# Investigating Host Biomarkers Associated with Cattle Tick Resistance

E. Mantilla Valdivieso[A,C], E. Ross[A], B. Hayes[A], N. Jonsson[B], P. James[A], A. Tabor[A]

[A]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia,
[B]Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, UK
[C]Email: e.mantilla@uq.net.au

*Rhipicephalus microplus* species complex are blood-feeding ectoparasites that adversely affect livestock health and production in tropical and subtropical regions. Beef cattle production in tick-endemic areas of Australia uses predominantly *Bos indicus* herds, which have a higher level of host resistance than *B. taurus* cattle. Crossbreeding is used to improve the genetic performance of cattle but there is no feasible method to account for tick resistance to date. The only validated method for assessment of this phenotype is by crush side examination of the animal's body for tick counts, which is time-consuming and potentially dangerous for the investigator. Therefore, elucidation of biomarkers that can assist in the identification of tick resistant animals will be a feasible option for assisted animal selection and breeding improvement. We predict that biomarkers of resistance can be assessed in different tissues of cattle through Next-Generation Sequencing approaches (RNA-Seq).

Thirty-five Brangus steers (~9 months old, ~250 kg) with no previous exposure to cattle ticks were sourced for this study. Prior to artificial tick infestation, ~2ml of blood from each animal was collected in EDTA tubes. White blood cell (WBC) pellets were isolated and stored in Qiazol reagent (QIAGEN) at -80°C until animals were fully phenotyped. Infestation protocol was as described in [1] using tick scores. RNA was extracted from WBCs of five resistant (R) and five susceptible (S) animals. Two RNA samples from R animals were pooled due to low sample concentration prior to sequence library preparation. For this sampling timepoint there were nine libraries (4 R and 5 S) sequenced as 100 bp single-end reads on the Illumina NovaSeq 6000 platform. The RNA-Seq bioinformatics pipeline has been described in [2]. Differential gene expression was conducted in R using the edgeR package [3] implementing the likelihood-ratio test between animals in the "S" vs. "R" group

A total of 34 genes were differentially expressed (DE) between susceptible and resistant animals prior to initial tick infestation. Among these DE genes, 19 genes were upregulated and 15 downregulated (FDR < 0.05). The top upregulated gene in S animals was *kallikrein 1* (*KLK1;* Figure 1). *KLK1* encodes a serine protease also known as tissue kallikrein which forms part of the kallikrein-kinin system involved in important signalling pathways that lead to the regulation of blood pressure, vascular permeability, inflammatory cascade and neutrophil chemotaxis, among others [4].

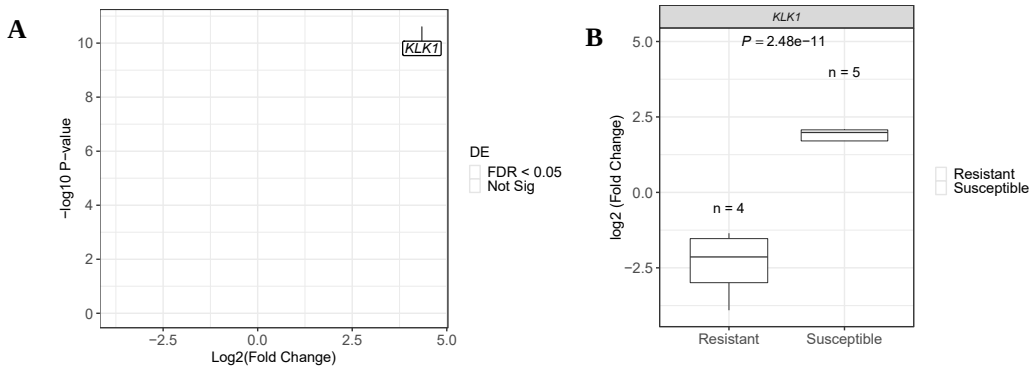


**Figure 1. A) Volcano plot displaying DE genes between tick-naïve resistant and susceptible Brangus cattle. B) Gene expression values showing upregulation of *KLK1* in the susceptible group compared to resistant (*P*< 0.05).**

RNA-Seq analysis revealed that there are genes likely associated with host resistance in cattle prior to tick exposure, particularly expressed in tissues that are relatively easy to access and have immunological importance such as WBCs. Although the KLK gene family is highly conserved in mammals, none of these genes has yet been associated with host resistance in bovines, but in humans some tissue KLKs have been already identified as cancer biomarkers. Thus, these results further support our hypothesis that investigation of high-throughput sequencing data can elucidate potential biomarkers for host resistance, however, further research and validation is needed.

**References**

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