j.Egypt.net.med.Assoc 77, no 3. 539 – 544 (2017)

SOME STUDIES ON THE POULTRY RED MITES "DERMANYSSUS GALLINAE"

By

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ABSTRACT

Dermanyssus gallinae is the most important ectoparasite affecting egg laying hens and it causes economic losses particularly among egg laying hens. The failure of the conventional control methods to eradicate *D. gallinae* necessitates more studies that can help in development of effective control strategies. In the present study the antigen profile of *D. gallinae* adult male and female parasite has been determined using SDS-PAGE stained with Commassie blue stain, where seven protein bands were identified that have a molecular weight range of 45 - 212kDa.

INTRODUCTION

Dermanyssus gallinae is a hematophagous parasite that infests many bird species. It costs the egg industry up to €130 million annually as a result of its deleterious impact on bird welfare, egg production, and difficulty in control **(Chauve**, **1998**). *D. gallinae* parasites rest during the day in any dark place around the framework of machinery and nest boxes of the poultry house. The mites infest birds and feed for approximately an hour and a half during the night before retreating to these dark hiding places to digest their blood meal after which they can moult, reproduce and lay eggs. Mites can survive for up to 8 months under natural conditions without a blood meal **(Kirkwood 1963)**. The ability of *D. gallinae* to survive for such long periods without a blood meal makes the de-infestation of barns and cages a problem that needs to be addressed before a new flock is introduced, especially in a commercial environment where rapid turnover is a priority. A fallow period of at least 4 weeks between housing separate flocks has been suggested to allow the mite population to decrease **(Beugnet *et al.*, 1997)**.Conventional mite control methods involve spraying of cages and housing with chemical acaricides. However, this is not always effective because mites in cracks and crevices escape acaricides treatment and resistance to pyrethroid has been reported in a number of European countries **(Beugnet *et al.,* 1997; Nordenfors *et al.,* 2001)**. Therefore new method for the control of *D. gallinae* should be explored through finding the

immunogenic protein of this parasite to act as potential vaccine candidates that is the aim of our work.

MATERIAL AND METHODS

Collection and identification of *D. gallinae*:

D. gallinae red mites were collected from chicken in an infested egg laying flock in Giza province. The collected mites were frozen at -20 °C for 24 -72 hours in order to kill the mites. The dead mites were spread over a Petri-dish and counted, separated into adult males, females and nymphs according to their body confirmation **(Baker, 1999)**.

Preparation of sonicated *D. gallinae* antigen (Pruett *et al.*, 2006).

Using tissue grinder 1 gram of poultry red mites was homogenized suspended in 10 mM sodium phosphate buffered saline, pH 7.2. The homogenate was sonicated using lab sonicator (fisher scientific®) for 5 min at 4 °C followed by centrifugation at 14,400 x g for 15 min at 4 °C. The supernatant was collected, pooled and stored frozen at - 20 °C.

Determination of protein content for *D. gallinae* antigens (Lowry *et al*., 1951):

50 ml of (2% sodium carbonate in 0.1 N sodium hydroxide) plus 1 ml of (0.5% copper sulphate, 5 H2O in 1% sodium potassium tartrate) were mixed together. The samples to be measured were diluted in 0.01 M PBS, pH 7.4 to a final volume of 200 ml. one ml from the mixture (2% sodium carbonate in 0.1 N sodium hydroxide and 0.5% copper sulphate. 5 H2O in 1% sodium potassium tartarte) was added to the sample and the different dilutions of standard (PBS) were allowed to stand for 20 minutes at room temperature. A 0.1 ml of folin reagent (1N) was added to each tube and mixed rapidly, then allowed to stand for 20 minutes at room temperature. The standard and the samples were read against blank in the spectrophotometer at 500 nm. A standard curve was plotted by the standard protein solution used. The unknown concentration of the protein was calculated from the slope of the curve.

Determination of D. gallinae antigens profile using SDS-PAGE:

According to **Laemmli (1970)** 6 ml H2O, 4.5 ml separating buffer, 6 ml acrylamyde, 1.5 ml glycerol, 25 µ1APS and 15 µ1 TEMED were mixed and poured to form the separating gel. Then, the stacking gel, formed of3.8 ml H2O, 1.5 ml stacking buffer, 0.7 ml acrylamide, 10 µ1APS, 10 µ1TEMED, was poured. Each sample (10 µ/well**)** was treated with the reducing buffer in the ratio of 1:2. The treated samples were immersed in a boiling water bath for 2 minutes to insure protein denaturation. Pre-stained protein molecular weight marker (Genedirex®) was applied to the first well and used as standard for SDS-PAGE. Avoltage of

100 V was applied and the run was terminated when the bromophenol blue reached the bottom of the gel. The gel was then ready to be stained.

Commassie staining of proteins in SDS-PAGE (Schägger *et al.*, 1988):

The Commassie stain was used to visualize protein in SDS-PAGE. The gel was socked in 150 ml solution (0.25 g Commassie /liter de-staining solution) for 1- 3 hour. The gel was washed several times with de-staining solution (40% methanol, 10% glacial acetic acid and 50% distilled water) till the back ground become completely clear.

RESULT

Concentration of proteins in *D. gallinae* whole parasite sonicates:

The protein concentration of sample (Adult males and females) is 0.76 g /dl.

Antigen profile of *D. gallinae*:

As shown in Fig. (1) seven protein bands have been identified from adult male and female sonicate. These bands were recorded at the following molecularweights: 45, 60, 63, 88.75, 93.75, 135,212KDa.

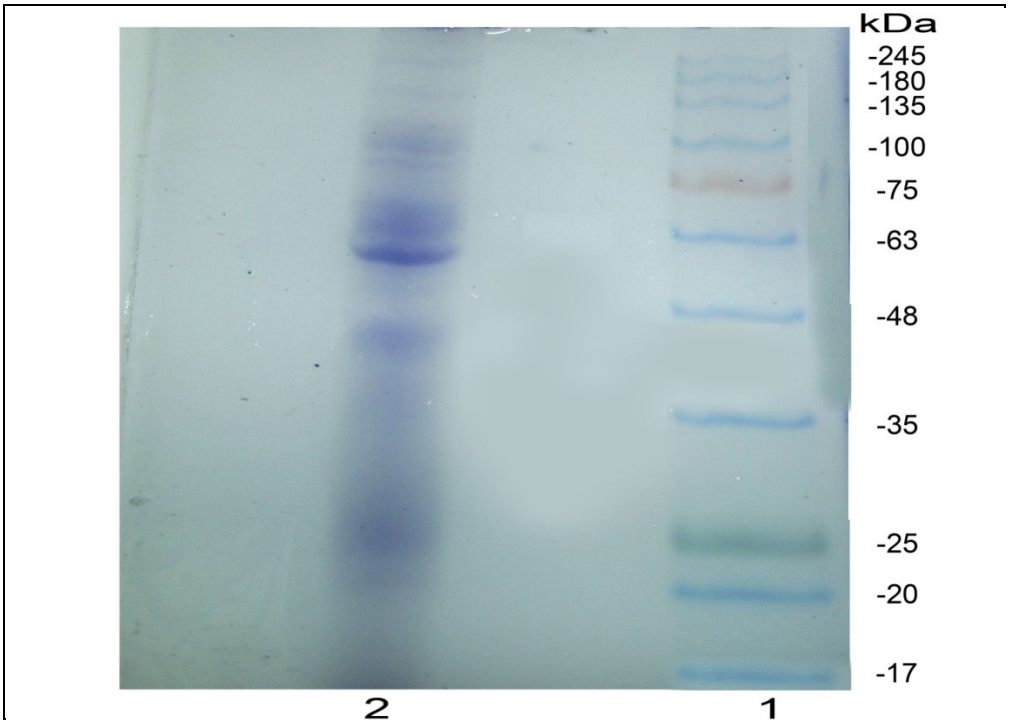

Fig. (1): SDS-PAGE electrophoresis of *D. gallinae* whole parasite sonicates.

DISCUSSION

The red poultry mite, *Dermanyssus gallinae*, is one of the most economically deleterious ecto-parasites of layer hen's worldwide **(Bruneau *et al.*, 2001)**. It can cause Irritation, Restlessness, Mild or severe anemia, occasionally resulting in death. Behavioral observations have also shown increases in cannibalistic feather pecking associated with poultry red mite infestation **(Kilpinen, 2000).** Conventional mite control methods involve spraying of cages and housing with chemical acaricides. However, this is not always effective because mites in cracks and crevices escape acaricides treatment and resistance to pyrethroid has been reported in a number of European countries **(Beugnet *et al.* 1997 and Nordenfors *et al.*, 2001).** Therefore several studies have been achieved to help discovery of alternative mite control methods. **Arkle, (2007)** made mini gel electrophoresis and approved that higher protein concentrations resulted in streaking, whereas lower concentrations failed to generate such an array of bands. Gel lanes containing 5 µg protein produced bands with a variety of molecular weights ranging from low (8 kDa) to high (220 kDa). At least 20 different sized bands were revealed, with several major bands identified at 20, 45, 60 and 120 kDa .Using an in vitro feeding assay allow the identification of protective *D. gallinae* antigens. **Wright *et al.*, (2009)** approved that hens that injected with four protein fractions in Quill A adjuvant revealed a strong antibody response against a range of injected proteins. **(Harry *et al.*, 2009)** showed protein profile of 4 protein fractions injected into hens in a vaccine trail by mini gel electrophoresis stained with simply blue (Invitrogen) stain that show different bands ranging from 14 to 188 kDa. Also **Makert *et al.*, (2016)** identified the mite protein antigens which elicit antibodies with anti-mite activity in the immunized chicken. Recently **Wright *et al.*, (2016)** produced and characterize recombinant forms of *D. gallinae* tropomyosin (Der g 10) and paramyosin (Der g 11) as vaccine candidate molecules. In our work we have demonstrated the antigen profile of *D. gallinae* adult male and female parasite using SDS-PAGE that stained with Commassie stain, where seven protein bands were identified from adult male and female sonicate at molecular weights ranging from 45 to 212 kDa. These findings will help in the future to know immunogenic band of *D. gallinae* and may help in the immunological control of poultry red mites.

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