The Importance Of Accounting For Cattle Fever Tick Genetic Diversity While Developing Robust Anti-Tick Vaccines

NAU Northern Arizona University
The Pathogen and Microbiome Institute

Grant Pemberton[1], Mackenzie L. Roberts[1], Rebekah E. Turner[1], Nathan E. Stone[1], Shelby M. Hutton[1], Juan Mosqueda[2], Guilherme Klafke[3], Donald B. Thomas[4], Pia U. Olafson[5], Massaro Ueti[6], Glen A. Scoles[7], David M. Wagner[1], and Joseph D. Busch[1]

[1]Pathogen and Microbiome Institute, Northern Arizona University, 1395 S. Knoles Dr. Bldg 56, Flagstaff, AZ 86011-4073, USA. [2]Facultad de Ciencias Naturales, Av. De las Ciencias s/n, Col Juriquilla, C.P 76230, Mexico.
[3]Instituto de Pesquisas Veterinárias Desidério Finamor, Estrada do Conde, 6000. Eldorado do Sul. Brazil. 92990-000. [4]USDA, ARS, SPA, Cattle Fever Tick Research Laboratory, Moore Air Base, Building 6419, 22675, N. Moorefield Road, Edinburg, TX 78541, USA.
[5]USDA, ARS, KBUSLIRL, 2700 Fredericksburg Rd., Kerrville, TX 78028-9184, USA. [6]USDA, ARS, ADRU, 3003 ADBF, Washington State University, Pullman, WA 99164-6630, USA.
[7]USDA, ARS, IIBBL, Beltsville Agricultural Research Center (BARC)-West, Bldg 007, 10300 Baltimore Ave., Beltsville, MD 20705

USDA United States Department of Agriculture | National Institute of Food and Agriculture

Funding by USDA-NIFA 2018-67015-28301

Purpose

The goal of this research is to prevent the spread of bovine babesiosis by improving future anti-tick vaccines for cattle.

Abstract

The highly invasive southern cattle tick (*Rhipicephalus microplus*) transmits single-celled parasites (*Babesia* spp.) that cause bovine babesiosis, a lethal disease of cattle. Both the disease and tick vector are endemic in Mexico and at risk of spreading into the US. Tick control in Mexico and a tick eradication quarantine area (TEQA) along the Texas border relies on the use of pesticides; however, extensive resistance to these chemicals is starting to develop. An alternative control method is being tested in Mexico and the TEQA, which uses an anti-tick vaccine to raise an antibody response in cattle that attacks the ticks when they take a bloodmeal. The current vaccine (Zoetis Immunomodulator™) targets a protein in the tick midgut (Bm86). Unfortunately, this vaccine is not universally effective against North American populations of *R. microplus*, possibly due to genetic diversity within the Bm86 protein. To increase the effectiveness of future vaccines, it is essential to account for the genetic diversity of vaccine targets, and we are sequencing thirteen tick genes under consideration as vaccine candidates. We screened tick populations from diverse locations in the Americas to identify mutations and quantify existing genetic diversity. This information will serve to increase the effectiveness of future vaccines by identifying highly conserved regions within each vaccine candidate and contribute to the long-term control of ticks in Texas and other parts of the world where *R. microplus* and babesiosis are now endemic.

Introduction

- **The highly invasive southern cattle tick (*Rhipicephalus microplus*) transmits a single-celled parasite (*Babesia* spp.) that cause bovine babesiosis, a lethal disease of cattle.**
- **Tick control in Mexico and southern Texas relies on the use of pesticides; however, resistance to these chemicals is evolving.**
- **The current vaccine (Zoetis Immunomodulator™) raises an antibody response in cattle blood that targets a midgut protein (Bm86) in the tick mid-gut. Unfortunately, this vaccine is not universally effective.**
- **To increase the effectiveness of future anti-tick vaccines, it is essential to account for the genetic diversity of vaccine targets.**
- **We screened tick populations from diverse locations in the Americas to quantify existing genetic diversity at Bm86 and two other tick genes being considered for vaccine development (Aquaporin-2 and Vitellogenin receptor).**
- **This information will serve to increase the effectiveness of future vaccines by identifying highly conserved regions within each vaccine candidate that will contribute to the long-term control of ticks in the US and other countries.**

Methods

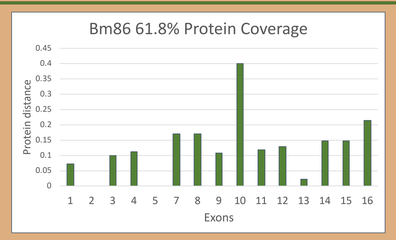
Figure 1. Map of *R. microplus* samples used in this study. A total of 91 DNA samples from diverse locations in the Americas are being used to quantify genetic diversity. We included one reference sequence from GenBank for each gene.

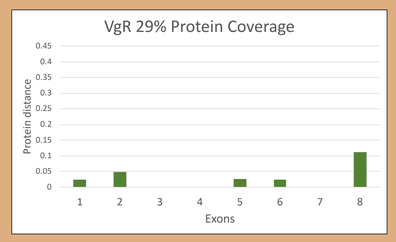
- **We used amplicon sequencing to amplify individual exons for each gene**
- **Genes included: Bm86, Aquaporin-2 (AQP2), Vitellogenin receptor (VgR)**
- **Sequencing of all three genes occurred simultaneously using a next-generation sequencing platform (Illumina MiSeq).**
- **Each DNA sequence was aligned and translated into amino acids using BioEdit; substitutions within each exon were identified and protein diversity was calculated.**

Results

- **Protein diversity was highest in the current vaccine (Fig. 2), and mutations were found within most of the specific vaccine epitopes for Bm86 (2 of 3) and VgR (3 of 3), while AQP2 was conserved (Fig. 3).**

Figure 2. Protein diversity partitioned by exon. The y-axis shows the proportion of amino acid substitutions per total number of amino acid positions encoded within each exon.





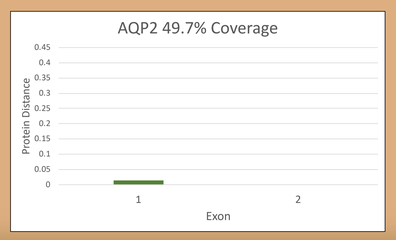


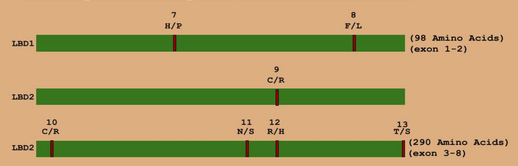
Figure 3. Protein diversity within specific vaccine epitopes. Amino acid mutations in the Americas are shown above each epitope sequence. The country where specific mutations are found is listed under each epitope set.

Bm86 epitopes developed in Brazil (Patarroyo et al. 2020)

1. SSICSDFGNEFCRNAC (exon 2)
2. CDCGEWGAMNMTTR (exon 4)
3. CLSKHVLRKLQACEH (exon 11)

Geographic location of mutations
1=US,MX,BR. 2=US,MX. 3,4,5,6=US,MX,BR,CO,PR

Vitellogenin Receptor (Hussein et al. 2019)

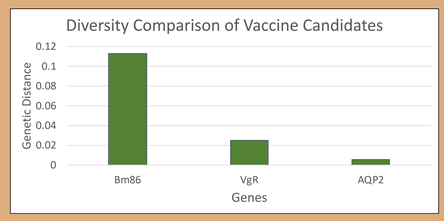


Geographic location of mutations
7=US,MX. 8=MX,CO,BR,PR. 9,11=US,MX,CO,BR,PR. 10,12,13=MX,BR.

Aquaporin-2 (Scoles et al. 2022)

1. AVFQLGSVGLAAAP (exon 1)
2. ADALSQVDVNLAIVYGTNATAPVFSCFPAPGV (exon 2)
3. MCGWGSAVFSFRSYNWFWV (exon 2)

Figure 4. Protein diversity combined across all exons. The y-axis shows the proportion of amino acid substitutions per total number of amino acid positions in our dataset. We did not sequence the entire protein of any candidate.



- **Bm86 (the current vaccine): We found a high proportion of amino acid substitutions in this protein (11.3%). None of the new sequences were identical to the Zoetis vaccine sequence, consistent with the inefficiency of the vaccine in North America.**
- **VgR: The two large peptides have an intermediate number of substitutions, and the combined rate (2.5%) is much lower than Bm86.**
- **AQP2: The most conserved vaccine candidate of the three, with protein diversity of <1%. Furthermore, AQP2 revealed zero substitutions within the three vaccine candidate epitopes (Fig. 3).**

Discussion

- **Our results support the hypothesis that genetic variation in Bm86 within *R. microplus* populations from North America may lead to the inefficiency of this anti-tick vaccine, which was designed for ticks in Australia.**
- **Future vaccines against *R. microplus* will likely need to target proteins (or epitopes) that are highly conserved across the Americas.**
- **An effective vaccine would target proteins vital for tick metabolism or reproduction.**
- **Based upon these considerations, the AQP2 gene appears to be a promising vaccine candidate.**
- **Future research will include the genetic analysis of 10 additional vaccine candidate genes, and in addition we plan to incorporate protein modeling to infer surface-expressed epitopes.**

References

1. Freeman J. M., et al. (2010) Parasites & Vectors 3:101
2. Joaquín H., et al. (2020) Bovine immunization 101461
3. Hussein H.E., et al. (2019) Parasites & Vectors. 2019;12(1):7.
4. Martinez-Arzate, J. C., et al. (2019) Genetic Diversity of Bm86 20:56
5. Patarroyo J.H., et al. (2020) Ticks and Tick-borne Diseases 11:101461
6. Schetters T., et al. (2016). Parasites & Vectors 9:105.
7. Scoles G. et al. (2022) Parasites & Vectors 15(1):49.

Acknowledgements

- **We thank USDA field inspectors for collecting tick samples, as well as APHIS and TAHC veterinarians for insightful discussions. Funding for this project was provided by USDA- NIFA 2018-67015-28301.**