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# Expression analysis of MHC class II DRB3 gene in mastitis affected indicus and crossbred cattle

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In cows, disorders of mammary gland functions often result from inflammation resulting mostly from the bacterial infections. Mechanisms of the immune response depend on different populations of immune cells and expression of their secreted mediators. MHC class II genes, DRB3 in particular has been found to be major immune response gene mediating antigen presentation to the cell surface of immune cells. Kumari *et al.* (2018) investigated the expression levels of DRB1 gene transcripts, which was found to be higher in low fecal egg count animals as compared to the high egg count using real-time based qPCR in Harnali sheep exposed to *Haemonchus contortus*. Major histocompatibility complex (DRB3) gene expression pattern has indicated differences in *Brucella abortus* S19 vaccine induced immune response in Karan Fries and Sahiwal cattle (Kumar *et al.* 2018). The differential expression of MHC class II genes for association with resistance to mastitis in Indian buffaloes (*Bubalus bubalis*) has been evaluated by Mishra *et al.* (2018). Purdie *et al.* (2019) have explored the gene expression profiles during subclinical *Mycobacterium avium* subspecies *paratuberculosis* infection in sheep and observed that members of several gene families were differentially regulated including MHC class I and class II. However, only few studies on the differential expression of immune response genes for association with disease resistance are reported in cattle. Mastitis being one of the economically important diseases in cattle, the objective of this study was to investigate the differential expression of DRB3 gene in the mastitis-affected and healthy indicus and crossbred cattle.

To analyze the expression of MHC class II DRB3 gene in animals comprising mastitis affected Sahiwal (7) and Karan Fries (6) cows; five samples each from healthy animals of both the breeds, the peripheral blood mononuclear cells (PBMCs) were purified from the blood collected in heparin-coated vacutainers by density gradient centrifugation using Histopaque (Sigma-Aldrich). RNA from purified PBMCs was isolated by TRIzol method and complementary DNA was synthesized from equal amount of RNA by reverse transcription (Thermo Fisher Scientific), before carrying out SYBR Green based real-time PCR assay (Roche Life Sciences). Real-time PCR amplification was performed in duplicate for each sample of the target and housekeeping genes in LightCycler® 480 Real-Time PCR System (Roche Life Sciences). Both forward and reverse primers for target gene (*BoLA*-DRB3) including house-keeping genes were designed using online primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) from conserved region of each locus. Melt curve based on Tm and standard curve were run to check the specificity and reaction efficiency, respectively. Melting curves were obtained following each sample amplification. The expression data was normalized using geometric means of Ct values of UXT and B2M house-keeping genes as control and for fold change in expression, relative quantification $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen (2001) was used. The Ct (cycle threshold) value based fold change in expression was calculated as shown below:

$$\Delta Ct = Ct\ (Target) - Ct\ (HKGs)$$
$$\Delta\Delta Ct = \Delta Ct\ (Test\ sample) - \Delta Ct\ (Calibrator\ sample)$$

One-way ANOVA was used to check the significance of expression variation across mastitis and non-mastitis animals within Sahiwal and KF groups.

The standard curve and the melting peaks indicated specific amplification of the product as well as good efficiency of the reaction as demonstrated by slope value of around 3.3. No significant fold change (more than 2 fold) for DRB3 gene expression, in mastitis and healthy animals of the two populations observed, but higher fold change in expression of the gene in healthy animals approaching double values (Table 1) indicates its possible impact on differential resistance to mastitis. This should be further validated by conducting experiments on more number of animals. Results also point towards polymorphism at this locus probably playing more significant role rather than expression of DRB3 in differential immune response.

This study highlights the comparative differential expression analysis of DRB3 gene in indicus (Sahiwal) and crossbred (Karan Fries) cattle. No significant difference was

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Table 1. Fold change in expression of *BoLA*-DRB3 gene after normalizing with housekeeping UXT and B2M genes in mastitis compared to healthy non-mastitis animals

| Group | Average ΔCt | ΔΔCt | Fold change |
|---|---|---|---|
| Mastitis SW | 0.223 | –0.213 | 1.79 |
| Healthy SW | 0.009 | | |
| Mastitis KF | 1.050 | –0.114 | 1.89 |
| Healthy KF | 0.935 | | |

observed in the gene expression among mastitis affected and non-affected animals in the present work, indicating gene polymorphism probably playing more significant role rather DRB3 gene expression in differential immune response. It further needs to be confirmed on large phenotypic data related to mastitis as well as other infectious diseases, since this particular gene holds a potential for differential immune response to fight against infectious diseases.

## SUMMARY

Expression analysis of immune response genes in the mammary gland tissues of both healthy and infected animals help to explain the genetic basis of differential immune response. This study was conducted to analyze the differential expression of MHC class II DRB3 gene, in mastitis-affected and healthy animals of indicus (Sahiwal) and crossbred (Karan Fries) cattle. Real-time PCR was performed on the cDNA synthesized from the RNA isolated from PBMCs of the mastitis and healthy animals. However, no significant difference was observed in the expression of DRB3 in the two populations. The results point towards polymorphism supposed to be playing major role in differential immune response, rather than the expression of DRB3 gene as far as state of udder health is concerned.

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