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Cytotoxic effects of permethrin in salivary glands of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) semi-engorged females

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ABSTRACT

Because of the medical and veterinary importance of ticks and the wide use of synthetic chemical substances such as permethrin (active ingredient of Advantage® Max3 – Bayer)for their control, this study evaluated the effects of different concentrations (206, 1031 and 2062 ppm) of the acaricide on the salivary glands of *Rhipicephalus sanguineus* semi-engorged females. Results showed that permethrin is a potent substance that acts morpho-physiologically in the tick glandular tissue, causing changes in the acini shape intense vacuolation in acinar cells, and disruption of the tissue by cell death process, with subsequent formation of apoptotic bodies, especially at higher concentrations, thus precluding the accurate identification of different types of acini. Importantly, it is demonstrated that permethrin acts on salivary gland tissue, as well as affecting the nervous system, accelerating the process of glandular degeneration, and interfering with the engorgement process of female ticks, preventing them from completing the feeding process.

1. Introduction

Ticks represent an arthropod group of medical and veterinary importance causing harm to hosts and transmitting pathogens to them (Walker, 1994).

Many studies, dealing mainly bio-ecology of ticks and demands for effective control methods especially those related to new vaccines are available in the literature (Kelly and Colley, 1988; Kaufman, 1989; Leal et al., 2003; Labruna, 2004). However, specific work on the cell biology of ticks are still scarce. A group of researchers from the Brazilian Central of Studies on Ticks Morphology (BCSTM) at São Paulo State University have performed several studies, primarily focusing on the morphology and histology of the main tick systems (Denardi et al., 2004; Saito et al., 2005; Oliveira et al., 2006; Nunes et al., 2006; Oliveira et al., 2007; Nunes et al., 2008; Furquim et al., 2008a,b,c, 2010; Roma et al., 2010). The relevance of structural and functional changes of *Rhipicephalus sanguineus* reproductive and glandular systems exposed to synthetic and natural chemicals has been given by Oliveira et al. (2008, 2009), Roma et al. (2009, 2010) and Arnosti et al. (2010).

Currently, field experiments have shown that the most effective method for tick control is still the use of synthetic acaricides despite their high cost, specialized labor requirements for application, and damage to the environment and public health related to the contamination induced by chemical residues (Freitas et al., 2005).

Among the synthetic acaricides widely used to control ticks, especially the dog tick *R. sanguineus*, there is permethrin (active ingredient of Advantage® Max3, Bayer), a chemical compound that causes nerve impulse disorders, as a result of disturbed sodium exchange in cell membranes. Thus, ectoparasites suffer excitement, indicated by tremors and spasms followed by paralysis and death (Mencke et al., 2003).

Literature on the direct influence of acaricides in tick systems, other than the nervous system, are still scarce. Mohamed et al. (2000) showed that permethrin would stimulate the increase of activity of salivary gland in *Hyalomma dromedary*, however, Pereira et al. (2009), studying the action of fipronil (Frontline®) in salivary glands of *R. sanguineus*, have revealed changes in this tissue, resulting in early gland degeneration.

Thus, this study aimed to analyze, using morphological and histological techniques, the action of permethrin on the salivary glands of *R. sanguineus* semi-engorged females subjected to permethrin in an attempt to provide new information to support the improvement and development of control methods' that are less aggressive to non-target organisms as well as to the environment.

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2. Material and Methods

2.1. Rhipicephalus sanguineus ticks

A total of 60 semi-engorged females of *R. sanguineus*, weighing 27 mg in average, supplied from the colony maintained at the Brazilian Central of Studies on Ticks Morphology (BCSTM) of São Paulo State University-UNESP, at the Biosciences Institute of Rio Claro, SP, Brazil, were used throughout the experiment. The ticks were kept under controlled conditions (28 ± 1 °C, 80% humidity and 12 h photoperiod) in an Eletrolab EL 202 BOD (Biological Oxygen Demand) incubator and fed on New Zealand White rabbits (Protocol n° 5442, approved by Comitê de Ética no Uso de Animal, UNESP, de Rio Claro/ CEUA-IB-UNESP).

The laboratory feeding conditions of *R. sanguineus* ticks in the hosts were followed (Bechara et al. 1995).

2.2. Dilution assays of permethrin (CAS n°: 52645–53-1)

Permethrin (3-phenoxybenzyl (1RS, 3RS, 1RS, 3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) used in this study was purchased from Fersol Indústria e Comércio S/A (Mairinque, SP, Brazil). The permethrin concentrations were based on LC_{50} of 2062 ppm determined previously in a pilot test by Roma et al. (2009). The doses correspond to 10% of the LC_{50} (206 ppm), 50% of the LC_{50} (1031 ppm) and the normal LC_{50} (2062 ppm). A control group was exposed only to distilled water.

R. sanguineus females, after being washed in a sieve with tap water, were dried on soft absorbent paper. Afterwards, 45 females were divided into three groups of 15 females each and immersed for 5 min in Petri dishes containing the different concentrations of permethrin above. The control group was also composed of 15 females that were immersed in distilled water for the same period. Ticks were then dried on absorbent paper and placed in the BOD incubator for 7 days. The observation period was established since the effect of the acaricide is often not immediate, acting slowly on the physiology of the ticks.

2.3. Scanning Electron Microscopy (SEM)

The salivary glands of *R. sanguineus* females were removed, fixed in Karnovsky medium for 24 h and dehydrated in a graded 70–100% acetone series. The material was processed by Critical Point Drying, sputtered with gold and examined by a Philips 505 SEM.

2.4. Histology

The *R. sanguineus* females were dissected on Petri dishes containing phosphate buffered saline-PBS solution (NaCl 0.13 M, Na_2HPO_4 0.017 M, KH_2PO_4 0.02 M, pH 7.2), and the salivary glands removed, fixed in 4% paraformaldehyde and 0.9% NaCl in 10% phosphate buffer (0.1 M – pH 7.5), and dehydrated in an alcohol series (70, 80, 90 and 95%) at 15 min intervals. The specimens were infiltrated with Leica resin and the material embedded in plastic moulds at + 4 °C to delay pre-polymerization. The moulds with material were filled and covered with Leica resin and the polymerization completed at room temperature (about 37 °C).

Sections 4 μm thick were mounted on glass slides, stained with hematoxylin & eosin (HE), examined and photographed in a Motic BA 300 photomicroscope. This device and other equipment were supplied by the Histology Laboratory of the Biology Department at the Biosciences Institute, UNESP, Rio Claro, SP, Brazil.

3. Results

3.1. Scanning Electron Microscopy (SEM)

The salivary glands of *R. sanguineus* semi-engorged females are paired structures that extend antero-laterally on the ventral body cavity and open into the oral cavity. They have a secretory and an excretory portion, and are devoid of a secretion storage reservoir. The secretory portion is composed of spherical acini with slightly wrinkled surface, while the excretory portion is formed by a branched duct system, where the largest one is the common excretory duct. Departing from this structure, intermediate or secondary (smaller size and diameter) ducts, are distributed along the length of the gland in small canaliculi or acinar ducts (Figs. 1A and B).

In female *R. sanguineus* treated with 206 ppm of permethrin, salivary glands had the same characteristics as those of the control group, except that some acini appear broken, and the presence of apoptotic bodies can be observed (Figs. 2A–C).

In individuals subjected to 1031 ppm of permethrin, the acini lost their original shape becoming smaller and irregular, presenting extremely wrinkled surface when compared to the control group individuals (Figs. 3A and B).

Salivary glands in individuals submitted to 2062 ppm of permethrin present similar changes (e.g., presence of irregular acini) (Fig. 4A).

3.2. Histology

3.2.1. Group I (control)

Histological techniques confirm the results on the salivary gland morphology in the control group which have acini **I**, **II** and **III** with regular shape and intact acinar cells (Figs. 1C–F).

The acini type **I**, which are agranular in ticks, present different shapes ranging from oval to rounded with a larger **central** cell and strongly stained nucleus and several smaller **peripheral** cells also with smaller nuclei (Fig. 1C).

The acini type **II** (granular) are spherical and present cells **c1**, **c2**, **c3** and **c4** (Figs. 1C–E) while the acini **III** (granular), also spherical, are larger than those of type **II** and are formed of **d** and **e** cells (Figs. 1E and F).

The different cell types of salivary gland acini (**I**, **II** and **III**) of *R. sanguineus* semi-engorged females were already described by Furquim et al. (2008b). In the present study, individuals of the control group were used only as reference to demonstrate the changes caused in the salivary glands by permethrin.

3.2.2. Group II (treated with 206 ppm of permethrin)

The tick females exposed to 206 ppm of permethrin had salivary glands with a few morphologically altered acini compared to the control group.

The acini **I** lost their original form, becoming irregular with a more enlarged lumen than those in the control group (Fig. 2D).

The acini **II** were the most affected by the action of permethrin. Changes in shape as well in **c1** and **c3** cells (intense vacuolation) were observed. However, most of the cells still had the same characteristics seen in the control group, and moreover, few showed cytoplasm vacuolation (Figs. 2E and F).

The acini **III** present **d** and especially **e** cell vacuolation, and some of these acini are irregular and disrupted (Fig. 2G).

3.2.3. Group III (treated with 1031 ppm of permethrin)

Individuals subjected to 1031 ppm of permethrin had salivary glands with severe morphological changes, e.g. acini transformed