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## Elucidating the Interplay between Sodium Selenite on the Tick *Amblyomma maculatum* Selenoprotein Gene Expression

Afnan M. Beauti  
University of Southern Mississippi

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The University of Southern Mississippi

**Elucidating the Interplay between Sodium Selenite on the Tick *Amblyomma maculatum*  
Selenoprotein Gene Expression**

By

Afnan M. Beauti

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Approved by:

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**Shahid Karim, PhD.**

Thesis Advisor

Department of Biological Sciences

---

**Sabine Heinhorst, PhD.**

Chair, Department of Chemistry and Biochemistry

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**Ellen Weinauer, PhD.**

Dean, Honors College

### **Abstract**

Selenium (Se) is an element recognized as an essential micronutrient in eukaryote organisms. Selenoproteins contain selenium as selenocysteine, the 21<sup>st</sup> amino acid. Selenium plays a role in cell growth and functioning. At low concentrations, it can induce growth and at high concentrations, it can cause a cell to stop growing and potentially have toxic effects on the cell and organism. When selenium levels are high, oxidative stress results by the production of reactive oxidative species. Selenoproteins, however, can aid the antioxidant response in the cell. Ticks are arthropods of interest, as they are one of few that contain many selenogenes, possibly the reason for their robust antioxidant system. Ticks are obligate hematophagous ectoparasite and require this strong and organized antioxidant system for when oxidative stress is induced during blood feeding and digestion. In this study, the effects of sublethal sodium selenite concentration on the female *Amblyomma maculatum* tick's antioxidant system were investigated. The oxidative stress analysis of ticks injected with sublethal concentration of sodium selenite (0.1 $\mu$ M Na<sub>2</sub>SeO<sub>3</sub>) compared with 1X PBS injected *Amblyomma* female ticks. Once tissues were obtained, transcriptional gene expression was performed for all antioxidants including selenogenes. The transcript level of selenogene M (SelM), and selenogene N (SelN) revealed up-regulation. A lipid peroxidation (MDA assay), and fluorescent assays were utilized to quantify the relative oxidative stress and visually examined oxidative stress effects on tick tissues. Oxidative stress was illustrated in the assay and confocal and gave insight on how tick antioxidant system responds to a sublethal sodium selenite concentration. The outcomes could lead to understanding tick physiology and give insight on tick antioxidant system.

**Key Terms:** *Amblyomma maculatum*, obligate hematophagous ectoparasite, oxidative stress, selenoprotein, sodium selenite, Selenium, gene expression



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## Chapter I: Introduction

The increase in tick-borne diseases is a significant threat to public health in the absence of preventive measures. Ticks are one of Earth's most essential obligate hematophagous ectoparasites due to their ability to act as vectors of different human and animal pathogens such as spirochetes, rickettsia, protozoa, nematodes and viruses (Bratton & Corey, 2005; Swanson et al., 2006; Flicek, 2007; Vorou et al., 2007). The feeding of ticks on blood generates toxic levels of reactive oxygen species (ROS) that could damage lipids, proteins, and DNA, thus promoting mutation, cellular dysfunction, and cell death. To successfully feed and survive, ticks must somehow prevent the detrimental and promote the beneficial aspects of ROS, which suggests that there are precise regulatory strategies for maintaining appropriate ROS levels within the tick. Previous studies from the lab have shown an adaptive coevolutionary process that has enabled ticks and tick-borne pathogen survival by manipulating an antioxidant system associated with selenium (Se), including a full set of selenoproteins and other antioxidants (Karim et al., 2011; Karim and Ribeiro 2015; Adamson et al., 2013; Adamson et al., 2014; Budachetri and Karim 2015; Kumar et al., 2016; Crispell et al., 2016; Budachetri et al., 2017). Selenoproteins are antioxidants due to their redox potential of selenium atom in selenocysteine amino acids. The tick shows most of selenoproteins in its genome. Selenoproteins provide survival advantages to ticks quenching reactive oxygen species generated during normal physiology.

### *Purpose and Hypothesis*

The purpose of this study is to elucidate role of selenium in the selenoprotein expressions and determine overall tick anti-oxidative stress level. We hypothesized that selenium supplementation would provide sufficient selenium level to activate and generate enough



selenoproteins, which thereby would increase the anti-oxidative capacity of ticks. To test the hypothesis, First, a sublethal dose of sodium selenite was determined, and second, the expression of selected tick antioxidants was determined upon sodium selenite exposure to the ticks.

## Chapter II: Literature Review

### *Ticks*

*Amblyomma maculatum* is an arthropod known as the Gulf Coast tick commonly found in the coastal areas of the southeastern region of the United States as well as Central and South American countries such as Guatemala, Costa Rica, and Venezuela. *Amblyomma maculatum* is a three-host species with the ability to feed on rodents, birds, and mammals. *Amblyomma maculatum* was first described in 1844, and over the last century, the tick has been further explored, as gaining attention for its damaging economic effect on livestock in the U.S.A. Further research on the ticks revealed novel tick-borne pathogen discoveries in the late 1900s, but the medical and veterinary significance of Gulf Coast ticks were only noticed once when it was labeled as a vector of the pathogen *Hepatozoon americanum*, the cause of American canine hepatozoonosis (Mathew et al. 1998).

Gulf Coast ticks are significant because of their large impact on livestock infestations. When bitten by a tick, prey can show inflammation, weakness, temporary paralysis, and changes in body composition (Teel et al. 2010). For instance, cattle become infected with a pathologic condition known as “gotch” ear, becoming less valued in the market (Hertz et al., 2014; Edwards, 2009). Ticks not only cause detrimental economic effects, but also pathogenic effects. Their ability to transmit different pathogens to humans and animals causes a larger issue. Prior to 2002, Rocky Mountain spotted fever was the only known tick-borne spotted fever caused by *Rickettsia rickettsii*. In 2002, the *Rickettsia parkeri* species, transmitted by the Gulf Coast ticks, was seen as a human pathogen that causes rickettsiosis (Paddock et al. 2004). Cases of the diseases were reported in Southeastern states of America, including in Mississippi (Walker et al. 2008;

Cragun et al. 2010; Parola et al. 2013). Rickettsiosis infection rates have increased up to 55% in some states, causing a public health epidemic (Nadolny et al., 2014; Paddock & Goddard, 2015). Other pathogens the Gulf Coast ticks carry include *Hepatozoon americanum* and *Leptospira Pomona* transmitted through other infected ticks or direct tick bites (Hertz et al., 2014). Gulf Coast ticks can be vectors of *Ehrlichia ruminantium*, which causes the fatal disease Heartwater in ruminants (Mahan et al., 2000). Regardless of the fact that no Heartwater diseases have been reported in the USA, main vectors of the disease have been detected on Florida's imported animals. These vectors have been exposed in the U.S. and can quickly introduce pathogens to uninfected hosts. When pathogens come in contact with the hosts (ruminants), they then enter the disease cycle that involves the Gulf Coast ticks.

The Gulf Coast tick has made recent headlines concerning its posed risk to the public health (Paddock & Goddard, 2015). When ticks feed, they release toxins, which can cause severe damage to a host's motor neurons, putting them at risk for paralysis and respiratory failure (Espinoza-Gomez et. al., 2011). My project aims to investigate the Gulf Coast ticks' oxidative stress machinery to better grasp the effect of antioxidant molecules on ticks' homeostasis. Through finding such potential antioxidant molecules and exploring the tick's antioxidant system, Gulf Coast ticks can be controlled.

### ***Selenium, antioxidants and Selenoproteins***

Selenium is an important trace element that is involved in human biological process such as brain functioning, immune system regulation, and muscle functioning (Rayman, 2012; Papp et al., 2010). Its presence in the body is necessary as it supports the immune system by decreasing the amount of damage done by free radicals in the body. Selenium, in the right amounts, can

also act synergistically with natural antioxidants, aiding the body in fighting oxidative stress, which in turn can aid in fighting cancer. If selenium proteins lack in the body, the development of cancers, cardiovascular diseases, diabetes, and immune system disorders could occur. Oxidative stress is a major factor in the causes of these diseases.

Superoxide dismutase (SOD), glutathione peroxidase, catalase, and selenoproteins are some of the antioxidant enzymes that cleanse the body of reactive oxygen species (ROS), harmful chemically reactive species that include hydroxyl radical, peroxide, and superoxide, and singlet oxygen. Selenoproteins exist in bacteria, archaea, and eukaryotes. There are twenty-five known selenoproteins, and out of those, a select number have been explored in terms of their functions. Characterized selenoproteins include peroxidases (GPxs) and thioredoxin reductases (TrxRs), both of which have antioxidant properties to defend the body and regulate redox states. Other known Se-proteins include iodothyronine deiodinases (DIOs), which aid the body in thyroid hormone metabolism, and selenophosphate synthetases 2 (SPS2), which helps in the biosynthesis of selenoproteins. Selenoproteins contain selenium, which uses the 21<sup>st</sup> amino acid, selenocysteine (Sec) to synthesize proteins (Roman et al, 2013). Selenocysteine is also involved in antioxidant defense, redox homeostasis, and redox signaling (Touat-Hamici et al., 2014).

Although it is known that Seleno-proteins are essential to biological systems, their mechanisms of action have not yet been fully investigated. Selenium is vital to the human body, so it can also be taken in supplement form. Either too much or too little Se in a human or other organism, however, can cause biological functions to deteriorate and can result in health disorders. Too much Se or sub-toxic levels of Se can cause harmful and potentially lethal effects on the body (Roman et al, 2013). For example, too much Se in the body can contribute to diabetes. Too little Se in the body can increase the risk of cancers such as prostate, lung, and colon cancer

(Touat-Hamici et al., 2014). On the other hand, Selenoproteins can have a positive impact on the body, where Selenoproteins' genes polymorphism has allowed Se to be used in anti-cancer medications (Cao et al, 2004).

Selenium is vital to biological functions and provides nutrient effects to the body. Se is essential to animals and a necessary factor in diet. The normal concentration in adult human blood serum is 70 to 150 ng/mL. Se is found in selenoproteins in the form of the 21<sup>st</sup> amino acid, selenocysteine (Reich and Handal, 2016). According to Vallentine et al (2014), when selenocysteine is inserted into proteins, genetic defects arise, thus leading to severe health issues. When selenoprotein is scarce, more consequences are present because the element is vital to health. Vallentine et al (2014) mentioned that a study using mouse models with different concentrations of Se helped uncover the functions of selenoproteins in rodents.

The first selenoprotein discovered, Glutathione peroxidase 1 (GPx1), was found in human erythrocytes, which protect hemoglobin from oxidative stress and damage and also includes Se in the form of selenocysteine. Cysteine has a high pKa than selenocysteine. Selenocysteine is a stronger nucleophile. Selenocysteine in selenoproteins activates the oxidoreductase function of the proteins. The human genome contains 25 selenoprotein genes. One known function of these proteins including their ability to help regulate cellular redox homeostasis, which include three thioredoxin reductases (TRs), five glutathione peroxidases (GPx1), methionine sulfoxide reductase (MsrB1), and three thyroid hormone deiodinases (DIs). Although many selenoproteins' functions are known, most of their functions and the specific reactions catalyzed by selenoproteins have not been explored. Oxidoreductase reactions are associated with intracellular redox homeostasis and antioxidant defense. GPxs can minimize the amounts of peroxides. TRs reduce disulfides and MsrB1 reduce methionine sulfoxide residues in proteins. DIs speed up

reactions removing iodine from thyroxine (T4)'s outer ring, creating thyroid hormones. There is information about selenoproteins SelP, SelM, SelH, SelS, SelK, SelN, SelT, and SelW. There has not been revealed enough information on SelV, SelO, and SelI. Comparing to selenoproteins' function as oxidoreductases, these proteins are most likely also oxidoreductases with a Sec active site. Many mammals have the fold similar to thioredoxin's in their selenoproteins. This new fold can also change the redox state of cysteine residues. Kasaikina et al (2012) stated that nearly 6 of the 25 selenoproteins (Sep15, SelK, SelM, SelN, SelS, and SelT) could be found in the ER lumen. In addition, a selenoprotein (D2 or Dio2) functions in the ER membranes as well as many secreted selenoproteins travel past the compartment. The ER is said to be plentiful with selenoproteins, suggesting selenoprotein function in pathways that take place in the ER. Some pathways include protein secretion/ modification and ER-associated protein degradation ERAD (Kasaikina et al, 2012).

To examine selenoprotein functions in the ER and their effect on physiology and pathology, Kasaikina et al (2012) used mature mouse models, one group with extreme amounts of selenium and another group with a manipulated selenoprotein biosynthesis pathway. Kasaikina et al (2012) found that SelP knockout mice dealt with transporting Se from the liver to peripheral tissues. With low levels of Se, the mice formed ataxia, seizures, and male infertility that could be prevented by consuming adequate amounts of dietary Se. ER associated protein degradation (ERAD) is a series of quality control pathways that dissociates, converts, or gets rid of harmful species in the ER. Essentially, ERAD cleans and clears the ER from any harm. The ERAD functions near ubiquitination systems in the cytoplasm and nucleus. The turnover of certain proteins is controlled by ubiquitination systems to form desired physiological states. In the future, ERAD will have to be further studied past the focus on quality control. When the ER is under

stress, it releases unfolded or improperly folded proteins. To resist this altered functioning, chaperones and modifying enzymes support membrane integration and folding. When proteins made within the ER are formed, they are transported to other parts of the cell or body to carry out a specific function. However, when new proteins are synthesized and fail to attain their native conformation, negative effects could occur. The misfolding can occur due to mutations, not enough binding partner, or a lack of chaperones. After being misfolded, proteins remain in the ER and are converted into substrates of the ERAD (Ruggiano et al, 2014).

When an excessive amount of misfolded proteins form in the ER's lumen and membrane, the ERAD mechanism inactivates. Too many misfolded proteins cause ER stress which in turn causes further damage to the ER and is caused by numerous diseases. The ERAD is essential to ER homeostasis in eukaryotes. Ruggiano et al (2014) found that sterol regulation by the ER is one of its first functions.

Vallentine et al (2014) studied the green alga *Chlamydomonas reinhardtii* to determine the role of UPP when the ER was stressed due to higher selenium concentrations. Theoretically UPP pathway is part of ERAD pathway. The ubiquitin–proteasome pathway (UPP) is involved in many plant and animal physiological processes.

The study found that UPP activity depended on time and dose of selenium. Selenite stress at moderate levels promoted proteasome activity and ubiquitination as well as the removal of deformed selenoproteins. Vallentine et al (2014) found that too much Se stress can reverse these functions and cause decreased proteasome activity, hindered protein ubiquitination, and the prevention of the removal of deformed selenoproteins. This is due to reactive oxygen species (ROS) accumulating in the cell. The UPP's role is to protect a cell from Se stress, but too much Se can cause toxicity and inhibit the functions of the UPP (Vallentine et al, 2014).

Valentine et al (2014) studied proteasome's role in mitigating selenite toxicity in plant cells. It was found that the UPP was inactivated at high concentrations of selenium, most likely due to ROS production and oxidative stress. In Valentine et al (2014)'s study, ROS were produced mainly in the mitochondria and chloroplast when under stress.

The proposed study should contribute to our understanding of the basic molecular mechanisms developed by blood-feeding ticks to mitigate oxidative stress, as well as how vector-borne pathogens use endogenous antioxidants of the vectors for their survival, colonization, and transmission. Our findings should facilitate the development of strategies for controlling tick-transmitted diseases.



## Chapter III: Methodology

### *Ticks*

Unfed adult female Gulf Coast ticks (*Am. maculatum*) were purchased from the tick rearing facility at Oklahoma State University's National Tick Research and Education Resource. The ticks were held in a laboratory incubator at 24-26°C and 90% relative humidity in a cycle of 14 hours light and 10 hours dark. All procedures were conducted utilizing The Institutional Animal Care and Use Committee's (IACUC) approved protocols from The University of Southern Mississippi. All tick-related experiments were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

### *Determination of SubLethal Concentration of Sodium Selenite*

To study the toxicity and effects of selenium on the antioxidant gene expression and lipid peroxidation, the lethal concentration of sodium selenite was quantified in adult female *A. maculatum* ticks by injecting using a 33-gauge needle with different concentrations of sodium selenite using a method described previously by Kumar et al., (2016). The sublethal concentration and normal concentration of sodium selenite in the ticks as well as one phosphate-buffered saline (PBS) control were used for injections. Unfed *A. maculatum* female tick's natural selenite concentration is 0.23 µg/g as estimated by inductively coupled plasma-mass spectrometry (ICP-MS) (unpublished data). Since sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) is easily soluble in water, 300 µg of sodium selenite was solubilized in 10 ml of PBS. After screening sodium selenite concentrations of 30, 3, 1, 0.5, and 0.125 µg/µl, all these doses caused lethality in ticks within less than 24 hours. Sodium selenite concentrations 0.01 µg/µl and 0.05 µg/µl were selected to inject in the ticks. A Hamilton syringe was used to inject 1 µl of each of the concentrations into the unfed tick

hemocoel through the posterior dorsal cuticle. Each group of 15 ticks was placed at room temperature surrounded by moist conditions for 24 hours following their injections. The ticks survived sublethal concentrations of sodium selenite.

### ***Tick Dissection***

Each of the three groups of ticks were dissected under a microscope, their salivary glands and midgut tissues being separated and washed in cold M199 buffer as described previously (Budachetri and Karim 2015). The tissues were stored in RNA later and protease inhibitors in -80° C in preparation for RNA extraction and transcriptional gene expression.

### ***RNA Isolation***

RNA isolation was then performed on the salivary gland and midgut tissues of each 0.1 ng/μl and 0.05 ng/μl, yielding sufficient concentrations of RNA. Frozen salivary gland and midgut tissues were thawed on ice. The RNA later solution was carefully pipetted from each sample. RNA isolation was performed on both tissue types from all three samples using the Illustra RNAspin Mini kit (GE Healthcare Life Sciences) and the included manufacturer's protocol. The isolated RNA's concentration in each sample was measured and recorded using Nanodrop analysis. Each sample was stored at -80° C.

### ***cDNA Synthesis***

From both sample concentrations of sodium selenite and 1X PBS RNA samples, 2 μg of RNA were added to individual 20 μL reverse transcription reactions from the iScript cDNA Synthesis Kit (Bio-Rad) using the included manufacturer's protocol. Each reaction

was heated in a Bio-Rad thermocycler under the following conditions: 25° C for 5 min, then 42° C for 30 min, then 85° C for 5 min, followed by a 4° C hold. The cDNA was made to a calculated concentration of 25 ng/ul. This was done to quantify RNA.

### ***qRT-PCR***

Each qRT-PCR reaction mixture used Universal SYBR green super mix (Bio-Rad Inc.) as described previously (Adamson et al., 2013; 2014; Budachetri and Karim 2015). The following thermo cycler protocol was used for the reactions: 50°C for 3 min, 95°C for 10 min, and the last entry of 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. All samples were run in triplicates including non-template controls (NTCs) for each set of primers.  $\beta$ -Actin was used as a housekeeping or reference gene for the purpose of normalization to calculate the relative expression of different antioxidant genes.

### ***Quantification of Oxidative Stress using MDA Assay***

Lipid peroxidation malondialdehyde (MDA) assay kit (Sigma-Aldrich, St. Louis, MO) was used to quantify the relative oxidative stress in midgut and salivary gland 0.1 ng/ $\mu$ L sodium selenite ticks and 1X PBS injected tick midgut and salivary gland tissues as described (Crispell et al., 2016). All of the instructions were followed within the manual provided by the manufacturer.

### **Oxidative stress estimation using CM-H2DCF-DA**

***In Plate reader assay:*** The protocol of the detection of reactive oxygen species by Owusu-Ansah et al (2008) was modified. The dissected tick salivary glands were rinsed into 1X PBS in 96 well plates with control and sodium selenite treatment separately. The salivary glands were treated with 1 $\mu$ M of the CM-H2DCF-DA, a chloromethyl derivative of H2DCFDA used as an indicator for reactive oxygen species. The dye diffuses into the cells where the acetate groups are cleaved by intracellular esterases and thiol-reactive chloromethyl group reacts with intracellular glutathione and other thiols, which yield the fluorescent adduct inside the cell. The fluorescent adducts between control and treatment groups of tick salivary glands were measured after cleaning excess dyes three times in 1X PBS at a kinetic mode of 485 nM excitation and 520 nM emissions.

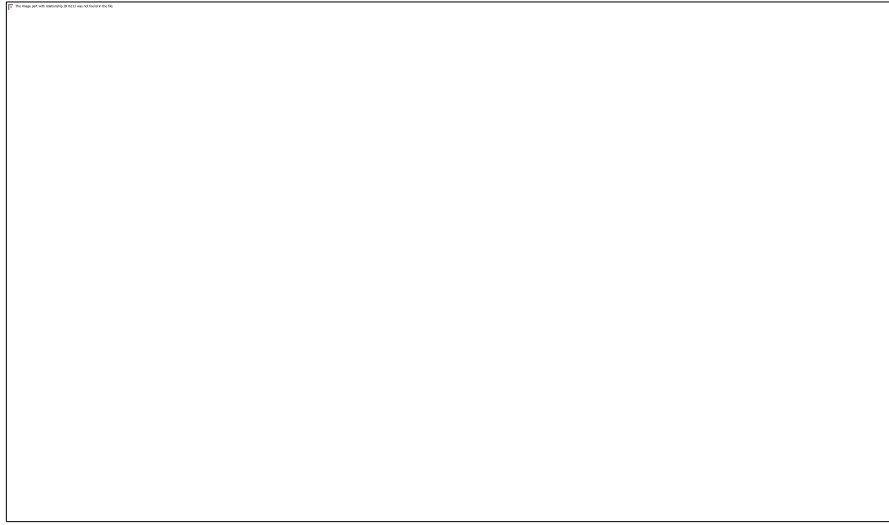
### ***Confocal Imaging***

To be able to view fluorescent signals, immunolocalization of dissected salivary glands of 0.1 ng/ $\mu$ L and 1X PBS injected from unfed female ticks. Dissected tissues of each group were washed three times in PBS. Salivary glands were permeabilized in 0.5% tritonX-100 for 30 minutes at room temperature and washed in PBS. Tissues were then incubated for 1 hour at room temperature. H2CFDA was used as an indicator to view the ROS. Tissues were soaked in Bovine serum albumin (BSA) and incubated overnight at 4°C. Tissues were washed in PBS. Salivary glands were incubated for thirty minutes at room temperature in the dark. Excess antibody was rinsed off with three PBS washes. Tissues were incubated in PROLONG Gold DAPI. Tissues were immediately viewed under a Zeiss LSM 510 META confocal microscope using the ZEN 2009 software (Zeiss, CA, USA).

## **Chapter IV: Results**

### **Sublethal dose estimation and its impact on tick selenoprotein expressions:**

The sublethal doses were determined to be 0.05 and 0.01  $\mu\text{g}/\mu\text{l}$  as standard concentration to be used to assess the interplay between exogenous selenium supplementation and its impact on the endogenous selenoprotein expressions (Fig 1). *SelM* antioxidant gene showed a 6-fold up-regulation of transcript level and *SelN* expression increased 8-fold upon exposure to the 0.01 $\mu\text{g}/\mu\text{l}$  dose. The 0.05  $\mu\text{g}/\mu\text{L}$  concentration did not affect the overall level of selenogenes except *SelT*, and *SelX* inside the tick tissues. As stated, ticks maintain a robust antioxidant system, which may have been stimulated to increase its antioxidants after injection.



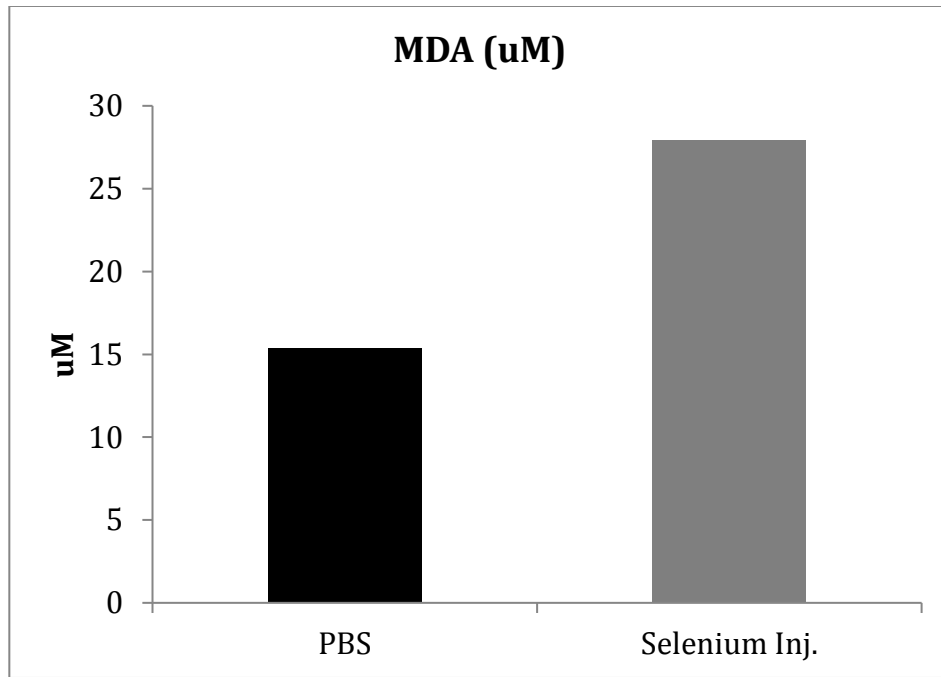
**Figure 1. Transcriptional gene expression of *Amblyomma maculatum* after the injection of Sodium selenite sublethal concentrations.** The qRT-PCR was used to determine transcriptional expression of tick antioxidants, and selenogenes using  $\beta$ -actin as reference gene to 1.

### **The impact on oxidative stress with injection of sublethal dose of sodium selenite:**

Only the higher dose 0.1 $\mu\text{g}/\mu\text{l}$  of sodium selenite was chosen for further study. Like with expression studies, 0.1 $\mu\text{g}/\mu\text{l}$  of sodium selenite was injected to ticks along with 1X PBS as control.

After the dissections, lipid peroxidation estimation protocol was followed to understand relative

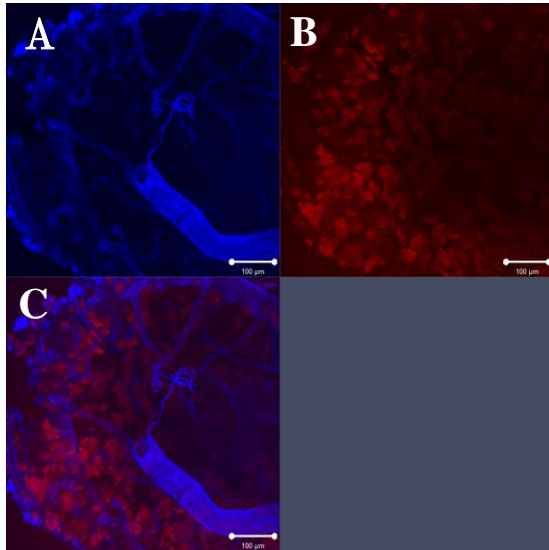
oxidative stress in tick with injections (Figure 2). We observed that sublethal dose of sodium selenite (0.1ug/ul) increased the lipid peroxidation as estimated by malondialdehyde in tick tissues. Sodium selenite injected ticks showed a nearly double lipid peroxidation of approximately 28  $\mu\text{M}$  compared to the PBS injected control ticks, which showed a lipid peroxidation of approximately 15  $\mu\text{M}$ .



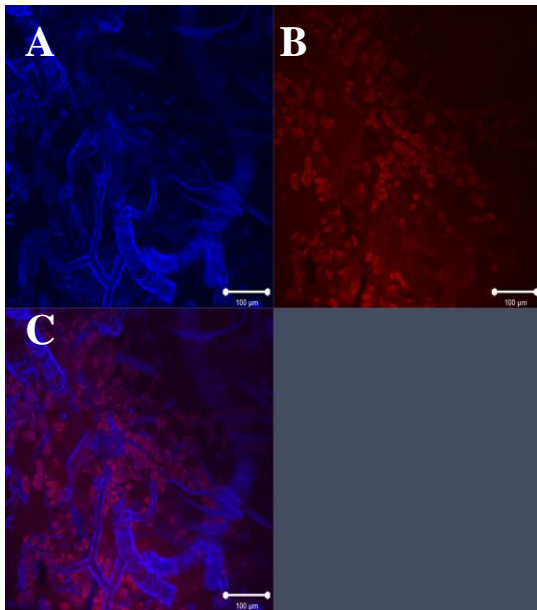
**Figure 2. ROS quantification by MDA assay.** Estimation of relative oxidative stress in *A. maculatum* tick tissues by lipid peroxidation malondialdehyde (MDA) assay.

Additionally, the role of the sodium selenite in the elevation of the oxidative stress was estimated using standard dye, Chloromethyl derivative of H<sub>2</sub>DCFDA (Figure 3-5). The plate-reading assay showed the increased fluorescent adducts with sodium selenite injections concurring the MDA assay results. The oxidative stress estimated by CM-H<sub>2</sub>DCFDA was visualized under confocal microscopy in control (Figure 3) and 0.1 $\mu\text{g}/\mu\text{l}$  sodium selenite injected tick salivary

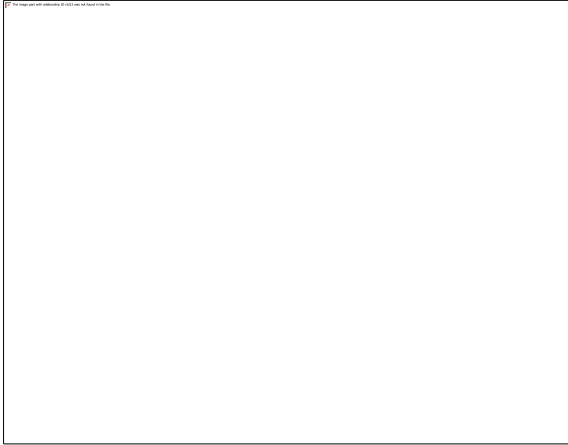
glands (Figure 4). The confocal results could not be distinctly differed the oxidative stress between two groups.



**Figure 3. Fluorescent microscopy image of (A) PBS salivary glands in DAPI, (B) PBS SG in H<sub>2</sub>DCF, (C) PBS salivary glands in DAPI and H<sub>2</sub>DCF images merged.**



**Figure 4. Fluorescent microscopy of (A) 0.1 ng/µl salivary glands in DAPI, (B) 0.1 ng/µl salivary gland tissue H<sub>2</sub>DCF, and (C) 0.1 ng/µl salivary glands in DAPI and H<sub>2</sub>DCF merged.**



**Figure 5. Protocol with H2DCF in microplate reader wavelength 520-545 nm of PBS injected salivary glands and 0.1 ng/ $\mu$ L sodium selenite injected salivary glands.**

Control salivary glands showed 100 relative fluorescence units while 0.1  $\mu$ M sodium selenite salivary glands showed double the relative fluorescence units at 200, signifying the induced and present oxidative stress in the tick as a result of toxic sodium selenite. The semi-quantitative value of oxidative stress confirms MDA results. More fluorophores were detected in treatment samples of ticks. This infers that more oxidative stress damage occurred in sodium selenite injected ticks than control ticks.



## Chapter V: Discussion and Conclusion

Oxidative stress induction was explored in unfed female *A. maculatum* ticks, also of which their potential gene targets by gene expression profile of antioxidant genes were examined. Assays were performed to explore the impact of selenium toxicity on tick antioxidant system. To measure and quantify ROS and oxidative stress, the MDA assay, CM-H<sub>2</sub>DCFDA, and confocal assays were performed.

The transcriptional gene expression of antioxidant genes in tick midgut when 0.1 µg /µL and 0.05 µg /µL sodium selenite was injected in separate group of unfed female *Amblyomma maculatum* ticks after 24-hours of incubation showed high expression in SelM and SelN antioxidant genes. These selenoproteins are ER resident proteins, which have a role in ER stress mitigation since ER stress is a result of oxidative stress. The SelM antioxidant gene showed a 6-fold up-regulation expression, and SelN showed an 8-fold up-regulation. As revealed by the transcriptional gene expression, ticks have a robust antioxidant system, which have been stimulated to increase its antioxidants after injection. Highly up-regulated genes can be further characterized to further comprehend how ticks respond to oxidative stress.

Reactive oxidative species were quantified using the MDA assay. Sodium selenite injected ticks showed a higher lipid peroxidation of approximately 28 µM, while PBS injected control ticks showed a lipid peroxidation of approximately 15 µM. The oxidative degradation of lipids in sublethal sodium selenite injected ticks resulted in cell damage and was seen by the assay results.

Confocal microscopy analyzed salivary glands of sodium selenite injected and control PBS injected ticks. The images (Figures 3 and 4) did not show a significant difference, where there should be a noticeable view in relation to the control and sodium selenite tissues. Only slightly do the sodium selenite tissues fluoresce due to oxidative stress, but does not show enough to make

a significant conclusion on the effects of sodium selenite analyzed by confocal. Salivary glands and midgut tissues will be more carefully obtained to perform confocal once again. The preliminary findings suggested that the MDA assay showed more lipid peroxidation. Detecting the definite increase in oxidative stress using confocal microscopy was not accomplished, however. A supplemented assay with modified cell culture protocol was used in support of the MDA findings and in lieu of the confocal results.

Quantitative confocal analysis of PBS SG showed 100 relative fluorescence units and of 0.1 $\mu$ M sodium selenite SG showed double the relative fluorescence units at 200. It was confirmed that sodium selenite induced ticks contained ROS by oxidative stress. This study provides a future direction for further experimentation with the tick gene profile and oxidative stress could enhance knowledge on ticks' antioxidant system.

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