Effect of Red Mite (*Dermanyssus gallinae)* Infestation on the Performance and Immune Profile in Vaccinated broiler breeder flocks

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Abstract: The article aimed to: (1) Investigate the impact of red mite infestation on performance of broiler breeders (egg production, mortality and egg- livability). (2) Assess the effect of red mite on immunological response of humeral antibodies (antibody levels) of vaccinated broiler breeders against Infectious bronchitis (IB), Infectious bursal disease (IBD), Avian Encephalomyelitis (AE), Chicken infectious anemia (CAV), Newcastle Disease (ND) and Avian Influenza (H5N1). According to the degree of infestation of the house, the obtained data were arranged into 4 groups: G1: houses of no infestation, G2: low, G3: houses of high and G4: houses of very high infestation. Results revealed that: (1) there were significant differences between the house infestation and mortality rate, egg production as well as egg livability percentage of breeders. (2) There were highly significant differences between the house infestation and the immune response (level of antibodies titre) in vaccinated breeders against:, Infectious bronchitis (IB), Infectious bursal disease (IBD), Avian Encephalomyelitis (AE) , Chicken infectious anemia (CAV) , Newcastle Disease (ND) and Avian Influenza (H5N2). [Journal of American Science 2010;6(8):72-78]. (ISSN: 1545-1003).

Keywords: Degree of infestation, Vaccination, Immune response, Egg performance

1. Introduction

The poultry red mite *Dermanyssus gallinae* is regarded as the most important external parasite of laying hens in organic as well as in conventional egg production in Europe (Maurer *et al.,* 1993; Höglund *et al.*, 1995; Kilpinen and Steenberg, 2009).It attacks the resting hens mainly at night for a short blood meal (Kirkwood, 1968; Lancaster & Meisch, 1986; Guy *et al.*, 2004; Amosova and Stanyukovich, 2008)). After feeding, the mites hide in cracks and crevices, where they also mate and lay their eggs (Hearle, 1938). Under favorable warm and moist conditions, the life cycle can be completed in less than 1 week (Kirkwood, 1968, Nordenfors and Chirico, 1999).

At high infestations, red mites can cause anemia; however low mite populations irritate the hens to an extent that they refuse to go into the henhouse or rest on the perches. The poultry red mite is currently almost impossible to control during the production cycle with traditional measure, and decrease their welfare significantly during egg production may result in poorer hen performance, associated with reduced production and cause decreased feed intake and weight loss (Williams, 2003). Scientific information on the effects of poultry red mite on hens is incomplete as information is mainly sourced from the industry and is not well documented. Researchers agree that there are indications for the following effects of poultry red mite, which include, increased water intake in infested hens and lower egg production from the flock overall(Mul *et al.*,2009).

Infested hens increase their production of new blood cells, but during periods of rapid mite population growth, blood loss exceeds blood production capacity resulting in severe anaemia (Kilpinen *et al.*, 2005). Other negative effects of poultry red mite include high mortality, stress behaviour (higher levels of preening, head scratching and gentle feather pecking), lower body weight and reduced egg quality due to blood spots (Chauve, 1998). The productivity link could be that a severe mite infestation can increase mortality, and as Arkle (2005) showed, there is a direct effect of the size of the mite population on bird mortality. This of course means lower hock productivity: however, lower egg production per hen has not been found as a result of a mite infestation (Kilpinen, 2005). Chicken mites may also act as carrier of several important disease-causing agents, e.g. Salmonella (Zeman *et al*, 1982), spirochaetosis (Hungerford & Hart, 1937), and encephalitis. Some of these survive in the mite for several months, thus forming a potential source of reinfection of new flocks, as the mite can live without feeding for up to 9 months (Nordenfors *et al*, 1999). Chicken mites are also known to cause puritic dermatosis in humans (Baselga *et al*,, 1997) and may create serious problems for workers in the poultry industry due to the nuisance of mites crawling on the skin. Furthermore, it is likely that the mite act as reservoirs for zoonotic bacteria since the mite will hide in the structure and thus be out of reach of the sanitation measures carried out between flocks (Steenberg *et al.*, 2005).

Red mite (*Dermanyssus gallinae* De Geer, 1778) is considered the most economically deleterious (Chauve, 1998). In commercial egg production, red mite is a serious problem, not only as a potential vector of several avian of pathogens, but more importantly as a direct parasite effecting both production and welfare (Nordenfors, *et al.*, 1999; Kilpinen, 2001). Exposure to red mite may induce a number of symptoms relative to the severity of infestation, including irritation, restlessness, anemia and occasionally death. This subsequently leads to reduced egg production (Axtell and Arends, 1990)., 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).

Therefore the aims of this study were: (1) to investigate the impact of red mite infestation on performance of breeders (including egg production, mortality and egg-livability). (2) to assess the probable effect of red mite-infestation on the immune response (antibody levels) of vaccinated breeders against: Infectious bronchitis (IB), Infectious bursal disease (IBD), Avian Encephalomyelitis (AE), Chicken infectious anemia (CAV), Newcastle Disease (ND) and Avian Influenza (H5N1).

2. Material and Methods

Birds and location

Seven red mite (*Dermanayssus gallinae*) infested commercial breeder farms were studied. The farms were environmentally controlled and the densities of farms were ranged between 15,000 to 50,000. Two farms of battery-cage system (five-tier wire battery cages, 5 hens\cage of Ross 308 breed and depended on artificial insemination) and 5 built up litter house system (Avian breed 48) as well as two non-infested commercial built up litter house breeder farms (Avian breed 48); as control (Environmentally controlled with 1:8 male to female ratio).The farms were in Wadi El-Natron, El- Behera and Alexanderia Governorates of Egypt. The farms were surveyed from October 2007 to October 2009.

Vaccination programme (Table1)

All flocks were received the same vaccination programme (Table 1).

Red mite population (Collection and counting of red mite)

Traps (10 traps\house) made of corrugated cardboard measuring 200 × 80 × 3 mm were placed on the floor of built up litter and battery-cage houses. The traps were collected every month to count mites in control and mite-infested houses. At low densities (below approximately 5000 mites/trap), mites were counted individually, but if they were abundant, the total number of mites were estimated by pouring them into a calibrated measuring cylinder and recording the number of mites.

Mites were counted by pouring them into a calibrated measuring cylinder and recording the number of mites (Nordenfors and Chirico, 1999). Score mite count: (1) 0 No evidence of infestation; 250 ≤ 5000 Low infestation; (2) > 5,000 ≤ 8,000 Moderate infestation; (3) >8,000 ≤ 15,000 High infestation and (4) < 15,000 Very high infestation.[Score mite counts (mite\trap)during the study period, were ranged 385- 4890;5588-7443;8227-14880 and 15983-17654 in low; moderate; high and very high infested farms, respectively.

Blood sampling

Venous blood was obtained from hens at 45, 47 and 49 wks of age. On each occasion, 1% randomly selected hens were bled directly from the wing vein to yield a volume of approximately 2 ml of blood. Blood was allowed to clot at room temperature and sera were obtained following centrifugation and stored -20°C, until required for further analysis. An enzyme linked immunosorbant assay (ELISA test-Synbiotics Corporation Company) to measure the antibody titre for Infectious bursal disease (IBD), Infectious bronchitis (IB), Chicken infectious anemia (CVA) and Avian Encephalomyelitis (AE) by using specific antigen for each one. Haemagglutination (HA) and Haemagglutination Inhibition (HI)) tests were performed to assess the immunological responses against both Newcastle Disease (ND) and Avian Influenza (AI). **Enzyme-linked immunosorbent assay** procedures were carried out according to manufactures´ protocol.

Hemagglutination and hemagglutination inhibition (HI) tests:

The recommended method use V-bottomed micro well plastic plates were applied. In which the final volume for both types of HA and HI test was 0.075 ml. The reagents required for these tests are isotonic PBS (0.1 M), pH 7.0-7.2 and RBCs. Positive and negative control antigens and antisera should be run with each test. The test was applied to quantify AIV and NDV antibodies in chicken sera according to OIE (2005)

Plate hemaggluntination inhibition (HI) test

(I) 25 µL of PBS was dispensed into each well of a plastic U-bottomed microtitre plate, except well 1 and 7 of each raw.

(ii) 25 µL of known positive antiserum (Newcastle Disease, Charles River SPAFAS ;) was placed into first and second wells of each raw of the plate.

(iii) From well two and eight of each raw two-fold dilution was made across the plate.

(iv) 25 µL of HA positive virus was added into each well and left for a minimum of 30 min at room temperature.

(v) 25 µL of 0.5% (v/v) chicken RBC was added to each well. After gentle mixing the plate was allowed to keep for about 40 min at room temperature.

(vi) In each raw two wells (six and twelve) were kept as control.

(vii) The hemagglutination inhibition activity was observed after 40 min and compared with control one. HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16 (24 or 4 log-2 when expressed as the reciprocal) or more against 4 HAU of antigen.

Plate Hemagglutination (HA) test

This test was done to detect the hemagglutinating viruses in the collected samples. The procedure of plate hemagglutination test was as follows:

(i) 25 µL of PBS was dispensed in each well of a plastic U-bottomed microtitre plate.

(ii) 25 µL of AF was placed in the first well and mixed well.

(iii) From first well 25 µL of mixture was transferred into second well to make two fold dilutions. This process was continued up to the last well (11th) and from there 25 µL of mixture was discarded. Well 12 was used as control.

(iv) 25 µL of 0.5% (v/v) chicken RBC was dispensed into each well.

(v) The plate was tapped gently and was allowed to keep at room temperature for about 15 min.

(vi) HA was determined by tilting the plate and observing the hemagglutination of the RBC.

(vii) A uniform layer of hemagglutination covering the bottom of well of the plate was considered as positive HA and a sharp buttoning of RBC at the bottom of well of the plate was considered as negative. The titration should be read to the highest dilution giving complete HA (no streaming); this represents 1 HA unit (HAU) and can be calculated accurately from the" initial range of dilutions.

Production performance

A number of additional weekly production parameters were recorded including egg production % (actual egg production), mortality %(actual mortality) and egg- livability %).

Statistical analysis (SPSS, 2006)

Data were analyzed by analysis of variance and Pearson's correlation, which calculated the relationships between serum antibody levels and degree of mite numbers (Infestation).

3. Results and Discussion

According to the degree of infestation of the house, the obtained data were arranged into four groups: - G1: houses of no infestation, G2: low, G3: houses of high infestation and G4: houses of very high infestation. By using the analysis of variance test, we found that, **(1)** there was a difference between the degree of house infestation and the mortality rate, the egg production as well as the egg-livability percentage of breeders, Table [2-a(built up litter and cage systems) and 2-b (built up litter)] showed that, with houses of high or very high infestation having higher mortality rate, lower egg production and lower egg-livability than those that had no or low degree of infestation ($P< 0.05$). The difference between bird mortality and total mite population, suggesting that an increase in mite number (very high infestation) contributes towards an increase in total bird losses. However, the difference is relatively moderate, indicating that the relationships are likely to be effected by other variables. Arends *et al.* (1984) reported decreased egg production and feed efficiency in broiler breeder flocks caused by NFM's (North fowl mites). During two separate 1-year trials, a significant reduction in egg production was produced by mites for only 1 month in one trial, and 2 months in the other. Internal quality of eggs is not affected by the presence of NFM's on hens ((DeVaney, 1979; DeVaney 1981). DeVaney (1978) estimated an annual loss of $66 million due to external parasites causing decreases in egg production; parasite prevention might cost as much as $1.1 million. High densities of mites during egg production may result in poorer hen performance, associated with reduced production and cause decreased feed intake and weight loss (Kirkwood, 1968). The birds become susceptible to other parasites and diseases, also, mortality rate increases especially in heavy infestation (Williams, 2003).

Cencek (2003) investigated the prevalence of *Dermanyssus gallinae* in 10 systems (battery cage, perchery system and deep litter), he found that 2-10 % reduction of egg production in heavy infested farms. The severity of infestation, including irritation, restlessness, anemia 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. (Axtell and Arends 1990;Chauve, 1998; Cosoroaba, 2001). In commercial egg production, red mite is a serious problem, not only as a potential vector of several avian of pathogens, but more importantly as a direct parasite effecting production and welfare (Nordenfors, *et al*,. 1999; Kilpinen, 2001), anemia (CAV), Newcastle Disease (ND) and Avian Influenza (H5N2).

In spite of there were two different systems of housing the results showed that [Tables (3-a and 3-b)], the houses of high and very high infestation had lower

immune titre, than those that had no (Control) or low infestation.

In houses of high (G3) and very high (G4) infestation which vaccinated against AE (Avian Encephalomyelitis), both had lower immune titre (2262±52 and 2440±77, respectively) than those that had no or low degree infestation (7371±73 and 7371±64 respectively) ($P < 0.001$).

Breeder houses (built-up litter system) of very high infestation (G4) which vaccinated against: IB, IBD, CAV, and AI (H5N2) had lower immune titre, than control group. Concerning the degree of infestation (ignoring the house system) ,low or high infestation, G1: houses (No evidence of infestation), G2: (low infestation) and G3: high infestation {(17956±72, 16258±67, 12258±43; 5374±44, 4873±33, 5374±67; 8632±55, 8661±44, 8632±44;8±0.4, 7.6±0.3, 7.6±0.34 and 4.2±0.7, 4±0.22, 4±0.33 ($P < 0.05$) respectively} have higher immune response than those of very high infestation,{ 9894±88, 5092±43, 6938±78, 5.4±0.44 and 3.8±0.34 ($P < 0.05$), respectively}.

Red mite shows preference for laying hens, although they have been known to engorge on a range of hosts, including humans (Bruneau *et al.*, 2001). In commercial egg production, red mite is a serious problem, not only as a potential vector of several avian of pathogens, but more importantly as a direct parasite affecting both production and welfare (Nordenfors, *et al*,. 1996).

In Summary, our data indicated that, (1) red mite infestation has deleterious impacts on the performance of broiler breeders (egg production, mortality and egg-livability). (2) it affects the immune response (Level of antibodies titre) in vaccinated breeders against:, IB(Infectious bronchitis), IBD(Infectious bursal disease),AE (Avian Encephalomyelitis),CAV (Chicken infectious anemia) , NDV(Newcastle Disease) and AV(Avian Influenza) and as a result ,it will probably have a negative effect on the transferred maternal immunity to baby chicks.

Table 1: Vaccination programmed.

Age in days	Type of vaccine	Method of vaccination
1	Infectious bronchitis (IB 120)	Spray
3	Coccidia (Coccivac B)	DW[1]
5	Chicken infectious anaemia (CVA)	S/C inj[2]. (0.2 mL/chieck)
7	Newcastle disease (HB1)	DW
8	Marek's disease	S/C inj. (0.2 mL / chieck)
10	Avian Influenza (H5N2)[a]	S/C inj. (0.2 mL / chieck)
14	Infectious bursal disease (IBD)	DW
19	Newcastle disease (LaSota)	DW
22	Infectious bursal disease (IBD)	DW
28	Newcastle disease (HB1)	DW
32	Infectious bursal disease (IBD)	DW
36	Chicken infection anaemia (CVA)	DW
44	Newcastle disease + Infectious bronchitis	I/M inj[3]. (0.5 mL / chieck)
50	Infectious bronchitis(IB)	DW
55	Fowl Pox	Wing web
58	Infectious Coryza	S/C inj. (0.2 mL / chieck)
65	Fowl Cholera	S/C inj. (0.2 mL / chieck)
77	H5N2	I/M inj (0.5 mL /poullet)
82	Infectious Coryza	S/C inj. (0.2 mL / pullet)
85	Newcastle disease (LaSota)	DW
90	Chicken infectious anaemia (CVA)	S/C inj. (0.5 mL / pullet)
95	Infectious laryngotracheitis (ILT)	Eye drop
105	Avian encephalomyelitis (AE)	DW
110	Infectious bursal disease (IBD)	S/C inj. (0.5 mL / poullet)
115	Fowl Cholera	S/C inj. (0.2 mL / poullet)
122	Infectious bronchitis(IB)	DW
128	Avian Influenza (H5N2) [a]	I/M inj. (0.5 mL/ pullet)

DW[1]: Drinking water; S/C Inj[2]. Subcutaneous injection; I/M Inj[3]: Intramuscular injection, all vaccines manufactured by Schering Plough except Avian Influenza vaccine (H5N2) [a] which produced by Ceva Sante´ company.

Asterisks indicate values that are significantly different from controls. *P <0.05, *: Significance ($P < 0.05$). [1]: 5 built up litter house system (Avian breed 48).

Asterisks indicate values that are significantly different from controls. *P <0.05. *: Significance ($P < 0.05$). [1]: (actual mortality %): the difference between bird mortality and total mite population, suggesting that an increase in mite number (high and very high infestation) contributes towards an increase in total bird losses. [2]: (actual egg production %), [3]: (actual egg livability %), [a]: built up litter house system, [b]: built up litter and cage systems.

Table 2-a. Effect of red mite infestation on Mortality %, Egg production % and Egg livability % in poultry breeders (Environmentally controlled built-up litter and battery- cage systems).

Performance (Mean SD)				
Group	Control(G1)[a]	Low(G2)[b1]	High(G3)[b2]	Very high(G4)[a]
	(n=2)	(n=2)	(n=3)	(n=2)
Parameters				
Mortality %[1]	0.38±0.04	0.45±0.08	0.55±0.1*	0.68±0.2*
Egg production %[2]	74±6.3	71±5.3*	67±6.5*	62±6.3*
Egg livability %	88±3.2	88±8.2	78±9.2*	68±7.2*

Table 2-b. Effect of red mite infestation on Mortality %, Egg production % and Egg livability %in environmentally controlled built-up litter broiler breeders[1]

Performance (Mean SD)				
Group	Control(G1)	Low(G2)	High(G3)	Very high(G4)
Parameters	(n=2)	(n=1)	(n=2)	(n=2)
Mortality %	0.38±0.04	0.42±0.09	0.54±0.2*	0.68±0.2*
Egg production	74±6.3	71.5±7.3*	65.2±8.5*	62±6.3*
Egg livability %	88±3.2	86±8.5	77± 9.4*	68±7.2

Table 3-a. Effect of red mite infestation on immune response in poultry breeders (Environmentally controlled built-up litter and cages).

	Immune responses (Mean SD)			
Group	Control(G1)[a] (n=2)	Low(G2)[b1] (n=2)	High(G3)[b2] (n=3)	Very high(G4)[a] (n=2)
Type of vaccine[1]				
IB	17956±72	16258±67	12258±43*	9894±88**
IBD	5374±44	4873±33	5374±67	5092±43
AE	7371±73	7371±64	2262±52**	2440±77**
CVA	8632±55	8661±44	8632±44	6938±7
ND	8±0.4	7.6±0.3	7.6±0.34	5.4±0.44*
AIV(H5N1)	4.2±0.7	4±0.22	4±0.33	3.8±0.34*

*: Significant ($P < 0.05$). **: Significant ($P < 0.001$). Asterisks indicate values that are significantly different from controls, [1]: three testing of antibody titre at; 45, 47 and 49 weeks of age. [a]: built up litter house system, [b1]: cage systems (1+1), [b2]: built up litter and cage systems (2+1, respectively).

Table 3-b. Effect of red mite infestation on immune response in poultry breeders (Environmentally controlled built-up litter system) [2].

Group	Immune responses (Mean SD) Control (n=2)	Low (n=1)	High (n=2)	Very high (n=2)
Type of vaccine[1]				
IB	17956±72	17254±63	12118±64*	9894±88**
IBD	5374±44	4972±27	5474±44	5092±43
AE	7371±73	7066±43	2252±44**	2440±77**
CAV	8632±55	87631±34	8552±84	6938±78*
ND	8±0.4	7.4±0.3	7.2±0.34	5.4±0.44*
AV(H5N2)	4.2±0.7	4.1±0.12	4±0.63	3.8±0.34*

Asterisks indicate values that are significantly different from controls, *: Significant ($P < 0.05$). **: Significant ($P < 0.001$). [1]: three testing of antibody titre at; 45, 47 and 49 weeks of age; [2]: 5 built up litter house system (Avian breed 48)

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