ANTIBODY DETECTION BY ELISA IN CHICKEN INFESTED WITH *DERMANYSSUS GALLINAE* *

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***RESUME**: L'arthropode* Dermanyssus gallinae *est responsable des pertes économiques les plus importantes dans les élevages de poules de nombreux pays.* D. gallinae *est un ectoparasite hématophage qui se nourrit pendant quelques heures durant la nuit. Le reste de son cycle de développement se passe caché dans les crevasses des murs ou des interstices des équipements de production, ce qui rend son éradication très difficile. De plus, de nouvelles réglementations européennes vont interdire l'usage de certains produits acaricides et les élevages en cage devront être remplacés dans quelques années par des systèmes d'élevage en liberté où les attaques de ces parasites sont plus importantes. Les problèmes économiques sont à plusieurs niveaux. Tout d'abord, comme tout parasite hématophage,* D. gallinae *entraîne des démangeaisons et une anémie, ce qui a aussi un impact sur la production des oeufs. Les oeufs des poules les plus attaquées ont des marques rouges sur leur coquille qui est aussi plus fragilisée et, de ce fait, de nombreuses pertes ou manques à gagner sont aussi enregistrées. En Grande-Bretagne, les pertes économiques en 2003 furent estimées à 5,5 millions d'euros. Le but de notre étude est de développer un vaccin contre ce parasite et tout d'abord d'étudier les réactions immunitaires des poules lorsqu'elles sont infestées. Un test ELISA a été développé sur les IgY (similaires aux IgG des mammifères) soit dans le jaune d'oeuf, soit dans le sérum. Les études par ELISA ont révélé une corrélation positive entre les niveaux d'anticorps et les niveaux d'infestation. Des études d'antigénicité ont aussi montré que certaines protéines pourraient être utilisées pour un nouveau vaccin.*

***Mots-clés: P**oulet, ectoparasite, sérologie, vaccin,* Dermanyssus gallinae*, IgY.*

***SUMARY:** The poultry red mite (*Dermanyssus gallinae*) is currently one of the most detrimental ectoparasites in laying birds across several countries. Symptoms of* D. gallinae *infestation include reduction in production, poor egg quality, increased mortality and also a compromise to welfare. Feeding on its host for only short periods of time, the red mite spends the vast proportion of its short life-cycle hidden deep within the house substructure. For this reason, prevalence of red mite is greater in free range or barn systems as opposed to cage systems, since a greater number of potential hiding places can be sought. The problem will therefore be amplified with the impending EU ban on battery cage production in 2012. This, in conjunction with concern over resistance to acaricides, toxicity risks and acaricide withdrawal, make control particularly problematic and financially draining for producers. Therefore alternative methods must be sought, such as vaccine development. However, in order for this to be achieved, an understanding of mite antigenicity must first be established. Thus, the purpose of this study was to assess immunological response of humoral antibodies to naturally occurring mite antigens, using enzyme-linked immunosorbent assay (ELISA) and SDS-PAGE.*

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* Texte de la communication présentée à la Journée AEEMA, 20 mai 2005

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Antibodies were derived from egg yolk and blood sera which were collected from commercial laying farms across the UK with varying levels of red mite infestation and using different production systems (caged, barn and free range). In addition, mites were trapped and counted periodically so as to follow population dynamics over a flock lifespan in conjunction with a series of production measures (eggs produced per bird per week, mortality and temperature). The results describe the effect of red mite infestation on production parameters, immunological response and the relationship between them.

Keywords: *Chicken, vaccine, serology,* Dermanyssus gallinae*, mite, IgY.*

I - INTRODUCTION

The poultry red mite is a temporary haematophagous ectoparasite as it feeds only for short periods on the hen during darkness, once every few days [Kilpinen, 2001]. Red mite shows a preference for laying hens, although they have been known to engorge on a range of hosts, including man [Bruneau *et al.*, 2001]. In commercial egg production, red mite is a serious problem, not only as a potential vector of several avian pathogens, but more importantly as a direct parasite effecting both production and welfare [Nordenfors *et al.,* 1999]. Exposure to red mite may induce a number of symptoms relative to the severity of infestation, including irritation, restlessness, anaemia and occasionally death. This subsequently leads to reduced egg production, from reduced egg weight and increased downgrading as a result of poor shell integrity and superficial blood staining (spotting) from engorged mites which are crushed on egg belts etc. [Chauve, 1998; Cosoroaba, 2001]. Control of red mite is typically undertaken by manual spraying of acaricides, although this method has limitations due to mite resistance and market withdrawal of many acaricides for environmental and toxicity concerns [Chirico and Tauson, 2002].

There is then an urgent need to develop alternative means of control, such as vaccination. Such strategies have been successfully implemented with other ectoparasite species, for example the tropical cattle tick (*Boophilus microplus)*. A concealed membrane-bound glycoprotein (BM86 antigen) was identified and successfully used in a commercial vaccine (TickGARD plus) [Willadsen, 1999]. In addition to this, it has been recently documented that egg yolk-derived chicken immunoglobulin-Y (IgY), the equivalent of mammalian IgG, can be used as a means of immunisation against infectious pathogens [Arasteh *et al.,* 2004]. Extraction of yolk-derived IgY is a non-invasive process and yields higher levels of antibody when compared to serum immunoglobulins [Karlsson *et al.,* 2004].

Therefore the aims of this study were:

1. to assess the immunological response of humoral antibodies, derived from both blood sera and egg yolk, to naturally occurring red mite antigens;
2. to investigate the impact of red mite infestation on egg production;
3. to determine the relationships between red mite population, production parameters and antibody levels.

II - MATERIALS AND METHODS

RED MITE POPULATION

Red mites were collected live using a trapping procedure, modified after Chirico and Tauson [2002]. However, plastic traps were used as a substitute for cardboard ones (ADAS mite monitors, ADAS Ltd., Oxon, UK), and were fixed along the length of each building, situated close to where abundant mite harvests were expected, i.e. close to joints/ openings in the house structure. Traps were left *in situ* for 30 days then removed and placed in individual sealable polythene bags. The traps were subsequently filtered and the mites preserved in alcohol. The number of mites within each trap was estimated by taking a sub-sample of this solution, counting the number of mites and multiplying up to total population. Four different mite stages were identified, and the mean number of eggs, larvae and adults and nymphs (fed and unfed) per sample recorded.

EGG COLLECTION

Between week 20 and 39 of age, eggs were collected every two weeks and thereafter at monthly intervals until week 54 of age. Eggs were processed in order to extract chicken immunoglobulin Y (IgY) via PBS-chloroform IgY extraction and stored at -20°C until required for further analysis.

BLOOD SAMPLING

Venous blood was obtained from a number of hens on occasions when blood samples were required for veterinary diagnostic purposes. On each occasion, 10 randomly selected birds were bled directly from the wing vein to yield a volume of approximately 1ml of blood. Blood was allowed to clot at room temperature and sera were obtained following centrifugation and stored at -20°C, until required for further analysis.

ENZYME-LINKED IMMUNOSORBENT ASSAY

96-well plates were coated with 100µL of unfed mite antigen (10µg/ml) in 0.1M $NaHCO_3$, pH 9.5 and incubated overnight at 4°C. The following day, the plates were washed 3 times with 200µL PBS-T (0.15 M NaCl, 0.02 M Na_2HPO_4, 0.01% Tween 20, pH 7.2). The plates were then blocked using either 5% or 1% BSA (150µL/ well) in PBS for 45 min at 37°C, for blood serum or yolk extract, respectively. Following another three washes in PBS-T, 100µL of blood serum/ yolk extract diluted in PBS-T (1:100) was added and left to incubate at 37°C for 1.5 hours in a water bath and then washed three times in PBS-T, as before. Each well then received 100 µL of a second antibody, which consisted of Anti-chicken IgG conjugated to peroxidase, diluted in PBS-T at concentrations of 1:30,000 and 1:10,000 for blood serum and yolk extract, respectively and once again incubated at 37°C for 1hour in a water bath and washed three times in PBS-T, as before. Finally, 100µL of TMB substrate (SIGMA, T 4444) was added to each well and colour was allowed to develop for 5 min. Optical density (OD) was read at 630nm at two minute intervals for 40 min using a plate reader (ELX 800 Universal Microplate Reader, Bio-tek Instruments, Winooski, USA).

SDS-POLY ACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Mite protein extracts were fractionated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, as described by Laemmli [1970]. Separating gels of 12% acrylamide-bisacrylamide (29:1) and stacking gels of 4% were used. Protein extract was mixed 2:1 with Laemmli sample buffer (SIGMA, S-3401) and boiled for 10 minutes. Approximately 25µg of extract and 5µL of Chemichrome Western Control Molecular weight marker (SIGMA, C-4236) were loaded into gel lanes. Electrophoretic fractionation was performed at 100V for 4 hours until dye front reached interface of stacking/separating gel and at 300V thereafter, for about 12 hours. Gels were washed three times for 5 minutes in deionised water and stained either with Comassie blue for 1 hour or silver stain (SIGMA Proteoprep™ Silver Stain kit, PROTSIL-1) to reveal protein bands. All procedures were carried out at room temperature.

STATISTICAL ANALYSIS

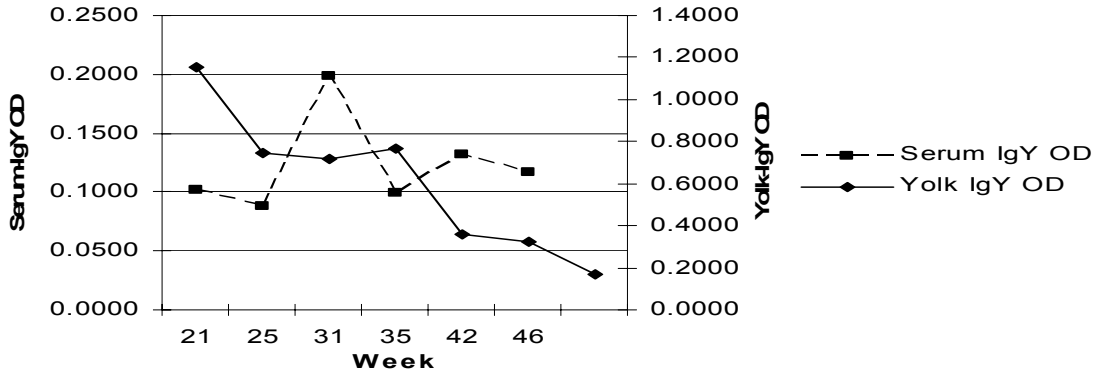
Data were analysed by analysis of variance and Pearson's correlation, which calculated the relationships between serum and yolk antibody levels, mite numbers and production variables, using the statistical package MINITAB for windows (V14).

III – RESULTS

When comparing results across all hen populations, significant differences were observed for mite population and immunoglobulin levels. The free-range and barn housing system had significantly higher total (P<0.001) mite population than either the cage or barn systems. Similarly, yolk IgY levels on Site 1 were significantly higher (P<0.001) in the free-range system compared to the other two housing systems (0.354, 0.760 and 0.550, respectively).

Figure 1

Mean weekly antibody titres (OD) of serum and yolk IgY (site 1)



IV – CONCLUSION

The level of production performance of laying hens used in this study is comparable with typical commercial egg production in the UK suggested by Nix [2005]. Similarly, the level of red mite infestation observed across three different housing conditions is comparable to that previously recorded for UK conditions [e.g. Arkle *et al.,* 2004; Arkle *et al*., 2005; Guy *et al*., 2004] and was significantly higher for birds kept free range. Under these conditions, birds mounted an immune response to red mite, which was significantly higher compared to those kept in either cage or barn systems. A higher mortality of free range birds could be due in part to blood loss, since infestation by red mite leads to hens becoming anaemic [Kilpinen, 2001; Kirkwood, 1967]. In the current study, there was a significant relationship between red mite population and mortality of hens (P<0.05), showing that an increased red mite burden leads to a rise in total bird mortality. This confirms the real economic and welfare consequence of predation by this parasite. Wojcik *et al.*, [2000], for example, estimated that red mite infestation could increase mortality by between 4 and 50% and Cosoroaba [2001] reported reduced egg production by as much as 20% with red mite infestation.

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Acknowledgements

This study was supported by the John Oldacre Foundation and the Yorkshire Agricultural Society.