GENOME-BASED SELECTION OF ANTI-CATTLE TICK VACCINE CANDIDATE ANTIGENS.

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Introduction

We have sequenced and assembled a significant portion of the cattle tick genome and transcriptome, presently at an overall 0.5X coverage. However, the coverage of the gene-rich regions of the genome are at ~2X coverage. This genomic resource will be hosted at Murdoch University, Perth, Western Australia as CattleTickBase (http://ccg.murdoch.edu.au/index.php/Main_Page). We have used this genomic information and additional proteomic and transcriptomic information to guide investigations to select antigens for evaluations in anti-cattle tick vaccine cattle stall trials.

Material and Methods

Genomic DNA from the f8 generation of the Deutsch strain of *R. microplus* was used to produce a 3X BAC library. This strain was developed from an outbreak in south Texas, USA. The genomic DNA was processed by Cot filtration experiments to enrich for single/low-copy DNAs that were subsequently sequenced by 454 methodologies (1). Transcriptome information was obtained by 454 Titanium pyrosequencing of RNA purified from various tissues and tick samples. Proteomic investigations utilized two-dimensional gel electrophoretic protocols on dissected adult female ovary and gut tissues. Cattle stall tests were performed in Campo Grande, Mato Grosso do Sul, Brazil using one-year-old Holstein calves assigned to control and treatment groups consisting of 6 animals per group. Each animal was given 3 intramuscular injections of 100 µg *Pichia pastoris*-expressed antigen + Montanide adjuvant spaced 2 weeks apart. Twenty-one days after the final immunization, each animal was challenged with 15,000 larvae from the Campo Grande strain of *R. microplus*.

Results

Based on proteomic, functional genomics, and sequence annotation approaches, we selected 8 *R. microplus* antigens for expression in *Pichia pastoris* and evaluation in cattle stall tests. Preference was given to proteins that were localized in the gut or ovary membrane fractions and that had sequence similarity to proteins in GenBank annotated as salivary- or salivary gland-associated proteins. Two of these antigens showed greater than 70% efficacy against *R. microplus* when presented in 3 injections of 100 µg *P. pastoris*-expressed antigen + Montanide adjuvant spaced 2 weeks apart.

Discussion and Conclusions

Six antigens are in various stages of evaluations singly and later tests will test the efficacy of different combinations of antigens and epitopes from different antigens. Our overall strategy is to identify proteins

from single- or low-copy gene families that encode membrane-bound or membrane-associated proteins with critical function to the tick. The two antigens that have shown >70% efficacy were isolated from protein coding regions from a Texas population of *R. microplus* and evaluated for efficacy on Brazilian cattle challenged with a Brazilian strain of *R. microplus*. Preparations are underway to evaluate these antigens on Texas cattle challenged with *R. microplus* ticks isolated from outbreaks along the border between Texas and Mexico.

References

1. Guerrero FD, et al. BMC Genomics 2010, 11:374.