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MODULATING ACTION FROM THE EXTRACT OF THE GLANDS SALIVARY OF THE TICK *Ornithodoros brasiliensis* (ACARI: ARGASIDAE) ABOUT SOME BACTERIA GRAM (-) E (+)

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Abstract: Arthropods have innate immunity that contributes considerably to an optimal defense mechanism, thus developing evolved responses against invasions by pathogenic microorganisms. Ticks are one of the most important groups of arthropods that are hematophagous parasites of domestic, wild and human animals. They belong to the subclass Acari of the class Arachnida, order Ixodida, and are distributed in three families: Argasidae, Ixodidae and Nuttalliellidae. Because they are vectors of diseases, there is a great concern when the vectorial capacity of ticks. During the period of hematophagy, ticks secrete saliva that contains pharmacologically bioactive molecules directed against hemostasis and the immune system to enable a long-lasting feeding without interrupting the host. Salivary glands are vital for the biological success of ticks, being one of the main routes of transmission of microorganisms to the host. However, in addition to the molecules that modulate the host's defense system, saliva may also contain molecules that promote the proliferation of some microorganisms in definitive hosts, making these hematophagous arthropods excellent vectors of pathogens. Neste contexto nos propusemos avaliar a atividade moduladora do Extrato das In this context, we proposed to evaluate the modulatory activity of the Salivary Gland Extract (EGS) Salivary glands (EGS) of the tick *Ornithodoros brasiliensis* (Acari:Argasidaeabout some pathogenic bacteria or not. Therefore, the modulatory activity of EGS was tested on four Gram-positive bacteria: *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus megaterium*, *Staphylococcus epidermidis, and four Gram-negatives*: *Escherichia coli*, *Salmonella enterica arizonae*, *Enterobacter cloacae*, *Serratia marcescens*, the liquid inhibition assay was used as described by Bulet et al. (1993). The reaction was evaluated by absorbance at 595 nm. Our results showed that EGS (at different concentrations) was able to promote cell proliferation in four of the studied strains, two showed growth inhibition and two others remained unchanged. It was concluded that EGS was able to promote some type of modification in both bacterial proliferation and inhibition in six of the eight microorganisms studied.

Keywords: Tick, epidemiological vector, *Ornithodoros brasiliensis* X salivary gland extract, gram-positive bacterium X *Bacilius megaterium*, gram-negative bacterium X *Enterobacter cloacae*, atividade moduladora.

INTRODUCTION

Arthropods are one of the oldest groups, due to their widespread distribution in ecosystems and habitats (SÖDERHÄLL et al., 1998). Like other invertebrates, arthropods have innate immunity, an important defense mechanism, unlike vertebrates that have innate and adaptive immune responses (HOFFMAN et al., 1999). As a response to pathogens, the innate immune system of arthropods evolved into a complex array of peptides with specific antimicrobial activities, favoring the primary defenses against the invasion of bacteria, viruses, fungi, destroying a diversity of eukaryotic and prokaryotic invading microorganisms (HANCOCK et al, 2000; ZASLOFF, 2002; KUHN-NENTWIG, 2003). During the period of hematophagy, ticks secrete saliva that contains pharmacologically bioactive molecules directed against hemostasis and the immune system, having an anticoagulant, anti-platelet, vasodilator, anti-inflammatory and immunomodulatory activity, in addition to having analgesic substances to prolong their feeding. without causing pain and/or irritation to the host (RIBEIRO et al., 1985; MARITZ-OLIVIER et al., 2007; GUGLIELMONE; NAVA, 2014; WIKEL, 2013). Salivary glands

are vital for the biological success of ticks, being one of the main routes of transmission of microorganisms to the host through saliva or co-feeding (MÁRQUEZ-JIMÉNEZ et al., 2005; BOWMAN et al., 2004). In addition to the molecules that modulate the host's defense system, saliva may also contain molecules that promote the proliferation of microorganisms in the hosts, making these hematophagous arthropods excellent vectors of pathogens.

The hypothesis of this work is that in the salivary gland extract (EGS) of ticks *Ornithodoros brasiliensis*, contain molecules capable of promoting the proliferation of certain microorganisms, since ticks are already vectors of pathogens through various routes, including saliva. They end up contaminating themselves when they feed on already infected vertebrates and end up transmitting the microorganism to another host during the next blood meal. During this period when the tick does not feed (between moulting), the pathogen remains dormant, reactivating itself when feeding occurs, replicating and relocating to the salivary glands to infect the host through the wound made by the chelicerae of the hypostome (mouthparts).) of the arthropod (PIESMAN, 1995; ALLEMAN, 2014).

Based on this knowledge about ticks, the objective of this work was to analyze the modulating action of the EGS of the *Ornithodoros brasiliensis* about bacteria (gram negative and gram positive) to evaluate whether the extract can cause any modification in the cell growth of these microorganisms.

OBJECTIVES

To test the modulatory activity of the tick salivary glands crude extract *Ornithodoros brasiliensis* on certain gram-positive and negative bacteria through the inhibition assay in liquid medium (Bulet, 1983).

SPECIFIC OBJECTIVES

- Extraction of salivary glands from semi-engorged adult ticks;
- Dosage of protein;
- Test EGS activity on Gram positive bacteria: *Staphylococcus aureus* ATCC 29213, *Micrococcus luteus* A270, *Bacillus megaterium* ATCC 10778, *Staphylococcus epidermidis* ATCC 12228;
- Test EGS activity on Gram-negative bacteria: *Escherichia coli* SBS 363, *Salmonella enterica arizonae* ATCC 13314, *Enterobacter cloacae* B12, *Serratia marcescens* ATCC 4112;

METHODOLOGY

COLONIES OF TICKS *O. BRASILIENSIS*

The colonies are established and maintained by the Parasitology Laboratory of the Butantan Institute under the responsibility of Dr. Simone M. Simons, in B.O.D. (Biochemistry and Oxygen Demand) with controlled temperature and humidity.

OBTAINING THE SALIVARY GLAND EXTRACT (EGS) OF *O. BRASILIENSIS*

After blood meal in previously sedated rabbits (**Anexo**, CEUA Nº 4487090320), the semi engorged ticks (males and females) were cleaned with running water and neutral liquid soap, then washed with 70% ethanol, and dried on paper towels, after which they were placed in the freezer briefly for a few minutes in order to anesthetize. them. To remove the salivary glands, an incision was made on the underlying end of the ticks, which were fixed with entomological pins to a paraffinized Petri dish. Visualization was performed under a stereoscopic microscope (5Z-STS, Olympus or MZ12, Leica), and then, after several washes with ice-cold PBS pH 7.4, the glands were exposed, with the aid of ophthalmic

scissors and tweezers, then transferred to a 1.5 ml microtube on dry ice, kept in a freezer at -80ºC until processing.

The EGS was obtained by macerating the salivary glands using a disposable plastic pestle, previously autoclaved maceration sand and ice-cold PBS pH7.4, in a 1.5 ml microtube, all maceration was carried out in an ice bath, then the extract was subjected to centrifugation for 3 minutes at 3,000 rpm, 4°C (Eppendorf 5810R Centrifuge). This step was repeated 5 times. Finally, the extract was pooled and filtered through membranes with a cut of 0.45 and 0.22 µm in a laminar flow hood. By this method, it is possible to remove pre-existing bacteria in the extract. The aliquots obtained were kept at -80°C until use.

PROTEIN DOSAGE

To measure the concentration of EGS proteins, the dosage in wavelength at A 280 nm in the NanoDrop 2000 - Thermo spectrophotometer was used.

DETERMINATION OF MODULATING ACTIVITY OF SAMPLES ON MICROORGANISMS

The microbial activity was evaluated through the liquid inhibition assay, as described by Bulet *et al.*, (1993) with modifications. Briefly, under laminar flow, the assay was performed in 96-well microplates, with a final volume of 200 µL. different concentrations of EGS (9,52, 19, 28,56 e 38 µg/ml) were applied to bacteria in culture medium PB (Poor Broth) in different dilutions (10, 20, 30 e 40µl). The plate was incubated for 18 hours at 30°C under agitation (Jenway ® 1000). after incubation, its activity was measured by the Victor 3 spectrophotometer (1420 Multilabel Counter/Victor3 Perkin Elmer) at 595 nm absorbance. Concomitantly, negative controls were performed on the same plate, only with PB medium, only water, bacteria in PB medium with streptomycin antibiotic (0,2mg) and as a positive control (100%) of the experiment, the bacteria were used in PB medium. The bacteria used were stored in the freezer at -80ºC, and the aliquots were removed half an hour before the experiment.

RESULTS AND DISCUSSION

In the test performed with Gram-positive bacteria, proliferation was observed in three bacteria, *S. aureus, S. epidermidis e B. megaterium,* with a clear progression as the EGS protein concentration increases (**Figure 1**). A *S. aureus* had an increase in absorbance demonstrating to be of the dose-response type. (de 0,246 a 0,372 nm). A *S. epidermidis presented* a growth in three EGS concentration, showing a slight inhibition in the last dose, possibly caused by the increase of the extract concentration. The highlight in the two bacterial tests was for B. *megaterium* by the exponential growth that the bacterium had at two concentrations, 28.56 and 38.08 ug/ml, where its cell number doubled, probably demonstrating a certain affinity with the EGS. The bacterium M. luteus was the only one of the Gram-positives that showed inhibition, caused by the increase in the concentration of EGS, especially at 9.52 and 19 ug/ml where there was an inhibition of 27% when compared to the average of the values of the experiments with 100% control, as shown in **Table 1**.

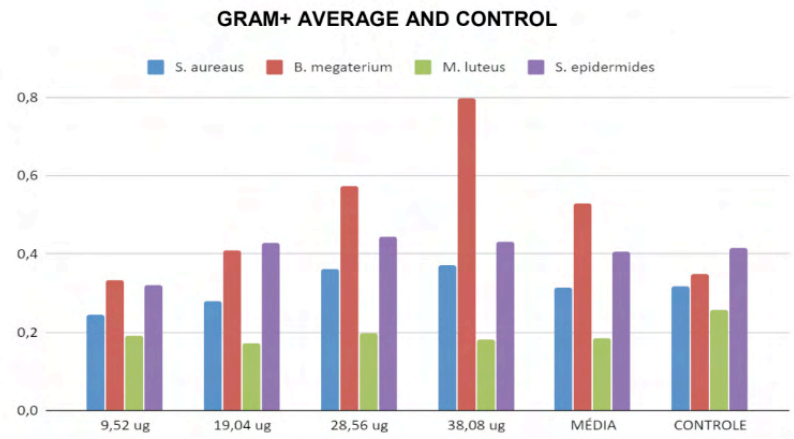
In relation to Gram-negative bacteria in **Figure 2**, it is shown that EGS was able to promote the inhibition of S. marcescens, where it had a decrease in its absorbance, with 19 ug/ml it had 27.3 % and with 28, it had a decrease in absorbance. 5 µg had 29.4% inhibition, . E. coli remained close to its control sample, having a small growth in the highest concentration of EGS, not being very significant. E. cloacae had a progressive proliferation in three concentrations, decreasing in the highest of the EGS, this

bacterium had an important proliferation of 128% more than the positive control. While *Salmonella* kept the values close to its control sample, having a small growth in the first three concentrations, decreasing slightly in the last one (**Table 1**).

Recent studies show that ticks *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae), have been found naturally infected with *Staphylococcus aureus* and *Serratia marcescens* (ANDREOTTI,2011; MIRANDA-MIRANDA, 2010), incredibly the *S. marcescens* did not proliferate as expected, showing a small inhibition in some specific doses of the extract. A study carried out in Hungary on the microbiota of ticks found mostly Gram-positive bacteria, the most frequent genera being *Staphylococcus* and *Bacillus*, where its numerical proportion increased with the instars (EGYED,2013). This demonstrates the affinity that the *S. aureus*, *S. epidermidi*s and *B. megaterium* have with the tick's internal biology so the increasing bacterial response as the EGS increased. *Escherichia coli* are naturally found in the female reproductive system of ticks, this could explain the growth that occurred at the highest concentration of the extract. *E. coli* It is used as a biological control against ticks to avoid losses in cattle raising, as they end up making it difficult to maintain the tick population. (JUNIOR,2012). Other pathogenic bacteria have already been isolated in Ixodidae ticks such as *Salmonella spp*. and *Enterobacter spp*., the two showed to have a response action in the face of a certain dose of EGS, where the two proliferatedIn a study done in Iraq on ticks that infest buffalo, *Enterobacteriaceae* was predominant in the microbiota of ticks (KHALAF,2018). Recent studies have detected the presence of an antimicrobial peptide that is located in the region between the ovary and the genital of female ticks, called microplusin, which has a reaction against Gram-positive strains, having an activity anti-*Micrococcus luteus*, which causes a respiratory deficiency in the bacteria (ESTEVES, 2009. SILVA, 2009).

Rickettsia rickettsii pathogen that causes Rocky Mountain spotted fever, has a dependency relationship with arthropods because this bacterium survives only in host cells and needs to be inside a tick to survive, some rickettsiae species are pathogenic for ticks that end up dying when infected (MONTEIRO,2006). Other ticks like *Ixodes ricinus, Ixodes scapularis*, *Rhipicephalus sanguineus* have a relationship with bacteria, in this case being of endossimbiose, some bacteria live lodged in the ovaries of the females, without harming them, the males found themselves with few or no microorganisms, possibly due to the fact that the bacteria prefer to lodge in the ovaries of the females. Endosymbiont relationships may benefit tick survival and fecundity, as some ticks play a key role in providing nutrients and essential cofactors absent in the blood. Environmental factors such as habitat, season and soil type influence the composition and diversity of the microbiota, other factors are the type of host, tick species, instar, sex and anatomical location. (NODA,1997;GREAY,2018).

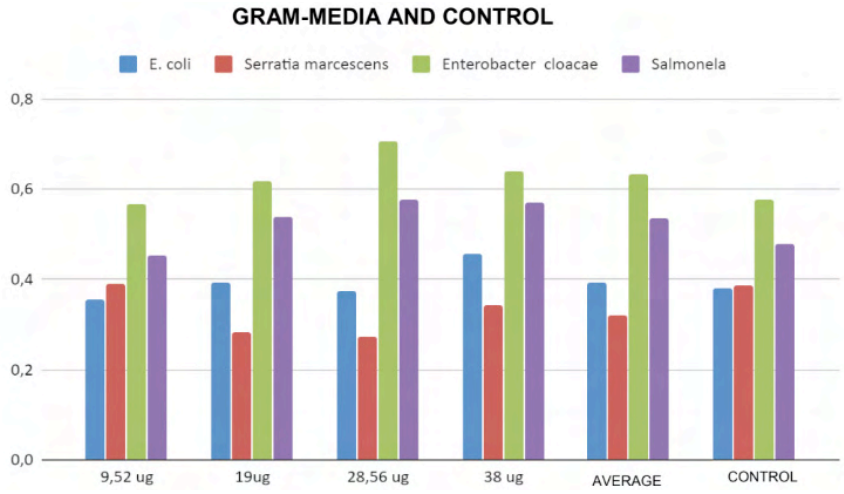
The extraction of salivary glands from ticks took place without external interference, as planned, there was no contamination of the samples before extraction and after it during the bacterial test; from the data it was possible to perform a protein profile through SDS-PAGE (data not shown). As discussed earlier, ticks are already known to have endosymbiont relationships with bacteria that can be commensal, mutualistic or parasitic. This bacterial community lives in the tick microbiota, by the studies already mentioned, most were not pathogenic for ticks. We can believe that the fact that they have an endosymbiotic relationship provided this response to the increase in the concentration



Axle y = Values obtained from the absorbances

Axle x = Protein/well. Positive control (100%)

FIGURE 1 - Mean and control of Gram-Positive bacteria



Axle y = Values obtained from the absorbances

Axle x = Protein/well. Positive control (100%)

FIGURE 2 - Gram-negative mean and control

EGS / Microorganism	9,52µg	19µg	28,56µg	38µg	AVERAGE	CONTROL
E.coli	0,354	0,394	0,375	0,455	0,3945	0,3816
Serratia marcescens	0,39	0,282	0,274	0,342	0,322	0,3882
Enterobacter cloacae	0,568	0,618	0,707	0,641	0,6335	0,5754
Salmonella	0,453	0,539	0,577	0,569	0,5345	0,48
S.aureaus	0,246	0,28	0,361	0,372	0,31475	0,317
B.megaterium	0,333	0,409	0,572	0,799	0,52825	0,349
M.luteus	0,191	0,173	0,196	0,181	0,18525	0,2588
S.epidemides	0,32	0,428	0,445	0,431	0,406	0,4144

TABLE 1 - Bacterial growth in the Inhibition Assay in Liquid Medium

of the extract. It is important to highlight the inhibition caused by Micrococcus luteus, possibly by a molecule that has antimicrobial action, this may suggest that this peptide somehow lodged in the salivary glands or moved to it. As there was inhibition at certain doses in different bacteria, it may have been caused by peptides that have a high concentration compared to other doses, or simply these peptides are selective in relation to their target bacteria. Studies related to the bacterial communities of ticks are necessary to have a better understanding of their biology and the interaction between the organisms involved (bacteria, tick and host).

It is possible to perceive from the analysis of the results that EGS was able to promote modulating activity in the growth of bacteria, some with dose-response reactions and others with a specific dose. In view of the pandemic caused by the SARs-COV-2 that we are experiencing, it was necessary to interrupt the experiments, therefore, no other bacterial tests were carried out so that it was possible to compare the data obtained and lead us to more conclusive results. In any case, a field of study of extreme relevance opens up, given the recent epidemics we are going through, such as dengue and yellow fever.

FINAL CONSIDERATIONS

Ticks are one of the most important groups of hematophagous parasitic arthropods because they are of great importance to public health and veterinary medicine. They have a diverse microbiota, where many microorganisms lodge in the ovaries and/or Malpighian tubules, having a beneficial relationship, but data on this bacterial community is little known in the scientific world. The results obtained in this work showed a tendency for bacteria to proliferate in the EGS samples. These data allow us to open a discussion about the proteins or molecules that facilitate these microorganisms, what their characteristics would be and whether they are the same ones that allow pathogens to lie dormant within these ticks. It must be taken into account that all the bacteria in the work were found in ticks in nature, with the exception of *Micrococcus luteus*, which showed considerable inhibition in the inhibition testThis may have occurred due to the non-relationship between the organisms or the antimicrobial action that ticks have. Studies of the tick microbiota and how it interferes with the biology of microorganisms allow us to understand the vectorial capacity of ticks, in addition to the modes of transmission. Understanding how the habitat, season of the year, humidity, instar, sex, type of host and the blood fed causes changes in ticks, allows us to develop drugs, create new ways of preventing or inhibiting these microorganisms and/or arthropods.

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REFERENCES

ALLEMAN, A. R.; ARMSTRONG, R. Compreendendo a Transmissão de Patógenos por Carrapatos Vetores. **Bravecto**. 2014 Disponível em:<https://www.bravecto.com.br/sou_vet/SEPARATA_TECNICA.pdf>. Acesso em 1 de Maio de 2020.

ANDREOTTI, R.; LEÓN, A.A.P.; DOWD, S.E.; GUERREIRO, F.D.; BENDELE, K.G.; SCOLES, G.A. Assessment of bacterial diversity in the cattle tick Rhipicephalus (Boophilus) microplus through tag-encoded pyrosequencing 2011. **BMC Microbiology**, 2011, 11:6. Disponível em:<http://www.biomedcentral.com/1471-2180/11/6>. Acesso em 2 de maio de 2020.

BOWMAN, A.S.; SAUER, J.R. Tick salivary glands: function, physiology and future. **Parasitology**, v. 129, 2004. Disponível em:<10.1017/s0031182004006468>. Acesso em 27 de março de 2020.

BULET, P.; DIMARCQ J.L.; HETRU C.; LAGUEUX, M.; HEGY G.; VAN DORSSELAER A.; HOFFMANN J.A. A novel inducible antibacterial peptide of Drosophila carries an O-glycosylated substitution. **J Biol Chem**. 1993, Jul,15; 268 (20): 14893-7.

EGYED, L.; MAKRAI, L. Cultivable internal bacterial flora of ticks isolated in Hungary. **Experimental & applied acarology**. 2013. Disponível em:< https://www.researchgate.net/publication/259456475_Cultivable_internal_bacterial_flora_of_ticks_isolated_in_Hungary >. Acesso em 2 de maio de 2020.

ESTEVES, E.V.S. **Células embrionárias BME26: modelo para o estudo da interação Anaplasma marginale e o carrapato Rhipicephalus (Boophilus) microplus**. 2009. Tese (Doutor em Ciências). Universidade de São Paulo. Disponível em:<https://teses.usp.br/teses/disponiveis/42/42135/tde-25032010-161130/publico/ElianeVirginiaSilvaEsteves_Doutorado.pdf>. Acesso em 2 de maio de 2020.

GREAY, T.L.; GOFTON, A.W.; PAPARINI, A.; RYAN, U.M.; OSKAM, C.L.; IRWIN, P.J. Recent insights into the tick microbiome gained through next-generation sequencing. Parasit Vectors. 2018.Disponível em:< 10.1186/s13071-017-2550-5>. Acesso em 2 de maio de 2020.

GUGLIELMONE, A.A.; NAVA, S. Names for Ixodidae (Acari: Ixodoidea): valid, synonyms, incertae sedis,nomina dubia, nomina nuda, lapsus, incorrect and suppressed names — with notes on confusions and misidentifications. **Zootaxa**, New Zealand, v. 3767 n. 1, p. 001–256, 2014. Disponível em:<10.11646/zootaxa.3767.1.1>. Acesso em 30 de março de 2020.

HANCOCK, R.E.W.; DIAMOND, G. The role of cationic antimicrobial peptides in innate host defences. **Trends Microbiol**, v. 8 p. 402–10, 2000. Disponível em:<10.1016/s0966-842x(00)01823-0>. Acesso em 2 de fevereiro de 2020.

HOFFMAN, J.A.; KAFATOS, F.C.; JANEWAY, C.A.; EZEKOWITZ, R.A.B. Phylogenetic perspectives in innate immunity. **Science**, v. 284, p. 1313–18, 1999. Disponível em:<10.1126/science.284.5418.1313>. Acesso em 29 de janeiro de 2020.

KHALAF, J.M.; MOHAMMED, I.A.; KARIM, A.J. The epidemiology of tick in transmission of *Enterobacteriaceae* bacteria in buffaloes in Marshes of the south of Iraq. Vet World, 11(12): 1677-1618, 2018. Disponível em:<10.14202/vetworld.2018.1677-1681>. Acesso em 2 de maio de 2020.

KUHN-NENTWIG, L. Antimicrobial and cytolytic peptides of venomous arthropods. **Cellular and Molecular Life Sciences**, v. 60 p. 2651–68, 2003. Disponível em:<10.1007/s00018-003-3106-8>. Acesso em 5 de abril de 2020.

JUNIOR, I.S.V.; SEIXAS, A.; MASUDA, A. **Pesquisa para uma Vacina contra o Carrapato**. Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular. 2012. Disponível em:< http://www.inctem.bioqmed.ufrj.br/images/documentos/biblioteca/Capitulo_17_Pesquisa_para_uma_Vacina_contra_o_Carrapato.pdf>. Acesso em 2 de maio de 2020.

MARITZ-OLIVIER, C.; STUTZER, C.; JONGEJAN, F.; NEITZ, A. W. H.; GASPAR, A. R. M. Tick anti-hemostatics: targets for future vaccines and therapeutics. **Trends in Parasitology**, v. 23, n. 9, p. 397–407, 2007. Disponível em:<10.1016/j.pt.2007.07.005>. Acesso em 5 de abril de 2020.

MÁRQUEZ-JIMÉNEZ, F.J.; HIDALGO-PONTIVEROS, A.; CONTRERAS-CHOVA, F.; RODRÍGUEZ-LIÉBANA, J.J.; MUNIAIN-EZCURRA, M.A. Ticks (Acarina: Ixodidae) as vectors and reservoirs of pathogen microorganism in Spain**. Enferm. Infecc. Microbiol. Clin**., v. 23, n.2, p. 94-102, 2005. Disponível em:<10.1157/13071613>. Acesso em 28 de janeiro de 2020.

MIRANDA-MIRANDA, E.; BAYUGAR-COSSIO, R.; QUEZADA-DELGADO, M.D.R.; SACHMAN-RUIZ, B; REYNAUD, E. Staphylococcus saprophyticus is a pathogen of the cattle tick Rhipicephalus (Boophilus) microplus. **Biocontrol Science and Technology**. 2010. p. 1055-1067. Disponível em:<10.1080/09583157.2010.505325>. Acesso em 2 de maio de 2020.

MONTEIRO, C.M.O.; RODRIGUES, A.F.S.F.; GUEDES, E. ASPECTOS GERAIS DA FEBRE MACULOSA BRASILEIRA. **CES revista**, 2006. Disponível em:<https://www.cesjf.br/revistas/cesrevista/edicoes/2006/febre_maculosa_brasileira.pdf>. Acesso em 2 de maio de 2020.

NAVA, S.; BEATI, L.; LABRUNA, M.B.; CÁCERES, A.G.; MANGOLD, A.J.; GUGLIELMONE, A.A. 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). **Ticks and Tick-Borne Diseases**, v. 5, n. 3, p. 252–276, 2014.

NODA, H; MUNDERLOH, U.G.; KURTTI, T.J. Endosymbionts of Ticks and Their Relationship to Wolbachia spp. and Tick-Borne Pathogens of Humans and Animals. **Applied and Environmental Microbiology**, p.3926–3932, Vol. 63, No. 10, 1997. Disponível em:< https://www.ncbi.nlm.nih.gov/pmc/articles/PMC168704/pdf/633926.pdf>. Acesso em 2 de maio de 2020.

PIESMAN, J. Dispersal of the Lyme disease spirochete Borrelia burgdorferi to salivary glands of feeding nymphal Ixodes scapularis (Acari: Ixodidae). **J Med Entomol**. 32:519-521. 1995. Disponível em:<https://doi.org/10.1093/jmedent/32.4.519>. Acesso em 1 de Maio de 2020.

RIBEIRO, J.M.C.; MAKOUL, G.T.; LEVINE, J.; ROBINSON, D.R.; SPIELMAN, A. Antihemostatic, Antiinflammatory and immunosuppressive properties of the saliva of a tick, Ixodes dammini. **J. Exp. Med.**, v. 161, n. 2, p. 332-344, 1985. Disponível em:<10.1084/jem.161.2.332>. Acesso em 3 de fevereiro de 2020.

SILVA, F.D.; REZENDE, C.A.; ROSSI, D.C.P.; ESTEVES, E.; DYSZY, F.H.; SCHREIER, S.; GUEIROS-FILHO, F.; CAMPOS, C.B.; PIRES, J.R.; DAFFRE, S.. Structure and mode of action microplusion, a copper II chelating antimicrobial peptide from the cattle tick Rhipicephalus (Boophilus) Microplus. **J. Biol. Chem**. 2009. Disponível em:<10.1074/jbc.M109.016410>. Acesso em 2 de maio de 2020.

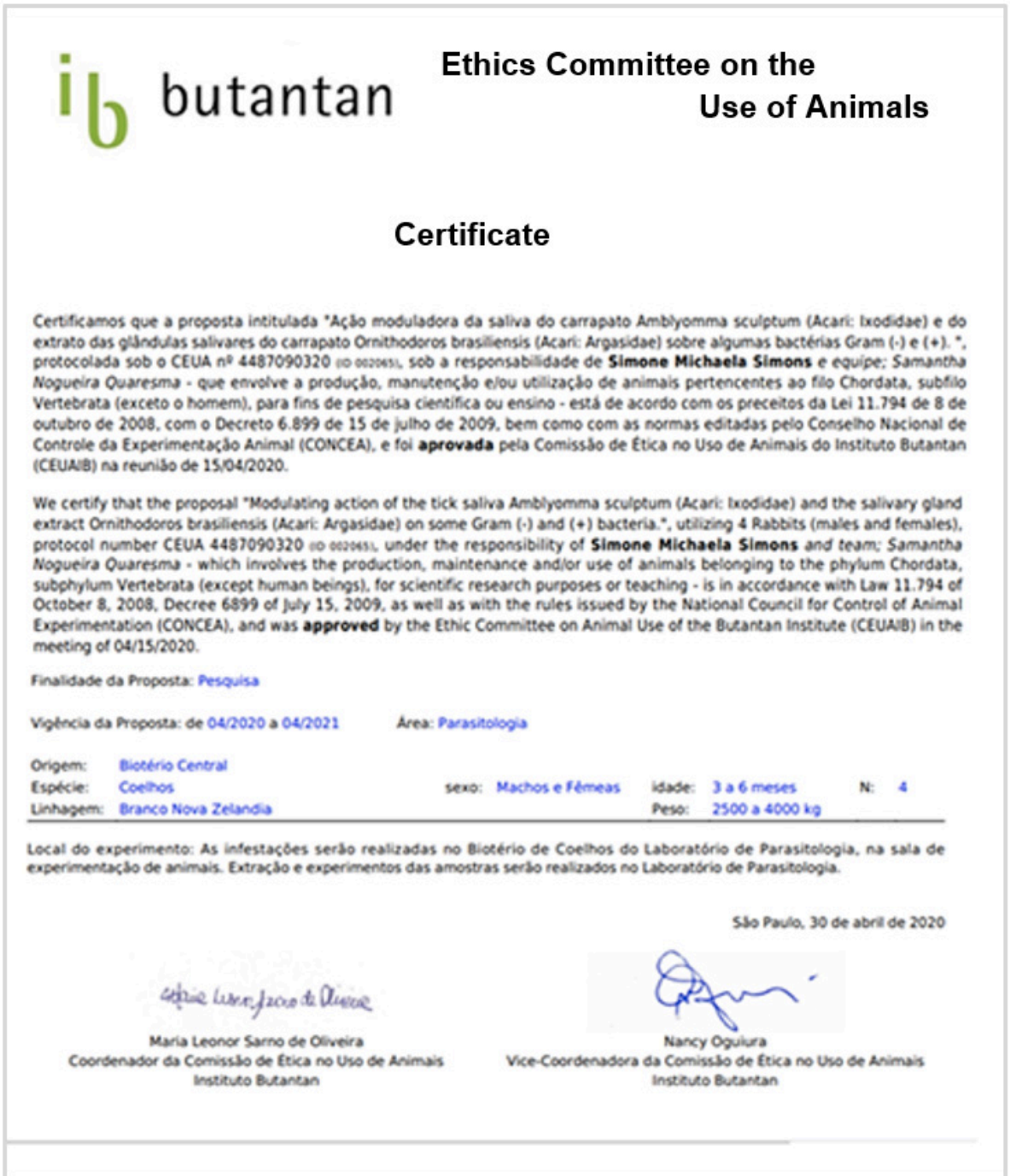
SÖDERHÄLL, K.; CERENIUS, L. Role of the prophenoloxidase-activating system in invertebrate immunity. **Curr Opin Immunol**, v. 10, p. 23–8, 1998. Disponível em:<10.1016/s0952-7915(98)80026-5>. Acesso em 29 de janeiro de 2020.

WIKEL, S. Ticks and tick-borne pathogens at the cutaneous interface: host defenses, tick countermeasures, and a suitable environment for pathogen establishment. **Front Microbiol**, v. 4, p. 337, 2013. Disponível em:< https://www.frontiersin.org/articles/10.3389/fmicb.2013.00337/full>. Acesso em 30 de fevereiro de 2020.

ZASLOFF, M. Antimicrobial peptides of multicellular organisms. **Nature**, v. 415, p. 389–95, 2002. Disponível em:<https://www.nature.com/articles/415389a>. Acesso em 14 de abril de 2020.

ANNEX A – APPROVAL OF THE ETHICS COMMITTEE

Documentation with the approval of the Ethics Committee on the Use of Animals of the Instituto Butantan (project CEUAIB n°4487090320):



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Ethics Committee on the Use of Animals

Certificate

Certificamos que a proposta intitulada "Ação moduladora da saliva do carrapato Amblyomma sculptum (Acari: Ixodidae) e do extrato das glândulas salivares do carrapato Ornithodoros brasiliensis (Acari: Argasidae) sobre algumas bactérias Gram (-) e (+). ", protocolada sob o CEUA nº 4487090320 (ID 002065), sob a responsabilidade de **Simone Michaela Simons** *e equipe; Samantha Nogueira Quaresma* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais do Instituto Butantan (CEUAIB) na reunião de 15/04/2020.

We certify that the proposal "Modulating action of the tick saliva Amblyomma sculptum (Acari: Ixodidae) and the salivary gland extract Ornithodoros brasiliensis (Acari: Argasidae) on some Gram (-) and (+) bacteria.", utilizing 4 Rabbits (males and females), protocol number CEUA 4487090320 (ID 002065), under the responsibility of **Simone Michaela Simons** *and team; Samantha Nogueira Quaresma* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Butantan Institute (CEUAIB) in the meeting of 04/15/2020.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 04/2020 a 04/2021 Área: Parasitologia

Origem:	Biotério Central				
Espécie:	Coelhos	sexo: Machos e Fêmeas	idade:	3 a 6 meses	N: 4
Linhagem:	Branco Nova Zelandia		Peso:	2500 a 4000 kg	

Local do experimento: As infestações serão realizadas no Biotério de Coelhos do Laboratório de Parasitologia, na sala de experimentação de animais. Extração e experimentos das amostras serão realizados no Laboratório de Parasitologia.

São Paulo, 30 de abril de 2020

Maria Leonor Sarno de Oliveira
Coordenador da Comissão de Ética no Uso de Animais
Instituto Butantan

Nancy Oguiura
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Instituto Butantan

Purpose of the proposal: **research**

Validity of the proposal: from **04/2020 to 04/2021** Area: **Parasitology**
Origin: **Central vivarium**
Species: **Rabbits** Sex: **Males and Females** age: **3 to 6 months**
Weight: **2500 to 4000 kg** N: **4**
Bloodline: **White New Zealand**

Place of the experiment: The infestations will be carried out in the Animal Facility of the Laboratory of Parasitology, in the animal experimentation room. Sample extraction and experiments will be carried out in the Parasitology Laboratory

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São Paulo, 30 April 2020

Maria Leonor Sarno de Oliveira
Coordinator of the Ethics Committee in the Use of Animals
Butantan Institute

Nancy Ogulura
Vice-Coordinator of the Ethics in the Use of Animals
Butatan Institute