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Research Article

Ricinoleic acid esters from castor oil modifying male reproductive system of *Amblyomma cajennense* (Fabricius 1787)

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Abstract

Ticks of the genus *Amblyomma* have medical and veterinary importance because they can transmit pathogens to humans, as well as domestic, wild and livestock animals. The management and control of this tick has led livestock farmers to an inadequate use of synthetic chemical acaricides, consequently creating environmental problems and selecting resistant populations. Thus, the search for new substances that can be efficient in tick control and have low toxicity to the environment and non-target organisms is latent. Therefore, the present study aimed to evaluate the effect of ricinoleic acid esters from castor oil as modifying agents of *A. cajennense* male reproductive system, seeking an alternative method to control these parasites. Hence, esters from castor oil were incorporated to the diet of rabbits, which were then infested with *A. cajennense*. Male ticks were collected from the hosts and their reproductive systems were removed and prepared for histological and histochemical techniques. The results showed that the effects of esters became more evident at the highest concentration available, leading to morphophysiological changes in the secretory cells of the accessory gland complex, altering the secretion content and causing morphological changes in spermatids. The esters changed development dynamics and gamete production, probably affecting the production of spermatophores and seminal fluid. Our results confirmed that these substances have the potential to interfere with reproduction, one of the most important biological processes for a species.

Keywords *Amblyomma cajennense*, esters, control, castor oil, testes

Introduction

Ticks of genus *Amblyomma* (Acari: Ixodidae) are widely distributed and have great medical and veterinary importance, mainly because they are related to the transmission of pathogens to humans, as well as domestic, wild and livestock animals, in addition to lacking preferred hosts throughout their ontogenic development (Alonso-Diaz et al. 2013, Senra et al. 2013, Krawczak et al. 2014).

In Brazil, *Amblyomma cajennense* is closely related to the human transmission of the bacterium *Rickettsia rickettsii*, the etiologic agent of the Brazilian spotted fever (BSF). The capybara (*Hydrochoerus hydrochaeris*) is an important wild host, which acts as an amplifier of infection within *A.cajennense* populations, especially in endemic areas in the State of São Paulo, Brazil (Ogrzewalska et al. 2011, Krawczak, et al. 2014).

A. cajennense, along with *Rhipicephalus annulatus* and *Ripicephalus microplus*, infests livestock in countries, such as Kenya and Tanzania, in West Africa, and Mexico, in North America. This high incidence hinders the management and control of these ticks, leading to an inadequate use of synthetic chemical acaricides, which in turn causes environmental problems and the selection of resistant populations (Alonso-Diaz et al. 2013, Senra et al. 2013).

Thus, the search for new acaricides that can be efficient in tick control and have low toxicity to the environment and non-target organisms is latent.

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Recent investigations have focused mainly on substances of vegetable origin, such as oils and extracts, which have shown good results. For example, eugenol and thymol isolated from *Thymus vulgaris* and *Dianthus caryophylus* oils, respectively, have been used on *A. cajennense*, *Dermacentor nitens* and *R. sanguineus* (Senra et al. 2013). Andiroba oil has been tested against *R. sanguineus* (Vendramini et al. 2012). *Tagetes patula* extract has been assessed against *R. sanguineus* larvae (Politi et al. 2012), while ricinoleic acid esters from castor oil have been tested on *R. sanguineus* (Arnosti et al., 2011a,b, Sampieri et al. 2012, Sampieri et al. 2013).

In addition to the environmental benefits of using substances of vegetable origin, the medical, veterinary and economic importance of *A. cajennense* has reinforced the need for new control methods. In this sense, the present study aimed to evaluate the effect of ricinoleic acid esters from castor oil as modifying agents of the morphophysiology of *A. cajennense* male reproductive system, thus confirming the potential of these substances for tick control.

Material and Methods

Bioassays

Three experimental groups were determined for this study: **Control Group (CG), Treatment Group 5 (TG5)** and **Treatment Group 15 (TG15).** A host rabbit (New Zealand White, Botucatu variety) was used for each group. The esters of ricinoleic acid from castor oil was produced and kindly provided by Prof. Dr. Gilberto Orivaldo Chierice from Department of Chemistry and Molecular Physics from USP – São Carlos, SP, Brazil.

The esters were added to the diet of the experimental groups (**TG5** and **TG15**). Standard rabbit food (Nutriara®) was crushed, the ricinoleic acid esters from castor oil were added before it was pelletized again, according to the methodology adopted by Arnosti et al. (2011a) and Sampieri et al. (2012). This diet was offered to the hosts for 15 days before *A. cajennense* infestation, in order to familiarize them with the new food. All three rabbits, from each group, were weighed at the beginning of the experiment and at its end. Weight data were collected and organized into a table, and then the differences between the groups (**GC**, **GT5 and GT15**) were calculated in ANOVA.

The food offered to each rabbit was also weighed daily, registered in a table to average calculation of its consumption. Those results are summarized in Table 1.

Table 1 Average feed intake by each rabbit from the three experimental groups during 30 days of experiment

Experimental Groups	Average Feed Intake
Control Group	149,79 g
Treatment Group 5	188,70 g
Treatment Group 15	169,88 g

The infestation in each experimental group is given as follows:

All hosts received the diet for 15 days before being infested with 16 pairs of *A. cajennense* ticks each. The period of blood feeding on the host lasted about 15 days, for which the host continued to receive the same diet.

Control Group (CG): received standard Rabbit food.

Treatment Group 5 (TG5): received a special diet prepared with standard rabbit food added with ricinoleic acid esters from castor oil (5 g/kg).

Treatment Group 15 (TG15): received a special diet prepared with standard rabbit food added with ricinoleic acid esters from castor oil (15 g/kg).

After the 12th day of infestation, when the germ cells are fully developed, male ticks from the three experimental groups were collected whenever they were in copulation position (ventral contact with the female in engorgement). Male ticks were cold-anesthetized in a refrigerator and dissected to remove the reproductive system, which underwent histological and histochemical analyses.

The present study was approved by the Ethical Committee in Animal Use (Comitê de Ética de Uso Animal-CEUA) from the Instituto de Biociências, UNESP, Rio Claro-SP, Brazil, under process number 017/2012 and protocol 1422.

Histology

The male reproductive system of 24 *A. cajennense*ticks, 8 in each group **(CG, TG5 and TG15),** were fixed in 4% paraformaldehyde for 24 h and dehydrated in increasing concentrations of ethanol (70, 80, 90 and 95%) for 15 min at each concentration. Then, the samples were embedded in Leica historesin for 72 h and polymerized in Leica

historesin. Later, the blocks were sectioned with a Leica microtome. The sections (3µm) were placed on glass slides and stained with hematoxylin-eosin (HE) for photo documentation using a Leica DM750 light microscope.

Histochemistry

Periodic acid-Schiff (PAS) staining for total polysaccharides detection

Eight male ticks from each group (**CG, TG5** and **TG15**) had their reproductive systems removed and fixed in aqueous Bouin's fluid for 72 h. The samples were dehydrated in increasing concentrations of ethanol (70, 80, 90 and 95%) for 15 min at each concentration. Afterwards, the samples were embedded in Leica historesin for 72 h and polymerized in Leica historesin. Later, the blocks were sectioned with a Leica microtome. The sections (3µm) were placed on glass slides and subjected to PAS staining for photo documentation using a Leica DM750 light microscope.

Results

Effect of Feed Intake on Rabbit weight

Rabbits from all three groups ate normally, showing that taste and texture of the special diet was well.

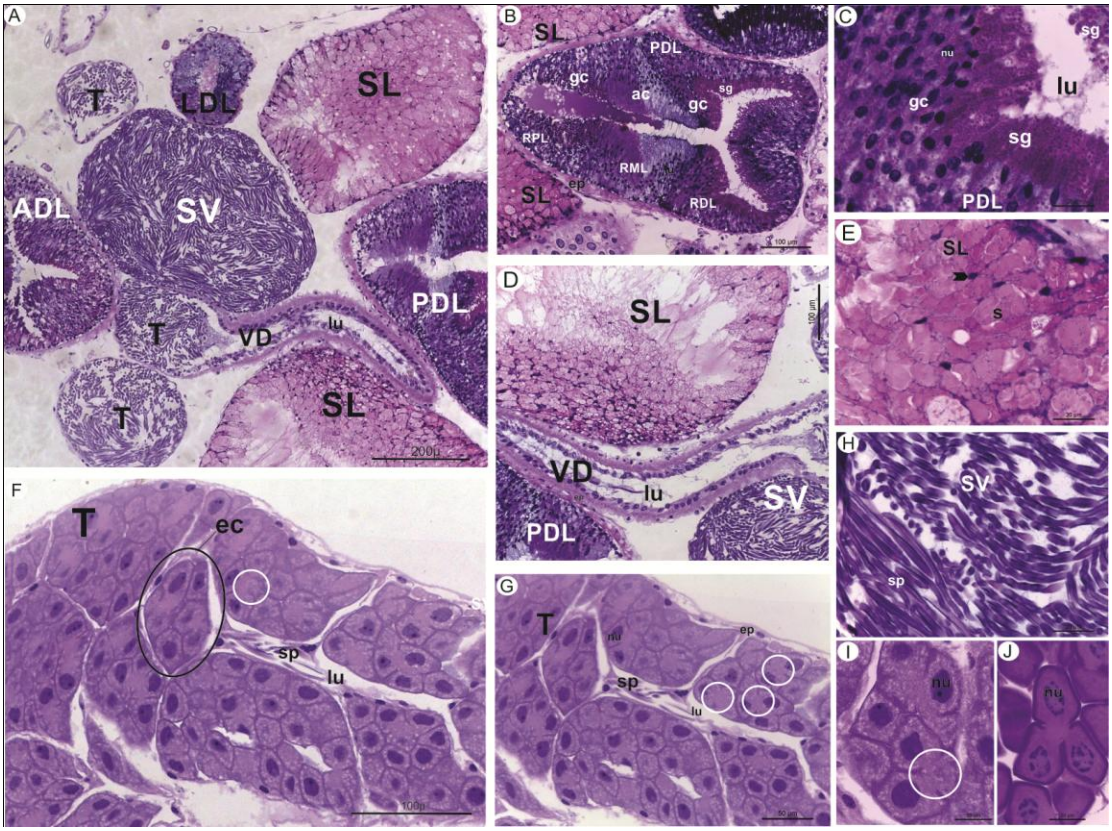

Figure 1 (A-J) Histological sections of male reproductive system of *Amblyomma cajennense* from Control Group (GC) stained with Hematoxylin-Eosin.

(A) Overview of the male reproductive system, where it can be observed the glandular complex lobes, the testes (**T**) connected to the vasa deferentia (**VD**) and the seminal vesicle (**SV**); (B-C) Detail of the postero-dorsal lobe (**PDL**) exhibiting granular (**gc**) and agranular cells (**ac**); (D-E) Detail of the spongy lobe (**SL**) with elongated cells full of secretion; (F-J) Testis exhibiting spermatocysts (**ec**) housing spermatids in development and seminal vesicle full of spermatozoa **(sp)**. **ADL**=Antero Dorsal Lobe; circle=cytoplasmic bridges; **ep**=epithelium; **lu**=lumen; **nu**=nucleus; **RDL**=Lobe Distal Region; **RML**=Lobe Midle Region; **RPL**=Lobe Posterior Region; **S**=secretion; **sg**=secretory granules.

accepted by the animals. All three groups showed no health problems as diarrhea or malnutrition consequences. The differences in weight gain between animals were not significant (P=0.30891), confirming the animals nutrition and health status.

Control Group (CG)

The male reproductive system of ticks from this group has general morphology identical to that described in literature, showing a multilobed accessory gland complex, a pair of seminal vesicles, a pair of vasa deferentia and a pair of elongated tubular testes containing germ cells.

A layer of epithelial cells lines the lobes of the accessory gland complex, being more evident in the postero-dorsal lobe (PDL). This lobe shows many secretory cells under the lining epithelium, with their nuclei strongly stained by hematoxylin and secretory granules weakly or strongly stained (Fig. 1A, B and C).

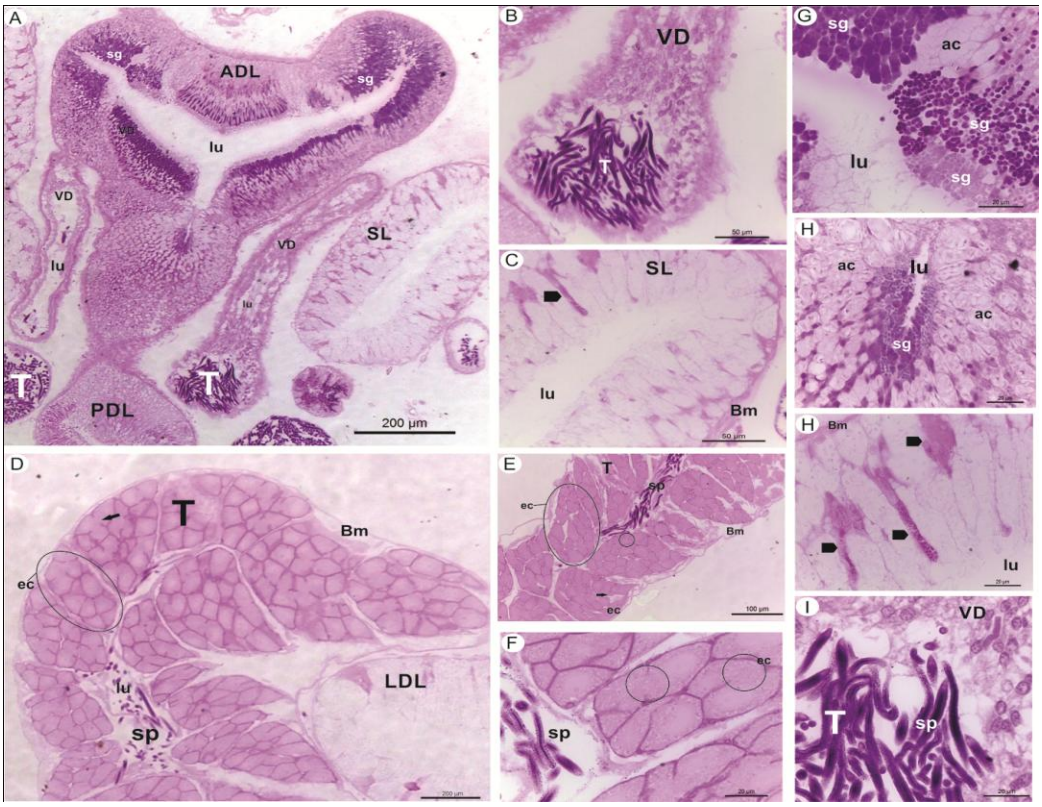

Fig. 2 - Histological sections of male reproductive system of *Amblyomma cajennense* from Control Group (**GC**) with PAS reaction. (A) Overview of the male reproductive system, where it can be observed the glandular complex lobes stained with variable intensity, the testes (**T**) connected to the vasa deferentia (**VD**) and the seminal vesicle (**SV**); (B) Distal portion of the testis connected to the VD, exhibiting spermatozoa strongly stained by the technique; (C) Spongy lobe (**SL**) exhibiting elongated cells negative to the PAS technique and intercellular spaces (**setae head**) housing positive-stained secretion; (D-F) Testis exhibiting spermatocysts (**ec**) housing spermatids in development and spermatozoa (**sp**) in its lumen. (H) Detail of spongy lobe, exhibiting elongated cells between intercellular spaces containing secretion positive-stained; (I) Detail of the proximal portion of the testis connected to the vasa deferentia, where it can be observed spermatozoa positive-stained. **ADL**=Antero Dorsal Lobe; **Bm**=basal membrane; **circle**=cytoplasmic bridges; **ep**=epithelium; **LDL**=Latero-Dorsal Lobe; **lu**=lumen; **nu**=nucleus; **PDL**= Postero-Dorsal Lobe; **S**=secretion; **sg**=secretory granules.

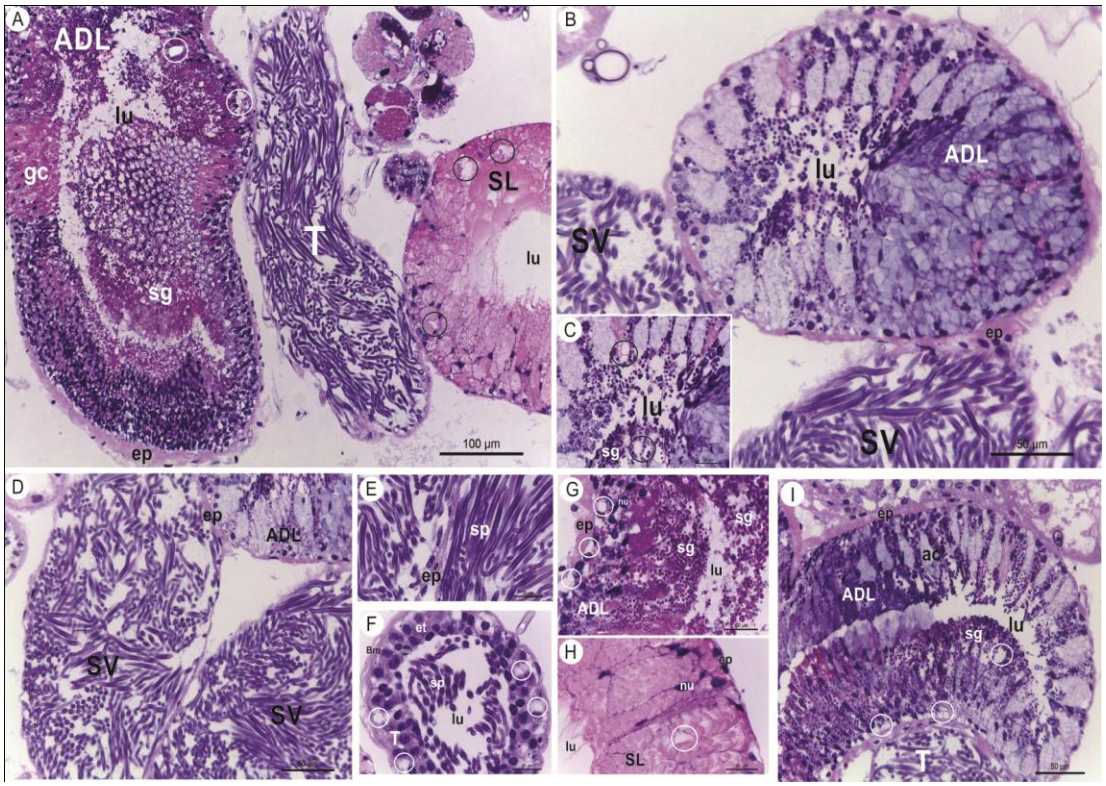

Fig. 3 - Histological sections of male reproductive system of *Amblyomma cajennense* from Treatment Group 5 (**GT5**) stained with Hematoxylin-Eosin. (A) Overview of the male reproductive system, where it can be observed the Antero Dorsal Lobe and the Spongy Lobe of the glandular complex exhibiting tissue disorganization and cell damages; (B-C) Antero Dorsal Lobe in detail showing vacuoles in its cells; (D-E) Seminal vesicles full of spermatozoa with none morphological alterations; (F) Transversal section of the testis exhibiting spermatids in development with vacuoles (circle) in its cytoplasm; (G-I) Detail of the ADL and the SL exhibiting cells with cytoplasmic damaged (vacuoles). **ac**=agranular cells; **ADL**=Antero Dorsal Lobe; **circle**=vacuoles; **ep**=epithelium; **lu**=lumen; **nu**=nucleus; **S**=secretion; **sg**=secretory granules; **SV**=seminal vesicle ; **sp**=spermatozoa; **T**=testis.

In the same lobe, it is possible to classify at least two cell types, based on the released secretion: a) granular cells, when the secreted material is seen in the cytoplasm, as granules; b) agranular cells, where secretion is seen dispersed in the cytoplasm. The granular cells occupy most of the antero-dorsal (ADL) and postero-dorsal lobes (PDL), being observed in the distal and proximal regions with purple secretory granules. Agranular cells are observed in the lobe median region, showing the secretion stained in light purple by hematoxylin (Fig. 1B).

The lateral-dorsal lobes (LDL) have elongated secretory cells, with barely evident nuclei and cytoplasm with large eosin-stained secretory granules. Thus, the LDL are here called spongy lobes (SL) (Fig. 1A, B, D and E).

The testes are covered by a squamous epithelium resting on a basement membrane, with large spermatids enveloped by the same epithelium, in the distal portion. Spermatids are seen in the spermatocysts at an advanced stage of development, with large and rounded nuclei, heterogeneous cytoplasm, evident cell boundaries and cytoplasmic bridges (Fig. 1F, G, I and J). Some spermatozoa at the final stages of maturation are observed in the testis lumen (Fig. 1F and G).

Spermatozoa reaching the lumen of the vasa deferentia are seen in the testes proximal region. The vasa deferentia are tubular and lined by

stratified cuboidal epithelium, with an outer layer facing the cavity of the tick body and an inner layer facing the lumen (Fig. 1A and D).

The spermatozoa seen reaching the lumen of the vasa deferentia are completely different from the spermatids observed in the testis distal region, and the cell as a whole is strongly stained by hematoxylin (Fig. 1A and H).

The seminal vesicles are parallel and have the appearance of bags lined by simple squamous epithelium, showing a lumen filled with mature spermatozoa (Fig. 1A, D and H).The accessory gland complex shows variation in PAS staining, according to the type of lobe analyzed. In the PDL and ADL, the cytoplasm of granular and agranular cells, as well as the epithelial cells, are general positively stained. However, the secretory granules show moderately to strongly positive staining (Fig. 2A, G and H).

The SL basement membrane is positively stained through the PAS method, but the secretory cells in these lobes are negative. Some SL regions

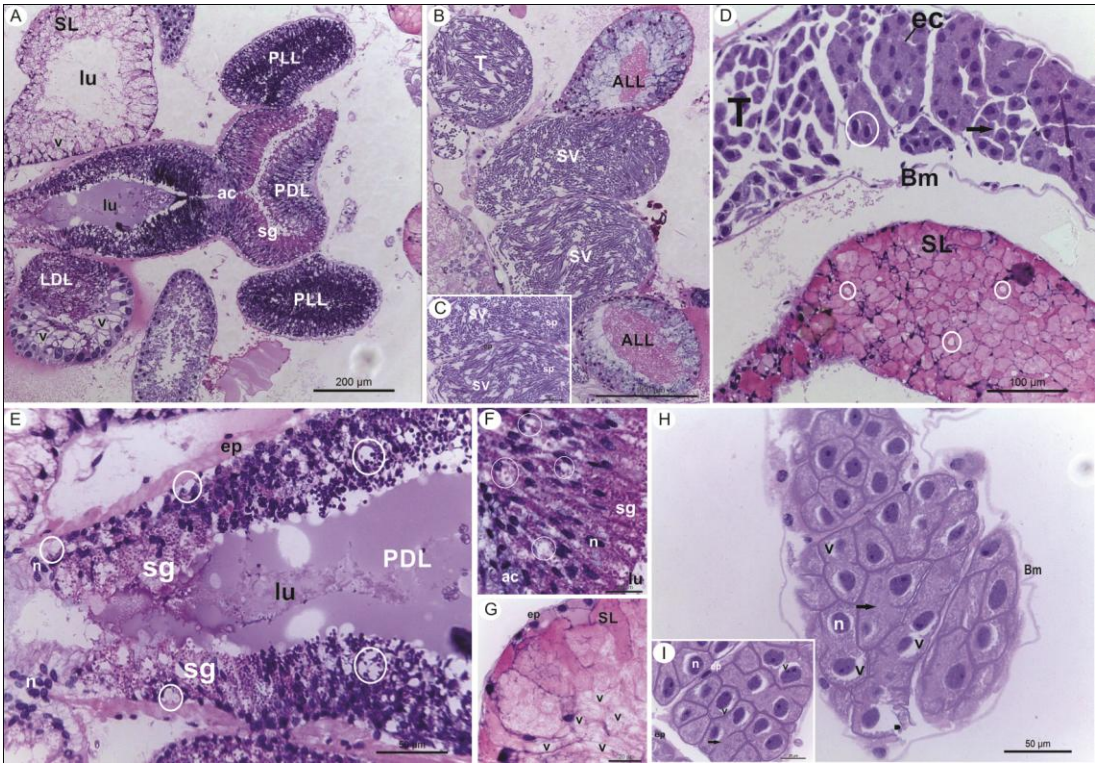

Fig. 4 - Histological sections of male reproductive system of *Amblyomma cajennense* from Treatment Group 5 (**GT5**) with PAS reaction(A) Overview of the male reproductive system, where it can be observed the Antero-Dorsal Lobe (**ADL**), Postero-Dorsal Lobe (**PDL**) and the Spongy Lobe (**SL**) of the glandular complex connected to a pair of Seminal Vesicles (**SV**); (B-C) Transversal section of the testis exhibiting spermatids in development with vacuoles (circle) in its cytoplasm which shows a medium-positive stained; (D-E) Detail of the ADL with negative-stained agranular cells and secretion granules in its lumen; (F) SL in detail with elongated cells negative-stained and intracellular secretion weakly-stained (ellipse); (E) Detail of spermatozoa inside the SV strongly-positive to the technique. **ac**=agranular cells; **Bm**=basal membrane; **ep**=epithelium; **lu**=lumen; **nu**=nucleus; **S**=secretion; **sg**=secretory granules; **sp**=spermatozoa; **T**=testis; **v**=vacuoles.

show intercellular spaces filled with PAS-stained secretion (Fig. 2A, C and I).

The testes basement membrane shows a positive staining, as well as the SL. Spermatids within spermatocysts present homogeneous

cytoplasm and cellular boundaries with positive staining. PAS-positive spermatozoa are seen in the lumen (Fig. 2D, E and F). Spermatozoa located in the testis proximal region are strongly PAS-positive (Fig. 2B and J).

Treatment Group 5 (TG5)

The male reproductive system of ticks from this group shows the accessory gland complex lined by an intact epithelium where morphological changes are not observed. Certain tissue disorganization is observed in the ADL, where secretory cells exhibit vacuolization around their nuclei and between the cytoplasmic secretory granules. Heterogeneous material different from that observed in the **CG** is seen in the lumen of this lobe, suggesting the presence of secretory cells from the glandular tissue (Fig. 3A, B, C and H).

The testes are almost fully developed and filled with spermatozoa. Germ cells organized in spermatocysts are not seen (Fig. 3A). However, in the testis transverse section is possible to note a region with widely dilated lumen containing some spermatozoa.

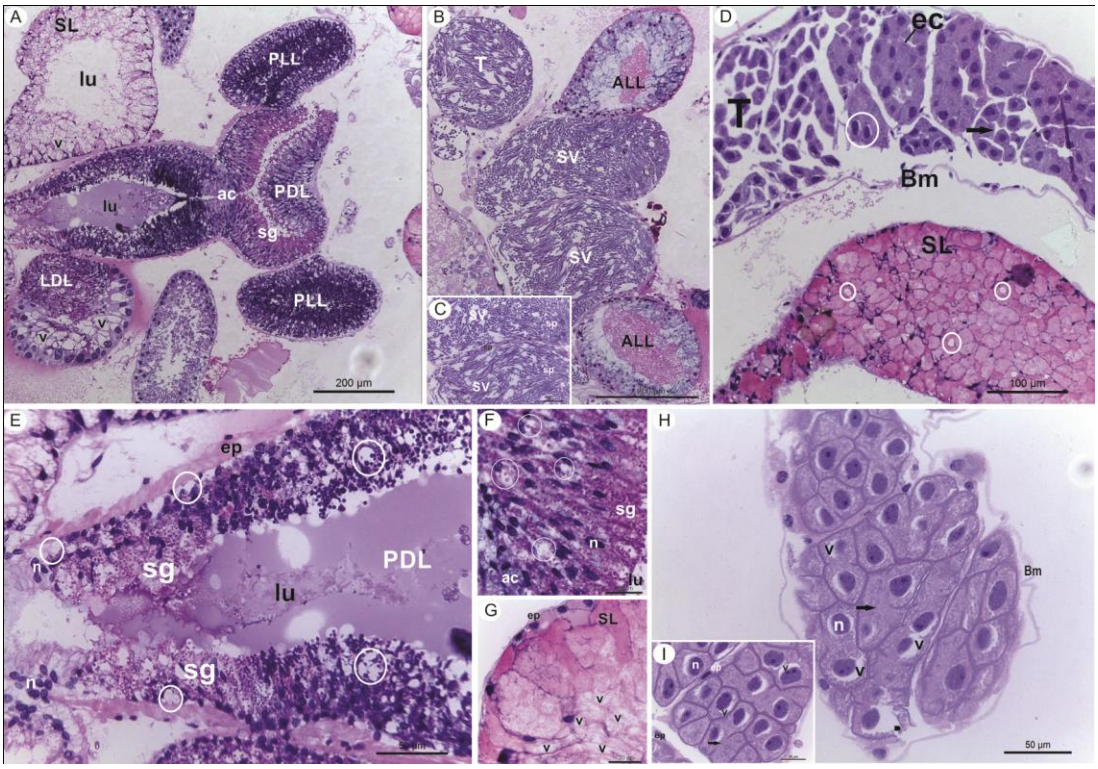

Fig. 5 - Histological sections of male reproductive system of *Amblyomma cajennense* from Treatment Group 15 (**GT15**) stained with Hematoxylin-Eosin. (A) Overview of the male reproductive system, where it can be observed the Postero-Dorsal Lobe (**PDL**), the Spongy Lobe (**SL**), the Lateral Dorsal Lobe (**LDL**) of the glandular complex with its cells damaged by the esters; (B-C) Detail of the Seminal Vesicles and the Antero-Lateral Lobe (ALL); (D) Testis (T) and SL in detail showing high level of cell damage and tissue disorganization; (E-G) Detail of the PDL and the SL exhibiting vacuolized cells (circles) and tissue damage; (H-I) Testis exhibiting spermatids with vacuoles around the nucleus. **ac**=agranular cells; **Bm**=basal membrane; **ep**=epithelium; **lu**=lumen; **n**=nucleus; **PLL**=Postero-Latero Lobe; **S**=secretion; **setae**=cell bridges; **SV**=Seminal vesicles; **sg**=secretory granules; **sp**=spermatozoa; **T**=testis; **v**=vacuoles.

The layers of germ cells are disorganized (under the basement membrane) and cells with round nuclei and vacuolated cytoplasm are observed. It is impossible to identify the spermatid developmental stage through this morphology (Fig. 3F).

The seminal vesicles show intact morphology, with spermatozoa weakly hematoxylin-stained, when compared with the **CG** (Fig. 3D and E).The polysaccharides detection method (PAS) in this group shows decreased staining intensity, mainly in the lobes of the glandular complex. ADL staining is moderately positive, with some negative areas being observed, which are probably agranular cells (4A, D and E).The secretory granules visible in the anterior and posterior lobes also show varying staining intensity (positive to moderately positive) (Fig. 4A, D and E).

The SL basement membrane and the few secretory granules found in the intercellular spaces are positively stained. The secretory cell cytoplasm is negative (Fig. 4F).The spermatozoa in the seminal vesicles are strongly stained, as in the **GC** (Fig. 4A-C and G). An area in the testes containing spermatids is positively stained, and some spermatozoa in the lumen show moderately positive staining (Fig. 4B, C and G).

Treatment Group 15 (TG15)

In this group, the morphological and histochemical changes observed in the male reproductive system are more pronounced than in the **TG5**. The accessory gland complex shows the PDL with secretory cells having strongly hematoxylin-stained nuclei and disorganized and vacuolated cytoplasm, especially around the nuclei (Fig. 5A, E and F). The lateral-dorsal lobe (LDL) shows secretory cells with changed morphology, containing large vacuolization areas in the cytoplasm (Fig. 5A).The testes contain completely disorganized spermatocysts sheltering spermatocytes at different stages of development, which are thus seen in varying sizes with heterogeneous cytoplasm and large areas of vacuolization around the nuclei. Several germ cells interconnected by cytoplasmic bridges are also visible (5D, H and I).The spermatozoa in the seminal vesicles are weakly stained by hematoxylin, in comparison with the groups previously described (Fig. 5B and C).

PAS staining resulted in a more intense color variation in the glandular complex lobes. Positive or strongly positive staining is observed. Cells in the PDL have positive cytoplasm, whereas secretory granules show strongly positive staining. Some areas of the cytoplasm are negatively stained, with a likely concentration of vacuoles. Fused secretory granules are strongly stained (Fig. 6A, E and G).Secretory cells show positive cytoplasm in the LDL, and areas with large vacuoles. The secretory granules that leave the secretory cell and reach the lumen are strongly positive (Fig. 6A and H).The testes are supported by a positive basement membrane. A detachment of the epithelium that lines the spermatocysts is observed. Spermatocysts are disorganized sheltering spermatids with moderately positive cell boundaries and heterogeneous, vacuolated cytoplasm (Fig. 6B, F and J). Moderately positive spermatozoa are observed in the lumen of the testes (Fig. 6B and F). Spermatozoa in the seminal vesicles are strongly positive (Fig. 6D and L).The results of the polysaccharide detecting method (PAS) are summarized in Table 2.

Table 2

Results summary from the application of hystochemical tests for polysaccharides detection in *Amblyomma cajenensse* reproductive system: control group (CG), treatment group 5 (TG5) and treatment group 15 (TG15).

	PAS (Polysaccharides)		
	GC	**GT5**	**GT15**
Granular cells	+	+	+
Agranular cells	+	−	−
Spongy Lobe	−	−	−
Spermatids	+	+	+
Spermatozoon (testis)	+++	++	++
Spermatozoon (seminal vesicle)	+++	+++	++

(−)negative; (+) positive; (++) medium positive; (+++) strongly positive.

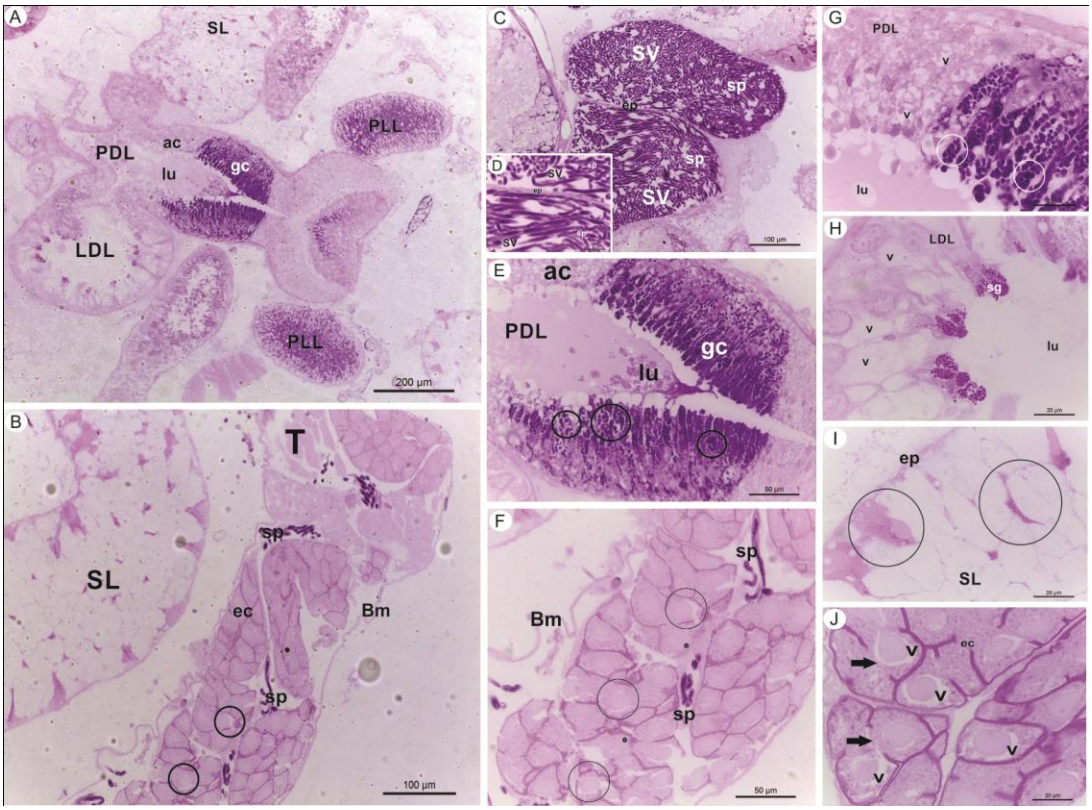

Fig. 6 - Histological sections of male reproductive system of *Amblyomma cajennense* from Treatment Group 15 (**GT15**) with PAS reaction. (A) Overview of the male reproductive system, where it can be observed the Postero-Dorsal Lobe (**PDL**), the Spongy Lobe (**SL**), the Lateral Dorsal Lobe (**LDL**), the Postero-Lateral Lobe (**PLL**) and the Spongy Lobe (**SL**) of the glandular complex, the last one highly damaged by the esters; (B) Detail of the SL and Testis (**T**) exhibiting spermatocysts with spermatids inside it weakly-positive for the technique; (C-D) Detail of the Seminal Vesicles full of spermatozoa, apparently with none morphological or histochemical alterations; (E) Detail of the **PDL** with agranular and granular cells releasing secretion granules in its lumen; (F) Testis exhibiting spermatids with vacuolized cytoplasm and disrupted cell boundaries; (G-I) Detail of secretion granules of granular cells from PDL fused and surrounded by an white halo (circle), the LDL with highly vacuolized granular cells and the SL showing its elongated cells negative for the technique. (J) Detail of spermatids exhibiting a heterogeneous cytoplasm with vacuolized areas. **ac**=agranular cells; **Bm**=basal membrane; **ep**=epithelium; **gc**=granular cells; **lu**=lumen; **n**=nucleus; **PLL**=Postero-Latero Lobe; **S**=secretion; **setae**=cell bridges; **sg**=secretory granules; **sp**=spermatozoa; **v**=vacuoles

Discussion

Ticks of the genus *Amblyomma* are currently important for the medical and scientific communities, as well as farmers around the globe, because they are closely related to losses in animal by-products industries and public health problems in tropical and subtropical regions (Rodriguez-Valle et al. 2012, Alonso-Díaz et al. 2013, Senra et al. 2013, Nava et al. 2014).

Recent studies have shown that *A. cajennense* populations in South America have developed resistance (mortality rates lower than 80%) to some acaricides, especially amitraz and deltamethrin, widely used by farmers for the control of *Rhipicephalus microplus* in cattle (Freitas et al. 2011, Alonso-Díaz et al. 2013, Rodriguez-Valle et al. 2013).

Therefore, given the importance of *A. cajennense* and the demand for new control methods, the results obtained in this study indicate that the esters of ricinoleic acid from castor oil, when incorporated to the host feeding, may act against hematophagous ectoparasites, being a

promising alternative for the management of rural and urban pests.

The data obtained from the **CG** allowed not only a comparison with **TG5** and **TG15**, but also to expand the knowledge on the male reproductive system of Ixodidae, which is basically composed of an accessory gland complex and a pair of testes. The accessory gland complex is responsible for the production and secretion of the spermatic fluid and spermatophore synthesis, both essential for the transfer of spermatozoa into the female reproductive tract. On the other hand, testes are responsible for the production and maturation of germ cells until they reach the spermatid stage and reach the lumen as spermatozoa, moving toward the seminal vesicles.

The accessory gland complex is morphologically composed of lobes that develop and secrete material, a process dependent on the tick feeding stage. Sonenshine (1970) classified the gland complex lobes as granular and agranular according to the type of material secreted by the cells. The granular type is most often found in the tick species analyzed to date. Sampieri et al. (2014) reported that the presence or observation of SL could be directly linked to feeding stage, which was confirmed in this study.

To our knowledge, *A. cajennense* SL had not been described previously. In this study, there was an indication that the secretion produced by this lobe was used in the final stages of the tick reproduction, probably when producing the spermatophores, because it has not been observed in individuals that fed less than 10 days on the host.This was confirmed by the PAS staining, which showed that the large volume of secretion in the cytoplasm of SL cells is not of polysaccharide nature. Instead, this secretion has a protein or glycoprotein nature, as large sacs containing proteinaceous material were found in the spermatophores of some tick species. This material is formed only at the final stages of the reproductive process, when the male detaches from the host to complete the synthesis of the spermatophore and transfer it into the female reproductive tract (Feldman-Muhsan et al. 1973).

From a morphological point of view, the SL found in the **TG5** did not undergo significant changes, except for the presence of some cytoplasmic vacuoles. However, SL cells showed extensive areas of vacuolization in the **TG15,** indicating that the increased concentration of esters in the diet also increases the damage to SL cells.The PDL, ADL and LDL of individuals from **TG5** and **TG15** showed morphophysiological changes, mainly cytoplasmic vacuolization, tissue disorganization and changes in the secretion process as a whole. In the **CG**, the PDL showed granular cells located in the anterior and posterior regions and agranular cells in the median lobe region. This organization seems to have changed as the concentration of esters increased, becoming more evident in the **TG15.**

Morphological changes certainly caused physiological damage, altering the production and release pattern of cellular secretion, compromising the quantity and composition of the seminal fluid and the spermatophore constituent material, which probably interfered or even delayed insemination. Furthermore, a protein of 12.000 DA seems to be involved in spermatids maturation already in the female tract and synthetized in the male glandular complex (Shepherd et al. 1981). Since the morphophysiology of the glandular complex appears to be altered, the synthesis of this protein and the process involved may be compromised.

The effect of exposure to esters became more evident in the spermatids and testes, perhaps because these cells are more sensitive to changes and more exposed to the medium elements. In **TG5,** most testes showed intact spermatozoa. Identification of spermatid developmental stage was hindered due to the action of the esters on the system morphology. Thus, it was confirmed in this group (**TG5**) that the esters acted on the testes and their cells: a) directly, causing extensive vacuolization in the cytoplasm of the few observed spermatids; b) indirectly, by inducing the individual to accelerate the maturation of spermatids, probably in an attempt to preserve these cells from the toxic effect of the product.

However, the concentration of esters did not alter germ cell maturation in **TG15**, as spermatocysts with developing spermatids were clearly observed in this group. Nevertheless, a complete disorganization was evident in spermatocysts, accompanied by the basement membrane detachment and disruption of the testis epithelial lining. Spermatids were also affected by the esters, showing a highly vacuolated cytoplasm, especially around the nuclei. In some cases, a reduction in the size of spermatids was observed, as

if they were in early stages of development. Some seemed to be at an advanced stage of degeneration, even showing cell lysis.

In **TG5** and **TG15,** the spermatozoa showed no significant morphological changes, except in staining with hematoxylin and PAS, when compared to the **CG**. Mainly in the **TG15**, the hematoxylin and the PAS staining were less intense than in the **CG**. This may indicate that a weak PAS staining was a result of the esters action, which was effective in the hydrolysis of polysaccharides. Leonardo et al. (2001), Ferreira et al. (2002) and Messeti et al. (2010), who showed the esters ability to hydrolyze the walls (proteoglycans) of the bacteria *Fusobacterium nucleatum*, *Prevotella nigrescens*, *Clostridium perfringens* and *Leuconostocmes enteroides*, have previously proved this.

Conclusions

Arnosti et al. (2011 a, b) and Sampieri et al. (2013) subjected *R. sanguineus* ticks to esters, reporting significant morphological and ultrastructural changes in the female reproductive system, with evident effect on the process of vitellogenesis in oocytes. However, the results obtained by these authors, added to those presented in this paper, confirm that the esters should be effectively tested as an acaricides, since it is clear that this product can interfere with reproductive parameters. Thus, ricinoleic acid esters from castor oil could be also used in the integrated management in combination with other methods of tick control in the field.

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References

A.Arnosti, P.D. Brienza, K.C.S. Furquim, G.O. Chierice, G.H. Bechara, I.B. Calligaris, M.I. Camargo-Mathias **(2011a)**. Effects of ricinoleic acid esters from castor oil of *Ricinus communis* on the vitellogenesis of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) ticks. Exp. Parasitol., **127**: 575–580.

A.Arnosti, P.D. Brienza, K.C.S. Furquim, G.O. Chierice, S.C. Neto, G.H. Bechara, B.R. Sampieri, M.I. Camargo-Mathias **(2011b)**. Effects of *Ricinus communis* oil esters on salivary glands of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). Exp. Parasitol., **127**: 569–574.

B. Feldman-Muhsan, S. Borut, S. Saliternik-Givant, C. Eden **(1973)**. On the evacuation of sperm from the spermatophore of the tick, *Ornithodoros savignyi*. J. Insect. Physiol.,**19**: 951-962.

B.R. Sampieri, A. Arnosti, P.H. Nunes, K.C.S. Furquim, G.O. Chierice, M.I. Camargo-Mathias **(2012)**. Ultrastructural changes in the ovary cells os engorged *Rhipicephalus sanguineus* female ticks trated with esters of ricinoleic acid from castor oil (*Ricinus communis*). Micros. Res. Techniq. **75**: 683-690.

B.R. Sampieri, K.C.S. Furquim, P.H. Nunes, M.I. Camargo-Mathias **(2013)**. *Rhipicephalus sanguineus* (Acari: Ixodidae) female ticks exposed to castor oil (*Ricinus communis*): an ultrastructural overview. Parasitol. Res., **12**: 611-619.

B.R. Sampieri, M.B. Labruna, O.C. Bueno, M.I. Camargo-Mathias **(2014)**. Dynamics of cell and tissue genesis in the male reproductive system of ticks (Acari: Ixodidae) *Amblyomma cajennense* (Fabricius, 1787) and *Amblyomma aureolatum* (Pallas, 1772): a comparative analysis. Parasitol. Res., **113**: 1511-1519.

C.M. Ferreira, O.P.S. Rosa, S.A. Torres, F.B.A. Ferreira, N. Bernardinelli **(2002)**. Activity of endodontic antibacterial agents against selected anaerobic bacteria. Braz. Dent. J.,**13**: 118-122.

D.E. Sonenshine**(1970)**. A contribution to the internal anatomy and histology of the bat tick *Ornithodoros kelleyi* Cooley and Kohls 1941. J. Med. Ent., **7**: 289-312.

D.E Sonenshine**(1991)**. Biology of ticks. Oxford University Press.

E.P.S. Freitas, M.T.A.G. Zapata, F.F. Fernandes **(2011)**. Monitoring of resistance or susceptibility of adults and larvae of

Amblyomma cajennense (Acari: Ixodidae) to synthetic acaricides in Goias, Brazil. Exp. Appl. Acarol., **53**: 189-202.

F.A. Politi, G.M. Figueira, A.M. Araújo, B.R. Sampieri, M.I. Camargo-Mathias, M.P. Szabó, G.H. Bechara, L.C. Dos Santos, W. Vilegas, R.C. Pietro **(2012)**. Acaricidal activity of ethanolic extract from aerial parts of Tagetespatula L. (Asteraceae) against larvae and engorged adult females of *Rhipicephalus sanguineus* (Latreille, 1806). Parasite. Vector. **5**: 295-300.

F.S. Krawczak, F.A. Nieri-Bastos, F.P. Nunes, J.F. Soares, J. Moraes-Filho, M.B. Labruna **(2014).**Rickettsial infection in *Amblyomma cajennense* ticks and capybaras (*Hydrochoerus hydrochaeris*) in a Brazilian spotted fever-endemic area. Parasite. Vector., **7**: 7.

M. Ogrzewalska, A. Uezu, M.B. Labruna **(2011)**. Ticks (Acari: Ixodidae) infesting wild birds in the Atlantic Forest in northeastern Brazil, with notes on rickettsial infection in ticks. Parasitol. Res., **108**: 665-670.

M. Rodriguez-Valle, A. Taoufik, M. Váldes, C. Montero, H. Ibrahin, S.M. Hassan, F. Jongejan, J. De La Fuente **(2012)**. Efficacy of *Rhipicephalus (Boophilus) microplus* Bm86 against *Hyalomma dromedarii* and *Amblyomma cajennense* tick infestations in camels and cattle. Vaccine, **30**: 3453-3458.

M.A. Alonso-Diaz, A. Fernández-Salas, F. Martínez-Ibáñez, J. Osorio-Miranda **(2013)**. *Amblyomma cajennense* (Acari: Ixodidae) tick populations susceptible or resistant to acaricides in the Mexican Tropics. Vet. Parasitol, **197**: 326-331.

M.A. Messetti, A.M. Santos, D.F. Angelis, G.O. Chierice, S.C. Neto **(2010)**. Estudo dos derivados do óleo de *Ricinus communisL.* (Mamona) como agente biocida e redutor da viscosidade produzida por *Leuconostoc mesenteroides* em indústrias sucroalcooleiras. Arq. Inst. Biol. **77**: 301-308.

M.C.R. Vendramini, M.I. Camargo-Mathias, A.U. De Faria, K.C.S. Furquim, L.P. De Souza, G.H. Bechara, G.C. Roma **(2012)**. Action of Andiroba Oil (Carapa guianensis) on *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) Semi-engorged Females: Morphophysiological Evaluation of Reproductive System. Micros. Res. Techniq., **75**: 1745-1754.

M.I. Camargo-Mathias **(2013)**. Guia Básico de Morfologia Interna de Carrapatos Ixodídeos. EDUNESP.

M.R. Leonardo, L.A.B. Silva, M. Tanomaru Filho, K.C. Bonifacio, I.Y. Ito **(2001)**. *Invitro* evaluacion of the antimicrobial activity of a castor oil-based irrigant. J. Endodont.**12**: 717-719.

S. Nava, L. Beati, M.B. Labruna, A.G. Cáceres, A.J. Mangold, A.A. Guglielmone**(2014)**. Reassessment of the taxonomic status of *Amblyomma cajennense* (Fabricius, 1787) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum* Koch 1844, and *Amblyomma sculptum* Berlese 1888 (Ixodida: Ixodidae). Tick. Tick. Borne. Dis., **5**: 252-276.

T.O.S. Senra, F. Calmon, V. Zeringóta, C.M.O. Monteiro, R. Maturano, R.S. Matos, D. Melo, G.A. Gomes, M.G. De Carvalho, E. Daemon **(2013)**. Investigation of activity of monoterpenes and phenylpropanoids against immature stages of *Amblyomma cajennense* and *Rhipicephalus sanguineus*(Acari: Ixodidae). Parasitol. Res. **112**: 3471-3476.

J. Shephered, J.H. Oilver Jr, J.D. Hall (1981). A polypeptide from male accessory glands which triggers maturation of tick spermatozoa. Int. J. Inver. Rep., **5**: 129-137.