Mol. Biochem. Parasitol.* 121, 107–118.
- Gnanasekar, M., Ramaswamy, K., 2007. Translationally controlled tumor protein of *Brugia malayi* as an antioxidant protein. *Parasitol. Res.* 101, 1533–1540.
- Hoglund, J., Nordenfors, H., Ugglä, A., 1995. Prevalence of the poultry red mite, *Dermanyssus gallinae*, in different types of production systems for egg layers in Sweden. *Poult. Sci.* 74, 1793–1798.
- Jasinskas, A., Jaworski, D.C., Barbour, A.G., 2000. *Amblyomma americanum*: specific uptake of immunoglobulins into tick hemolymph during feeding. *Exp. Parasitol.* 96, 213–221.
- Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G., Gibson, T.J., 1998. Multiple sequence alignment with Clustal X. *Trends Biochem. Sci.* 23, 403–405.
- Kang, H.S., Lee, M.J., Song, H., Han, S.H., Kim, Y.M., Im, J.Y., Choi, I., 2001. Molecular identification of IgE-dependent histamine-releasing factor as a B cell growth factor. *J. Immunol.* 166, 6545–6554.
- Kemp, D.H., Pearson, R.D., Gough, J.M., Willadsen, P., 1989. Vaccination against *Boophilus microplus*: localization of antigens on tick gut cells and their interaction with the host immune system. *Exp. Appl. Acarol.* 7, 43–58.
- Kilpinen, O., Roepstorff, A., Permin, A., Norgaard-Nielsen, G., Lawson, L.G., Simonsen, H.B., 2005. Influence of *Dermanyssus gallinae* and *Ascaridia galli* infections on behaviour and health of laying hens (*Gallus gallus domesticus*). *Br. Poult. Sci.* 46, 26–34.
- Kubiak, J.Z., Bazile, F., Pascal, A., Richard-Parpaillon, L., Polanski, Z., Ciemerych, M.A., Chesnel, F., 2008. Temporal regulation of embryonic M-phases. *Folia Histochem. Cytobiol.* 46, 5–9.
- MacDonald, S.M., Rafnar, T., Langdon, J., Lichtenstein, L.M., 1995. Molecular identification of an IgE-dependent histamine-releasing factor. *Science* 269, 688–690.
- MacDonald, S.M., Bhisutthibhan, J., Shapiro, T.A., Rogerson, S.J., Taylor, T.E., Tembo, M., Langdon, J.M., Meshnick, S.R., 2001. Immune mimicry in malaria: *Plasmodium falciparum* secretes a functional histamine-releasing factor homolog in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* 98, 10829–10832.
- Maurer, V., Baumgartner, J., 1992. Temperature influence on life table statistics of the chicken mite *Dermanyssus gallinae* (Acari: Dermanyssidae). *Exp. Appl. Acarol.* 15, 27–40.
- McDevitt, R., Nisbet, A.J., Huntley, J.F., 2006. Ability of a proteinase inhibitor mixture to kill poultry red mite, *Dermanyssus gallinae* in an in vitro feeding system. *Vet. Parasitol.* 141, 380–385.
- Minoura, H., Chinzai, Y., Kitamura, S., 1985. *Ornithodoros moubata*: host immunoglobulin G in tick hemolymph. *Exp. Parasitol.* 60, 355–363.
- Moro, C.V., Chauve, C., Zenner, L., 2005. Vectorial role of some Dermanyssoid mites (Acari, Mesostigmata, Dermanyssoidea). *Parasite* 12, 99–109.
- Moro, C.V., Desloire, S., Chauve, C., Zenner, L., 2007a. Detection of *Salmonella* sp. in *Dermanyssus gallinae* using an FTA filter-based polymerase chain reaction. *Med. Vet. Entomol.* 21, 148–152.
- Moro, C.V., Fravalo, P., Amelot, M., Chauve, C., Zenner, L., Salvat, G., 2007b. Colonization and organ invasion in chicks experimentally infected with *Dermanyssus gallinae* contaminated by *Salmonella Enteritidis*. *Avian Pathol.* 36, 307–311.
- Mulenga, A., Macaluso, K.R., Simser, J.A., Azad, A.F., 2003a. Dynamics of Rickettsia-tick interactions: identification and characterization of differentially expressed mRNAs in uninfected and infected *Dermacentor variabilis*. *Insect Mol. Biol.* 12, 185–193.
- Mulenga, A., Macaluso, K.R., Simser, J.A., Azad, A.F., 2003b. The American dog tick, *Dermacentor variabilis*, encodes a functional histamine release factor homolog. *Insect Biochem. Mol. Biol.* 33, 911–919.
- Mulenga, A., Azad, A.F., 2005. The molecular and biological analysis of ixodid ticks histamine release factors. *Exp. Appl. Acarol.* 37, 215–229.
- Nakai, K., Horton, P., 1999. PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization. *Trends Biochem. Sci.* 24, 34–36.
- Nisbet, A.J., Huntley, J.F., Mackellar, A., Sparks, N., McDevitt, R., 2006a. A house dust mite allergen homologue from poultry red mite *Dermanyssus gallinae* (De Geer). *Parasite Immunol.* 28, 401–405.
- Nisbet, A.J., Mackellar, A., Wright, H.W., Brennan, G.P., Chua, K.Y., Cheong, N., Thomas, J.E., Huntley, J.F., 2006b. Molecular characterization, expression and localization of tropomyosin and paramyosin immunodominant allergens from sheep scab mites (*Psoroptes ovis*). *Parasitology* 133, 515–523.
- Nordenfors, H., Hoglund, J., Tauson, R., Chirico, J., 2001. Effect of permethrin impregnated plastic strips on *Dermanyssus gallinae* in loose-housing systems for laying hens. *Vet. Parasitol.* 102, 121–131.
- Page, R.D.M., 1996. TreeView: an application to display phylogenetic trees on personal computers. *Comput. Appl. Biol. Sci.* 12, 357–358.
- Pettit, D., Smith, W.D., Richardson, J., Munn, E.A., 2000. Localisation and characterisation of ovine immunoglobulin within the sheep scab mite, *Psoroptes ovis*. *Vet. Parasitol.* 89, 231–239.
- Prevot, P.P., Couvreur, B., Denis, V., Brossard, M., Vanhamme, L., Godfroid, E., 2007. Protective immunity against *Ixodes ricinus* induced by a salivary serpin. *Vaccine* 25, 3284–3292.
- Rand, K.N., Moore, T., Skiranthan, A., Spring, K., Tellam, R., Willadsen, P., Cobon, G.S., 1989. Cloning and expression of a protective antigen from the cattle tick *Boophilus microplus*. *Proc. Natl. Acad. Sci. USA* 86, 9657–9661.
- Rao, K.V., Chen, L., Gnanasekar, M., Ramaswamy, K., 2002. Cloning and characterization of a calcium-binding, histamine-releasing protein from *Schistosoma mansoni*. *J. Biol. Chem.* 277, 31207–31213.
- Schwartz, L.B., Austen, K.F., Wasserman, S.I., 1979. Immunologic release of beta-hexosaminidase and beta-glucuronidase from purified rat serosal mast cells. *J. Immunol.* 123, 1445–1450.
- Smith, W.D., Bates, P., Pettit, D.M., Van den Broek, A., Taylor, M.A., 2002. Attempts to immunize sheep against the scab mite, *Psoroptes ovis*. *Parasite Immunol.* 24, 303–310.
- Sonenshine, D.E., 1991. Biology of Ticks, vol. 1. Oxford University Press, New York.
- Thind, B.B., Ford, H.L., 2007. Assessment of susceptibility of the poultry red mite *Dermanyssus gallinae* (Acari: Dermanyssidae) to some acaricides using an adapted filter paper based bioassay. *Vet. Parasitol.* 144, 344–348.
- Tracey-Patte, P.D., Kemp, D.H., Johnston, L.A., 1987. *Boophilus microplus*: passage of bovine immunoglobulins and albumin across the gut of cattle ticks feeding on normal or vaccinated cattle. *Res. Vet. Sci.* 43, 287–290.

- Vitale, A.M., Calvert, M.E., Mallavarapu, M., Yurttas, P., Perlin, J., Herr, J., Coonrod, S., 2007. Proteomic profiling of murine oocyte maturation. *Mol. Reprod. Dev.* 74, 608–616.
- Vonakis, B.M., Macglashan Jr., D.W., Vilarino, N., Langdon, J.M., Scott, R.S., MacDonald, S.M., 2008. Distinct characteristics of signal transduction events by histamine-releasing factor/translationally controlled tumor protein (HRF/TCTP)-induced priming and activation of human basophils. *Blood* 111, 1789–1796.
- Walker, D.J., Pitsch, J.L., Peng, M.M., Robinson, B.L., Peters, W., Bhisutthibhan, J., Meshnick, S.R., 2000. Mechanisms of artemisinin resistance in the rodent malaria pathogen *Plasmodium yoelii*. *Antimicrob. Agents Chemother.* 44, 344–347.
- Wheeler, D.L., Chappey, C., Lash, A.E., Leipe, D.D., Madden, T.L., Schuler, G.D., Tatusova, T.A., Rapp, B.A., 2000. Database resources of the National Center for Biotechnology Information. *Nucleic Acids. Res.* 28, 10–14.
- Willadsen, P., Riding, G.A., McKenna, R.V., Kemp, D.H., Tellam, R.L., Nielsen, J.N., Lahnstein, J., Cobon, G.S., Gough, J.M., 1989. Immunologic control of a parasitic arthropod. Identification of a protective antigen from *Boophilus microplus*. *J. Immunol.* 143, 1346–1351.