

## **Acaricidal and Immunological Studies on Fowl Tick *Argas persicus* Infecting Commercial Balady Chickens Flock**

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The present work aimed to study the acaricidal effect of deltamethrin and ivermectin as well as immunization of chicken by whole crude *Argas persicus* larvae extract against *A. persicus* infestation in commercial balady chicken flock. Two experiments were carried out. The first experiment; 120 commercial balady chicken flock of 140 days old naturally infested by *A. persicus* belonged to private poultry farm in Meest- Kenena village Kalubya Governorate were classified into 3 equal groups. Group I was administered ivermectin orally at a dose of 100 mg/kg body weight. Group II was dipped in 0.05% deltamethrin. Group III was kept without treatment as a control group. Birds were kept for 4 weeks and examined weekly at night for counting different stages of the infected ticks. In the second experiment; twenty of 120 days old naïve chickens were classified into two equal groups. The first group (group A) was injected subcutaneously with initial dose of whole crude *A. persicus* larvae extract followed by booster dose after 12 days later. The second group (group B) was injected subcutaneously with physiological saline as a control group. After 7 days from the booster dose, all chicken groups were challenged by natural infection by *A. persicus* infection through keeping them in the same infected poultry house for 28 days. The total number of *A. persicus* larvae on each chicken was estimated weekly for 4 weeks. The present study showed that oral administration of ivermectin caused 100% reduction in the number of *A. persicus* burden in chicken in the 1<sup>st</sup> week post dosing while, dipping of chicken in deltamethrin 0.05% caused prolonged complete (100%) reduction in the number of ticks till the end of the 3<sup>rd</sup> week post dipping. Immunized chickens showed 85.6% reduction in the mean number of infective *A. persicus* larvae and the moulting percentage of larvae was significantly reduced (26.7%) compared to (80%) in non immunized control group. It was concluded that dipping birds in 0.05% deltamethrin or immunization by using double doses of crude extract are better than oral administration of ivermectin

**Key words:** *Argas persicus*, Ivermectin, Deltamethrin, Immunization

### **Introduction**

*Argas persicus* is an important ectoparasite of domestic and wild birds in the world. It causes severe irritation to poultry which has an adverse effect on birds quality and cause heavy economic losses in poultry industry (21 and 24). Heavy infestation causes loss of blood leading to anemia and eventually

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death (4). *A. persicus* also is an efficient reservoir and /or vector for many viruses. rickettsia, spirochaete and mycoplasma (17). As pullorum (27), Mycoplasma gallisepticum and Mycoplasma meleagridis (25), Salmonella gallinarum (9), Aegyptinella pullorum (29), avian encephalomyelitis and leucocytozoonosis (20), West Nile virus (1) and Borrelia anserina (6). *A. persicus* harbours different types of bacteria including those of genus Salmonella, Aerobacter, Escherichia, Proteus, Staphylococcus, Flavobacterium, Pseudomonas and Streptococcus (5). *A. persicus* infestation causes paralysis to heavily infested chickens (26). *A. persicus* larvae have been responsible for simultaneous occurrence of Infectious Bursal Disease and Spirochaetosis (2).

The control of *A. persicus* using chemical insecticides poses health risks and causes environmental problems (18). Additionally, the use of chemicals alone leads to rapid development of resistance (11 and 24)). So the present study aimed to evaluate the acaricidal effect of two new acaricides; deltamethrin and ivermectin against fowl tick as well as immunization of the chicken by repeated injections of laboratory prepared crude *A. persicus* antigen.

## Material and Methods

### Acaricide

1-Butox (Deltamethrin) (1R 3R) – (2, 3 dipromoving) 2-2- diethyl-cyclopropane carboxylate 5 - \* - Cyano-3 phenoxy- benzyl. It is produced by Hoechst Roussel Vet.

2- Ivermectin is a member of the ivermectin group of macrocyclic lactones.

### Preparation of whole crude *A. persicus* larvae extract according to (31).

One hundred and fifty of semi- engorged *A. persicus* larvae were collected from chicken, washed with physiological saline and used for preparation of crude extract. The washed ticks were disrupted and homogenized by using automatic tissue homogenizer in phosphate buffer saline (PBS) PH 7.2 at 4C then filtered by filter paper. The filtrate was centrifuged at 4000/min. in cooling centrifuge, the supernatant was collected. The protein concentration of the extract were estimated and stored at -20C till use according to (7).

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**Experiment I: Effect of Deltamethrin and ivermectin on tick burden of treated chicken.**

One hundred and twenty commercial balady chickens of 140 days old naturally infested by *A. persicus* (some of them showed tick paralysis; Plate I, B) belonged to private poultry farm in Meet- Kenena village Kalubya Governorate were classified into 3 equal groups each consists of 40 hen and treated as follow:

**Group I** was given ivermectin orally at a dose of 100 mg / kg body weight according to (16).

**Group II:** were dipped once in Butox (Deltamethrin) 0.05% according to (19).

**Group III.** Kept without treatment as a control group. All bird groups were left in the same infected poultry house for 4 weeks post treatment and fed on balanced rations and clean source of water.

Birds were examined weekly at night for detection and counting of different stages of *A. persicus* starting from the day of treatment (0 day). Total number of ticks on each bird were estimated by counting the different stages of *A. persicus* ticks attached to the left side and the result was multiplied by two according to (14).

**Experiment II: Effect of immunization of chicken by crude *A. persicus* larvae extract on tick burden:**

Twenty of 120 days old naïve chickens obtained from clean private poultry farm and free from *A. persicus* infection were classified into two equal groups (group A and Group B) and treated as follow: group A were injected subcutaneously with initial dose of whole crude *A. persicus* larvae extract. Booster dose was injected after 12 days later. Group B were injected subcutaneously with physiological saline and used as control group. After 7 days from the booster dose, all chicken groups were challenged by *A. persicus* infection through keeping them in the same infected poultry house for 28 days according to (10). The total number of *A. persicus* larvae on each chicken was estimated every 7 days by counting the larvae attached to the left side and the result was multiplied by two according to (14). Thirty engorged larvae (felling off from chickens and wandering on feathers) were collected from immunized and control chicken groups at the end of the 3<sup>rd</sup>

week Larvae were incubated at 27C and 95% R.H for 12 days according to (12) to evaluate the effect of immunization upon its ability to moult.

### Results

Results in table (1) showed that oral administration of ivermectin caused reduction in the number of *A. persicus* burden in chicken in the 1<sup>st</sup> week post dosing but tick burden increased again at the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week post injection Dipping of chickens in Butox 0.05% caused prolonged and complete reduction in the number of ticks( starting from the first week till the end of the 3<sup>rd</sup> week post dipping.

Table (2) showed that ivermectin caused comparatively lower reduction in the mean number of larvae, nymphs and adults (62.89, 58.92, and 61.84%, respectively) compared to Butox which achieved high reduction (95.06, 98.30, and 97.34%) in the mean number of larvae, nymphs and adults respectively. The acaricidal efficacy of ivermectin against larvae was 100, 92.59, 36.01 and 10.08 during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week respectively compared to 100, 100, 100 and 87.51% reduction caused by deltamethrin during the same weeks. The mean number of *A. persicus* nymphs moderately decreased (the reduction % was 100, 100, 29.18 and 0.00 % during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week, respectively) under the effect of ivermectin administration but dipping in Butox 0.05% caused prolonged and high effect (100, 100, 100 and 92.89 % during the same period, respectively). Similarly, the acaricidal efficacy of ivermectin against adult *A. persicus* lasted for the first two weeks only post- treatment while Butox dipping lasted 4 weeks

Results in table (3) indicated that subcutaneous injection of two doses of whole crude *A. persicus* larvae extract resulted in 85.6% total reduction in the mean number of larvae. Tick burden in immunized chickens in the first week of challenge showed 95.09% reduction compared to non immunized control group (PlateI; C, D and E). Tick burden increased gradually again from the 2<sup>nd</sup> week till the end of the 4<sup>th</sup> week post injection. The moulting percentage of larvae collecting from immunized chickens was significantly reduced (26.7%) compared to (80%) in non immunized control group (Table, 4).

**Table (1): Efficacy of ivermectin and Deltamethrin on the mean number of *A. persicus* burden in chicken for 28 days.**

Days Group	Mean number Before Treatment.	1 <sup>st</sup> week Red. %	2 <sup>nd</sup> week Red. %	3 <sup>rd</sup> week Red. %	4 <sup>th</sup> week Red. %	Total Red. %
Ivermectin	276.65	0.00 100	14.52 94.71	189.78 31.40	267.59 3.27	471.9 61.3
Deltamethrin	281.42	0.00 100	0.00 100	0.00 100	49.73 71.67	49.7 95.9
Control	284.37	290.9 0.00	282.1 0.00	288.23 0.00	351.33 0.00	1215.6 0.00

Red.% = Reduction%

Reduction % =  $\frac{\text{Number of ticks before treatment} - \text{Number of ticks after treatment}}{\text{Number of ticks before treatment}} \times 100$

Table (2): Effect of ivermectin oral dosing and butox0.05% immersion on different developmental stages of tick *A. persicus* infecting chicken for 28 days

week Group	Before Treatment	1 <sup>st</sup> week Red. %	2 <sup>nd</sup> week Red. %	3 <sup>rd</sup> week Red. %	4 <sup>th</sup> week Red. %	Total ticks number	Total Red. %
<b>GI</b>							
Larvae	196.00	0.00 100	14.52 92.59	125.43 36.01	176.23 10.08	316.18	62.89
Nymphs	65.00	0.00 100	0.00 100	46.03 29.18	66.19 0.00	112.22	58.92
Adults	21.65	0.00 100	0.00 100	18.32 15.38	25.17 0.00	43.49	61.84
<b>GII</b>							
Larvae	195.76	0.00 100	0.00 100	0.00 100	42.07 78.51	42.07	95.06
Nymphs	65.13	0.00 100	0.00 100	0.00 100	4.63 92.89	4.63	98.30
Adults	20.53	0.00 100	0.00 100	0.00 100	3.03 85.24	3.03	97.34
<b>GIII</b>							
Larvae	188.71	190.83 0.0	182.2 0.00	193.15 0.00	199.47 0.00	852.22	0.00
Nymphs	67.35	63.49 0.0	70.8 0.00	70.03 0.00	73.88 0.00	273.23	0.00
Adults	28.16	36.58 0.0	25.1 0.00	25.05 0.00	28.25 0.00	113.98	0.00

GI: ivermectin treated GII: immersed in 0.005 deltamethrin solution GIII: control group

% = Reduction%

Reduction % =  $\frac{\text{Number of ticks before treatment} - \text{number of ticks after treatment}}{\text{Number of ticks before treatment}} \times 100$

Red.

**Table (3) Efficacy of whole crude *A.persicus* larvae extract on the mean number of larvae infesting chickens for 28 days**

Weeks groups	1 <sup>st</sup> w. Red. %	2 <sup>nd</sup> w. Red. %	3 <sup>rd</sup> w. Red. %	4 <sup>th</sup> w. Red %	Total Red. %
GroupA Immunized	15.54 95.09	28.43 90.74	39.67 87.3	97.55 69.36	181.2 85.6
GroupB control	316.72 0.00	307.21 0.00	312.60 0.00	318.41 0.00	1254.9 0.0

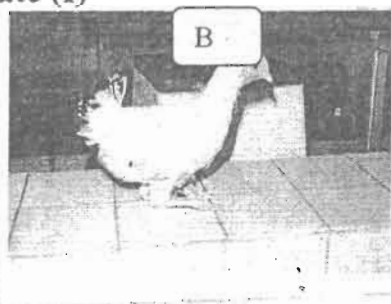
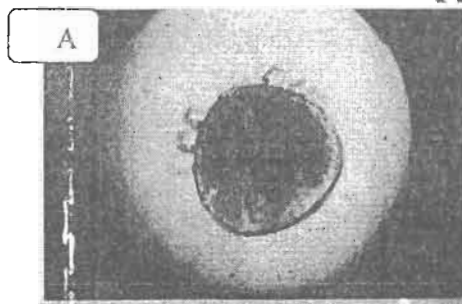
Reduction % =  $\frac{\text{Number of ticks in control group} - \text{Number of ticks immunized group}}{\text{Number of ticks in control group}} \times 100$

Number of ticks in control group

**Table (4): Efficacy of whole crude *A.persicus* larvae extract on moulting percentage of collected larvae from immunized birds**

Group	Stages of ticks	No. of collected larvae	Number. of moulted nymphs	% of moulting
GroupA	Immunized	30	8	26.7
Group B	control	30	24	80

Plate (I)



A = *Argus persicus* larva      B= Chichen showed tick paralysis  
C & D= Immunized chichen showed ligh infestation  
E= Non immunized chichen showed heavy infestation



## Discussion

Most studies dealt with control of ticks infesting birds were carried out in Vitro and few were carried out in vivo (28). In Egypt, control measures against were carried out traditionally by veterinarians depending their experience which lead to development of resistant strains of ticks to traditionally applied organophosphorous acaricides and caused environmental problems. In the present study two synthetic acaricides of low mammalian toxicity; ivermectin and deltamethrin were applied separately to chicken flocks of 140 days old naturally infected by *A. persicus*. Concerning the efficacy of oral administration of ivermectin against fowl tick, the present data indicated that it caused 100% reduction in the mean number of ticks infesting chicken during the first week post administration and 94.71% reduction at the end of the second week while during the 4<sup>th</sup> weeks its acaricidal efficacy was reduced to 3.27%. These results agreed with (16) who mentioned that population of engorged *A. persicus* showed 80, 80, 100, 90 and 75% paralysis in after 3, 15, 24, 48 and 72 hrs respectively when fed on chickens fed a single oral dose of 100 microgram ivermectin per kg body weight. Also (13) recorded that ivermectin had an acaricidal effect against *A. persicus* and achieved 82.96% for its control. Ivermectin also have an adverse effect on *A. persicus* by extensive alteration in its testes and prevent the development of new spermatid and break down of the cell membrane and cytoplasmic organelles of the spermatozoa so it can reduce the number of ticks in the long run (15). Deltamethrin is a synthetic chemical based on Pyrethrin with greater stability and low mammalian toxicity (22) and acts on the insect central nervous system to give a rapid knockdown but it is not used yet in destruction of tick *A. persicus* in commercial chicken flocks (30). The present study dealt with the acaricidal efficacy of deltamethrin dipping against *A. persicus* infection and showed that, it was more effective and had prolonged acaricidal effect compared to ivermectin as it achieved 100% reduction in *A. persicus* burden from the first week to the end of the third week post dipping. This result was agreed with (28) who reported in bioassays against *A. persicus* that deltamethrin was 106 times more effective than chlorvos and dipping of chicken legs in 0.05% deltamethrin resulted in death of 80,90, 100% of adult ticks nymphs and

larvae respectively within one day and its acaricidal effect lasted for 5 days. The more prolonged acaricidal effect of deltamethrin in the present study may attribute to change in the methods of application of the acaricide as (28) dipped the legs of chickens. The prolonged activity of deltamethrin was explained by (30) who noted that the microencapsulation of the pyrethroids prolonged their activity to weeks with greater stability as the microcapsules adhere to the insect skeleton and the pyrethroids absorbed through the chitin to produce its toxic effect.

The present study indicated that subcutaneous injection of two doses of whole crude *A. persicus* larvae extract resulted in total reduction (85.6%) in the mean number of *A. persicus* larvae. The evaluation of whole crude extract prepared from *A. persicus* larvae was carried out by challenged the immunized chickens to natural infection by housing it in infested poultry house. Such challenge permits the immune system of immunized chickens to react with the infested larvae resulting in its destruction. All previous studies dealt with immunization of chicken against *A. persicus* depend on using salivary gland extract or experimentally repeated infestation by adult ticks (23, 10, 7 and 8). Larvae burden infesting immunized chickens showed 95.09% reduction in the first week of challenge compared to non immunized control group. Our result was higher than that of (7) who recorded repeated infestation of spring fowl with *A. persicus* females stimulated 35.9% resistance to tick feeding compared to 20.4% in control. Such high reduction percentages may be attributed to the change of immunization and challenge programs. The present data showed that, the premoult period in larvae collected from immunized chicken was prolonged compared to control group. In this respect, (12) mentioned that *A. persicus* larvae falling off after 5 days attachment, followed by 8 days before moulting to the first nymph. The recorded higher larval mortalities, prolonged premoult period and lowered moulting percentage in our results were explained by (3) who attributed such mortalities to gut damage and the digest cells were sloughed off leaving the basal lamina. Such damage allowed host leukocytes to enter the haemocoel and attack tick muscles, malpighian tubules and blocking synthesis of vitelligenin by the gut cells.

#### **Conclusion**

It was concluded that:

1- Dipping in deltamethrin 0.05% were effective than oral dosing of 100 ug/kg.b.w/ ivermectin in control of *A.persicus* in naturally infested chickens.

2- Whole crude extract prepared from *A. persicus* larvae was effective in reduction of *A. persicus* larvae that infect immunized chickens and consequently reduce the number of developed nymphs and adult ticks in infested poultry house.

Further immunological studies are necessary to study the effect of other safe acaricides, prepare and evaluate a polyvalent vaccine prepared from larvae, nymphs and adults on all stages of *Argas persicus* to minimize the use of harmful acaricides

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دراسات على بعض مبيدات الاكروسات والمناعة ضد قراد الدجاج (ارجس بيرسكس) الذي يصيب  
قطعان الدجاج البلدي التجارية

محمد يوسف رمضان

قسم الطفيليات كلية الطب البيطري بمشتهر جامعة بنها

الملخص العربي

أجريت هذه الدراسة بهدف تقييم كل من الايفرمكتين والبيوتوكس كمبيدي اكروسات وكذلك تقييم المناعة المكتسبة المستحدثة في الدجاج ضد قراد الدجاج. ولهذا الغرض تم اجراء تجربتين:  
- التجربة الاولى وفيها تم اختيار عدد ١٢٠ دجاجة عمر ١٤٠ يوم من قطيع واحد مصاب بالقراد الناعم تم تقسيمهم إلى ثلاث مجموعات متساوية؛ المجموعة الاولى: تم علاجها بمادة الايفرمكتين عن طريق الفم بمعدل ١٠٠ مج /كجم وزن حي مرة واحدة .  
المجموعة الثانية: تم تغطيسها في محلول ٠.٠٥ % من مادة البيوتوكس مرة واحدة . المجموعة الثالثة تركت بدون علاج كمجموعة ضابطة .  
- التجربة الثانية وفيها تم إنتاج وتقييم لقاح مستخلص من يرقات قراد أرجس بيرسكس وقد تم اختيار ٢٠ دجاجة عمر ١٢٠ يوم لم يسبق اصابتها بالقراد الناعم وتم تقسيمهم إلى مجموعتين ؛ المجموعة الاولى حققت بمستخلص اليرقات تحت الجلد جرعة اولي ثم اعيد حقنها مرة ثانية بعد ١٢ يوم . المجموعة الثانية تركت كمجموعة ضابطة. وتم تعرض المجموعتين للعدوي الطبيعية بالقراد وذلك بتربيتهما في حظيرة مصابة بالقراد الناعم و تم عد يرقات القراد بعد كل اسبوع ولمدة اربع اسابيع .  
وجدت الدراسة أن العلاج بالايفرمكتين ادي إلى انخفاض عدد القراد بنسبة ١٠٠ % في الاسبوع الاول فقط أما تغطيس الدجاج في محلول ٠.٥ % من مادة البيوتوكس فقد ادي انخفاض بنسبة ١٠٠ % في عدد القراد من الاسبوع الاول إلى نهاية الاسبوع الثالث. ما وجدت الدراسة أن حقن مستخلص يرقات القراد الخام تحت الجلد في الدجاج ادي إلى انخفاض عدد يرقات التراد التي اصابت الدجاج المحصن بنسبة ٨٥,٦ % . كما ادي استخدام هذا المستخلص إلى انخفاض نسبة انسلاخ هذه اليرقات إلى ٢٧,٦ % مقارنة بنسبة ٨٠ % في الدجاج غير المحصن .