Egypt. J. Vet. Sci., pp. 1-10

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Biological and Histological Studies on Tick-Borrelia Interaction During Reproduction and Transovarial Transmission of *B. Crocidurae* in Female *Ornithodoros erraticus*

Amira E. Abdel-Hamid[1], Ayat Yousery[1], Nawal M. Shanbaky[1], Nadia Helmy[1], Doaa E. Soliman[1], Hala M. Khater[2] and Shimaa S. Ahmed[1]*

[1] *Entomology Department, Faculty of Science, Ain Shams University, Cairo. Egypt.*

[2] *Biochemistry Researcher, Research and training center on vector of Disease, Ain Shams University, Cairo. Egypt.*

Abstract

Borrelia crocidurae infection of female *Ornithodoros erraticus* reduced the fecundity and fertility of the infected female tick, decreasing its egg production and viability. Microscopic examination of the semithin sections of the infected female ovary revealed five developmental stages of the oocyte as in the uninfected female. However, in the infected females, *Borrelia spirochetes* were apparently transported to the ovary via its surrounding hemolymph (HL) to invade oocytes during all their stages of development. The borrelial spirochetes were localized adhering to and directly penetrating oocytes of the early stages ɪ and ɪɪ having no chorion and protruding into HL. Also, B. spirochetes penetrated the ovary epithelial wall to be localized inside the pedicels then moved to enter the ovary lumen and attached oocytes including those not fully chorionated such as the vitellogenic oocyte stage ɪɪɪ. *Borrelia spirochetes* seemed to pass through the unchorionated points of oocytes contact with pedicels to spread throughout the developing oocytes. *Borrelia spirochetes* were detected adhering to and inside the closely packed large yolk granules in the mature oocyte stage V. The noticeable disappearance of chorion in some infected ovulating oocyte stage V subjected them to penetration and further invasion by B. spirochetes which have entered the ovary lumen.

Keywords: Softtick, Transovarial, Chorion, Yolk granules.

Introduction

The small race of *Ornithodoros* (*Pavlovskyella*) *erraticus* Lucas is widely distributed in Egypt [1], harbors and transmits *Borrelia crocidurae*, the etiologic agent of the North Africa relapsing fever [2]. *B*orrelia theileri the etiologic agent of bovine borreliosis has been demonstrated in the blood of cattle and live stock Europe, North America, and Asia [3,4] . The tick becomes infected by feeding on spirochetemic animals and transmits the spirochetes to man and animals via saliva and coxal fluid during tick bites [5]. Shortly after feeding the spirochetes migrate from the digestive tract of the tick to invade the hemolymph [6] to be transported to almost all internal organs including ovary where they may be transmitted transovatially (Tov) via the infected eggs to the offspring [7]. Physiological, biological and ecological studies on the vector argasid tick, *O. erraticus* infected with the spirochete, *B. crocidurae*, have shown evident interrelationships and interactions between the tick vector and the pathogen [2,6-10,11-15)].The parameters defining vector competence of ticks for pathogens such as acquisition, maintenance and transmission to the vertebrate host have been studied by several authors [16-19].

The interaction may influence the tick reproduction by direct invasion of the tick ovary as in the Tov transmission of *B. crocidurrae* from the infected female *O. erraticus* to its progeny [7]. However, indirect effects of the spirochetal infection on reproduction of the female tick might result from an interference with some other physiological processes in such a way that deprives the tick from enough or essential proteins [14,15,20] which are required for oocyte maturation and completion of egg development [21,22].

*Corresponding authors: Shimaa S Ahmed, E-mail: dr.shimaa.salah77@gmail.com, Tel.: 01212023356
(Received 08 November 2024, accepted 19 December 2024)
DOI: 10.21608/EJVS.2024.334411.2479

The reproductive potential and the Tov transmission of the spirochetal infection from one generation to another are two of several factors affecting the tick vector potential in spreading disease [5, 23]. The study of structural and physiological peculiarities of the tick reproduction and interaction between a pathogen and vector tick is necessary to understand the bases underlying its success as a vector of disease which may help in the tick and disease control.

The present study investigates the effect of *B. Crocidurae* infection on some biological parameters of reproduction of female *O. erraticus*. Also, it histologically investigates effects of the borrelial infection on oocytes development and localizes *B. Crocidurae* spirochetes in the tick ovary and oocytes during development, maturation and ovulation, in order to elucidate the spirochete dynamics and interactions in the tick ovary during reproduction and Tov transmission of the pathogen.

Material and Methods

Tick collection and rearing:

The argasid tick *Ornithodoros* (Pavlovskyella) *erraticus* Lucas, was collected from burrows of the Nile grass rat *Arvicanthis niloticus* in fields or in canal dykes in Monofeya governorate, Egypt. The collected ticks were kept in laboratory at 28±1˚C, 75% R. H. and 16 hr. light, fed on the hamster *Mesocricetus auratus* and reared as described by [24].

Tick colonies:

Samples of the collected tick were examined for spirochetal infection with *Borrelia crocidurae* using Fontana stain [15,25] and direct immunoflouresenct technique [26)]. Uninfected F1 adults of field collected ticks showed no spirochetes in hemolymph (HL) were used to start the uninfected stock for the laboratory colonies. Ticks found to be naturally infected with *Borrelia crocidurae* were fed on hamsters. By 4-7 days later the hamsters developed spirochetemia [15] were used as a source for infection by feeding the uninfected ticks on them. Uninfected and infected ticks colonies and hamsters were carefully separated away from each other. The infection rates in the females and unfed larvae were regularly inspected to make sure of the existing high levels of infection and transovarial transmission (Tov) in the infected colony [15].

Effect of *B. Crocidurae* infection on reproduction of female *O. erraticus*:

In order to assess the effect of *B. Crocidurae* on the fecundity and fertility of female *O. erraticus*, 15 virgin females of each uninfected and infected ticks were fed and placed with freshly engorged uninfected males as pairs in separate rearing tubes and observed daily for oviposition. Eggs were collected and counted daily until the female ceased oviposition. The preoviposition, oviposition and incubation periods, mean egg production/ oviposition/ female, daily egg output /female and viability of the eggs were recorded for uninfected and infected female ticks.

Histological studies:

Ornithodoros erraticus females uninfected and infected with *B. Crocidurae* were dissected and the ovaries were removed on the 4th and 9th day after feeding (daf), where oocyte maturation and oviposition have started, respectively [15]. Ovaries were fixed for 24 hrs in 3% cold fresh glutaraldhyde (40 ml 25% glutaraldhyde, 100 ml 40% formaldhyde, 2.7 g sodium hydroxide and 11.6 sodium dihydrogen phosphate all in 1000 ml distilled water). The ovaries were washed three times for 20 minutes each, in phosphate buffer (pH 7.2), post fixed in cold 1% osmic acid for 2 hrs and washed again in three changes of fresh buffer for another 20 minutes in each. Then ovaries were dehydrated in ascending series of ethanol (50%, 70%, 80%, 90% each for 15 min and two changes of 100% for 30 min each) infiltrated by pure acetone for 30 min, transferred through acetone-epoxy resin at room temperature overnight, and then embedded in epoxy resin in plastic molds. Polymerization processed at 60˚C for 24 hrs. Semithin sections (1µm) from blocks were prepared using a glass knife on an ultra-cut microtome, stained with methylene blue and examined microscopically.

Statistical analysis:

The obtained biological data were manipulated statistically with statistic stat soft program version 10 using one-way ANOVA and student test for comparison.

Results

Biological effects of B. Crocidurae infection on reproduction of female O. erraticus:

The preoviposition, oviposition periods, the mean daily egg output, mean egg number/ oviposition/ female (fecundity) and the egg incubation period and percent hatching (fertility) were used as biological parameters to assess the effect of *B. Crocidurae* infection on the egg production and viability in the infected female *O. erraticus* (Table 1).

Preoviposition and oviposition period:

The infection of the female *O. erraticus* with *B. Crocidurae* caused a insignificant ($p>0.05$) increase of the preoviposition period (9.87±0.40 days) and decrease ($p>0.05$) of the oviposition period (7.60±0.46 days) as compared with the uninfected female

Fecundity and daily egg output:

During the oviposition period, eggs were laid in separate 2-6 batches in both uninfected (4-60 egg/batch) and infected (4-48 eggs/batch) female. *Borrelia crocidurae* infection of female *O. erraticus* caused a highly significant decrease ($p < 0.001$) of fecundity (81.00±5.34 eggs/oviposition/female) and a significant decrease ($p<0.05$) of the mean daily egg output (10.92±0.65 eggs) as compared with uninfected female.

Egg viability and incubation period:

The infection of the female *O. erraticus* with *B. Crocidurae* caused a significant increase ($p<0.05$) of the egg incubation period to reach a mean of 10.33±0.16 days and a decrease of the percent egg hatching of the total number of eggs laid per oviposition of the infected female (86.01±2.19%) as compared with the uninfected female.

Histological studies on oocyte development and localization of B. Crocidurae in the ovary of infected O. erraticus:

-Oocyte development in uninfected and B. Crocidurae −infected female:

The ovary in both uninfected and B. crocidurae-infected female *O. erraticus* is a single tubular U-shaped structure which is composed of a lumen delimitated by a wall of small epithelial cells with large round nuclei. In some regions, the epithelial wall thickens by multicellular protrusions of pedicel cells which attach oocytes of different sizes and developmental stages to the ovary and protrude into hemocoel (Fig. 1).

Histological examination of the ovaries of both uninfected and *B. Crocidurae* –infected females showed five different developmental stages of the oocyte which were classified according to the size, presence of the germinal vesicle, cytoplasm appearance, presence of yolk granules and chorion following [38]:

Stage ɪ, are small, round or elliptical shaped oocytes which have homogeneous cytoplasm with no granulation and a central germinal vesicle (nucleus) with a prominent large nucleolus (Fig. 2&4).

Stage ɪɪ, are oval or elongated oocytes, larger than oocyte ɪ (nearly twice as large), have large germinal vesicle with a nucleolus. The cytoplasm shows fine granulation and small vacuoles (Fig. 2&4).

Stage ɪɪɪ, is round and much larger than stage ɪ &ɪɪ. Evident yolk granules are distributed throughout the cytoplasm with the smaller toward the centre and the larger yolk granules toward the periphery. It has a large nucleolated germinal vesicle which has been shifted to the periphery of cytoplasm. The chorion is well identified except at the pedicel-oocyte interference (Fig.2&3).

Stage ɪV, is a large oocyte with evident chorion. The cytoplasm is filled with dense small and large yolk granules masking the germinal vesicle (Fig. 2, 3&4).

Stage V, is the largest oocyte with cytoplasm is filled with closely packed large yolk granules. The oocytes V are surrounded by stretched but still evident chorion (Fig. 3&4). Disappearance of chorion was observed in some B. infected ovulating oocyte V (Fig. 4&5).

On day 4 after feeding (daf) of uninfected and *B. Crocidurae* – infected females most oocytes were of stage ɪ-ɪɪɪ but sometimes stage ɪV oocytes were also observed. However, oocytes of stage V were only observed on the 9th daf beside oocytes of other stages (Fig. 3&4).

Localization of *B. Crocidurae* in the ovary of the infected female *O. erraticus*:

Borrelia crocidurae spirochetes were detected in the hemolymph (HL) and inside the ovary of the infected female *O. erraticus* during oocytes development and ovulation (Fig.4). Some of spirochetes were found adhering to and penetrating oocytes of early stages (ɪ&ɪɪ) having no chorion and protruding into HL. Also, spirochetes penetrated the ovary wall and were localized in pedicels holding and representing points of contacts with the attached oocytes of early stages ɪ&ɪɪ and oocytes covered with chorion except at their points of contact with pedicels such as oocyte stage ɪɪɪ (Fig. 4). Apparently, at these points, spirochetes passed into the oocyte where no chorion was detected in oocyte of stage ɪɪɪ. In this stage, B. spirochetes were localized near the attached apices and around the peripherally shifted nucleus of this oocyte stage to spread in the developing oocytes. *Borrelia spirochetes* were localized throughout the mature oocyte of stage V adhering to and inside the closely packed large yolk granules within the oocyte (Fig.5). Moreover, some spirochetes in pedicels crossed the ovary wall to enter the ovary lumen (Fig. 4). The chorion was lacking around some of the infected fully mature stage V oocytes which were released into the ovary lumen at ovulation on the 9th daf. The disappearance of chorion subjected these oocytes and hence their enclosed large yolk granules to penetration and further invasion by *B. Crocidurae* spirochete which have entered into the ovary lumen (Fig.4).

<u>Discussion</u>

Ornithdoros erraticus female ticks with high infection rates and well defined transovarial transmission of *Borrelia crocidurae* [15] were used to study effects of borrelial infection on some biological parameters of reproduction in this tick species. Also, dynamics of the spirochete in the ovary and effects on the oocyte development in the ovary of the infected female tick were investigated.

Effects of B. Crocidurae infection on reproduction and oocyte development in female O. erraticus:

Borrelia crocidurae infection of the female *O. erraticus* reduced the fecundity and fertility of the infected females by decreasing their egg production and viability. This result conforms to findings in the B. crocidurae–infected female *O. erraticus* where the total protein, weight of the ovary and number of mature oocytes were decreased as compared with the uninfected controls during most of the pre- and oviposition periods [13]. Also, various effects of pathogens on reproduction of their arthropod vectors have been reported in ticks and insects. The engorged Ixodes dammini female failed to oviposit or laid small batches of eggs when had a massive infection with *B. burgdorferi* in the ovary [27]. However, no difference in fecundity and fertility have been found between uninfected and *B. coriaceae*−infected females of *O. coriaceus* [28] or the uninfected and B. burgdorferi−infected *I. pacificus* [29]. Laboratory studies showed different effects of the tick infection with the protozoan Babesia bovis [30] and *Babesia bigemina* [31] on some biological parameters of reproduction of their vectors of the ixodid ticks, *Boophilus microplus* and Boophilus annulatus, respectively. However, a natural babesial infection with a *Babesia* sp. showed no detrimental consequences to the reproduction of the infected female *Boophilus microplus* [32]. In insects, ovaries of the malaria vectors, *Anopheles stephensi* and *An. gambiae* infected with *Plasmodium yoelii nigeriensis* and *P. falciparum*, respectively, exhibited a significant reduction in fecundity of both mosquitoes [33,34]. In the infected *An. stephensi*, the reduction was associated with ovary follicular apoptosis and follicular resorption [35,36)].

Histological studies showed no apparent difference in the ovary structure and oocyte development in both uninfected and B. crocidurae−infected female *O. erraticus*. Similar to other studied ticks the ovary of *O. erraticus* is U-shaped structure which consists of an elongated lumen delimitated by an epithelial wall [37-42]. Oocytes with different sizes and developmental stages (I-IV according to [38,43] are attached to ovary wall by clusters of protruding cells called pedicels (funicles by Balashov). The oocyte development is classified into three phases, previtellogenic, vitellogenic and ovulation. The previtellogenic phase refered to the early stages have no evident yolk granules and included stage I and II oocytes of Balashov classification [21]. These two oocyte stages (I and II) were observed together with the vitellogenic oocyte stages III and sometimes IV during the early (4th daf) and late time (9th daf) intervals tested in the present study which reflected the asynchronous nature of the oocyte development in ticks [21]. The vitellogenic phase of oocyte development begins with an evident appearance of yolk granules (Balashov stage III) and ends with ovulation (Balashov stage V). In the present study, oocytes of stage III were attached to pedicels, ensheathed by chorion (egg shell) except at their points of attachment and evidently contained small central and large peripheral yolk granules and shifted germinal vesicle. The fully mature stage V oocytes detached to be released into ovary lumen. The ovulated stage V oocytes appeared on the 9th daf and were fully chorionated and packed with large yolk granules. In ticks, yolk granules have been found to be synthesized endogenously by the oocyte or exogenously in other tissues as fat body and gut cells [44,45]. Yolk precursors of exogenous origin are transported via hemolymph to be taken up into oocytes by endocytosis [45]. Some authors suggested that pedicel cells may serve as a source of exogenous yolk precursors [39-41] in addition to the pedicel role as a mechanical support for oocytes.

Dynamics of B. Crocidurae in the ovary of infected female O. erraticus:

The microscopic examination of the HL and semithin sections of the ovary of *B. Crocidurae* infected *O. erraticus* localized B. spirochetes outside and inside the ovary wall, oocytes and yolk granules. Apparently, B. spirochetes were transported into the ovary via its surrounding HL, by direct penetration of the ovary wall and oocytes of early stages I and II. *Borrelia spirochetes* were observed adhering to and penetrating oocyte I and II having no chorion and protruding into HL. It is expected that *B. spirochetes* invaded these early stages lacking chorion by direct entry through the basement lamina (lamina propria) demarcating the oocytes from HL [38, 21]. This suggestion may conform to the presence of numerous *B. burgdorferi* spirochetes in the space between the lamina propria and the oocyte plasma membrane in the infected female *Ixodes dammini* [27]. Also, in ticks, the basement lamina was found to be permeable to large molecules such as hemoglobin, yolk proteins and some microorganisms in the HL of ticks [21]. However, the fully formed chorion with continuous chitin plaques [43] seemed to represent a serious barrier to pathogen invasion of developing oocytes [21] where different pathogens as *B. duttoni* [46)], *Coxiella burneti* [38] *Ricketsia rickettsi* [47] and *Babesia major* [48] could only invade younger but not older oocytes having complete egg shells in the corresponding tick vectors (*O. moubata, Hyalomma asiaticum, Dermacentor andersoni* and *Haemaphysalis punctate*, respectively). Many of the microorganisms which are transmitted transovarially have evolved mechanisms for the penetration of the basement lamina but probably not the egg-shells of older oocytes [21].

In the present study, *B. Crocidurae* spirochetes penetrated the epithelial wall of the ovary of the infected female *O. erraticus* were mainly localized in the pedicels where they moved to enter into the ovary lumen and attached oocytes including those chorionated stages except at their points of attachment as stage III oocytes. Similarly, in previously studied ticks [21,38,42] it was found that chorion (egg-shell) synthesis by vitellogenic oocytes began during stage III and was completed during stage IV oocyte. The chorion material was discharged extracellulary forming discontinuous patches of chorion plaques separated from adjacent microvilli of the oocytes by narrow spaces of plasma membrane which allowed the passage and uptake of large yolk protein precursors along with deposition of chorion material. Apparently, *B. Crocidurae* spirochetes in the infected *O. erraticus* have passed through the pedicel points of contact with oocyte III having no chorion or with discontinuous plaques of chorion. The invading *B. Crocidurae* spirochetes were localized at the apecies of the attached oocyte of stage III to spread throughout the developing oocyte. In the mature ovulating oocyte stage V *B. Crocidurae* spirochetes were localized adhering to and inside the closely packed large yolk granules in the oocyte. The adhesion and penetration of the spirochete at the yolk granules might be associated with a probable consumption of yolk or its chemical components. In developing oocytes this deprives the infected females from enough and essential protein for oocyte maturation [15,49] and embryonic development [50)]; hence caused the observed decrease in the egg production and viability in the infected female *O. erraticus*. Infection of female *O. erraticus* with *B. Crocidurae* reduced the number of mature oocytes, egg vitellins and vitellogenic protein fractions and their percent amount in HL and ovary of the infected female as compared with the uninfected female noticeably during vitellogensis [15].

In the present study, chorion was lacking around some infected ovulating mature oocyte stage V which enabled their penetration and further invasion by B. spirochetes entering the ovary lumen. Similarly, [27] found that the massive spirochetal presence of *B. burgdorferi* around the oocytes in the ovary of *Ixodes dammini* destroyed the microvilli, prevented egg maturation and interfered with the transport of chitin required for the chorion formation which has resulted in failure of the infected female to oviposit. Also, [51] localized a tick vitellogenin (Vg) receptor on the oocyte plasma membrane in *Haemaphysalis longicornis* which was essential for Vg uptake and oocyte maturation and the transovarial transmission of *Babesia* parasites.

In the present study, the entry of *B. Crocidurae* inside the developing oocytes directly or through their supporting pedicels has led to its transovarial transmission specifically [11] from the female *O. erraticus* to its larval progeny [7,15].

Conclusion

We could conclude that *Borrelia crocidurae* infection of female *Ornithodoros erraticus* reduced the fecundity and fertility of the infected female tick, decreasing its egg production and viability. And the entry of *B. Crocidurae* inside the developing oocytes directly or through their supporting pedicels has led to its transovarial transmission specifically from the female *O. erraticus* to its larval progeny.

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This research paper was approved by the research ethics committee from Faculty of Science, Ain

Shams University (ASU-SCI/ENTO/2024/2/2).

TABLE 1. Effect of *B. Crocidurae* infection on preoviposition period, oviposition period, daily eggoutput, fecundity, fertility, and eggs incubation period of *O. erraticus* females.

Infection state	Preoviposition period (day) mean±SE (range)	Oviposition Period (day) mean±SE (range)	Daily egg output mean±SE (range)	Total no. of eggs/oviposition/female mean±SE (range)	Eggs incubation period (day) mean±SE (range)	% hatched eggs mean±SE (range)
Uninfected	9.47±.22[a] (8-10)	8.00 ±.72[a] (2-12)	15.56±2.66[a] (7.2-49.5)	103.8±6.21[a] (65-140)	9.80±.28[a] (8-11)	90.46±1.13[a] (83.01-97.22)
Infected	9.87±.40[a] (8-13)	7.60±.46[a] (4-10)	10.92±.65[b] (4.75-14.14)	81.00±5.34[b] (38-105)	10.33±.16[b] (9-11)	86.01±2.19[b] (65.31-97.09)

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

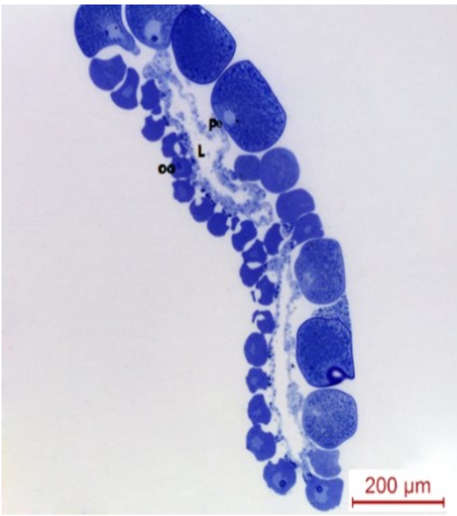

Fig.1. Semi-thin section through ovary of mated fed female *O. erraticus* on 4d.a.f showing structure of the ovary. L, lumen; pe, pedicel; oo, oocyte Bar = 200 µm .

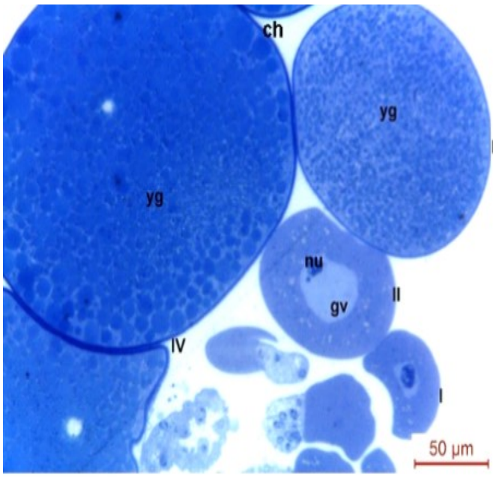

Fig. 2. Semi-thin section through ovary of uninfected mated fed female *O. erraticus* 4d.a.f .

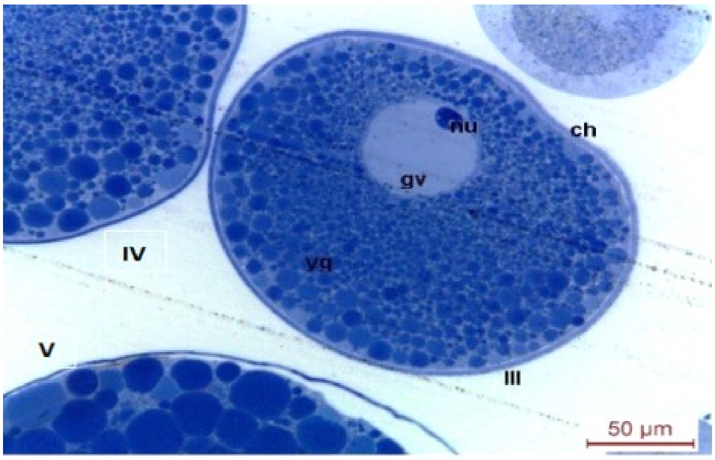

Fig. 3. Semi-thin section through ovary of uninfected mated fed female *O. erraticus* 9d.a.f showing stage III - V oocyte. gv, germ vesicle; Nu, nucleolus; yg , yolk granules; ch, chorion; oocyte. Bar = 50 µm.

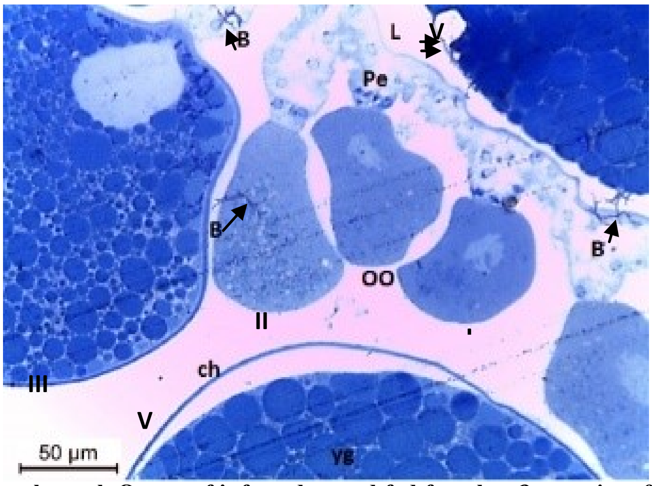

Fig. 4. Semi-thin section through Ovary of infected mated fed females *O. erraticus* 9 d.a.f showing the oocyte developmental stages I – V, and localization of *Borrelia* (B) and lack of chorion on a mature ovulating oocyte V (double arrows). L, lumen; pe, pedicel; oo, oocyte B, *Borrelia* (arrows) Bar= 50 µm.

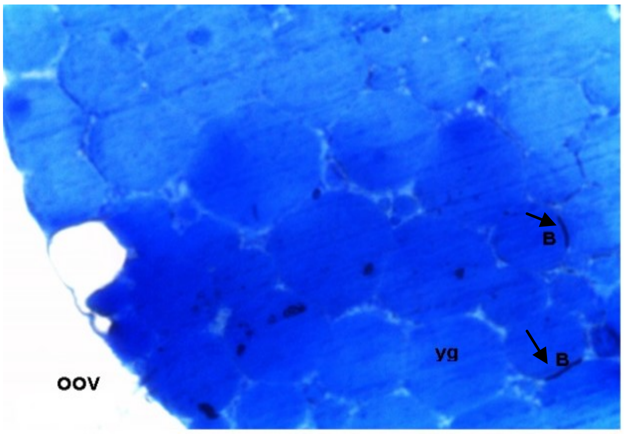

Fig.5. Semi-thin section through ovary of infected mated fed females *O. erraticus* 9 d.a.f showing infected ovulated oocyte stage V. yg, yolk granules; B, *Borrelia* (arrows); OOV, ovulated c oocyte stage V.

References

1. Hoogstraal, H., Salah A.A. and Kaiser, M.N. Summary of the known distribution and biology of *Ornithodoros erraticus* (Lucas, 1849) (Ixodoidea, Argasidae) in Egypt. *J. Egypt. Publ. Hlth Assoc.*, **29**, 127-138 (1954).
2. Davis, G. E. and Hoogstraal, H. The relapsing fevers: a survey of the tick-borne spirochetes of Egypt. *J Egyptian Public Health Assoc*.; **29**,139 – 143 (1954).
3. Hoogstraal, H. Argasid and nuttalliellid ticks as parasites and vectors. *Adv. Parasitol.*, **24**, 135-238 (1985).
4. Uilenberg, G., Hinaidy, H.K., Perié, N.M. and Feenstra, T. Borrelia infections of ruminants in Europe. *Veterinary Quarterly,* **10**(1), 63-67 (1988).
5. Khan, M., Almutairi, M.M., Alouffi, A., Tanaka, T., Chang, S.C., Chen, C.C. and Ali, A. Molecular evidence of Borrelia theileri and closely related Borrelia spp. in hard ticks infesting domestic animals. *Frontiers in Veterinary Science,* **10**, 1297928 (2023).
6. Helmy, N.A. Lotfy, N. and Abd El-Mohsen, A. Dynamics of *Borrelia crocidurae* infection in the midgut and hemolymph of *Ornithodoros (P.) erraticus* and localization of the spirochete in the midgut. *Ain. Shams. Sci. Bull.,* **34**, 405 – 423(1996).
7. Gaber, M.S., Khalil, G.M., and Nasr, A.E. *Borrelia crocidurae* localization and transmission in *Ornithodoros erraticus* and *O. savignyi*. *Parasitology,* **88**, 403-413(1984).
8. Helmy, N.A., Khalil, G.M. and Hoogstraal, H. Hyper parasitism in *Ornithodoros erraticus*. *J. Parasitol.,* **69**(1), 229-233 (1983).
9. Khalil, G.M., Helmy, N., Hoogstraal, H. and El-Said, A. Seasonal dynamics of *Ornithodoros (Pavlovskyella) erraticus* (Acari: Ixodoidae: Argasidae) and the spirochete *Borrelia crocidurae* in Egypt. *J. Med. Entomol*., **21** (5), 536 – 539 (1984).
10. Helmy, N. Prevalence of *Borrelia crocidurae* in *Ornithodoros (pavlovskyella) erraticus* (Acari: Argasidae) and rodent hosts in Egypt. *J. Union Arab Biol. Cairo.* **13** (A), 193 – 212 (2000).
11. Shanbaky, N.M., and Helmy, N. First record of natural infection with Borrelia in *Ornithodoros (Ornithodoros) savignyii*. Reservoir potential and specificity of the tick to *Borrelia*. *J. Egypt. Soc. Parasitol.,* **30** (3),765-780 (2000).
12. Shanbaky, N.M., Helmy, N., Khater, H.M., Soliman, D.E.A. and Youssry, A. Proteolytic digestion of blood meal in *Ornithodoros erraticus*, a vector of *Borrelia crocidurae* causing relapsing fever in Egypt. *Egypt. Acad. J. Biolog. Sci.,* **3**(1),37-50 (2011).
13. Shanbaky, N.M., Helmy, N., Khater, H.M., Soliman, D.E.A. and Youssry, A. Effect of Borrelial infection on Haemolymph and ovarian protein concentrations during vittellogensis in *Ornithodoros erraticus*, a vector of relapsing fever in Egypt. *Egyptian Acad. J. Biol. Sci.,* **9**(1), 15-25 (2017).
14. Yousery, A. Digestion of blood proteins in an ornithodorine tick vector of borreliosis (*Ornithodoros erraticus*). M.Sc., Faculty of science, Ain Shams University (2011).
15. Yousery, A. Tick-*Borreli*a interaction during reproduction of *Ornithodoros erraticus*, a vector of relapsing fever in Egypt. Ph.D. thesis, Faculty of Science, Ain Shams University (2017).
16. Piesman, J. and Sinsky, R.J. Ability of Ixodes scapularis, Dermacentor variabilis, and Amblyomma americanum (Acari: Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (Borrelia burgdorferi). *Journal of Medical Entomology*, **25** (5),336 – 339 (1988).
17. Lane, R.S., Brown, R.N., Piesman, J. and Peavey, C.A. Vector competence of Ixodes pacificus and Dermacentor occidentalis (Acari: Ixodidae) for various isolates of Lyme disease spirochetes. *Journal of Medical Entomology,* **31**(3),417-424 (1994).
18. Rollend, L., Fish, D. and Childs, J.E. Transovarial transmission of *Borrelia spirochetes* by Ixodes scapularis: a summary of the literature and recent observations. *Ticks and Tick-borne Diseases*, **4** (1-2), 46-51 (2013).
19. Lynn, G.E., Breuner, N.E., Hojgaard, A., Oliver, J., Eisen, L. and Eisen, R.J. A comparison of horizontal and transovarial transmission efficiency of Borrelia miyamotoi by Ixodes scapularis. *Ticks and Tick-borne Diseases,* **13**(5), 102003(2022).
20. Lunke, M.D. and Kaufman, W.R. Effects of the avermectin analogue MK-243 on vitellogenesis and reproduction in the ixodid tick, *Amblyomma hebraeum*. *Experimental and Applied Acarology,* **13**, 249-259(1992).
21. Diehl, P.A., Aeschlimann, A. and Obenchain, F. D. Tick reproduction: Oogenesis and oviposition. In: Obenchain F. D and Galun, R. Eds., Physiology of ticks. Pergamon press, Oxford, VK, pp. 277 – 350(1982).
22. Chinzei, Y. and Taylor, D. Regulation of vitellogenesis induction by engorgement in the soft tick (*Ornithodoros moubata*). *Advances in Invertebrate Reproduction*, **5**, 565–570 (1990).

23. Spielman, A., Wilson, M. L., Levine, J.F. Ecology of *Ixodes da*mmini- borne human Babesiesis and Lyme disease. *Annu. Rev. Entomol*., **30**, 439-460 (1985).

24. Khalil, G. M. Biochemical and physiological studies of certain ticks (Ixodoidea). Gonad development and gametogenesis in *Argas (Persicargas) arboreus* Kaiser, Hoogstraal, and Khols (Argasidae). *The Journal of Parasitology*, **55**,1278 – 1297(1969).

25. Conn, H.J., Darrow, M.A. and Emmel, V.M. Staining procedure used by the biological stain commission. 2nd ed., The Williams and Wilkins Company. Baltimore. 289 pp. (1960).

26. Piesman, J., Mather, T. N., Donahue, J. G., Levine, J. F., Campbell, J. D. Karakashian, S. J. and Spielman, A. Comparative prevalence of *Babasia microti* and *Borrelia burgdorferi* in four populations of *Ixodes dommini* in eastern Massachusetts. *Acta Trop.,* **43**, 263-270(1986).

27. Burgdorfer, W. and Hayes, S.F. Vector-spirochete relationships in Louse-borne and tick-borne borrelioses with emphasis on Lyme disease. *Advances in Disease Vector Research,* **6**,127-150 (1989).

28. Lane, R.S. and Manweiler, S.A. *Borrelia coriaceae* in its tick vector, *Ornithodoros coriaceus* (Acari: Argasidae), with emphasis on transstadial and transovarial infection. *J. Med. Ent.,* **25**, 172 – 177 (1988).

29. Schoeler, G.B. and Lane, R.S. Efficiency of transovarial transmission of the Lyme disease spirochete, *Borrelia burgdorferi*, in the western blacklegged tick, *Ixodes pacificus* (Acari: Ixodidae). *J. Med. Entomol.,* **30**(1), 80 – 86 (1993).

30. Davey R.B. Effects of *Babesia bovis* on the ovipositional success of the Southern cattle tick, *Boophilus microplus*. *Annals of the Entomological Society of America,* **74**, 331 – 333(1981).

31. Ouhelli, H., Pandey, V. S. and Aboughal, A. Effect of infection by *Babesia* spp. on the development and survival of free-living stages of *Boophilus annulatus*. *Vet. Parasitol.,* **23**, 147 – 154(1987).

32. Cen-Aguilar, J. F., Rodríguez-Vivas, R. I., Domínguez-Alpizar, J. L. and Wagner, G. G. Studies on the effect of infection by *Babesia sp.* on oviposition of *Boophilus microplus* engorged females naturally infected in the Mexican tropics. *Vet. Parasitol.,* **78**(4), 253- 257(1998).

33. Hogg, J.C. and Hurd, H. *Plasmodium yoelii nigeriensis*; the effect of high and low intensity of infection upon the egg production and blood meal size of *Anopheles stephensi* during three gonotrophic cycles. *Parasitology,* **111**, 555-562(1995).

34. Hogg, J.C. and Hurd, H. The effect of natural *Plasmodium falciparum* infection on the fecundity and mortality of *Anopheles gambiae* s.i. in north east Tanzania. *Parasitology,* **114**, 325-331(1997).

35. Carwardine, S.L. and Hurd, H. Effect of *Plasmodium yoelii nigeri*ensis infection in *Anopheles steph*ensi egg development and resorption. *Med. Vet. Entomol*., **11**, 265-269(1997).

36. Hopwood, J. A.; Ahmed, A.M.; Polwart, A. Williams, G.T. and Hurd, H. Malaria –induced apoptosis in mosquito ovaries: a mechanism to control egg development. *J. Exp. Biol.,* **204**, 2773-2780 (2001).

37. Aeschlimann, A. and Hecker, H. Observations preliminaires sur l'ultrastructure de l'ovocyte en development chez *Ornithodoros moubata*, Murray (Ixodoidea: Argasidae). *Acta Trop.,* **24**, 225-243 (1967).

38. Balashov, Y. S. Bloodsucking ticks (Ixodoidea) - vectors of disease of a man and animals. *Miscellaneous Publications of the Entomological Society of America,* **8**, 161–376 (1972).

39. Denardi, S.E., Bechara, G.H., Oliveira, P.R., Nunes, E. T., Saito, K. C. and Camargo-Mathias, M. I. Morphological characterization of the ovary and vitellogenesis dynamics in the *Amblyomma cajennense* (Acari: Ixodidae). *Veterinary. Parasitology,* **125**, 379–395(2004).

40. Oliveira, P.R., Bechara, G.H., Denardi, S.E., Saito, K. C., Nunes, E. T. and Camargo-Mathias, M. I. Morphological characterization of the ovary and oocytes vitellogenesis of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). *Exp. Parasitol.,* **110**, 146 – 156 (2005).

41. Oliveira, P.R., Camargo-Mathias, M.I. and Bechara, G.H. *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae): Morphological description of the ovary and vitellogenesis. *Exp. Parasitol.,* **113**, 179 – 185 (2006).

42. Sanches, G.S., Bechara, G. H. and Camargo-Mathias M. I. Ovary and oocyte maturation of the tick *Amblyomma brasiliense* Aragao, 1908 (Acari: Ixodidae). *Micron.,* **41**, 84 – 89(2010).

43. Balashov, Y. S. An atlas of ixodid tick ultrastructure. Society of America publications, 289p. (1979).

44. Araman, S.F. Protein digestion and synthesis in ixodid females, pp.385-395. In I. G. Rodriguez [ed.], Recent advances in acarology. Academic, New York. (1979)

45. Ricardo, A.J., Oliveira, P.R.; Bechara, G.H., Camargo-Mathias, M.I. Ultrastructural detection of protein, lipids and carbohydrates in oocytes of Amblyomma triste (Koch 1844) (Acari: Ixodidae)

during the vitellogenesis process. *Tissue and Cell* **39**, 203-215(2007).

46. Aeschlimann, A. Dēvelopment embryonnaire d'*Ornithodoros moubata* (Murry) et transmission transovairienne de *Borrelia duttoni*. *Acta. Trop.,* **15**,15-64(1958).

47. Burgdorfer, W. and Brinton, N.P. Mechanisms of transovarial infection of spotted fever rickettsia in ticks. *Ann. N.Y. Acad. Sci.,* **266**, 61-72(1975).

48. Morzaria, S.P., Bland, P. and Brocklesby, D.W. The ultrastructure of penetrating stages of *Babesia major* infecting the ovary of *Haemaphysalis punctata*. *Parasitology,* **75**, 125-130(1977).

49. Chinzei, Y., Chino, H. and Takahashi, K. Purification and properties of vitellogenin and vitellin from a tick, *Ornithodoros moubata*. *J. Comp. Physiol.,* **152**,13 – 21 (1983).

50. Kamel, M.Y., Shalaby, F.Y. and Ghazy, A.M. Biochemical studies of tick embryogenesis DNA, RNA, Hemoprotein, Guanosine and Guanine in developing eggs of *Hyalomma dromedarii*. *Insect. Biochem.,* **12** (1), 15 – 28(1982) .

51. Boldbaatar, D., Battsetseg, B., Matsuo, T., Hatta, T., Umemiya-Shirafuji, R., Xuan, X. and Fujisaki, K. Tick vitellogenin receptor reveals critical role in oocyte development and transovarial transmission of *Babesia* parasite. *Biochem. Cell Biol.,* **86** (4), 331 – 344(2008).

دراسات بيولوجية وهستولوجية علي تفاعل القراد والبوريليا أثناء التكاثر والنقل المبيضي لبوريليا كروسيديوري في أنثي أورنيثودوروس إيراتيكاس.

اميرة السيد عبد الحميد[1]، آيات يسري محمد عبدالعظيم [1]، نوال محمود شنبكي[1]، نادية حلمي أحمد[1]، دعاء السيد عبدالكريم سليمان[1]، هالة محمد محمد خاطر[2] و شيماء صلاح احمد[1]

[1] قسم علم الحشرات، كلية العلوم، جامعة عين شمس، القاهرة، مصر.

[2] مركز الأبحاث والدراسات والتدريب لناقلات الأمراض، جامعة عين شمس، القاهرة، مصر.

الملخص

تسبب إصابة أنثي القراد أورنيثودوروس إيراتيكاس بالبوريليا كروسيديوري نقص خصوبتها بتقليل إنتاج البيض وحيويته. وقد تبين بالفحص المجهري للدم ولشرائح المبيض شبه الرقيقة في الأنثي المصابة أن هناك خمسة مراحل لنمو البويضات كما في الأنثي غير المصابة. ولكن في وجود السبيروكيتات بدم الإناث المصابة يبدو ان غزو سبيروكيتات البوريليا للمبيض قد تم عبر نقلها في الدم المحيط بالمبيض لتغزو جميع مراحل نمو البويضات بداخله. وبتحديد أماكن تواجد سبيروكيتات البوريليا تبين التصاق بعضها بسطح البويضات وإختراقها مباشرة للمراحل المبكرة منها كالمرحلة الأولي والثانية حيث لا يوجد حولها غلاف الكوريون مع بروزهما في تجويف الجسم وبه الدم. كذلك أنتقلت السبيروكيتات إلي داخل الجدار الخلوي للمبيض لتتواجد في أجزائه الحاملة والمتصلة بالبويضات (السويقات) ومنها تنتقل لتدخل تجويف المبيض والبويضات المحمولة بالسويقات بما فيها البويضات المحاطة بغلاف الكوريون فيماعدا عند نقاط إتصاله بالمبيض حيث يحتمل دخول السبيروكيتات من خلالها لتظهر بالقرب من هذه النقاط عند أعلي البويضات في المرحلة الثالثة لتنتشر بداخل البويضات أثناء نموها. وقد لوحظ التصاق وأختراق سبيروكيتات البوريليا لحبيبات المح كبيرة الحجم المكدسة بداخل البويضات الناضجة بالمرحلة الخامسة كما لوحظ أختفاء الكوريون حول بعض البويضات التي أنفصلت عن السويقات أثناء التبويض مما عرضها للإختراق والغزو بالبوريليا المتواجدة بتجويف المبيض.

الكلمات الدالة: بوريليا، قراد، نقل مبيضي، كوريون، حبيبات المح.