DETERMINING THE GENE EXPRESSION PROFILES OF 17 CANDIDATE GENES FOR HOST RESISTANCE TO TICKS IN SOUTH AFRICAN BEEF CATTLE

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SUMMARY

Beef production is under threat from tick infestation problems, which have so far not been successfully controlled because of shortcomings in chemical and vaccine usage. The variation in resistance to ticks among breeds provides an opportunity to determine the mechanisms that underlie resistance to ticks. Brahman, Nguni and Angus animals were used to study gene expression following artificial infestation with *Rhipicephalus* ticks. Skin biopsies were collected, and RNA extracted for gene expression analyses. Variation in gene expression was observed in genes involved in discouraging long-term supply of blood meal to the tick and those associated with immune responses.

INTRODUCTION

Cattle ticks pose the risk of inflicting deleterious effects on production traits by hindering the growth and weight gain, productivity, fertility, as well as the meat quality of cattle (Marufu *et al.* 2011). The profitability of the beef cattle industry may be compromised as many beef enterprises maximise their profit margins by concentrating more on fertility and a high weaning weight off the veld (Mapholi 2014). Current tick control methods include grazing practices and use of acaricides and vaccines, which have however not been successful in completely eradicating ticks. The widespread use of acaricides to control tick burdens places strong evolutionary pressure towards the emergence of new chemical-resistant strains of ticks, faster than new chemicals can be produced (Gasbarre *et al*. 2009). Ticks also mutate the targeted epitopes into unfamiliar forms and nullify the effect of a particular vaccine. There is also increasing public concern about chemical residues in animal products and the environment (Mapholi *et al*. 2014). A relatively simple and cheap method of reducing the effects of parasite infestation would be use of genetically tick-resistant animals. Resistance to ticks among cattle breeds is variable (Muchenje *et al*. 2008) and this presents an opportunity to exploit the host's resistance to ticks in developing more cost-effective and sustainable tick control programs. Tick bites trigger immune responses in the host animal's body by releasing specific proteins that fight infection at the site, suggesting that response to tick infestation may be under genetic control (Marufu *et al*. 2014). Thus, a better insight into the mechanism of resistance to ticks may be achieved by identifying the genes expressed as a result of tick infestation. The objective of the study was therefore to evaluate the genetic expression differences in different cattle breeds in response to infestation by two different tick species.

MATERIALS AND METHODS

Thirty-six cattle, comprising of 12 Nguni bulls, 12 Brahman bulls, six Angus heifers and six bulls aged between 12 and 15 months were artificially infested with unfed *Rhipicephalus* tick larvae. Half of the animals per breed were infested with *R. microplus*, while the other half were infested with *R. decoloratus*. The Angus groups were further divided in terms of sex, with three animals of each sex being infested with *R. microplus* and the remaining three being infested with *R. decoloratus* larvae. Skin biopsies were collected pre-infestation and the animals' mid-back area was shaved and a calico bag was attached, after which the tick larvae were placed inside. Twelve hours post-infestation, the bags were opened and skin biopsy samples were collected from the tick bite sites. The biopsies were preserved in 5 ml RNAlater® RNA stabilization Reagent (Qiagen) and stored at -80°C. About 50-100 mg of each biopsy sample was used for RNA extraction, which was conducted following the TRIzol® Reagent protocol. Samples showed separation of the 28S and 18S bands with partial smearing after running the 1% agarose gel. Purity test was done using the NanoDrop spectrophotometer to ensure that all samples had 260/280 values ≥ 1.70. Samples which were below this value were then repurified.

cDNA was synthesised using equal amounts of total RNA and the RT^2 First Strand Kit was used according to manufacturer's protocol. To obtain optimal results, 400ng of total RNA per sample was used to obtain a total cDNA volume of 30µl. Then, genetic analysis was done, where threshold cycle (C_T) values generated were used to calculate the expression levels of a panel of 17 candidate genes using the RT^2 Profiler PCR Array Data Analysis Webportal (SABioscience - Qiagen). The panel of genes included cytokines (*TLR5, TLR7, TLR 9, TRAF6, CD14*), chemokines and their receptors (*CCR1, CCL2, CCL5*), toll-like receptors (*IL-1β, CXCL8, IL-10, TNF*) and other candidate genes (*BDA20, OGN, TBP, LUM, B2M*). The fold change value of each gene, normalised against the reference genes Ribosomal protein, large, P0 (*RPLP0*), 18S ribosomal RNA (*RN18S1*), Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and Beta-actin-like (*LOC616410*). Fold change was calculated using the $\Delta\Delta C_T$ method described by Livak and Schmittgen (2001). An analysis of variance for two-way factorial designs was used to test the interaction between the main effects, breed and tick species, for each of the genes. The primers for each of the genes of interest were custom designed by Qiagen using forward and reverse primer sequences associated with the GenBank and UniGene reference sequence numbers.

Ninety-six-well RT^2 Profiler PCR arrays were used for the real-time PCR analyses and facilitated high-throughput focused expression analysis on the genes of interest. Each plate enabled the analyses of four samples at a time to generate amplification data for 17 genes of interest and four reference genes per sample. The gene expression profiles of selected genes were examined using the ABI 7500 real-time PCR thermocycler. A PCR components mix was prepared in a 5ml tube for each sample according to manufacturer's protocol. The arrays were also fitted with primers designed to amplify three Qiagen recommended quality control parameters, namely Bovine Genomic DNA Control (BGDC), Reverse Transcription Control (RTC) and Positive PCR Control (PPC).

RESULTS AND DISCUSSION

Four reference genes, namely *RPLP0*, *RN18S1*, *GAPDH* and *LOC616410* were chosen to normalise the data. The average C_T values for the reference genes were 24.153, 15.717 and 25,399 for *RPLP0*, *RN18S1* and *GAPDH*, respectively. There was no significant interaction between the main effects, breed and tick species, observed for any of the genes, which may suggest similar responses to both tick species' infestations by these breeds. While the expression of most of the genes did not differ significantly according to breed, the expression profiles of genes *TRAF6, TBP, LUM* and *B2M* were significantly different among breeds. There were differences between the Nguni and Angus in the expression levels of *TBP* and *TRAF6* ($P < 0.05$), as well as between the Brahman and Angus in the

expression levels of *LUM* and B2M ($P < 0.01$). Increases in the expression levels of six genes (*CCL2, CCL26, CD14, OGN, LUM,* and *B2M*) post-infestation for all breed × tick species treatment groups were observed. Five genes (*CCR1, TLR5, TRAF6, TBP, BDA20*) increased expression or remained approximately equal after infestation with ticks for all groups. Mixed results were obtained in the breed × tick species groups for expression levels for the genes *IL1-β, TLR7* and *TLR9*, while the expression levels of three genes (*CXCL8, IL10, TNF-α*) decreased or remained the same after tick challenge in all breed × tick species groups.

The results of this study were broadly consistent with previous work (Wang *et al.* 2007; Piper 2010). The genes encoding the extracellular matrix constituents, most importantly, *LUM* and *B2M*, were upregulated at much higher levels in the high (Brahman) and intermediate (Nguni) resistance breeds than the genes involved in immune system regulation and inflammatory responses. This was in agreement with the observation by Piper *et al.* (2010), where there was upregulation of genes encoding constituents of the extracellular matrix in the tick-resistant Brahman in comparison to the susceptible Holstein-Friesian cattle. Kongsuwan *et al.* (2010) attributed resistance to ticks to the epidermal permeability barrier of the skin, which is associated with the heightened expression of these genes in the tick-resistant Brahman cattle. The genes*, LUM, B2M* and *TBP* induced resistance to ticks by promoting continued cellular regeneration, tissue repair and detoxification of the tick bite site, instead of initiating host immune responses. This activated the mechanism required to discourage long term supply of blood meal to the tick. These genes, except *TBP*, were upregulated in all treatment combination groups, excluding the Angus-*R. microplus* group.

The highest upregulation values were detected for *LUM* in the Brahman treatment groups and Nguni-*R. microplus.* As a gene that encodes a member of the small leucine-rich proteoglycan (Weizmann Institute of Science 2016a), *LUM* serves in conjunction with *OGN* to induce immune responses. The gene *OGN* similarly presented higher upregulation values than the rest of the genes of interest. Both *LUM* and *OGN* are capable of regulating fibril organisation and circumferential growth as well as epithelial cell migration in the process of tissue repair at the tick bite site (Weizmann Institute of Science 2016a). The significantly high expression level of *LUM* in the Brahman more than the Angus suggested that the Brahman had a stronger capacity to prevent tick feeding through continuous tissue repair than the Angus. This was true for both tick species. The results suggest that *LUM* can be used as a biomarker for resistance to both *R. microplus* and *R. decoloratus* tick species.

Unlike *LUM*, the significant differences in the expression levels of *TBP* and *B2M* in different treatment groups were unexpected. *TBP* is a component of the RNA polymerase III; hence it was expected to behave like a housekeeping gene exhibiting stable expression levels in all treatment combinations to facilitate continued cell growth. While *TBP* was upregulated in most treatment groups, the gene displayed a downregulated but stable expression level in Angus-*R. microplus* group. This may be attributed to the stressful conditions inflicted by the tick infestations, which might have resulted in the regulatory protein *Maf1* repressing RNA polymerase III activity (Vannini *et al*. 2010). The *B2M* gene is a component of the MHC class I that is responsible for presenting peptide antigens (including tick antigens) to the immune system, while simultaneously forming amyloid fibrils in pathological challenges (Weizmann Institute of Science 2016b). Therefore, the significantly low *B2M* expression levels produced by the Angus animals may imply that this breed's nucleated cells had a poor capacity to detect the tick antigens to prompt host immune responses.

CONCLUSIONS

The differences in the expression profiles of different genes in breeds of different levels of resistance to ticks may provide an insight into the mechanism of resistance to ticks. Genes that show variation in responses to tick infestation among breeds are involved in discouraging long term supply of blood

meal to the tick, although there was some variation in the genes associated with immune responses. The gene *LUM* may be used as biomarker for resistance to ticks. Given that resistance to ticks is a polygenic trait, deep sequencing may reveal more genes associated with this trait. Further studies should be conducted to investigate the association between skin permeability, genes expressed and resistance to ticks.

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