“Genetic polymorphism and expression profiling of candidate genes associated with tick resistance in Indian native cattle”

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SUMMARY

Ticks are the most harmful ecto-parasites of cattle in tropical and sub-tropical areas and are responsible for severe economic losses worldwide. They transmit a wide variety of infectious agents, more than any other group of arthropods, including bacterial, protozoan, rickettsia, and viral diseases. Tick control is largely based on the use of chemical agents like acaricides. However, its uses increase production costs and result in the accumulation of chemical residues in milk, meat hides, and surroundings. In India, the most prevalent tick is Rhipicephalus (Boophilus) microplus, which causes significant financial losses in the dairy industry by damaging hides, reducing milk quality and quantity, and transmitting pathogens that cause babesiosis and anaplasmosis. The yearly cost of controlling ticks and tick-borne diseases in India has been evaluated at 498.7 million US dollars. To maintain the unique gene pool of native cattle breeds in India, it is essential to characterize their important and unique traits, such as their superior tick resistance quality compared to European counterparts.

A cross-sectional study was conducted from March 2017 to December 2018 in Lucknow, India, to investigate the prevalence of tick infestation in cattle of all breeds, ages, and sexes. Ticks were collected, labeled, preserved, and identified through microscopic examination. The study covered three seasons and suburban areas surrounding Lucknow district, which is situated in the middle Indo-Gangetic Plain and has a humid subtropical climate. Study was conducted in a tropical environment to investigate the impact of tick infestation on physiological and molecular factors in dairy cows. Epidemiological study was conducted in 2017 and 2018 on tick

infestation in different breeds of cattle in the Lucknow region of Uttar Pradesh, India, and it involved observing the prevalence of tick infestation in indigenous cattle from an organized farm, exotic crossbred cattle from both organized and unorganized farms, and non-descriptive cattle from an unorganized farm, during different seasons. Prevalence of tick infestation was recorded according to sex, age, and breed, and the data was analyzed using the Chi-square test.

Physiological parameters such as rectal temperature, respiration rate, and pulse rate were collected from a random sample of Sahiwal and crossbred cows, along with blood samples for genomic DNA and RNA extraction. Blood samples were collected and processed to assess antioxidant parameters. Serum Ferric reducing/antioxidant power (FRAP), DPPH, and Glutathione (GSH) levels were estimated. FRAP and DPPH assays were carried out using standard reagents, while GSH estimation was done using DTNB and Na2HPO4. Results were calculated using standard curves and reported in units of mg/dL.

Isolation of peripheral blood mononuclear cells (PBMCs) from whole blood was done using the Histopaque-1077 reagent and density gradient centrifugation, followed by RNA isolation from PBMCs using the TRIzol method, and subsequent evaluation of RNA quality/integrity. Finally, the preparation of cDNA from the isolated RNA using the Revert AidTM First Strand cDNA synthesis kit was done for further qPCR gene expression analysis. For qPCR, cDNA samples were amplified using a SYBR Green/ROX qPCR master mix and specific primers for target genes and reference genes. Amplification conditions were 40 cycles at 95°C for 15 seconds, 60°C for 1 minute, and a dissociation protocol was employed to detect potential primer dimers. For genomic DNA isolation, blood samples were subjected to lysis of red and white blood cells using RBC lysis buffer. DNA was extracted using phenol:

chloroform extraction and then precipitated using sodium acetate and ethanol. The DNA pellet was then reconstituted in Tris-EDTA buffer, and the concentration was determined using a spectrophotometer. PCR products were electrophoresed on agarose gels with ethidium bromide, and genotypes were scored by analyzing restriction patterns. Statistical analysis was performed.

The study found that crossbred cattle are more susceptible to tick infestation than indigenous cattle, with a higher prevalence rate of tick infection in humid seasons. The prevalence rate of tick infestation was also found to be higher in productive animals maintained in unorganized farms as compared to organized farms. Three different tick species were identified across all seasons in both breeds of cattle. The resistance of cattle to tick infestation is affected by various factors, including morphological, physiological, and behavioral characteristics.

The study concludes that Sahiwal cattle (Bos indicus) were more resistant to tick infestation than Bos taurus dairy cattle. The findings of the study suggest that better dairy practices and medical care can reduce the tick infestation in productive animals. The study also recommends the need for further research to identify the different tick species infesting cattle in the region and the factors that contribute to their infestation.

The study compared physiological parameters (rectal temperature, pulse rate, and respiration rate) in tick-infested and non-infested Sahiwal and crossbred cattle during different seasons. The results showed that tick infestation had a greater impact on crossbred cattle than Sahiwal cows. The highest values of physiological parameters were observed in tick-infested cattle during the hot and humid season, while the lowest values were recorded in winter. The study highlights the importance of considering tick infestation in assessing the physiological responses of cattle and suggests further research to investigate the association of tick prevalence and physiological parameters.

During summer, antioxidant parameters were evaluated in Sahiwal and crossbred cows. DPPH scavenging activity and FRAP were higher in crossbred cows due to higher tick burden, while serum GSH levels were higher in Sahiwal cows, indicating lower oxidative stress.

PCR-RFLP, used to analyze the genetic variation in two different genes in cattle breeds. Technique is used to genotype the TLR2 gene using five different restriction enzymes. The genotyping results showed that there were some differences in the restriction patterns between Sahiwal (indigenous) and crossbred cattle. However, overall, the genotyping results using PCR-RFLP were more or less similar in both cattle breeds.

PCR-RFLP used to genotype the BoLA-DRB3.2 gene, which is a part of the major histocompatibility complex (MHC) in cattle. The genotyping results using PCR-RFLP showed that there were certain alleles that were exclusively present in Sahiwal or crossbred cattle. The genotyping results also showed that the RFLP patterns were quite distinct in Sahiwal and crossbred cattle, with specific patterns being restricted to either of the two cattle types.

The results are significant as they provide insights into the genetic variation in these two genes in different cattle breeds. The differences in the genetic variation in these genes could possibly be the reason for the differential resistance ability of Sahiwal cattle to ticks and other diseases as compared to crossbred cattle. The PCR-RFLP technique used in these studies is a useful tool for genotyping and identifying the genetic variation in different genes in cattle breeds.

A study on the TLR2 gene across different mammalian species found high similarity in major dairy species, and least similarity in pig gene sequence. Polymorphic sites were identified in the TLR2 exon II in Indian native and exotic cattle, with one non-synonymous SNP at position 119 predicted to affect protein function. The low frequencies of the SNPs could be due to small sample size, but could be validated and used for genotype-phenotype association studies for tick resistance in cattle. TLR2 has previously been associated with mastitis resistance in dairy animals and infection with Borrelia afzelii in wild bank voles.

The study analyzed the expression of heat shock proteins (HSPs) in tick-infested and non-infested cows using real-time qPCR. The expression of HSF1 and HSP90 did not differ significantly in tick-infested and non-infested Sahiwal cows, but it was significantly higher in tick-infested crossbred cows. HSP70 and HSP60 had significantly higher expression in tick-infested Sahiwal cows compared to non-infested individuals, but their expression did not differ significantly in tick-infested and non-infested crossbred cows. These findings suggest a differential response to tick infestation in different breeds of cows. Gene expression of heat shock proteins, TLR2, and ATP1A1 were measured in tick-infested and non-infested Sahiwal and crossbred cows. While ATP1A1 expression did not differ significantly between tick-infested and non-infested cows, TLR2 expression was significantly higher in tick-infested crossbred cows, indicating an inflammatory response to tick infestation. The lower induction of TLR in Sahiwal cows suggests a non-significant role of TLR-driven inflammatory response in tick resistance in native cows. These findings highlight the potential differences in tick resistance mechanisms between different breeds of cows.