Isolation of uniquely recognized salivary gland antigens to interfere with feeding performance of ixodid ticks

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Background

- Tick-borne diseases are currently controlled with the use of antibiotics and pesticides, which can have harmful consequences such as development of resistance, residues in the host and environmental contamination
- Previous work involving immunization with certain extracts of tick salivary gland or midgut have demonstrated a distinct effect on tick feeding or fecundity with canine and bovine models[1,2]

Figure 1. Male *Dermacentor andersoni*

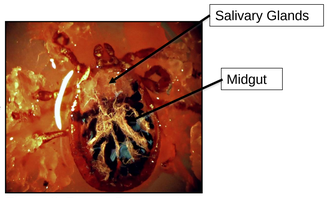


Figure 2. Female *Dermacentor andersoni* with carapace removed

Figure 3. Midgut removed from a tick

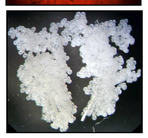


Figure 4. Salivary glands removed from a tick[3]

Objective

To isolate specific tick salivary gland molecules that are only reactive to protective anti-salivary gland sera. Isolation with 2-dimensional electrophoresis will allow use of mass spectrometry to identify uniquely reactive proteins as targets for further evaluation as defined anti-tick salivary gland vaccine candidate antigens.

Significance

- Hosts that become resistant to ticks after multiple infestations display immune reactivity to molecules in tick saliva, and it has been proposed that immunization with salivary gland extract can induce resistance by mimicking the immune response that occurs after multiple infestations[2]
- Another hypothesis proposes that “novel” or “concealed” antigens associated with the midgut, do not reach the host under natural feeding circumstances, but are viable candidate antigens for an ant-tick vaccine, because they induce immunity that reduces tick fecundity[2]
- Midgut and salivary glands dissected from multiple unfed *Dermacentor andersoni*, the “Rocky Mountain Wood Tick,” were used for this study
- This ixodid tick species was chosen as part of a well-established experimental vector-pathogen-host model system; *D. andersoni* has been identified as a vector of *Rickettsia rickettsii* and *Anaplasma marginale* to people and cattle, respectively

References

[1]Jittapalapong, S., W. Jansawan, O. O. Barriga, and R. W. Stich. 2004. Reduced incidence of Babesia bigemina **infection in cattle immunized against the cattle tick, Boophilus microplus. Ann N Y Acad Sci 1026:289-297.**
[2]**Jittapalapong**, S., R. W. Stich, J. C. Gordon, T. E. Wittum, and O. O. Barriga. 2000. Performance of female Rhipicephalus sanguineus (Acari: Ixodidae) fed on dogs exposed to multiple infestations or immunization with tixck salivary gland or midgut tissues. J Med Entomol 37:601-611.
[3]Sathaporn Jittapalapong, DVM, PhD, Dean, Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand

Preliminary Proteomic Analysis

Bradford Assay

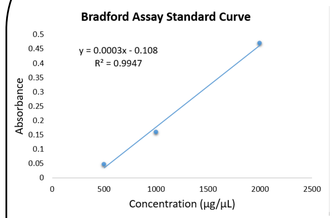


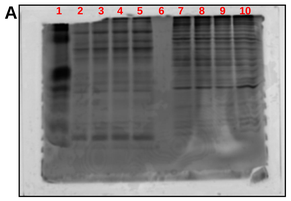
Figure 5. Bradford assay standard curve using bovine serum albumin (Absorbance at 595 nm).

- Unfed adult stage *D. andersoni* were obtained from the colony maintained at Oklahoma State University
- Midgut and salivary glands, tick organs which directly interface with vertebrate hosts, were removed
- Female and male midgut and salivary glands were homogenized
- A Bradford assay technique was used to calculate protein concentrations

Table 1. Protein concentrations of tick-tissue samples as calculated with the equation in Figure 5

Tick-Tissue Sample	Protein Concentration (µg/µL)
Male Salivary Gland	6.74
Male Midgut	9.76
Female Salivary Gland	3.89
Female Midgut	3.43

SDS-PAGE of tick midgut and salivary glands



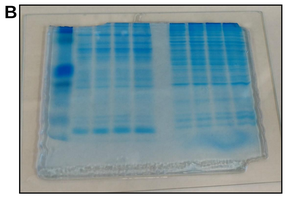


Figure 6. A. Image of denaturing gel after SDS-PAGE and protein staining with Page Blue (invitrogen), taken with a FluorChem Q System. Lane 1 contains BioRad Kaleidoscope Ladder, lanes 2-5 contain 30µg, 40µg, 50µg and 70µg of male tick midgut protein respectively, lane 6 is blank and lanes 7-10 contain 30µg, 40µg, 50µg and 70µg of female tick salivary gland protein respectively. **B.** Color image of the same SDS-PAGE gel.

- To confirm protein concentrations for isoelectric focusing, tick-tissue samples were denatured/reduced and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) gel with different protein amounts

All four protein amounts (30, 40, 50 and 70 µg) were visible on a polyacrylamide gel (Figure 6), without overloading the gel

- Isoelectric focusing was done with 50 µg of protein from each tick-tissue sample and run with a Novex ZOOM® IPGRunner System using non-linear immobilized pH gradient (IPG) strips

Two-Dimensional Electrophoresis

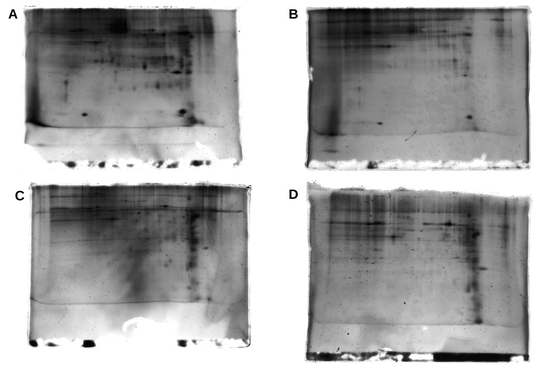


Figure 7. Images of silver-stained 2-D gels. A. Male tick midgut (50 µg of protein). **B.** Female tick midgut (50 µg of protein). **C.** Male tick salivary glands (50 µg of protein). **D.** Female tick salivary glands (50 µg of protein). All images acquired with a FluorChem Q System.

- After isoelectric focusing on non-linear pH 3-10 gradients, 12% SDS-PAGE was performed
- The gels were first fixed and stained with Page Blue stain, but the proteins were not visible
- Gels were then silver-stained and protein spots were visualized (Figure 7)

Future Directions

- In order to ensure our two-dimensional protocol is valid we will re-run each tick-tissue sample and compare the silver stain images to those in Figure 7 to confirm similarities in protein distribution
- Once the 2-D electrophoresis protocol is optimized, we will use 1-D and 2-D Western blot analyses to compare salivary gland and midgut proteins reactive to antisera associated with reductions in different tick performance parameters

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