


Deltamethrin as inductor agent of precocious ovarian degeneration in *Rhipicephalus sanguineus* s.l. (Acari: Ixodidae) ticks

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Abstract The cosmopolitan species *Rhipicephalus sanguineus* s.l. is one of the most widely distributed ticks all over the world. These ectoparasites are vectors of several pathogens and cause significant direct damage to their hosts. The biological success of these ectoparasites has been attributed to their ovaries and salivary glands, organs that ensure their survival in various environmental conditions. The importance of the ovaries in ticks is that, after mating, the individuals are able to lay approximately three thousand eggs. The present study had the objective to demonstrate the effects of deltamethrin obtained from the product Butox P CE 25[®] (MSD Saúde Animal, São Paulo, Brazil) on the ovarian development of *R. sanguineus* s.l. females. The chemical was tested in the concentrations of 25, 50, 100 and 200 ppm (respectively 80, 40, 20 and 10 times lower than the recommended by the manufacturer). Through the application of histological techniques and HE staining, the results showed that the deltamethrin was potentially able to modify the morphophysiology of the oocytes in all developmental stages, interfering in the vitellogenesis, causing intense vacuolation, cytoplasmic disorganization, and alterations in the chorion secretion. In addition, the chemical affected the germ vesicle of some oocytes, causing damages and hypertrophy, fragmenting the chromatin and forming bodies strongly stained by hematoxylin. Therefore, this study confirmed that the deltamethrin had an important action on the reproductive system of the *R. sanguineus* s.l. females, causing the precocious structural disorganization of the germ cells, consequently preventing the generation of new individuals.

Keywords Histology · Morphophysiology · Vitellogenesis · Reproductive system

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Introduction

Ticks are blood-feeding ectoparasites (Arthropoda: Arachnida: Acari) of vertebrate hosts, such as cattle, horses, camels, sheep, bats, terrestrial birds, and the human being as well (Flechtmann 1973). These ectoparasites find a host while in their immature stages (larva and nymph) and remain attached and blood feeding until they reach their adult developmental stage (Freitas et al. 1982; Walker 1994).

Rhipicephalus sanguineus s. l. ticks (Dantas-Torres et al. 2013) are popularly known as “brown ticks” or “dog ticks” and this species is one of the most widely distributed worldwide (Hoskins and Woody 1991). These arthropods affect their hosts causing blood loss and transmitting infectious agents. The dog is the preferential host for this species (Dantas-Torres 2008), once these mammals do not present an efficient immunological response against the fixation of the ectoparasite (Ferreira and Silva 1998).

These arthropods are vectors of several microorganisms, agents of diseases that affect mainly their preferential hosts. Examples of such diseases are the canine monocytic ehrlichiosis, transmitted by *Ehrlichia canis* and canine babesiosis, caused by *Babesia canis* (Blagburn and Dryden 2009). Occasionally, the human beings are affected, as in the case of babesiosis, caused by *Babesia microti*. These agents are transmitted when an infected tick perforates the host skin to start the feeding process, allowing the microorganisms to reach the blood stream.

The biological success of the ticks is mainly attributed to the ovaries and the salivary glands, organs that allow them to resist under different environmental conditions, ensuring their survival. The importance of the ovary is in the fact that, after mating, these individuals are able to lay a considerable number of eggs, estimated in an average of three thousand (Koch 1982).

Morphologically, *R. sanguineus* s. l. ovaries are U-shaped tubular organs constituted by epithelial cells. The epithelial wall formed by these cells attach the oocytes through a pedicel, acellular structure that synthesizes and provides yolk precursors (Oliveira et al. 2005). The oocytes are responsible for the generation of new individuals, and are classified in stages (I–V) according to their developmental stages (Oliveira et al. 2005).

The classification of the tick oocytes is based on their morphology, which includes shape, presence/size and location of the germ vesicle (nucleus of the germ cell), yolk aspect (thin or thick granulation), and the presence or absence of the chorion, a membrane that protects the oocyte (Camargo-Mathias 2013).

Given that ticks in general have adapted well to the environment, and that dogs are the most common pet companion to humans, finding methods to control the proliferation of these ectoparasites is of critical importance. Some methods are under scientific scrutiny, such as vaccines (Péter and Brossard 1998), natural predators as the cattle egret, *Bubulcus ibis* (Alves-Branco et al. 1983), the application of pheromones (De Bruyne and Guerin 1994), fungi *Metarhizium anisopliae* (Costa et al. 2002; Tellam et al. 1992; Willadsen 1997) and, most frequently, the use of synthetic or natural substances with acaricide action. Examples of synthetic acaricides are the cypermethrin, amitraz (Bicalho et al. 2001) and the permethrin (Melo et al. 2008). The most important natural acaricides are the neem oil (Denardi et al. 2010; Remedio et al. 2015), andiroba oil (Roma et al. 2013, 2015; Vendramini et al. 2012) and the castor oil esters (Arnosti et al. 2011).

The deltamethrin is a pyrethroid insecticide, or a type II semisynthetic pyrethrin, used as the active ingredient of tickcides as the Butox P CE 25[®] (MSD Saúde Animal, São Paulo, Brazil). This synthetic chemical has been widely used to control spiders, fleas, ticks,

carpenter ants, bees, cockroaches and bedbugs, causing immediate paralysis and death (ETN 1995).

Considering the importance of ticks in public health, the difficulty to control these ectoparasites, and the economic losses caused by them, the present study had the objective to analyze the effects of the synthetic chemical deltamethrin at the concentrations of 25, 50, 100 and 200 ppm (respectively 80, 40, 20 and 10 times lower than the ones recommended by the manufacturer) on the ovary morphophysiology of *R. sanguineus* s. l. adult ticks. The results showed that this chemical product is able to modify the reproductive processes of this species, once the generation of new individuals depends on the production of viable eggs.

Materials and methods

Obtaining the ticks

For this experiment, 25 semi-engorged *R. sanguineus* s. l. were used. The specimens were supplied by the Brazilian Central of Studies on Ticks Morphology (BCSTM), UNESP, campus Rio Claro, SP, Brazil, and maintained under controlled conditions (28 ± 1 °C, 80% humidity, photoperiod 12 h) in BOD incubator. The ticks fed on naive host rabbits provided by the Genetics Group Botucatu. The ticks were placed in feeding chambers and the engorgement process occurred according to the methodology described by Bechara et al. (1995). The ticks were observed every 3 h until the sixth semi-engorgement day. After this period, the ticks were removed with tweezers, washed in distilled water, and submitted to the bioassay.

Deltamethrin bioassay

This step was performed using the deltamethrin concentration of 25 g/L (25,000 ppm). From this initial concentration, several dilution in distilled water were performed until the concentrations of 200, 100, 50 and 25 ppm of deltamethrin were obtained. Five *R. sanguineus* s. l. females were immersed in each concentration for 5 min and dried in absorbent paper. The same procedure was performed for the females from the control group, which had been immersed in distilled water. Each group was mounted on labelled Petri dishes, covered with permeable plastic film and kept in BOD incubator Eletrolab EI 202 at 27 ± 1 °C and UR $80 \pm 10\%$ for 5 days. The ticks belonging to the control group were maintained in a different incubator to avoid possible interference caused by the evaporation of deltamethrin.

Harris hematoxylin/aqueous eosin technique

The ticks were dissected in saline buffer (7.5 g of NaCl, 2.38 g of Na₂HPO₄, 2.72 g of KH₂PO₄ and 1 L of distilled water), and fixed in paraformaldehyde 4% for 7 days (Junqueira and Junqueira 1983). After, the samples were transferred to sodium phosphate buffer for 30 min and dehydrated in graded ethanol series at 70, 80, 90 and 95% (30 min each bath). The material was then embedded in resin (JB-4 Polaron Instruments/Bio Rad) for 7 days, included in historesin and kept in BOD incubator for 7 days. After drying, the samples were sectioned using microtome Sorvall JB-4/Bio Rad (3 μm thickness), mounted

on slides and stained with Harris hematoxylin for 10 min and then with aqueous eosin for 5 min. Posteriorly, the slides were allowed to dry at room temperature and mounted in Canada balsam to be analyzed and photographed with a photomicroscope LEICA DM750.

Results

In this study, the oocytes of the individuals belonging to the control group, in the five developmental stages described by Oliveira et al. (2005), were intact, with all the morphological characteristics preserved, and so were the germ vesicle and yolk content (Fig. 1). In the oocytes in the final developmental stage (V), the chorion was deposited, preserved and intact.

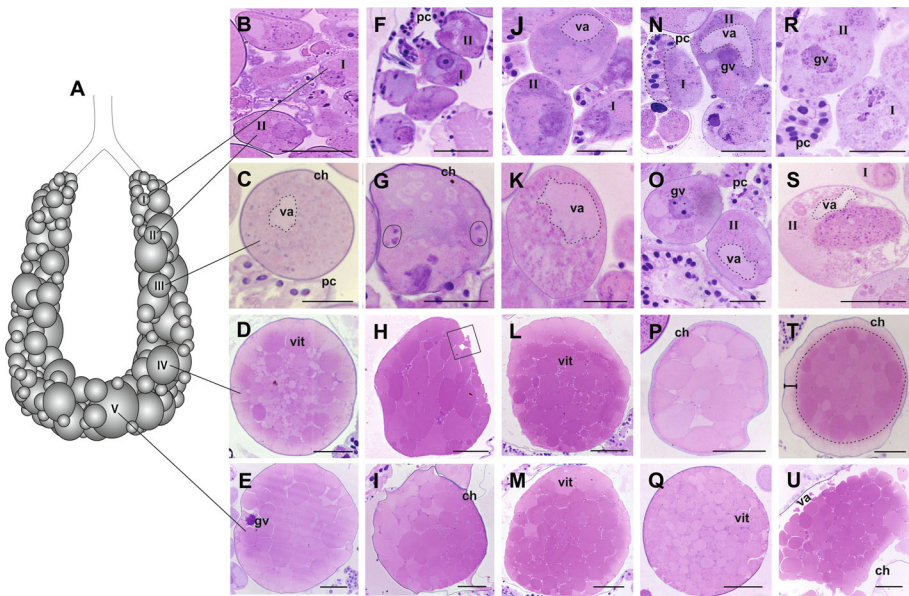


Fig. 1 Vitellogenesis alterations histology of *Rhipicephalus sanguineus* s. l. ticks caused by the exposure to deltamethrin. **a** Scheme showing oocytes in different stages of development (I–V) adhered to the ovary wall. **b–e** Histology of the ovary of ticks belonging to the control group where oocytes are observed at different stages of development and with preserved morphology. **f–i** Histology of the ovary of ticks belonging to the group exposed to the concentration of 25 ppm of deltamethrin in which it is evident the beginning of the loss of original rounded morphology of oocytes as well as noticeable damage to its germ vesicle (gv) (shown with *solid circles* in the yolk). Notice in **H** a disruption of the chorion (ch) (shown by a *square*). **j–m** Histology of the ovary of ticks belonging to the group exposed to the concentration of 50 ppm of deltamethrin in which it is evident the loss of yolk granules in the periphery of the oocyte in (**l**), and fusion of yolk granules in the center of the oocyte in (**m**). **n–q** Histology of the ovary of ticks belonging to the group exposed to the concentration of 100 ppm of deltamethrin in which it is evident the increase of cytoplasmic vacuolation (va) of the oocytes, extensive damage to the DNA of the pedicel cells (pc) in (**n**). Notice the existence of evident fusion of yolk granules. **r–u** Histology of the ovary of ticks belonging to the group exposed to the concentration of 200 ppm of deltamethrin in which it is observed the loss of original morphology of the oocyte, besides damage to the DNA of the oocytes I and II and of the pedicel cells. Extensive vacuolation is still observed as well as the loss of yolk granulation showed in (**s**). In (**t**) it is observed loss of yolk granules and in (**u**) total loss of oocytes form, as well as loss of granulation and fusion of the granules. Bars **b, d, e, f, h, i, l, m, p, q, t, u** = 0.01 mm; **c, g, j, k, n, o, r, s** = 0.05 mm

Group treated with deltamethrin 25 ppm: TG25

The *R. sanguineus* s. l. females exposed to the concentration of deltamethrin at 25 ppm presented morphophysiological alterations in the oocytes, in the pedicel cells and in the cells forming the ovary wall as well. One of the most noticeable alterations was the change in the shape of the oocytes, from round to irregular. The cytoplasm in all oocytes underwent alterations, especially concerning the yolk granulation; e.g., in oocytes II the fusion of the granules caused the emergence of giant granules. These modifications were observed in some stage III oocytes as well.

Group treated with deltamethrin 50 ppm: TG50

In the females exposed to 50 ppm of deltamethrin, the oocytes showed more evident morphological alterations, once oocytes in initial developmental stages (I, II) displayed vacuolated cytoplasm and fusion of yolk granules. The oocytes III presented intense cytoplasmic vacuolation and the germ vesicle was completely irregular. The oocytes in which the beginning of chorion deposition was observed (mainly oocytes IV), this membrane was thicker and located in the interior of the oocyte, especially in the peripheral region in contact with the interior portion of the plasma membrane, an evident eosinophilic band. The morphology of the oocytes in general was modified.

Group treated with deltamethrin 100 ppm: TG100

This concentration of deltamethrin was extremely harmful for the *R. sanguineus* s. l. germ cells. The immature oocytes displayed intense vacuolation, yolk granules fusion, and some of them showed extensive damages in the germ vesicle. Such damages were lethal to the oocytes, once they presented nuclear fragmentation.

Group treated with deltamethrin 200 ppm: TG200

The oocytes of the females exposed to deltamethrin at the concentration of 200 ppm displayed morphological alterations, including changes in shape and in the cytoplasmic content. Some oocytes in the final developmental stages presented chorion disruption and others the complete disorganization of the cytoplasm, characterizing degeneration; i.e., loss of the characteristics that allow the cell viability and the development of a new individual.

Discussion

The search for new acaricide products has been intensified, once ectoparasites as *R. sanguineus* s. l., whose preferential host is the dog, are in close contact with humans, causing direct damages and transmitting important diseases (Arnosti et al. 2011; Oliveira et al. 2008, 2009; Denardi et al. 2010, 2011; Roma et al. 2010, 2011).

In this sense, several synthetic and natural chemicals are currently available in the market. Despite their efficacy, the synthetic acaricides leave residues, affecting the environment, and nontarget organisms as well (Borges et al. 2007).

The primary function of the acaricides is to inactivate the nervous system of the ectoparasites, once it controls all the organism functions (Pereira et al. 2017). In addition to

the central nervous system, the acaricides can act on other systems, inhibiting the development and affecting fundamental biological processes associated with the survival the ticks. The generation of new individuals depends on the complete development of the oocytes, therefore, the female reproductive system is of critical importance (Arnosti et al. 2011; Oliveira et al. 2008, 2009; Denardi et al. 2010, 2011; Roma et al. 2010, 2011).

The histology and vitellogenesis of the *R. sanguineus* s. l. ovary have been described by Oliveira et al. (2005), who reported that this organ is panoistic, formed by an epithelial wall where oocytes in five developmental stages are attached through a pedicel, corroborating (Denardi et al. 2004) for *Amblyomma cajennense* and (Saito et al. 2005) for *R. (Boophilus) microplus*.

Thus, this study brings relevant data on how acaricides primarily developed to inhibit the central nervous system can affect the morphology of other systems, such as the reproductive one. The deltamethrin (pyrethroid of broad-spectrum synthetic activity and active ingredient of the chemical Butox[®]) concentrations of 25, 50, 100 and 200 ppm used in this experiment were potentially efficient in inhibiting the ovary development of *R. sanguineus* s. l. females.

The damages in the female germ cells exposed to the deltamethrin were dose-dependent; i.e., the most significant alterations were caused by the concentrations of 100 and 200 ppm; and the damages intensified as the concentration increased (Camargo-Mathias 2013). Modifications in the shape of the oocytes (from round-shaped to irregular), in the composition and organization of yolk granules (fusion resulting in giant granules that lost the original characteristics), and an intense cytoplasmic vacuolation in all oocytes (including those in initial developmental stages) were the most noticeable alterations. The occurrence of cytoplasmic alterations, mainly the emergence of vacuoles in the oocytes exposed to chemical products is considered a strategy to preserve the viable and active portions of the cells, and an attempt to separate damaged portions of the cytoplasm and inactive organelles in form of vacuoles (Denardi et al. 2012). Still in these concentrations, the chorion was completely secreted and thinner, which probably caused the oocyte to lose the original shape. Oliveira et al. (2009) found the same results studying *R. sanguineus* s. l. females exposed to the acaricide fipronil, where the chorion became thinner after the treatment. It is known that the chorion is secreted when the oocytes reach advanced developmental stages, and this membrane have the functions of preserving the structure of the future embryo, protecting the eggs against mechanical shocks and environmental variations, preventing the embryo dehydration, minimize predation and promoting gases exchange (Oliveira et al. 2008). Thus, this study confirmed the high vulnerability of the oocytes facing chorion secretion faults caused by the exposure to different concentrations of deltamethrin.

The results confirm the potential of deltamethrin to modify the oocyte morphophysiology in *R. sanguineus* s. l. ticks in all developmental stages. The product affected the vitellogenesis, causing intense vacuolation, cytoplasmic disorganization, alterations in the chorion secretion and serious damages to the germ vesicles of some oocytes, leading to their hypertrophy, fragmenting the chromatin and forming bodies strongly stained by hematoxylin. These are signs that the cells would be inviable and would not be able to advance to further developmental stages, and, consequently, would be incapable to generate new individuals. Similar morphological alterations were observed in *R. sanguineus* s. l. exposed to the synthetic chemicals fipronil and permethrin, and to the natural acaricide andiroba oil (Oliveira et al. 2008, 2009; Vendramini et al. 2012). A study by Oliveira et al. (2009), exposing *R. sanguineus* s. l. females to fipronil demonstrated through an ultrastructural analysis of the oocytes that the cell components were significantly damaged, and

that such damages were more evident in the plasma membrane, mitochondria and yolk protein granules. The authors also detected alterations in the defense mechanisms by the increase in the number of cytoplasmic microtubules and the presence of several digestive vacuoles, which showed that the acaricide agent had affected the fertility of the females exposed to the chemical.

This study confirmed that, in addition to acting on the target organ (the central nervous system) of *R. sanguineus* s. l. ticks, deltamethrin had an important effect on the female reproductive system, disorganizing the structure of the germ cells and preventing them from generating new individuals.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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