

Vaccination with concealed antigens to control *D. gallinae* infestations in laying hens

David Harrington^{1*}, José de la Fuente^{2,3}, Mario Canales², Jonathan Guy¹,
Karen. Robinson⁴, Olivier. Sparagano¹

¹ School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK,

² Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain

³ Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA

⁴ Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

* Corresponding author: david.harrington@newcastle.ac.uk

Abbreviated title: Immunisation of *Dermanyssus gallinae*

Summary

Heavy infestations of the haematophagous poultry red mite *Dermanyssus gallinae* can result in severe economic losses in egg-laying poultry e.g. decreased egg production and down-graded eggs. The increased structural complexity of alternative laying systems provides a greater number of refuges for *D. gallinae* to evade the cleaning and disinfection cycle, thereby creating the possibility of even greater *D. gallinae* infestations. Vaccines against *D. gallinae* could provide an alternative to chemical control, although their development is hampered by technical issues. In our laboratory we have immunized laying hens with concealed antigens (both somatic proteins and recombinant arthropod proteins) and used an in vitro feeding assay using antibodies extracted from eggs both immunized and control birds to determine efficacy against *D. gallinae*. Immunization of hens with somatic proteins and recombinant proteins from mosquitoes resulted in significant increases ($P < 0.05$) in mortality of *D. gallinae* compared to controls (50.6 % and 35.1 % respectively), whilst immunization with recombinant proteins from ticks gave a numerical increase

in mite mortality of 23 %. The results show that immunological control of *D. gallinae* is possible and further research is needed to develop a vaccine against this economically important mite which is effective under field conditions.

Keywords: *Dermanyssus gallinae*; Bm86; Subolesin; vaccination; ectoparasite; poultry

Introduction

The haematophagous poultry red mite, *Dermanyssus gallinae* (De Geer), is a member of the order Parasitiformes, containing the ticks and mites. *D. gallinae* is considered to be the most serious and economically significant ectoparasite affecting poultry in Europe (Sparagano et al., 2009), adversely affecting bird welfare (anaemia, restlessness) and egg production (reduced number of eggs, increased downgrading),

In most European countries, acaricides for the control of *D. gallinae* can only be used when the poultry house is empty, although some newer compounds are licensed for use when birds are *in situ* (Keita et al., 2006).

Contamination of eggs with acaricides in some production systems can occur (Hamscher et al., 2003) and the documentation of acaricide resistance to *D. gallinae* (Thind and Ford, 2007) suggests that alternative control methods are required. Alternative strategies to chemical control have been applied to other arthropod ectoparasites and agricultural pests including biological control (Van der Geest et al., 2000; de Oliveira Vasconcelos et al., 2004) and physical control such as sorptive dusts (Kirkwood, 1974), whilst the use of plant-derived products is a current area of research for *D. gallinae* control (George et al., 2009), but currently there are no vaccines available for the control of *D. gallinae* in poultry.

Research on the immunological control of *D. gallinae* has met with mixed success, although immunisation of hens with either somatic or recombinant *D. gallinae* proteins has shown promise (Bartley et al., 2008; Harrington et al., 2009; Wright et al., 2009). Consequently highly conserved antigens from other arthropods, but non-*D. gallinae* in origin, might provide an alternative source of antigen candidates for a *D. gallinae* vaccine. A number of recombinant tick proteins have demonstrated some efficacy in controlling tick infestations, but the gut membrane protein Bm86 is the only one that has formed the basis for a commercial vaccine. The efficacy of Bm86 vaccines against *Boophilus microplus* infestation is well documented (reviewed by de la Fuente et al. 2007) and they have shown variable efficacy for the control of non-*Boophilus* tick species (Willadsen, 2004). More recently, the recombinant protein subolesin

(4D8) identified by cDNA expression library from *Ixodes scapularis* has been shown to affect blood meal digestion, development and reproduction in ticks (Almazán et al., 2005; de la Fuente et al., 2006; Kocan et al., 2007; Nijhof et al., 2007; de la Fuente et al., 2008) and has been demonstrated as an orthologue of akirins, a group of proteins that act as transcription factors in insects and mammals (Galindo et al., 2008).

The objective of the current study was to determine whether immunisation of domestic fowl using the recombinant proteins Bm86 and subolesin derived from *B. microplus* ticks and *Aedes albopictus* mosquito, respectively, or urea-extracted *D. gallinae* proteins could control *D. gallinae* using an *in vitro* feeding model.

Material and Methods

Preparation of vaccines

The recombinant *A. albopictus* subolesin orthologue protein vaccine was prepared as described by (Canales et al., 2009). The Bm86 vaccine consisted of the commercial product Gavac™ (Revetmex S.A., Mexico City, Mexico). Protein was extracted from *D. gallinae* using a series of detergents, as described by Harrington et al. (2009). Briefly, mites were frozen in liquid nitrogen, crushed and suspended in phosphate buffered saline (PBS) containing 10% v/v proteinase inhibitor (Sigma, St Louis, USA) and 1% Triton X-100. The suspension was spun at 18,620 *g* for 90 minutes at 4 °C, the supernatant removed and the pellet resuspended in a urea and thiourea based solution, Cellular and Organelle Membrane Solubilising Reagent (COMSR) (Sigma, St Louis, USA). Following sonication on ice and centrifugation, urea and thiourea were removed by a series of ultrafiltration steps using spin columns until a neutral pH was achieved. The mite antigen extract was then passed through a 0.22 µm filter. All proteins were aseptically mixed with the adjuvant Montanide ISA 50 V (Seppic, France), to provide a protein concentration of 50 µg/1 ml dose (Bm86 and subolesin) or 200 µg/1 ml dose *D. gallinae* proteins (DGE). Vaccine for control birds comprised sterile physiological saline and Montanide ISA 50 V, blended aseptically 1:1 saline:adjuvant. All vaccine doses were kept at 2-8 °C until required.

Animals and housing

A total of 48, 16-week old laying hens (Shaver Brown) with no prior exposure to *D. gallinae* (confirmed by ELISA) were used, sourced from a commercial poultry breeder. The birds had been vaccinated according to the typical laying hen

vaccination programme in the UK. Birds were individually identified by numbered leg rings and housed in groups of two in metal cages (0.75m²/bird), whilst water was provided *ad libitum* and birds were fed a commercial non-medicated pelleted ration. Lighting was provided on a cycle of 16 hours light and 8 hours dark. All procedures and bird maintenance were conducted according to the prevailing national legislation on the use of animals in research.

Treatment groups, immunisation schedule and sampling

Birds were randomly allocated to one of four treatment groups (12 birds/group) which consisted of a subcutaneous immunisation on Day 0 (20 weeks of age) and Day 21 with a 1.0 ml vaccine dose made up of Montanide ISA 50V adjuvant plus physiological saline (Control), subolesin (Subolesin) or Bm86 (Bm86) recombinant proteins or DGE. Birds were observed twice daily on the days of vaccination and daily for the rest of the study as part of general health observations. The study was terminated on Day 49. One bird each from the Bm86, Subolesin and DGE groups died (on days 22, 42 and 22 respectively). Birds were subject to a *post mortem* examination by an experienced poultry technician, and cause of death was non-specific in the Bm86 and Subolesin birds, whilst a tear in the uterus was found in the DGE bird. Following discussion with a veterinary surgeon, all deaths were considered unrelated to vaccination. Eggs were collected weekly and retained for use in the feeding assay. IgY was extracted from eggs using Dulbecco's phosphate buffered saline and chloroform following the method of (Hagan *et al.*, 2004) and samples frozen for later analysis by ELISA and use in the *in vitro* feeding assay.

Evaluation of vaccine efficacy

Evaluation of vaccine efficacy was performed using a *D. gallinae in vitro* feeding assay as described by Harrington *et al.* (2009). Briefly, the chamber comprised a clear glass vial (32 x 11.6 mm, length x width) and day-old chick skin (scraped of fat) was stretched across a modified 11 mm silicon/PTFE disc so that the outer surface of the skin was facing into the vial. The skin was secured in place by a snap ring plastic cap. A 0.5 ml tube with the end removed was secured to the top of the lid using modeling clay and acted as a reservoir for fresh blood.

D. gallinae were obtained from a commercial poultry farm and kept at 22 °C in polythene bags for 7–10 days followed by 14–28 days at 4 °C in the dark to allow digestion of the blood meal. Only unfed mites (20 females/feeding chamber) were used. Fresh blood was collected into tubes containing anticoagulant from 20 week old hens with no exposure to *D. gallinae*. Mite chambers were kept in an incubator in the dark at 36 °C and relative humidity 75–85%.

Mites were counted 17 hours after being allowed to feed and the number of mites fed; number of mites unfed; number of dead unfed mites and number of dead fed mites observed. Mites were considered to have fed if they were engorged and/or had changed colour from pale grey/brown to bright red and considered dead if they were unresponsive to stimulation with a needle as described by McDevitt et al. (2006).

IgY extract was added to fresh blood at a ratio 1:4, IgY extract: blood, the blood mixed vigorously briefly and then added to the mite feeding apparatus, 200 µl blood per chamber. IgY extracted from eggs on Day 35 was used for the Subolesin group, whilst IgY extracted on Day 43 was used for Controls, DGE and Bm86 group corresponding to peak egg IgY level in the Subolesin and Bm86 groups. Twelve replicates (6 individual cages repeated twice) per treatment group were used for the feeding assay.

Statistical analysis

Data were tested for normality and where the assumptions were not met, data were transformed if possible. All statistical analyses were performed using Minitab (V 15.0 for Windows, Minitab Inc, State College, USA). General Linear Model command (GLM) was used to analyse the proportion of fed and dead mites, fitting replicate and treatment group as factors in the model. *Post hoc* testing was performed using Tukey's *t*-test. The effect of and Bm86, Subolesin and DGE egg IgY level on *D. gallinae* mortality was analysed by linear regression. Unless stated otherwise, means are presented as arithmetic mean \pm 1 standard deviation (S.D.), shown as non-transformed data. Statistical significance was declared at $P \leq 0.05$.

Results

The mean percentage of fed *D. gallinae* 17 hours after placement in the feeding chambers ranged from 25.8 to 67.0% (Table 1). The mean percentage of fed mites was significantly different between treatments ($F_{3,40} = 5.44$, $P = 0.003$), where it was lower in the Control than Subolesin treatment (34.1 versus 64.5%, respectively,) and also lower in the DGE than Subolesin treatment (42.7 versus 64.5%, respectively). The mean percentage of fed mites in the Bm86 treatment was intermediate, 52.8%.

There was a significant effect of treatment on fed mite mortality ($F_{3,40} = 8.77$, $P < 0.001$), where both the Subolesin and DGE treatments had a significantly higher mortality than the Controls (53.6, 69.2 and 18.5%, respectively), whilst

mortality in the DGE group was also significantly higher than the Bm86 group (41.5%). *D. gallinae* mortality was numerically higher in the Bm86 treatment than Controls, although the difference was not significant.

Regression analysis of mite mortality with Day 43 Bm86 egg IgY level was not significant. However, mite mortality was significantly associated with Day 35 Subolesin egg IgY level (mortality = 0.089 + 0.730 x Subolesin level, $r^2 = 42.9\%$, $P = 0.021$) and Day 43 DGE egg IgY level (mite mortality = 0.258 x DGE level + 0.392, $r^2 = 68.6\%$, $P < 0.01$).

Table 1. Effect of immunisation of birds with recombinant tick or mosquito antigens and DGE on in vitro feeding and mortality of *D. gallinae* after 17 hours. Data shown are arithmetic mean (± 1 S.D.)

Treatment ^a	Replicate	<i>n</i>	% Total Fed	% Dead of Total Fed
Control	1	6	25.8 ^a (± 13.3)	27.1 (± 27.6)
Subolesin	1	6	64.4 ^b (± 22.8)	50.9 (± 16.3)
Bm86	1	6	67.0 ^b (± 13.7)	41.8 (± 27.5)
DGE		6	44.5 ^{a,b} (± 19.7)	71.9 (± 29.8)
Control	2	6	42.5 ^a (± 17.0)	10.0 ^a (± 8.9)
Subolesin	2	6	64.7 ^b (± 17.2)	56.3 ^{b,c} (± 17.7)
Bm86	2	6	38.7 ^{a,b} (± 25.8)	41.2 ^{a,c} (± 33.5)
DGE		6	41.8 ^{a,b} (± 21.9)	66.5 ^{b,c} (± 28.5)
Mean (\pm S.D.)				
Control		6	34.1 ^a (± 10.6)	18.5 ^a (± 16.1)
Subolesin		6	64.5 ^b (± 13.0)	53.6 ^{b,c} (± 7.4)
Bm86		6	52.8 ^{a,b} (± 15.5)	41.5 ^{a,b} (± 21.7)
DGE		6	42.7 ^{a,b} (± 15.1)	69.2 ^c (± 24.7)

Within a column, and within replicate or Mean, means with different superscripts are significantly different at $P < 0.05$

Discussion

The identification of potential antigens for use in a *D. gallinae* vaccine has until recently largely relied upon fractionating whole mite proteins. The study described in this paper reports a comparison of the use of recombinant tick proteins subolesin and Bm86 with known anti-tick activity and proteins extracted from *D. gallinae* itself to immunise poultry for the possible control of *D. gallinae*.

The *in vitro* feeding of mites on blood containing egg-extracted IgY from both subolesin and DGE immunised birds resulted in a significant increase in *D. gallinae* mortality of 35.1% and 50.6% compared to controls, whilst the use of

Bm86 resulted in a numerical 23% increase in mortality, although this was not significant. In addition, both increasing anti-subolesin and anti-DGE antibody level were positively correlated with increased mite mortality. Despite lower levels of IgY found in the egg yolk compared to the hen's plasma, the pattern of IgY response in the hen is still observed in the egg (Hamal et al., 2006), hence egg antibodies used in the feeding assay were representative of those circulating in the hen's plasma. These data confirm that birds can generate protective antibodies against *D. gallinae* following subcutaneous immunisation with the recombinant protein subolesin and somatic proteins from *D. gallinae* when tested in an *in vitro* feeding assay. Anti-subolesin antibodies have been shown to adversely impact upon oviposition in ticks (de la Fuente et al., 2006). In the current study, observations on mite oviposition and egg-hatchability were not performed, but further research in this area could determine the impact of anti-subolesin or *D. gallinae* antibodies on both completion and development of other mite life cycle stages.

There are few reports in the literature on the use of *D. gallinae* proteins to immunise birds against mites, and the data are conflicting. Whilst the current study found a significant increase in *D. gallinae* mortality using somatic *D. gallinae* proteins, work reported by Wright et al. (2009) reported contrasting findings. Wright et al. (2009) found no significant effect on mite mortality using *D. gallinae* proteins extracted with urea and only PBS-extracted proteins resulted in significant increases in mite mortality. In contrast, Arkle et al. (2009) reported no significant effect on *D. gallinae* mortality when mites were fed blood from hens immunised with PBS-extracted mite proteins. However, there were considerable methodological differences between the different studies. Harrington et al. (2009) and Wright et al. (2009) used different egg IgY dilutions and methods of urea-based *D. gallinae* protein extraction, whilst all three authors used slightly different *D. gallinae* feeding assays.

The small size of *D. gallinae* precludes dissection as a means of targeting individual tissues for potential antigen candidates, hence the approach taken in one of the treatment groups in the current study and other reports in the literature to use proteins extracted from whole crushed *D. gallinae*. However the identification of individual proteins which might prove useful as suitable antigens for use in a *D. gallinae* vaccine is difficult. Consequently, the use of either existing or newly defined and specific recombinant proteins is an ideal approach. Currently, there is only one promising report of the use of a recombinant protein, an orthologue of a tick Histamine Release Factor (HRF), and when tested *in vitro* the protein demonstrated a significant 7% increase in mite mortality compared to controls (Bartley et al., 2008). The current study provides data that suggest that existing arthropod recombinant proteins, mosquito derived subolesin, has potential to be used as an antigen in a *D. gallinae* vaccine. The mosquito subolesin orthologue was selected for the current study because it is ancestral to tick subolesin and was therefore more likely to contain protective epitopes conserved between insects and acari (de la Fuente et al., 2006; Canales et al., 2009).

Whilst it is unclear whether the entire subolesin protein is conserved, or if there is similarity of protein epitopes between the recombinant protein and native *D. gallinae* proteins, the *in vitro* mite mortality data described in the current study suggests that the recombinant proteins used in this study are present in some form in *D. gallinae*. The use of existing recombinant tick or mosquito proteins has the potential to shorten the length of time to bring a *D. gallinae* vaccine to market, since early development work such as antigen production methods have already been established. Further research is required to optimize many aspects of both the immunisation procedure and the *in vitro* testing assay described herein. However, this study provides further evidence that immunisation is a real alternative to chemical control of *D. gallinae*.

In summary, the results demonstrated that immunisation of laying hens with either recombinant subolesin or somatic *D. gallinae* proteins can confer significant protective immunity against *D. gallinae* when assessed *in vitro*, whilst immunisation with recombinant Bm86 results in numerically higher mite mortality compared to controls. In the absence of defined antigens for *D. gallinae* control, further research on the use of subolesin and Bm86 as *D. gallinae* vaccine candidates should be explored.

Acknowledgments

This work was supported by a grant from UK funding bodies the Biological and Biotechnology Research Council (BBSRC) and the Yorkshire Agricultural Society and from the INIA, Spain (project FAU2008-00014-00-00). We wish to thank all those at Newcastle University who helped in the conduct of the trial, especially Mr Hatem Mohi El Din, Mr Jim Wightman and finally Dr Robert Shiel for statistical advice.

References

- ALMAZÁN, C., KOCAN, K.M., BLOUIN, E.F. and DE LA FUENTE, J. (2005) Vaccination with recombinant tick antigens for the control of *Ixodes scapularis* adult infestations. *Vaccine* 23: 5294-5298.
- ARKLE, S., HARRINGTON, D., DE LUNA, C., GEORGE, D., GUY, J. and SPARAGANO, O.A. (2009) Immunological control of poultry red mite: the use of whole mite antigens as a candidate vaccine. *Annals of the New-York Academy of Sciences* 1149: 36-40.
- BARTLEY, K., NISBET, A.J., OFFER, J.E., SPARKS, N.H.C., WRIGHT, H.W. and HUNTLEY, J.F. (2009) Histamine Release Factor from *Dermanyssus gallinae* (De Geer): Characterization and *in vitro* assessment as a protective antigen. *International Journal for Parasitology* 39: 447-456.
- CANALES, M., NARANJO, V., ALMAZÁN, C., MOLINA, R., TSURUTA, S.A., SZABÓ, M.P.J., MANZANO-ROMAN, R., PÉREZ DE LA LASTRA, J.M., KOCAN, K.M., JIMÉNEZ, M.I., LUCIENTES, J., VILLAR, M. and DE LA FUENTE, J. (2009) Conservation and immunogenicity of the mosquito ortholog of the tick protective antigen, subolesin. *Parasitology Research* doi10.1007/s00436-009-1368-2.
- DE LA FUENTE, J., ALMAZÁN, C., BLAS-MACHADO, U., NARANJO, V., MANGOLD, A.J., BLOUIN, E.F., GORTAZAR, C. and KOCAN, K.M. (2006) The tick protective antigen, 4D8, is a conserved protein involved in modulation of tick blood ingestion and reproduction. *Vaccine* 24: 4082-4095.
- DE LA FUENTE, J., ALMAZÁN, C., CANALES, M., PERÉZ DE LA LASTRA, J.M., KOCAN, K.M. and WILLADSEN, P. (2007) A ten-year review of commercial vaccine performance for control of tick infestations. *Animal Health Research Reviews* 8: 23-28.
- DE LA FUENTE, J., MARITZ-OLIVIER, C., NARANJO, V., AYOUBI, P., NIJHOF, A.M., ALMAZÁN, C., CANALES, M., PÉREZ DE LA LASTRA, J.M., GALINDO, R.C., BLOUIN, E.F., GORTAZAR, C., JONGEJAN, F. and KOCAN, K.M. (2008) Evidence of the role of tick subolesin in gene expression. *BMC Genomics* 8: 372-387.
- DE OLIVEIRA VASCONCELOS, V., FURLONG, J., DE FREITAS, G.M., DOLINSKI, C., AGUILLERA, M.M., RODRIGUES, R.C.D. and PRATA, M. (2004) *Steinernema glaseri* Santa Rosa strain (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* CCA Strain (Rhabditida: Heterorhabditidae) as biological control agents of *Boophilus microplus* (Acari: Ixodidae). *Parasitology Research* 94: 201-206.
- GALINDO, R.C., DONCEL-PÉREZ, E., ZIVKOVIC, Z., NARANJO, V., GORTAZAR, C., MANGOLD, A.J., MARTÍN-HERNANDO, M.P., KOCAN, K.M. and DE LA FUENTE, J. (2008) Tick subolesin is an ortholog of the akirins described in insects and vertebrates. *Developmental and Comparative Immunology* 33: 612-617.
- GEORGE, D.R., SPARAGANO, O.A.E., PORT, G.R., OKELLO, E., SHIEL, R.S. and GUY, J.H. (2009) Environmental interactions with the toxicity of plant essential oils to the poultry red mite *Dermanyssus gallinae*. *Medical and Veterinary Entomology* doi:10.1016/j.vetpar.2009.01.010.
- HAGAN, J.C., ASHTON, N.J., BRADBURY, J.M. and MORGAN, K.L. (2004) Evaluation of an egg yolk enzyme-linked immunosorbent assay antibody test and its use to assess the prevalence of *Mycoplasma synoviae* in UK laying hens. *Avian Pathology* 33: 93-97.
- HAMAL, K.R., BURGESS, S.C., PEVZNER, I.Y. and ERF, G.F. (2006) Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poultry Science* 85: 1364-1372.
- HAMSCHER, G., PRIEB, B., HARTUNG, J., NOGOSSEK, M.I., GLÜNDER, G. and NAUA, H. (2003) Determination of propoxur residues in eggs by liquid chromatography–diode array detection after treatment of stocked housing facilities for the poultry red mite (*Dermanyssus gallinae*). *Analytica Chimica Acta* 483: 19-26.
- HARRINGTON, D., MOHI EL DIN, H., GUY, J., ROBINSON, K. and SPARAGANO, O. (2009) Characterization of the immune response of domestic fowl following immunization with proteins extracted from *Dermanyssus gallinae*. *Veterinary Parasitology* 160: 285-294.

- KEITA, A., PAGOT, E., POMMIER, P., BADUEL, L. and HEINE, J. (2006) Efficacy of Phoxim 50% EC (ByeMite) for treatment of *Dermanyssus gallinae* in laying hens under field conditions. *Revue De Médecine Vétérinaire* 157: 588-592.
- KIRKWOOD, A.C. (1974). Sorptive dusts for the control of the poultry red mite *Dermanyssus gallinae*. *International Pest Control* 16: 12-15.
- KOCAN, K.M., MANZANO-ROMAN, R. and DE LA FUENTE, J. (2007) Transovarial silencing of the subolesin gene in three-host ixodid tick species after injection of replete females with subolesin dsRNA. *Parasitology Research* 100: 1411-1415.
- MCDEVITT, R., NISBET, A.J. and HUNTLEY, J.F. (2006) Ability of a proteinase inhibitor mixture to kill poultry red mite, *Dermanyssus gallinae* in an *in vitro* feeding system. *Veterinary Parasitology* 141: 380-385.
- NIJHOF, A.M., TAOUFIK, A., DE LA FUENTE, J., KOCAN, K.M., DE VRIES, E. and JONGEJAN, F. (2007) Gene silencing of the tick protective antigens, Bm86, Bm91 and subolesin, in the one-host tick *Boophilus microplus* by RNA interference. *International Journal for Parasitology* 37: 653-662.
- SPARAGANO, O., PAVLIĆEVIĆ, A., MURANO, T., CAMARDA, A., SAHIBI, H., KILPINEN, O., MUL, M., VAN EMOUS, R., LE BOUQUIN, S., HOEL, K. and CAFIERO, M.A. (2009) Prevalence and key figures for the poultry red mite *Dermanyssus gallinae* infections in poultry farm systems. *Experimental and Applied Acarology* 48: 3-10.
- THIND, B.B. and 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. *Veterinary Parasitology* 144: 344-348
- VAN DER GEEST, L.P.S., ELLIOT, S.L., BREEUWER, J.A.J. and BEERLING, E.A.M. (2000) Diseases of mites. *Experimental and Applied Acarology* 24: 497-560.
- WILLADSEN, P. (2004) Anti-tick vaccines. *Parasitology* 129: S367-387.
- WRIGHT, H.W., BARTLEY, K., NISBET, A.J., MCDEVITT, R.M., SPARKS, N.H.C., BROCKLEHURST, S. and HUNTLEY, J.F. (2009) The testing of antibodies raised against poultry red mite antigens in an *in vitro* feeding assay; preliminary screen for vaccine candidates. *Experimental and Applied Acarology* 48: 81-91.