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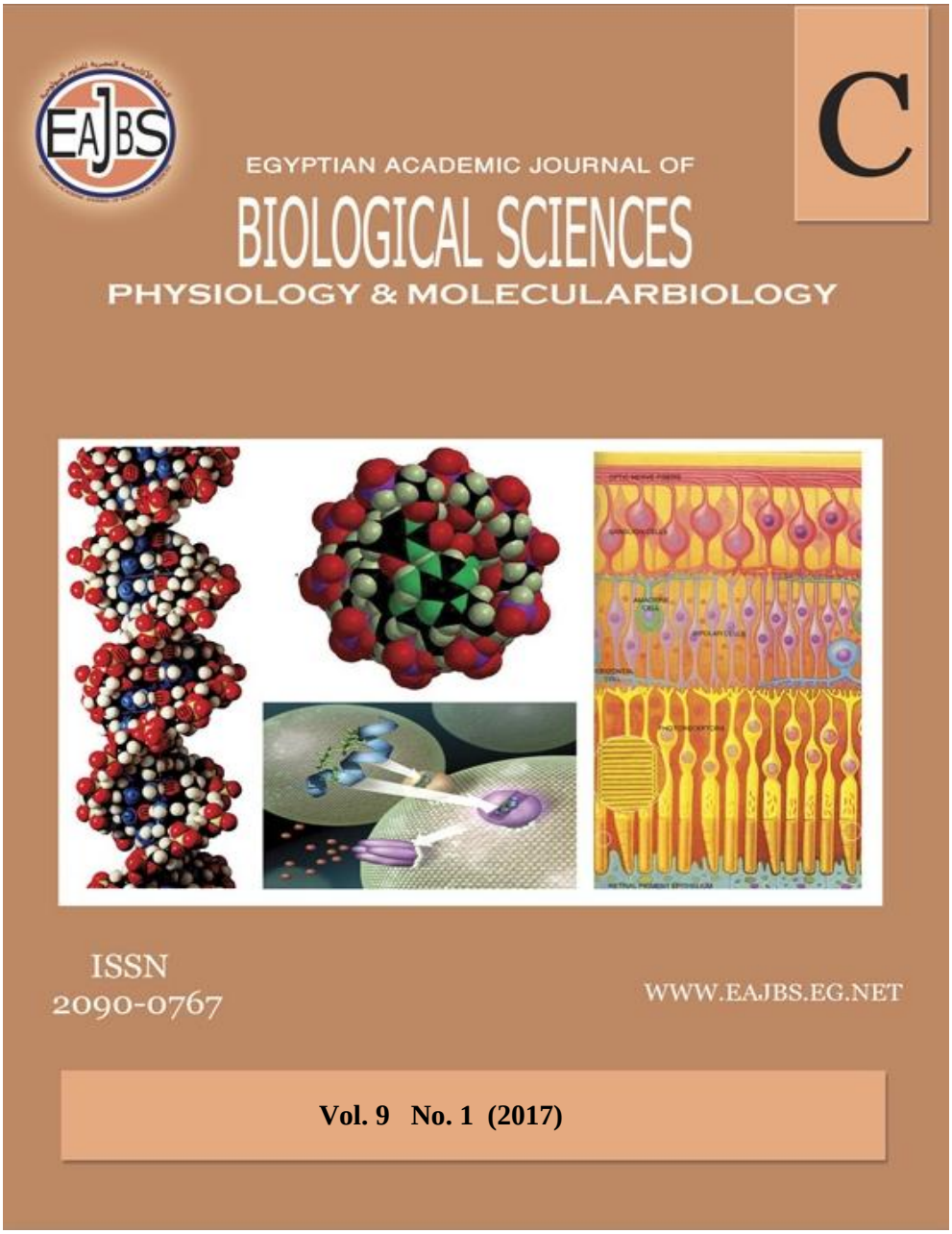

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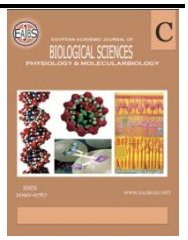

Effect of Borrelial Infection on Haemolymph and Ovarian Protein Concentrations During Vitellogenesis in *Ornithodoros erraticus,* a vector of Relapsing Fever in Egypt

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ABSTRACT

Changes in the total protein concentration of haemolymph and ovaries in *Ornithodoros erraticus* mated fed females uninfected and infected with *Borrelia crocidurae* were studied during vitellogenesis and at the end of oviposition. In the uninfected mated females, engorgement was followed by a decrease in the haemolymph total protein concentration immediately and for the following 2 days after feeding, then the level of haemolymph protein gradually increased on the following days to reach maximum levels on 5 – 9 d.a.f. The ovary total protein level increased gradually on the 1st d.a.f, then the increase continued evidently on the following days to reach a maximum on 9 d.a.f (day of oviposition).At the end of oviposition the level of haemolymph and ovaries total protein largely decreased. The increase in the level of haemolymph proteins was positively correlated with the increase in the ovary total protein, ovary weight and number of mature oocytes inside the ovary.

Changes in the haemolymph and ovaries total protein concentration of mated females infected with *Borrelia crocidurae* followed the same pattern of the uninfected ones. However, borrelial infection reduced the level of haemolymph and ovaries total protein during most of the preoviposition period (3 – 9 d.a.f). Also the weight of ovary and number of mature oocytes were lower in the infected than uninfected on the 5th- 9th and 3rd-9thd.a.f, respectively. Freshly deposited eggs of the infected females had lower total protein concentration than those deposited by uninfected females.

INTRODUCTION

Ornithodoros erraticus is one of three ornithodorine tick species which have been recorded in Egypt as reservoirs and vectors of the etiologic agents of borreliae of endemic relapsing fever (Hoogstraal *et al*., 1954; Hoogstraal, 1985; Shanbaky and Helmy, 2000). The small race of *O. (Pavlovskyella) erraticus* is widely distributed in Egypt (Hoogstraal *et al*., 1954), harbors and transmits *Borrelia crocidurae*, the etiologic agent of the North African relapsing fever (Davis and Hoogstraal, 1954). Ticks become infected by feeding upon spirochaetemic animals and transmit the spirochaetes to man and animals via saliva and coxal fluid during tick bites (Hoogstraal, 1985). Shortly after feeding, the spirochaetes migrate from the midgut of the tick to invade hemocoele (Helmy *et al*., 1996) and almost all tissues including the reproductive system where they multiply and may be transmitted transovarially to the offspring (Gaber *et al*., 1984).

However the dynamics of borrelial infection and penetration of spirochaetes into the ovary and developing oocytes have never been investigated

In ticks, it has been reported that egg yolk proteins originate from both exogenous and endogenous sources (Shanbaky *et al*., 1990b) being synthesized extraovarially in the fat body and gut epithelium and intraovarially in the ovary by oocytes (Araman, 1979). The extraovarial proteins are released into haemolymph as vitellogenins (Diehl, 1969, 1970) to be incorporated into the developing oocytes (Aeschlimann and Hecker, 1967, 1969; Jenni, 1971). Araman (1979) demonstrated that haemolymph vitellogenins and other proteins involved in vitellogenesis utilize a large portion of the digestive products of bloodmeal proteins.

Interaction between *Borrelia* and the vector tick may involve changes in proteins of the spirochaete (Schwan *et al*., 1995) and the tick (Yousery, 2011). Differential gene expression of the outer surface protein has been demonstrated in *B. burgdorferi* during transmission from vector-tick to mammalian host and vice versa. On the other hand, borrelial infection of *O. erraticus* has reduced digestion of the bloodmeal protein in the whole midgut and protease activity in the midgut wall (Yousery, 2011; Shanbaky *et al*., 2011).

The study of structural and physiological peculiarities of the tick reproduction and interaction between pathogen and vector tick is necessary to understand the bases underlying its success as a vector of diseases. The present study provides basic information on changes of protein level in haemolymph, ovary and eggs in mated female *O. erraticus* uninfected and infected with *B. crocidurae* during vitellogenesis and oviposition. Also, the ovary weight and number of mature oocytes inside the ovary are examined.

MATERIALS AND METHODS

Tick rearing:

The argasid tick *Ornithodoros erraticus,* was collected from burrows of the Nile grass rat *Arvicanthis niloticus* in fields or in canal dykes in Monofeya governorate, Egypt. To establish a laboratory colony, the collected ticks were maintained at 28±1 ºC and 75% RH. The hamster, *Mesocricetus auratus* was used as a laboratory host.

Tick colonies:

Samples of the collected ticks were examined for spirochaetal infection with *Borrelia crocidurae* using Fontana stain (Conn *et al*., 1960), or by direct immunofluorescent antibody procedure (Peisman *et al*., 1986). Uninfected F1 adults of field collected ticks, showing no spirochaetes in haemolymph (HL) were used to start the uninfected stock for the laboratory colonies. *O. erraticus* ticks found to be naturally infected with *B. crocidurae* were fed on hamsters. By 4- 7 days later the hamsters developed spirochaetemia were used as the infection source for ticks by feeding the uninfected ticks on them. Uninfected and infected tick colonies were carefully separated. Also hamsters infected with *Borrelia* and uninfected hamsters used for feeding ticks were kept in separate cages.

Experimental groups and selection of ticks:

Two groups of *O. erraticus* ticks were used in this study. The first group was uninfected ticks and the other group was infected with the spirochaete. Virgin females from each group were obtained by keeping engorged 3rd, 4th and 5th nymphal instars individually in separate rearing tubes until molting. Mating was allowed by placing pairs of males and females in the rearing tubes.

Tick dissection and collection of samples:

Samples of haemolymph, ovary and eggs of mated uninfected and *Borrelia crocidurae*-infected adult

females were studied at different physiological stages. The samples were collected from five ticks and each experiment was replicated three times.

Haemolymph (HL):

Haemolymph collection was facilitated by immobilizing the tick by a double- sided tape with the ventral surface exposed. The base of capitulum was punctured with a fine needle and, by application of a moderate pressure on the ventral surface of the tick, the exuding haemolymph was collected by mean of micropipettes of 3 µl capacity. Traces of EDTA (Ethylenediaminetetraacetic acid) were added to HL to prevent coagulation. The HL was centrifuged at 3000 rpm and 4º C for about 10 minutes and the supernatant was collected and stored at -20° C until used.

Ovaries:

The ovaries of 5 female ticks of each physiological state were dissected out, the number of mature oocytes inside the ovary was counted, then the ovary was weighed and homogenized in 50 µl of phosphate buffered saline (PBS). The cell detritus was centrifuged off at 3000 rpm for 20 minutes and the supernatant was stored at - 20ºC.

Eggs:

Freshly deposited eggs were weighed (90 – 100 mg) and homogenized in one ml of PBS and the cell detritus was centrifuged off at 3000 rpm for 20 minutes and the supernatant was stored at - 20ºC.

Total protein determination:

The protein assay method of Bradford (1976) was used in determining the total protein content of the haemolymph, ovaries and eggs samples. Bovine serum albumin used as protein standard.

Five hundred µl of protein dye reagent were added to 10 µl of each sample of the haemolymph, extracts of ovaries and eggs of each physiological stage in the eppendorf and the reagents were mixed thoroughly by vortexing. After 10 minutes the samples were measured spectrophotometrically at 595 nm verses the blank sample. From the standard curve the O. D. was converted to mg protein.

Statistical analysis:

Means of total protein concentration, ovary weight and mature oocytes number were calculated and their statistical significance were determined using Statistica software version 10. Also, the correlation between changes in protein concentration in the ovaries, ovarian weight, and number of oocytes was determined.

RESULTS

Changes of total protein Concentration in the haemolymph of *O. erraticus* female uninfected and infected with *B. crocidurae*:

The haemolymph total protein concentration of uninfected mated fed female *O. erraticus* (Table1) dropped significantly ($p<0.01$) immediately after feeding (4.33 ± 0.29 mg/ml) and to a lesser extent during the 1st and 2nd d.a.f (5.68 ± 0.23 and 8.81 ± 0.50mg/ml, respectively) as compared with that of the unfed females (13.31 ± 0.26 mg/ml). An increase in the haemolymph total protein concentration was observed on 3d.a.f (12.29 ± 0.54 mg/ml) to reach that of the unfed female ($p>0.05$). Then the level of haemolymph total protein increased gradually on the 4th d.a.f ($p<0.05$) and reached maximal levels on 5 -9 d.a.f. On 20 d.a.f (end of oviposition) the level of haemolymph total protein decreased again ($p<0.01$) to approach the level in unfed female ($P>0.05$).

Changes in the haemolymph total protein concentration of mated fed infected female (Table 1) followed the same pattern of that in the uninfected one. The level of haemolymph total protein concentration dropped significantly ($p<0.01$) immediately after feeding (4.10 ± 0.31mg/ml) and to

Table 1: Changes in the total protein concentration in the haemolymph of female *O. erraticus* mated uninfected and *B. crocidurae*-infected on different days after feeding.

Tick physiological state	Uninfected Total protein concentration (mg/ml) mean ± SE (range)	Infected Total protein concentration (mg/ml) mean ± SE (range)
Unfed	13.31 ± 0.26[a] (12.86 – 13.77)	13.11 ± 0.34[a] (12.55 – 13.73)
imm.a.f	4.33 ± 0.29[b] (3.82 – 4.84)	4.10 ± 0.31[b] (3.51 – 4.59)
1 d.a.f	5.68 ± 0.23[b] (5.27 – 6.07)	5.33 ± 0.38[bd] (4.62 – 5.90)
2 d.a.f	8.81 ± 0.50[c] (7.97 – 9.70)	7.81 ± 0.59[d] (6.99 – 8.96)
3 d.a.f	12.29 ± 0.54[a] (11.30 – 13.13)	10.52 ± 0.84[e] (9.21 – 12.09)
4 d.a.f	15.40 ± 0.77[d] (13.86 – 16.29)	13.57 ± 0.58[a] (12.87 – 14.72)
5 d.a.f	21.22 ± 1.12[e] (19.30 – 23.19)	18.62 ± 0.60[f] (17.54 – 19.61)
6 d.a.f	24.81 ± 0.61[f] (23.91 – 25.97)	21.46 ± 0.35[g] (20.83 – 22.02)
7 d.a.f	25.48 ± 0.51[f] (24.75 - 26.46)	23.40 ± 0.34[h] (22.92 – 24.05)
8 d.a.f	24.01 ± 0.52[fg] (23.30 – 25.02)	21.59 ± 0.76[gh] (20.51 – 23.05)
9 d.a.f	22.49 ± 1.41[eg] (19.91 – 24.75)	19.30 ± 0.54[f] (18.36 – 20.21)
20 d.a.f	13.99 ± 0.81[ad] (12.51 – 15.27)	12.96 ± 0.61[ae] (11.93 – 14.03)

*Means bearing different letters (within columns) are significantly different.

a lesser extent during the following three days after feeding compared with that of the unfed female (13.11 ± 0.34 mg/ml).The level of haemolymph total protein concentration increased on the 4th d.a.f to reach the level of unfed female ($p>0.05$). Then the increase continued evidently and reached maximal levels ($p<0.01$) on the following days (5-9 d.a.f). After completion of oviposition on 20 d a.f the level of haemolymph proteins decreased again ($p<0.01$) to reach the level of unfed females ($p>0.05$).

The level of haemolymph total protein concentration of both uninfected and infected females were almost similar up to the 2nd d.a.f ($p>0.05$). However, on 3 -9 d.a.f the level of haemolymph protein in the uninfected females was higher ($p<0.05$) than that on their corresponding infected ones. The level of haemolymph protein approached each other ($p>0.05$) on 20 d.a.f in both females.

Changes of the ovary total protein concentration, weight and number of mature oocytes in *O. erraticus* female uninfected and infected with *B. crocidurae*:

The ovary total protein concentration of uninfected *O. erraticus* female (Table 2) began to increase significantly ($p<0.05$) on the 1st d.a.f (21.82 ± 0.84) mg/gm tissue) as compared with the level of the unfed female (17.05 ± 1.84 mg/gm tissue). Then the increase continued evidently ($p<0.01$) on the next days to reach a maximum level ($p<0.01$) on the 9th d.a.f (109.52 ± 0.70 mg/gm tissue). The level of ovary total protein concentration largely decreased ($p<0.01$) at the end of oviposition on 20 d.a.f (38.17 ± 0.66 mg/gm tissue).

The changes of the ovary weight and in the number of mature oocytes of the uninfected females (Table 2) paralleled the changes of the ovary total protein. These two parameters increased significantly ($p<0.05$) on the 2nd and 3rd d.a.f for the ovary weight and oocytes number, respectively.

Table 2: Changes in the total protein, weight of ovaries and number of mature oocytes in mated uninfected female *O. erraticus* on different days after feeding.

Physiological states	Ovary total protein (mg/g tissue) mean ± SE (range)	Weight of one ovary (mg) mean ± SE (range)	Number of mature oocytes inside the ovary mean ± SE (range)
Unfed	17.05 ± 1.84[a] (14.36 - 20.56)	1.55 ± 0.16[a] (1.34 - 1.86)	0.00 ± 0.00[a] (0 - 0)
imm.a.f	17.17 ± 0.57[a] (16.26 - 18.23)	1.54 ± 0.04[a] (1.48 - 1.62)	0.00 ± 0.00[a] (0 - 0)
1 d.a.f	21.82 ± 0.84[b] (20.96 - 23.49)	1.81 ± 0.04[a] (1.74 - 1.86)	0.00 ± 0.00[a] (0 - 0)
2 d.a.f	31.38 ± 1.25[c] (28.912 - 32.985)	2.06 ± 0.06[b] (1.96 - 2.16)	1.60 ± 0.88[a] (0 - 10)
3 d.a.f	43.19 ± 1.14[d] (41.342 - 45.267)	2.36 ± 0.03[c] (2.3 - 2.42)	24.13 ± 1.31[b] (15 - 32)
4 d.a.f	56.34 ± 1.38[e] (54.389 - 59.01)	2.57 ± 0.04[c] (2.52 - 2.64)	32.67 ± 1.73[c] (20 - 42)
5 d.a.f	70.16 ± 0.69[f] (68.772 - 70.903)	2.88 ± 0.08[d] (2.76 - 3.02)	41.73 ± 1.36[d] (31 - 50)
6 d.a.f	85.14 ± 0.41[g] (84.433 - 85.856)	3.19 ± 0.09[e] (3.04 - 3.36)	43.67 ± 1.43[d] (35 - 53)
7 d.a.f	92.89 ± 0.54[h] (91.859 - 93.666)	3.37 ± 0.04[ef] (3.3 - 3.44)	52.47 ± 2.06[e] (39 - 65)
8 d.a.f	101.77 ± 0.86[i] (100.322 - 103.29)	3.58 ± 0.03[fg] (3.52 - 3.64)	57.27 ± 1.87[f] (45 - 69)
9 d.a.f	109.52 ± 0.70[j] (108.286 - 110.71)	3.73 ± 0.06[g] (3.62 - 3.84)	66.87 ± 1.99[g] (55 - 79)
20 d.a.f	38.17 ± 0.66[k] (36.892 - 39.082)	2.07 ± 0.10[b] (1.9 - 2.26)	2.40 ± 0.61[a] (0 - 8)

*Means bearing different letters (within columns) are significantly different.

Also, the increase continued on the following days after feeding ($p<0.01$) to reach a maximum at the beginning of oviposition (9 d.a.f). A decline ($p<0.01$) in the weight of ovary and number of mature oocytes was observed at the end of oviposition (20 d.a.f). A significant correlation ($p<0.01$) was observed among the three parameters. The correlation coefficients between changes in the ovary total protein concentration versus ovary weight and number of oocytes were 0.996 and 0.978, respectively.

Changes in the ovary total protein concentration of the infected females (Table 3) followed the same pattern of the uninfected ones. The ovary total protein level increased significantly ($p<0.05$) on the 1std.a.f (21.33 ± 0.74mg/gm tissue) as compared with the level of the unfed female (15.93 ± 0.54mg/gm tissue). The increase of ovary total protein level continued on the following days ($p<0.01$) to reach maximum level on the 9th d.a.f (104.79 ± 0.27 mg/gm tissue). The level of ovary total protein concentration largely decreased ($p<0.01$) on 20 d.a.f (35.18 ± 0.76 mg/gm tissue).

The changes in the ovary weight and in the number of mature oocytes inside the ovaries of the infected females (Table 3) paralleled the changes of the ovary total protein. These two parameters increased significantly ($p<0.05$) on the 2nd and 3rd d.a.f for the ovary weight and oocytes number, respectively. Also the increase continued on the following days ($p<0.01$) to reach a maximum at the beginning of oviposition (9 d.a.f). The weight of ovary and number of mature oocytes greatly declined ($p<0.01$) at the end of oviposition (20 d.a.f). A significant correlation ($p<0.01$) was

observed among the three parameters. The correlation coefficients between changes in the ovary total protein concentration versus ovary weight and number of oocytes were 0.995 and 0.970 respectively.

Table 3: Changes in the total protein, weight of ovaries and number of mature oocytes in mated *B. crocidurae*-infected female *O. erraticus* on different days after feeding

Physiological states	Ovary total protein (mg/g tissue) mean ± SE (range)	Weight of one ovary (mg) mean ± SE (range)	Number of mature oocytes inside the ovary mean ± SE (range)
Unfed	15.93 ± 0.54^{a} (14.878 - 16.68)	1.52 ± 0.14^{a} (1.3 - 1.78)	0.00 ± 0.00^{a} (0 - 0)
imm.a.f	16.59 ± 0.42^{a} (15.982 - 17.391)	1.53 ± 0.15^{a} (1.26 - 1.78)	0.00 ± 0.00^{a} (0 - 0)
1 d.a.f	21.33 ± 0.74^{b} (19.897 - 22.348)	1.78 ± 0.10^{ab} (1.66 - 1.98)	0.00 ± 0.00^{a} (0 - 0)
2 d.a.f	27.03 ± 0.86^{c} (25.88 - 28.702)	1.91 ± 0.08^{bh} (1.78 - 2.04)	0.00 ± 0.00^{a} (0 - 0)
3 d.a.f	38.28 ± 1.81^{d} (34.658 - 40.199)	2.28 ± 0.11^{c} (2.1 - 2.48)	20.27 ± 1.63^{b} (10 - 30)
4 d.a.f	48.02 ± 1.25^{e} (45.629 - 49.825)	2.42 ± 0.05^{cd} (2.32 - 2.5)	27.07 ± 1.40^{c} (18 - 36)
5 d.a.f	55.53 ± 1.02^{f} (54.026 - 57.479)	2.53 ± 0.03^{d} (2.49 - 2.58)	36.00 ± 1.60^{d} (25 - 45)
6 d.a.f	68.93 ± 1.50^{g} (66.55 - 71.693)	2.84 ± 0.04^{e} (2.76 - 2.89)	39.87 ± 1.45^{e} (30 - 50)
7 d.a.f	83.19 ± 0.58^{h} (82.404 - 84.322)	3.13 ± 0.07^{f} (2.98 - 3.21)	43.80 ± 1.38^{f} (35 - 53)
8 d.a.f	97.31 ± 1.50^{i} (95.002 - 100.115)	3.34 ± 0.04^{fg} (3.27 - 3.4)	53.33 ± 1.37^{g} (43 - 63)
9 d.a.f	104.79 ± 0.27^{j} (104.414 - 105.301)	3.57 ± 0.06^{g} (3.48 - 3.67)	57.80 ± 1.83^{g} (45 - 69)
20 d.a.f	35.18 ± 0.76^{k} (33.849 - 36.498)	1.96 ± 0.08^{h} (1.84 - 2.12)	2.73 ± 0.67^{a} (0 - 8)

*Means bearing different letters (within columns) are significantly different.

Changes in the ovary total protein concentration of uninfected and infected females are almost similar up to the 2nd d.a.f. The levels of ovary total protein concentration are significantly greater ($p<0.05$) in the uninfected than the infected females on the 3rd to the 9th d.a.f, then the levels of protein approached each other at 20 d.a.f. Also the weight of ovary and number of mature oocytes of both females are similar ($p>0.05$) up to the 4th and 2nd d.a.f, respectively. However, the weight of ovary and number of mature oocytes are higher ($p<0.05$) in the uninfected than the infected on 5-9 d.a.f for ovary weight and 3-9 d.a.f for oocytes number. At the end of oviposition (20d.a.f) the weight of ovary and number of oocytes of the uninfected and infected females became similar ($p>0.05$).

Total protein of the eggs uninfected and infected with *B. crocidurae*:

Freshly deposited eggs laid by uninfected females had a total protein of 26.64±1.26 mg/g tissue. However, *B. crocidurae* infected females laid eggs with lower ($p<0.05$) total protein of 22.53±1.43mg/g tissue.

DISCUSSION

Total protein in the haemolymph, ovary and eggs of *O. erraticus* mated females:

The level of haemolymph total protein concentration of uninfected *O. erraticus* females largely decreased immediately after blood feeding and to a lesser extent on the 1st and 2nd days after

feeding. A similar decrease was observed in the haemolymph of *Argas hermanni* (Shanbaky *et al.*, 1990a) and *Argas persicus* (Radwan *et al.*, 2015). This decrease was mainly attributed to dilution of haemolymph during concentration of the ingested blood meal inside the gut (Hefnawy, 1972; Hesse, 1984).Both authors observed an increase in the volume of haemolymph of *Argas persicus*, *Argas arboreus* and *Ornithodoros moubata* for a few days after feeding then the haemolymph volume gradually decreased until oviposition.

In the present study, the observed decrease in haemolymph protein titer imm.a.f was followed by a gradual increase on the next days to reach the level of the unfed female on the 3rd d.a.f. Then the increase continued evidently on the next days to reach a maximum on the 5th – 9th d.a.f. This period of increase coincided with the rapid phase of digestion reported in argasids (Tatchell, 1964; El Shoura, 1988) and could be attributed to the large demand of protein for vitellogenesis. An increase in the vitellogenin (major haemolymph yolk protein precursor) concentration in the haemolymph was observed in *Ornithodoros moubata* and reached a peak on the 5th d.a.f (Chinzei, 1983). Similarly the level of vitellogenin concentration of *Hyalomma dromedarii* begins to increase 2 days after feeding with a peak on day 4 (Schriefer, 1991).

In the present study the level of haemolymph total protein concentration largely decreased at the end of oviposition (20d.a.f) to reach the level of the unfed female. This decrease might be due to consumption of large amounts of protein by the ovary for vitellogenesis (Shanbaky *et al*. 1990a).

Total protein concentration in the ovaries of *O. erraticus* uninfected mated females gradually increased on the 1st d.a.f and the increase continued evidently on the following days to reach maximum on the 9th d.a.f. The increase in the ovary total protein coincided with an increase in its weight and the number of oocytes inside. The increase in the ovary total protein was positively correlated with increase of haemolymph total protein. A similar correlation was observed in the Argasid tick *Argas hermanni* (Shanbaky *et al*., 1990a). Also a positive correlation between haemolymph vitellogenin concentration and ovary vitellin was observed in the ixodid tick *Ixodes scapularis* (James and Oliver, 1996). Diel (1969; 1970) and Jenni (1971) demonstrated the endogenous protein synthesis by the oocyte and the uptake of the exogenous haemolymph vitellogenin inside the oocytes by micropinocytosis in female *O. moubata* during vitellogenesis. Also the ability of the pedicel cells of the ovary to provide yolk precursors for oocytes development during vitellogenesis was observed in *Amblyomma cajennense* (Denardi *et al*., 2004) and *Amblyomma triste* (Olivera *et al*., 2007). Thus the increase in the ovary total protein, ovary weight and the number of mature oocytes inside it could be attributed to either synthesis of yolk proteins inside the ovary or the uptake of vitellogenin from the haemolymph. On 20 d.a.f the level of ovary total protein largely decreased as the tick ceases oviposition. At this time there were a few oocytes, if any, in the ovary. The weight of the ovary also decreased in a parallel way.

The effect of *B. crocidurae* infection on the total protein in the haemolymph, ovary and deposited eggs of *O. erraticus* mated females:

The level of haemolymph, ovary and eggs total protein concentration of the infected mated females followed the same pattern of the uninfected ones. However, infection of *O. erraticus* with *B. crocidurae* caused significant decrease in the level of haemolymph and ovary total protein on the 3rd – 9th d.a.f. Also the weight of ovary and number of

mature oocytes inside it decreased on the 5^{th} – 9^{th} and 3^{rd} – 9^{th}d.a.f, respectively. *Borrelia crocidurae* ingested with the blood meal migrated from the midgut to the haemolymph by penetrating the midgut wall either intercellularly by penetrating the spaces between midgut epithelial cells or intracellularly by entering the midgut epithelium during engulfment of blood meal (Helmy *et al*., 1996). A decrease in bloodmeal protein digestion (Yousery, 2011) and proteolytic activity (Shanbaky *et al*., 2011) was observed in the gut of *Ornithodoros erraticus* infected with *B. crocidurae*. The authors attributed this decrease to the impairment of the function of the midgut epithelial cells during the penetration of *B. crocidurae*. Vitellogenin, the main yolk protein precursor constitutes a large proportion of the haemolymph protein to reach about 85% of the total protein in the haemolymph of *Argas hermanni* during vitellogenesis (Helmy, 1988). This protein is synthesized mainly by the fat body and midgut, released in the haemolymph then transported to the ovary as observed in *O. moubata* (Horigane *et al*., 2010). Thus the observed decrease in the haemolymph total protein concentration might be a result of the reduction of digestion and the impaired function of the midgut cells which caused a decrease in vitellogenin production by the gut cells. As a result of the decrease of the level of haemolymph proteins, less nutrients were transported to the ovary as was observed in the decrease of the ovary total protein level. The decrease in the level of ovary total protein was accompanied by a decrease in the ovary weight and number of mature oocytes.

Borrelial infection caused the female to lay eggs with a lower total protein levels than eggs laid by the uninfected females. The decrease in the level of eggs total protein could be attributed to less nutrients obtained by the developing oocyte. A similar observation of lower level of yolk protein production was observed in *Rhipicephalus microplus* infected with *Babasia bovis* (Rachinsky *et al*., 2007). Yousery (2017) detected *Borrelia crocidurae* in the ovary of infected female *O. erraticus* and localized the spirochaete histologically inside the oocyte which might have adversely affected or interfered with the embryonic development and hatchability of the egg.

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ARABIC SUMMERY

تأثير الإصابة *بالبوريليا* علي بروتينات الهيموليمف والمبيض خلال فترة تكوين البيض في *الأورنيثودورس إيراتيكاس*, الناقل للحمي الراجعة في مصر.

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تمت دراسة التغيرات في تركيز البروتين الكلي في هيموليمف ومبايض إناث *الأورنيثودورس إيراتيكاس* المتزاوج والمغذي غير المصاب والمصاب *بالبوريليا كروسيديوري* خلال فترة تكوين البيض وبعد وضع البيض. تبعت تغذية الإناث المتزاوجة غير المصابة بنقص في تركيز بروتينات الهيموليمف مباشرة ولمدة يومين بعد التغذية. ثم بدأ مستوي بروتينات الهيموليمف يزداد تدريجيا في الأيام المتتالية لتصل إلي أعلي مستوي من اليوم الخامس إلي التاسع بعد التغذية. وقد زاد مستوي بروتينات المبيض في اليوم الأول بعد التغذية واستمرت الزيادة في الايام التالية لتصل إلي أعلي مستوي في اليوم التاسع بعد التغذية (بداية وضع البيض). ثم انخفض مستوي بروتينات الهيموليمف والمبيض بعد إنتهاء وضع البيض بصورة كبيرة. وقد كانت الزيادة في مستوي تركيز بروتينات الهيموليمف متزامنة مع الزيادة في مستوي بروتينات المبيض ومع وزن المبيض عدد البويضات الناضجة داخل المبيض.

وقد تشابه نمط التغيرات في مستوي تركيز بروتينات الهيموليمف والمبيض في الإناث المصابة *بالبوريليا كروسيديوري* بالإناث غير المصابة. ولكن كان مستوي بروتينات الهيموليمف والمبيض أعلي في الإناث غير المصابة عنها في الاناث المصابة في معظم فترة ما قبل وضع البيض (من اليوم الثالث الي التاسع). أيضا كان وزن المبيض وعدد البويضات الناضجة داخل المبيض أعلي في الإناث غير المصابة عن المصابة. وقد كان تركيز بروتينات البيض الذي وضعته الإناث المصابة اقل من تركيز بروتينات البيض الذي وضعته الإناث غير المصابة