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Dinotefuran-induced morphophysiological changes in semi-engorged females *Rhipicephalus sanguineus* Latreille, 1806 (Acari: Ixodidae) ticks: Ultra-structural evaluation

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ABSTRACT

The present study demonstrated the effects of dinotefuran (active ingredient of the acaricide Protetor Pet®) on the ovary and midgut cells of semi engorged *R. sanguineus* females exposed to different concentrations of this chemical. For this, 120 semi-engorged females were divided into four treatment groups with 30 individuals each: group I or control (distilled water), group II (5000 ppm), groups III (6250 ppm) and group IV (8334 ppm of dinotefuran). All the ticks were immersed in the different concentrations of dinotefuran or in distilled water for 5 min and then dried and kept in BOD incubator for 7 days. The results showed alterations mainly regarding the damaged cell structures, such as yolk granules, organelles and the plasma membrane of the germ cells. In addition, structures related with defense mechanisms were found, such as vacuoles, cytoskeletal filaments, and myelin figures in the germ cells. Damages in the generative cells of the midgut, alterations in the size of digestive cells, the number of endosomes, digestive vacuoles, digestive residues, lipid drops and organelles in the cytoplasm of the digestive cells and the presence of microvilli in the plasma membrane of these cells also demonstrate the progressive damages caused by the action of dinotefuran in the midgut and germ cells of *R. sanguineus* semi-engorged females. The concentrations applied partially impaired the digestive processes; and, without proper nutrition, all the ectoparasite's physiologic events are prevented from occurring, leading the individual to death. The germ cells were also damaged, and probably would not be able to advance in their development (I–V) and complete the vitellogenesis, which would affect the fertility of the female and consequently impede the formation of a new individual.

1. Introduction

Rhipicephalus sanguineus is probably the kind of tick that is most widely distributed in the entire world (González et al., 2004; Labruna and Pereira, 2001; Soares et al., 2006; Szabó et al., 2001).

This arthropod is the main vector of *Ehrlichia canis,* being also responsible for the transmission of other pathogens such as *Babesia canis*, *B. caballi, B. equi* (Sexton et al., 1976), *Hepatozoon canis* (Craig, 1990), *Anaplasma platys* (French and Harvey, 1983) and *Haemobartonella canis* (Woldehiwet and Ristic, 1993). Some studies have reported its participation in the transmission of canine visceral leishmaniasis (Coutinho et al., 2005). Others stated that *R. sanguineus* would be the vector of *Coxiella burnetii* – causing agent of the human Q fever (Stephen et al., 1980), *Rickettsia rickettsii* causing agent of spotted fever, *Rickettsia conori* causing agent of Boutonneuse fever (Merle et al., 1998), of *simile* Lyme borreliosis (Yoshinari et al., 1997) and of the *Francisella tularensis* bacteria, the causing agent of tularemia (Walker et al., 2000).

The main form to control these parasites still is with chemicals (through synthetic acaricides). Selection of acaricide-resistant strains of ticks and contamination of non-target organisms, as well as the environment, are factors that have encouraged investigations

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that aim at improving recent acaricides and/or developing new anti-parasite products through the identification of new pesticide molecules (Crampton et al., 1999; Nolan, 1985; Oliveira et al., 2009, 2008; Pruett, 1999). With that approach, there is also the intention of decreasing or even replacing ineffective or inappropriate synthetic products that are currently in use.

A class of substances that has the potential of controlling several plagues and that has most increased in the market since the commercialization of pyrethroids is the neonicotinoids. This promising class presents excellent chemical and biological properties, as well as low toxicity for mammals (Nauen and Bretschneider, 2002).

Among the neonicotinoids is the dinotefuran, the most recently synthetized, belonging to the third generation (Wakita, 2011; Wakita et al., 2005, 2003). The dinotefuran is unique in its structure, once it is based on the acetylcholine molecule, not on nicotine as the other neonicotinoids (Wakita, 2011). As the other neonicotinoids, it is highly toxic for the insects, mainly Hemiptera, Coleoptera, Diptera, Dictyopera and Thysanoptera (Tomizawa and Casida, 2005). Toxicological and ecotoxicological studies have demonstrated that the dinotefuran presents low toxicity for birds, aquatic animals (Kagabu, 1997; Uneme et al., 1999; Wakita et al., 2005), and the environment (EPA, 2009; Wakita, 2011; Wakita et al., 2005). Still regarding its toxicology, it was demonstrated that the dinotefuran does not have genotoxic, teratogenic or carcinogenic effects on mice, rats, rabbits of guinea pigs (Wakita et al., 2003). Its excellent chemical-physical, biological and toxicological properties make the dinotefuran a promising option to control plagues and vectors of public importance (Wakita et al., 2005; Zaim and Guillet, 2002), confirming its important role in the present context and motivating the development of studies focusing on its effects and mechanisms of action.

For *Ctenocephalides felis* fleas, dinotefuran has been a very efficient product (Dryden et al., 2011). However, until now, there are only few studies in literature on how to use this chemical to control the *R. sanguineus* tick.

Considering the above information, this study aimed at determining the effects of different dinotefuran concentrations in germ and midgut cells of semi-engorged *R. sanguineus* females ticks through an ultra-structural study, comparing the results obtained from the Control Group individuals, in order to understand the activity of this product in different cells and, therefore, allow the accomplishment of essential information that will help the development of new control methods of *R. sanguineus* ticks and/or improve usual and more specific control methods, which do not induce tick-resistance and are less toxic and less damaging to the environment and non-target organisms.

2. Material and methods

2.1. Chemical substance

2.1.1. Synthetic: dinotefuran (CAS 165252-70-0)

Dinotefuran is a compound of the neonicotinoid chemical class, molecular formula $C_7H_{14}N_4O_3$. The chemical was obtained from the commercial acaricide Protetor Pet®, produced by "Ouro Fino Saúde Animal", Cravinhos, SP, Brazil, in tubes of 0.48 mL, concentration 25%, for animals up to 5.0 kg.

2.2. Rhipicephalus sanguineus *ticks (Latreille, 1806)*

Semi-engorged *R. sanguineus* females, weighing 27 mg on average (about five days of feeding), were used throughout the experiment. They were supplied by the tick colony maintained under controlled conditions (28 °C, 85% humidity, and 12-h photoperiod) in a BOD (Biological Oxygen Demand) incubator, in a room of the Animal Facility of the Department of Biology – UNESP, Rio Claro Campus/São Paulo, Brazil. Semi-engorged females were obtained after unfed *R. sanguineus* couples (25 couple/infestation) were allowed to feed on naive Botucatu genetic group rabbits following Bechara et al. (1995). The semi-engorged stage of the females was chosen due to the high parasitary efficiency in this phase.

2.3. Hosts

Botucatu genetic group rabbits, weighing between 3 and 3.5 kg, were used as hosts. Rabbits were obtained from the Animal Facility of UNESP – Botucatu Campus/São Paulo – Brazil and housed in the Animal Facility of UNESP – Rio Claro Campus/São Paulo – Brazil. Animals did not have prior contact with ticks or acaricides and were kept under controlled conditions. During the entire experiment, animals were maintained in cages and received water and rabbit food *ad libitum*.

The Ethics Committee for Animal Experimentation of UNESP/SP/Brazil, protocol n°6334/2014, approved this study.

2.4. Dinotefuran dosage

The initial concentration of dinotefuran was defined based on the recommendations of manufacturer – product label of Protetor Pet®. Several doses were evaluated in preliminary tests (pilots) by diluting dinotefuran (Protetor Pet®) in distilled water (0 to 50%). After this bioassay, the efficacy of dinotefuran and the level of susceptibility of the semi-engorged females were evaluated, and the lethal concentration LC_{50} determined was 10,182.253 ppm. In this study, the concentrations corresponded to 5000 ppm, 6250 ppm and 8334 ppm of dinotefuran. All the concentrations of dinotefuran were kept in labeled volumetric flasks until the tests. Each treatment was conducted in duplicate.

2.5. Experimental model

R. sanguineus semi-engorged females were divided into three treated groups: group II (5000 ppm of dinotefuran), group III (6250 ppm of dinotefuran) and group IV (8334 ppm of dinotefuran). The control group was exposed only to the placebo (distilled water).

The 120 semi-engorged females of *R. sanguineus*, after being washed in a sieve with tap water, were dried on soft absorbent paper. After that, 90 females were divided into three groups of 30 females (30 females for each concentration – 2 groups with 15 individuals – duplicates) and immersed for 5 min in Petri dishes containing the above different concentrations of dinotefuran. The control group was also composed of 30 females that had been immersed in distilled water for the same period. Ticks were then dried in absorbent paper and placed in the BOD incubator (28 ± 1 °C, 80% relative humidity and 12 h photoperiod) for 7 days. The observation period was established because frequently the effect of acaricides is not immediate, but acts slowly on the physiology of the individual analyzed (Roma et al., 2010).

After 7 days of monitoring, all the semi-engorged females were forwarded to ultrastrucure techniques.

2.6. Methods

2.6.1. Transmission electron microscopy

All semi-engorged females were fixed in 2.5% glutaraldehyde for two hours, rinsed twice for 15 min each with 0.1 M sodium cacodylate buffer, and post-fixed with 1% osmim tetroxide for two hours. The material was then rinsed twice with 0.1 M sodium cacodylate for 15 min each.