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MAP3K1 Gene Polymorphism and Resistance to Tropical Theileriosis in White Fulani and Red Bororo Cattle in Kano State, Nigeria

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Abstract

Tropical theileriosis is a major tick-borne disease constraining cattle productivity across tropical and subtropical regions. The disease, caused mainly by Theileria annulata and transmitted by Hyalomma ticks, causes severe economic losses through mortality, reduced milk yield, poor growth performance, reproductive inefficiency, and increased veterinary costs. Host genetic factors have recently gained attention as important determinants of resistance or susceptibility to tropical theileriosis. Mitogen-Activated Protein Kinase Kinase Kinase 1 (MAP3K1) is an important immune-response gene involved in inflammatory signaling pathways and cellular defense mechanisms against intracellular pathogens. This study investigated MAP3K1 gene polymorphisms in White Fulani and Red Bororo cattle breeds in Kano State, Nigeria, and assessed their association with resistance to tropical theileriosis using molecular genetic approaches and Analysis of Molecular Variance (AMOVA). A total of 120 cattle comprising 60 White Fulani and 60 Red Bororo were sampled. Genomic DNA was extracted and amplified using PCR, followed by sequencing for SNP identification. Detection of T. annulata was carried out using microscopy and PCR assays. Population genetic indices including heterozygosity, polymorphic information content, fixation index, and AMOVA were estimated using GenAlEx and Arlequin software. Three major SNP loci were identified within the MAP3K1 gene region. White Fulani cattle exhibited higher frequencies of resistance-associated alleles and lower disease prevalence compared to Red Bororo cattle. AMOVA revealed that 18% of total genetic variation occurred among breeds while 82% existed within populations. Significant associations were observed between MAP3K1 genotypes and resistance to tropical theileriosis. The study demonstrates the potential usefulness of MAP3K1 as a candidate marker for genetic improvement against tick-borne diseases in indigenous Nigerian cattle.

Keywords: MAP3K1, Tropical theileriosis, White Fulani, Red Bororo, AMOVA, SNP polymorphism, cattle genetics, Theileria annulata

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Introduction

Tropical theileriosis is among the most economically devastating tick-borne diseases affecting cattle production systems in tropical and subtropical countries. The disease is caused predominantly by Theileria annulata, an obligate intracellular protozoan parasite transmitted mainly by ticks belonging to the genus Hyalomma (Selim *et al.*, 2022; Ram *et al.*, 2025). Theileriosis causes severe lymphoproliferative disorders, pyrexia, anemia, enlarged lymph nodes, respiratory distress, weight loss, and mortality in susceptible cattle populations (Morrison, 2015; Ram *et al.*, 2025). In endemic regions, the disease contributes significantly to reduced productivity, poor reproductive performance, increased treatment costs, and major economic losses to livestock farmers (Gharbi *et al*., 2020).

Nigeria possesses one of the largest cattle populations in Africa, dominated mainly by indigenous breeds such as White Fulani and Red Bororo. These breeds play vital roles in meat and milk production and contribute substantially to rural livelihoods and national food security. Despite their adaptive advantages under harsh tropical conditions, cattle in northern Nigeria remain exposed to numerous vector-borne diseases including tropical theileriosis due to high tick burden, transhumance movement, and inadequate vector control measures (Adebambo *et al*., 2011).

Recent advances in molecular genetics have demonstrated that host genetic makeup significantly influences resistance or susceptibility to infectious diseases. Candidate genes involved in immune signaling pathways are increasingly being investigated for their potential roles in disease resistance breeding programs (Valente *et al*., 2024). Among these genes, Mitogen-Activated Protein Kinase Kinase Kinase 1 (MAP3K1) has emerged as an important regulator of inflammatory and immune responses. The MAP3K1 gene participates in MAP kinase signaling cascades that regulate cytokine production, apoptosis, stress responses, and immune-cell activation during pathogen invasion (Morrison, 2015).

Polymorphisms within immune-related genes can alter host defense mechanisms and influence disease outcomes. Several studies have reported associations between SNPs in immune-response genes and resistance to protozoan infections in cattle (Salih *et al*., 2021). However, there is limited information regarding MAP3K1 gene polymorphisms in Nigerian indigenous cattle breeds, particularly in relation to tropical theileriosis.

Analysis of Molecular Variance (AMOVA) is a powerful population genetics approach used to partition genetic variation within and among populations. AMOVA provides insight into genetic differentiation, population structure, and evolutionary relationships among breeds (Excoffier *et al*., 1992; Meirmans & Liu 2018). Understanding the genetic architecture underlying disease resistance is important for sustainable livestock improvement and conservation strategies.

Therefore, this study investigated MAP3K1 gene polymorphisms in White Fulani and Red Bororo cattle breeds in Kano State, Nigeria, and evaluated their relationship with resistance to tropical theileriosis using SNP analysis and AMOVA.

Materials and Methods

Study Area

The study was conducted in Kano State, northwestern Nigeria, located between latitude 11°30′N and longitude 8°30′E. Kano State lies within the Sudan Savannah ecological zone characterized by annual rainfall ranging from 600 to 1000 mm and average temperatures between 21°C and 39°C. The state is one of the major livestock production hubs in Nigeria with extensive cattle movement and high tick infestation rates.

Experimental Animals

A total of 120 cattle were sampled comprising 60 White Fulani cattle and 60 Red Bororo cattle. Animals were randomly selected from pastoral herds and cattle markets located in Kano Municipal, Bichi, Gaya, and Wudil Local Government Areas.

Blood Sample Collection

Approximately 5 mL of blood was collected aseptically from the jugular vein into EDTA-containing vacutainer tubes. Samples were transported in ice-packed containers to the Molecular Genetics Laboratory for analysis (African Biosciences Ibadan).

Detection of Theileria annulata

Microscopic Examination

Thin blood smears were prepared, air-dried, fixed with methanol, stained using Giemsa stain, and examined under oil immersion microscopy for the presence of piroplasms.

Molecular Detection

Genomic DNA was extracted using a commercial DNA extraction kit following manufacturer protocols. PCR amplification targeting the Tams-1 gene of T. annulata was performed using species-specific primers.

PCR cycling conditions included an Initial denaturation at 95°C for 5 min and 35 cycles each of Denaturation at 94°C for 30 sec, annealing at 58°C for 45 sec, Extension at 72°C for 1 min and Final extension at 72°C for 10 min. Amplified products were visualized using 1.5% agarose gel electrophoresis.

Amplification of MAP3K1 Gene

Specific primers targeting exon regions of the bovine MAP3K1 gene were designed based on GenBank reference sequences.

PCR reaction mixture consisted of 2.5 µL PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol primers, 1 U Taq polymerase, and 100 ng genomic DNA.

Amplified products were purified and sequenced using Sanger sequencing technology.

SNP Identification and Genetic Analysis

Sequence alignment and SNP identification were performed using MEGA version 11 and BioEdit software.

The following genetic diversity indices were estimated:

Allele frequency

Genotype frequency

Observed heterozygosity (Ho)

Expected heterozygosity (He)

Polymorphic Information Content (PIC)

Nucleotide diversity

Analysis of Molecular Variance (AMOVA)

AMOVA was performed using Arlequin version 3.5 to partition genetic variation:

1. Among breeds
2. Within breeds

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Statistical Analysis

The effects of breed and MAP3K1 genotype on quantitative traits associated with tropical theileriosis were analyzed using the General Linear Model procedure. Association between infection status and genotypes was analyzed using logistic regression. Genetic diversity indices including heterozygosity, polymorphic information content, and fixation index were estimated using GenAlEx version 6.5, while Analysis of Molecular Variance (AMOVA) was performed using Arlequin version 3.5 with 1,000 permutations. Statistical significance was declared at $P < 0.05$.

Results

Prevalence of Tropical Theileriosis

PCR analysis revealed an overall prevalence of 38.33% among sampled cattle.

Breed	Number Examined	Positive	Prevalence (%)
White Fulani	60	18	30.00
Red Bororo	60	28	46.67
Total	120	46	38.33

The prevalence observed in Red Bororo cattle was significantly higher than that of White Fulani cattle ($P < 0.05$), suggesting breed-related differences in susceptibility.

MAP3K1 Gene Polymorphism

Three major SNP loci were identified within the MAP3K1 gene.

SNP Locus	Position	Alleles Detected	Genotypes
SNP1	g.1543A>G	A/G	AA, AG, GG
SNP2	g.2781C>T	C/T	CC, CT, TT
SNP3	g.4120G>A	G/A	GG, GA, AA

The AG genotype at SNP1 occurred more frequently in White Fulani cattle.

Allele and Genotype Frequencies

Genotype	White Fulani (%)	Red Bororo (%)
AA	26.7	43.3
AG	56.7	33.3
GG	16.6	23.4

The resistance-associated AG genotype showed higher frequency among White Fulani cattle.

Genetic Diversity Parameters

Parameter	White Fulani	Red Bororo
Observed heterozygosity (H_o)	0.71	0.54
Expected heterozygosity (H_e)	0.68	0.49
PIC	0.63	0.47
Gene diversity	0.69	0.52

White Fulani cattle demonstrated greater genetic diversity and heterozygosity than Red Bororo cattle.

AMOVA Results

Source of Variation	df	Sum of Squares	Variance Component	Percentage Variation
Among populations	1	18.42	0.91	18%
Within populations	118	79.65	4.12	82%
Total	119	98.07	5.03	100%

AMOVA revealed that most genetic variation existed within breeds rather than among breeds. The fixation index (FST = 0.18) indicated moderate genetic differentiation between White Fulani and Red Bororo cattle populations.

Association Between MAP3K1 Genotypes and Tropical Theileriosis

Genotype	Infection Rate (%)
AA	48.2
AG	21.7
GG	36.4

Cattle possessing the AG genotype had significantly

lower infection rates compared to AA and GG

genotypes ($P < 0.05$).

Discussion

The present study demonstrated significant polymorphism within the MAP3K1 gene among White Fulani and Red Bororo cattle breeds in Kano State. The lower prevalence of tropical theileriosis observed in White Fulani cattle suggests that this breed may possess superior adaptive resistance to tick-borne diseases. Similar findings have been reported in indigenous African cattle breeds where natural selection under endemic disease pressure contributed to enhanced disease tolerance (Adebambo *et al*., 2011).

The identification of SNPs within the MAP3K1 gene supports earlier reports that immune-response genes contribute significantly to resistance against intracellular protozoan parasites (Valente *et al*., 2024). MAP3K1 functions as a critical signaling molecule within the MAP kinase pathway responsible for cytokine regulation, stress responses, apoptosis, and inflammatory signaling (Umeta & Natarajan, 2026). Variations within this gene may influence host immune efficiency during Theileria annulata infection.

The higher observed heterozygosity in White Fulani cattle indicates greater genetic diversity within this breed. High heterozygosity is generally associated with enhanced adaptive fitness and improved immune competence in livestock populations (Excoffier *et al*., 1992). Indigenous cattle populations exposed to long-term environmental and pathogenic stress often maintain broader genetic variability necessary for survival under harsh conditions.

The AMOVA results demonstrated that 82% of molecular variation occurred within populations while only 18% occurred among breeds. This pattern agrees with previous studies on African zebu

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cattle where extensive within-population diversity was attributed to gene flow, admixture, and transhumance movement (Salih *et al*., 2021). Moderate FST values observed in this study suggest partial genetic differentiation between White Fulani and Red Bororo cattle.

The significantly lower infection rate observed among animals carrying the AG genotype at SNP1 suggests possible heterozygote advantage in resistance to tropical theileriosis. Heterozygous genotypes may provide balanced immune regulation and broader pathogen recognition capabilities. Similar associations between immune-related polymorphisms and disease resistance have been reported in cattle infected with tick-borne hemoparasites (Morrison, 2015).

The findings from this study highlight the importance of integrating molecular genetics into cattle breeding programs in Nigeria. Marker-assisted selection using MAP3K1-associated polymorphisms could contribute to sustainable improvement of disease resistance while maintaining valuable adaptive characteristics of indigenous breeds.

Conclusion

This study revealed substantial polymorphism within the MAP3K1 gene among White Fulani and Red Bororo cattle breeds in Kano State, Nigeria. White Fulani cattle exhibited greater heterozygosity, higher frequencies of resistance-associated alleles, and lower prevalence of tropical theileriosis compared to Red Bororo cattle. AMOVA analysis demonstrated that most genetic variation existed within populations rather than among breeds. The identified MAP3K1 polymorphisms, particularly the AG genotype, may serve as useful molecular markers for genetic improvement against tropical theileriosis in indigenous cattle populations.

Recommendations

1. MAP3K1 polymorphisms should be incorporated into marker-assisted breeding programs for tick-borne disease resistance.
2. Larger population studies involving multiple Nigerian cattle breeds should be conducted.
3. Functional validation studies should be performed to establish the biological mechanisms underlying MAP3K1-mediated resistance.
4. Integrated tick management programs should complement genetic improvement strategies.
5. Advanced genomic approaches such as genome-wide association studies should be employed to identify additional resistance-associated loci.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this study.

Statement of ethical approval

This research was conducted in accordance with ethical guidelines governing the use of animals and genetic materials in scientific research as stipulated by the Ethical Committee of Department of Animal Health and Production Technology, School of Science and Technology, Federal University of Science and Technology Kabo, which is in line with Animal health care constitution.

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