Neuronal in vitro impact of *Amblyomma americanum* salivary glands extracts

Madi Leierer [1,2], Lidia Montenegro-Cadena [1], and Paulina Maldonado [1] Berlin Londono-Renteria [1]

1. Vector Biology laboratory, Department of Entomology, Kansas State University, Manhattan, KS, USA.
2. Department of Biochemistry and Molecular Biophysics, College of Arts and Sciences, Kansas State University

Abstract

When a tick feeds off a host, the salivary glands of the tick excrete saliva to assist the tick in feeding (1).
The reason saliva assists the tick in consuming its blood meal is due to its immunosuppressive, anti-inflammatory, and anti-coagulant properties (2).Tick salivary glands also play an important role in the transmission of tick-borne pathogens (3). We wanted to investigate the effect of tick salivary gland extracts (SGE) on human neurons. For our experiment, the organism whose salivary gland extract we used was the *Amblyomma americanum*. We compared the effects of salivary gland extract from ticks collected in the field and ticks reared in a lab colony. Our results showed that the salivary gland extract from the two types of ticks affected the neurons differently, and the salivary gland extract of the field tick was more damaging to the neurons. This indicates that SGE from field ticks, together with infectious agents, could result in a more severe neuronal impairment in a human host than the infectious agent alone.

Purpose

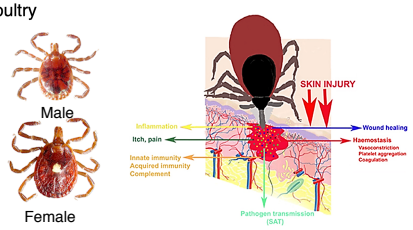
The purpose of this research is to determine the impact of both field *A. americanum* salivary gland extract and colony *A. americanum* salivary gland extract on human neurons.

Questions, Hypotheses, and Predictions

Question: How do the field and colony SGE samples from *A. americanum* affect the gene expression in neurons?
Hypothesis: Salivary glands extract collected from field ticks will have a bigger impact on neuronal damage as compare with the colony ones.

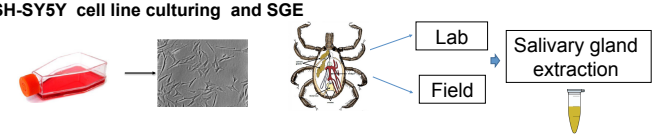
Study System

The *Amblyomma americanum (*Order: Ixodida, Family: Ixodidae*),* also known as the lone star tick, is found in Mexico and southeastern and eastern regions of the United States. The *Amblyomma americanum* can transmit the following disease causing bacteriums: *Ehrlichia chaffeensis* and *Ehrlichia ewingii,* which cause ehrlichiosis; *Rickettsia parkeri,* which causes Rickettsiosis; and *Francisella tularensis*, which causes tularemia. The *Amblyomma americanum* can also transmit *Theileria cervi* – a protozoan parasite that attacks white-tailed deer (4). *A. americanum's* life cycle can take two years to complete. It feeds on a different host during each of its developmental stages (which include larval, nymphal, and adult). Hosts of the *Amblyomma americanum* include humans, large mammals, and poultry

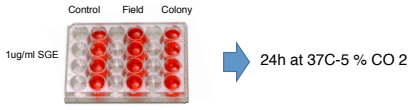


Methods and Experimental Design

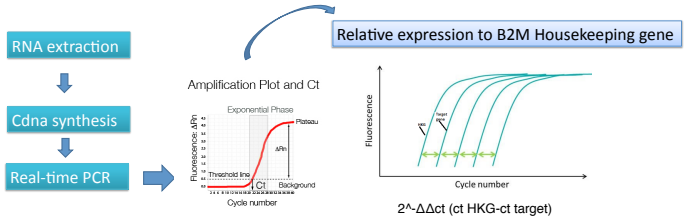
SH-SY5Y cell line culturing and SGE



SH-SY5Y exposure to Amblyomma americanum SGE (Field and Colony)



Relative gene expression: Fox, Enolase 2, Caspase 3 (Neuronal damage) and Tollip, MYD88 (Inflammation)



Results

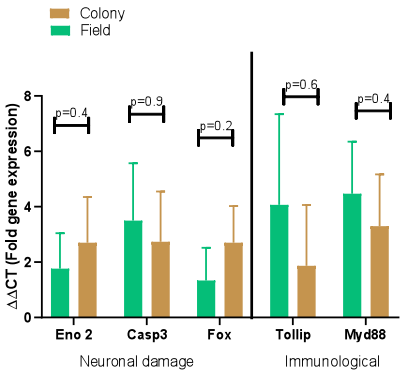
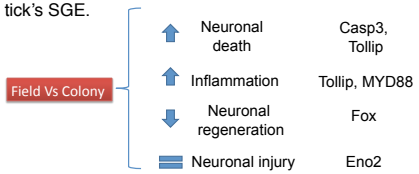


Figure 1. Damage and immunological gene related expression on Neurons treated with *A. americanum* SGE field (green) and lab (brown) collected. Statistical significance p<0.05.

Conclusions

The data from our experiment showed that the genes Casp3, Tollip, and Myd88 in the neurons exposed to SGE from the field tick were more upregulated than they were in the neurons exposed to the SGE from the colony tick. However, the contrary was found with Eno2 and Fox. The genes Eno2 and Fox were more upregulated in the neurons exposed to the colony tick's SGE than in the neurons exposed to the field tick's SGE.



The field SGE induces higher damage and inflammation on the neurons than the colony tick SGE. This may be because the field tick more frequently comes in contact with wild and human hosts, which could increase its virulence against the hosts compared to the ticks not exposed to hosts. In summary, tresult in a he SGE from field ticks, together with infectious agents, could more severe neuronal impairment in a human host than the infectious agent alone.

Tick saliva + Infectious agents = Damage enhanced
Worse patient outcomes

Future Directions

The next step could be to isolate the proteins from the SGE, to repeat the experiment but with individual and known proteins. Since the brain environment not only has neurons, we could test other cells like immune cells along with the neurons and the salivary proteins.

References

1. Francischetti, I. M., Sa-Nunes, A., Mans, B. J., Santos, I. M., & Ribeiro, J. M. (2009). The role of saliva in tick feeding. *Frontiers in bioscience (Landmark edition), 14*, 2051-88.
2. Šimo, L., Kazimirova, M., Richardson, J., & Bonnet, S. I. (2017). The Essential Role of Tick Salivary Glands and Saliva in Tick Feeding and Pathogen Transmission. *Frontiers in cellular and infection microbiology, 7*, 281. doi:10.3389/fcimb.2017.00281
3. Kazimírová, M., & Štibrániová, I. (2013). Tick salivary compounds: their role in modulation of host defences and pathogen transmission. *Frontiers in cellular and infection microbiology, 3*, 43.doi:10.3389/fcimb.2013.00043
4. Holderman, C. J., & Kaufman, P. E. (2013, November). Amblyomma americanum. Retrieved November 15, 2018, from http://entnemdept.ufl.edu/creatures/urban/medical/lone_star_tick.htm

Acknowledgements

Special thanks to Dr. Montenegro and Dr. Londono for giving me this amazing experience and learning opportunity.