PRECEPITATING ANTIBODY CONCENTRATIONS AND VISUAL ESTIMATES CORRELATED TO ABSOLUTE NORTHERN FOWL MITE (ACARI: MACRONYSSIDAE) DENSITIES FROM WHITE ROCK HENS

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ABSTRACT

Fifty-four White Rock hens were infested with 2,000 northern fowl mites/bird and six hens were sampled at nine time periods during the 28-week study. Hens supported an average of 15,028 mites when mites were collected from infested bird carcasses digested with a modification of the Cook’s technique. Mite-specific precipitating antibodies were quantified from hen serum samples with rocket electrophoresis. Seventy-four percent of the serum samples gave quantifiable results. A reduction in antibody concentrations was observed with increased mite population densities suggesting that immunosuppressive or tolerance mechanisms may play a role in the host’s humoral immune response to a mite infestation. A visual mite density rating index was more accurate in assessing mite population densities than quantitative antibody measurements.

Key Words: Northern fowl mite, host immunity, rocket electrophoresis, population density.

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The northern fowl mite, Ornithonyssus sylvirum (Canestrini and Fanzago), is one of the most serious arthropod pests affecting poultry production in the United States (DeVaney 1978). As obligate, hematophagous parasites, mites complete their entire five stage (egg, larva, protonymph, deutonymph and adult) life cycle on an avian host. Only protonymphs and adults are known to feed (Sikes and Chamberlain 1954). Large populations (> 50,000 mites/bird) have the potential of consuming 6% of the total blood volume/day of a 1.5 kg hen (DeLoach and DeVaney 1981).

Matthysse et al. (1974) and DeVaney and Ziprin (1980b) detected the presence of mite-specific, precipitating antibodies in the sera of infested White Leghorn chickens with an agar-gel diffusion technique and showed a positive correlation between percentage of positive agar-gel tests and mite infestation severity. Quantitative measurements of mite-specific, precipitating antibody concentrations and comparisons with absolute mite numbers have not been made to date.

Precipitating antibody concentrations ([Ab]) and antigen volumes have been correlated with rocket electrophoresis (RE) (Laurell 1966), and [Ab] can be quantified with this technique. Electrophoretic migration of antigen into antibody-

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Impregnated agarose produces a rocket-shaped precipitate at the site of optimum antibody and antigen concentrations. The area under the precipitate is proportional to antigen concentration ([Ag]) and inversely proportional to [Ab] (area = (K) [Ag]/[Ab], where K is a constant).

The purpose of this study was to quantify mite-specific [Ab] in northern fowl mite-infested White Rock hens with RE and to correlate changes in host's [Ab] with mite density changes. The accuracy of visual estimates and [Ab] measurements were compared with absolute mite densities to determine the reliability of each as a mite monitoring method.

MATERIALS AND METHODS

Fifty-four White Rock hens (Case Farm Hatcheries, Brodbecks, PA) were randomly divided among three similar, adjacent shelters (7.3 x 7.3 m) at The Pennsylvania State University Poultry Research Farm. Hens were caged individually in single deck, double row cage banks and received feed and water ad libitum. At 36 weeks of age (10 February 1983), each hen was infested with 2,000 northern fowl mites/bird. Feathers containing mites were removed from infested birds and held for 24 h in 3.8-liter freezer bags. Individual feathers were then placed on a white enamel tray, and active mites were mechanically aspirated into Pasteur pipets. Collected mites were mechanically blown from the pipet into the vent feathers of a previously uninfested hen.

Mite population densities on all hens were estimated weekly for 28 weeks and prior to sacrifice by parting feathers on the vent, breast and legs. The following visual rating scale was used for mite population estimates: 0 = 0 mites/bird, 1 = 1-2 mites, 2 = 3-9 mites, 3 = 10-31 mites, 4 = 32-99 mites, 5 = 100-315 mites, 6 = 316-999 mites, 7 = 1,000-3,159 mites, 8 = 3,160-9,999 mites, 9 = 10,000-31,586 mites, and 10 = > 31,586 mites/bird. Endpoints for the index were from 0.5-intervals on a log scale that were then converted to real numbers. Birds were selected randomly to avoid any bias resulting from systematic counting.

Blood samples were collected from two randomly selected hens/shelter 2, 4, 6, 8, 12, 16, 20, 24 and 28 weeks after infestation (six hens/sample period). Blood was obtained from a wing vein at 2 and 4 weeks after infestation and by cardiac puncture during the remaining sample periods. Cardiac puncture samples were taken within 1 minute. Collected sera were stored at -20°C.

Following blood sample collection, hens were killed with chloroform and stored at -40°C in 15.1-liter plastic bags. Bird carcass digestion was performed with the Cook's technique (Cook 1954) as modified by Kim (1972) and Lemke et al. (1988). Immature (larvae, protonymphs and deutonymphs), adult and gravid female mites from each hen were counted with a binocular microscope (10X), and the life stage counts were combined for comparisons with antibody concentrations and visual estimates.

Each serum sample (0.5 ml) was analyzed for mite specific precipitating antibody concentrations using RE (Laurell 1966). Plate preparation, duration of electrophoresis run, washing and staining of plates, measurements of precipitates and mite antigen preparation were done according to Burg et al. (1988).

A regression line \( Y = a + bX \) was fitted to the five precipitate area measurements (1,2,3,4 and 5 µl of mite antigen) from each sample (Steel and Torrie 1980), \( Y \) was calculated for \( X = 1 \) and \( 1/Y \) was used as a measure of antibody
concentration. Absolute mite densities were compared with analysis of variance (Steel and Torrie 1980) for shelter and time effects. Differences with $P < 0.05$ were considered significant. Analyses were conducted with StatView 512+™ statistical software (Feldman et al. 1986).

RESULTS AND DISCUSSION

The mean number of mites/hen for the nine sampling periods were not significantly different and no effects due to shelter were observed. White Rock hens supported an average of $15,028 \pm 3,100$ mites/bird. Sixteen hens (29.6%) supported between 16 and 1,942 mites with an average of $669 \pm 165$ mites/bird.

RE analysis for mite-specific antibodies resulted in measurable precipitates in 40 of 54 serum samples (74.1%). Distinct precipitates could not be differentiated from the background stain in six serum samples. Baseline migration during electrophoresis made accurate tracing difficult in 16 cases. Six samples also had faint second peaks, with incomplete bases, indicating the presence of an additional mite-specific antibody. Precipitating antibodies were not detected in 14 serum samples; however, mite densities were not related to the results of the RE analyses. There were no significant differences ($P = 0.72$) in absolute mite densities between hens that showed positive ($16,735 \pm 3,998$ mites) or negative ($10,154 \pm 3,408$ mites) RE results when the data were analyzed with Student's t-test (Steel and Torrie 1980).

DeVaney and Ziprin (1980a) demonstrated a degree of acquired resistance in White Leghorn hens that had been previously infested, then cleaned of northern fowl mites. Their results suggested that circulating antibodies or a cellular immune response may have been responsible for the observed resistance.

Mite population densities were negatively correlated ($P < 0.003$) to antibody concentration (Fig. 1) which suggested a decline in antibody concentration as mite numbers increased. This observed antibody reduction may indicate the presence of partial immunological tolerance or mite-induced immunosuppression. A significant negative correlation between mite density and antibody concentration from the first sample period suggested that precipitating antibody suppression was established within 2 weeks of mite activity on White Rock hens.

White Rock hens that received intramuscular injections of a crude mite extract antigen prior to a northern fowl mite infestation supported similar mite numbers as untreated hens (Burg et al. 1988). DeVaney et al. (1984) bursectomized and splenectomized White Leghorn roosters and demonstrated that a reduction in antibody activity had no effect on northern fowl mite development. Additional immunological work on the localized response to mite feeding in chickens should be studied to determine the effects of cell-mediated immune effector mechanisms on mite population biology.

RE analyses of serum samples was not an acceptable method for assessing mite population numbers due to a small correlation coefficient ($R = 0.46$). There was a significant correlation ($R = 0.91$) between visual mite density rating values and absolute mite densities (Fig. 2). No obvious time-dependent bias was observed with the half log rating scale making it an accurate and reproducible method of estimating northern fowl mite population densities. Adherence to the specific endpoints for each division of the scale is not critical since it is impossible to
Fig. 1. Correlation of absolute northern fowl mite densities and precipitating antibody concentrations (1/Y) from mite-infested White Rock hens. Blood samples were collected prior to hen sacrifice and mite densities were determined from hen carcasses digested with potassium hydroxide (modified Cook's technique).

Fig. 2. Correlation of visual northern fowl mite index values and absolute mite densities from White Rock hens initially infested with 2,000 mites/bird. The index value endpoints were computed from a half log scale (see text for endpoints) and absolute mite densities were determined from hen carcasses digested with potassium hydroxide (modified Cook's technique).
visually distinguish between individual mites on heavily infested birds. A practical visual index could be established using a log scale as a base with the division endpoints rounded off. Divisions at the lower end of the scale (1 through 5) are important for their separation since mites on an infested hen can be counted individually up to at least 100.

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LITERATURE CITED


