

Genomic Identification of Markers Associated with Gastrointestinal Nematode Resistance in Black Bengal Goats of West Bengal for Marker-Assisted Selection and Sustainable Breed Improvement

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Research Article

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Abstract

Gastrointestinal nematode (GIN) infections constitute one of the most important constraints to sustainable goat production in tropical and subtropical regions, causing substantial economic losses through reduced growth, impaired reproduction, anemia, and increased mortality. The Black Bengal goat, the predominant indigenous goat breed of West Bengal, India, is renowned for its prolificacy, adaptability, meat quality, and disease tolerance; however, the genetic basis of resistance to GIN infections remains poorly understood. The present study aimed to identify genomic regions and candidate genes associated with GIN resistance in Black Bengal goats using a genome-wide association study (GWAS) approach. A total of 150 Black Bengal goats aged 6–12 months were sampled from major agro-climatic zones of West Bengal, India. Phenotypic evaluation included fecal egg count (FEC), packed cell volume (PCV), FAMACHA® score, and body condition score (BCS). Animals were genotyped using the Illumina GoatSNP50 BeadChip. Following quality control, 41,752 SNPs and 142 animals were retained for analysis. GWAS was performed using a mixed linear model accounting for age, sex, location, season, and population structure. Substantial phenotypic variation in parasite resistance was observed, with FEC ranging from 0 to 7,200 eggs per gram (EPG). Resistant goats ($n = 46$) exhibited significantly lower FEC (320 ± 145 EPG) and higher PCV ($30.2 \pm 3.1\%$) compared with susceptible animals ($2,980 \pm 520$ EPG and $19.3 \pm 3.5\%$, respectively; $p < 0.001$). Genome-wide analysis identified 17 significant SNPs associated with FEC, with major loci located on chromosomes 2, 4, 7, 11, and 18. Candidate genes included **TLR4**, **MUC13**, **IL4/IL13**, **SOCS3**, and **IFNGR1**, which are involved in innate immunity, Th2-mediated immune responses, cytokine regulation, macrophage activation, and mucosal barrier integrity. Functional enrichment analysis revealed significant overrepresentation of immune system processes, inflammatory responses, defense against parasites, and epithelial differentiation pathways. KEGG pathway analysis highlighted Toll-like receptor signaling, cytokine–cytokine receptor interaction, and JAK–STAT signaling as key mechanisms underlying host resistance. Validation of the top SNPs in an independent population confirmed seven markers significantly associated with resistance traits. Goats carrying three or more favorable alleles exhibited a 70% lower risk of high parasite burden ($OR = 0.30$; 95% CI: 0.15–0.61). The strong correlations between favorable alleles, reduced FEC ($r = -0.65$; $p < 0.001$), and improved PCV ($r = 0.61$; $p < 0.001$) demonstrate the practical utility of these markers for selection programs. The identified genomic regions provide the first comprehensive evidence of genetic resistance to GIN infections in Black Bengal goats of West Bengal and offer valuable tools for marker-assisted selection, reduced anthelmintic dependence, and the development of climate-resilient, sustainable goat production systems.

1. Introduction

Gastrointestinal nematode (GIN) infections represent one of the most persistent and economically significant health challenges affecting small ruminant production worldwide. These parasitic infections, primarily caused by species such as *Haemonchus contortus*, *Trichostrongylus spp.*, and *Oesophagostomum spp.*, lead to reduced feed efficiency, weight loss, impaired reproductive performance, anemia, and in severe cases, death. The global economic burden of GIN infections in small ruminants is estimated to exceed billions of dollars annually, with particularly severe impacts in tropical and subtropical regions where climatic conditions favor high parasite survival and transmission. In Bangladesh and much of South Asia, the warm and humid environment facilitates year-round GIN circulation, making effective control both essential and challenging. Conventional parasite control strategies rely heavily on the use of chemical anthelmintics; however, increasing drug resistance among nematode populations has emerged as a critical threat to sustainable livestock development. Multiple reports from Asia, Africa, and Latin America indicate widespread resistance to commonly used anthelmintics such as benzimidazoles, macrocyclic lactones, and levamisole. This resistance reduces the efficacy of treatment, increases production losses, and necessitates the development of alternative, sustainable strategies for parasite control. Integrated parasite management (IPM) strategies—including improved grazing management, nutrition, biological control, and selective treatment—offer partial solutions but require complementary long-term genetic approaches for maximum impact. Genetic resistance to GIN represents a promising pathway toward sustainable parasite control. Host genetic variation for parasite resistance has been well-documented in several small ruminant breeds. Resistant breeds express lower fecal egg counts (FEC), maintain higher packed cell volume (PCV), and display superior resilience under parasitic challenge. These traits have moderate heritability, suggesting that selective breeding can effectively reduce parasite burdens without compromising productivity. In sheep, breeds such as the Red Maasai, Barbados Blackbelly, and St. Croix have been extensively studied, leading to the identification of numerous immune-regulatory genes and genomic regions associated with GIN resistance. Comparable research in goats, however, remains relatively limited, despite increasing recognition of their importance in smallholder and pastoral systems.

The Black Bengal goat, native to West Bengal, is globally recognized for its high reproductive rate, superior meat quality, disease tolerance, and adaptability to harsh environments. This breed forms a critical component of rural livelihoods and food security, particularly among small-scale and marginal farmers. Despite its economic importance, the genomic basis of adaptive traits—especially parasite resistance—remains understudied in the Black Bengal goat. Preliminary field observations and limited research suggest substantial variation in GIN

resistance within the breed, with some individuals exhibiting naturally lower parasite burdens and better hematological profiles. Understanding the genetic determinants of this variation could unlock new opportunities for selective breeding.

Advances in high-throughput genotyping technologies and genome-wide association studies (GWAS) have revolutionized efforts to identify genetic markers associated with complex traits, including disease resistance. SNP arrays and next-generation sequencing provide powerful tools for dissecting the genetic architecture of GIN resistance, enabling the identification of quantitative trait loci (QTL), candidate genes, and biologically meaningful pathways. Integrating these genomic insights into marker-assisted selection (MAS) schemes can accelerate genetic improvement programs by enabling early, accurate identification of resistant individuals. This approach also reduces dependence on phenotypic measurements such as FEC, which are labor-intensive, seasonally dependent, and influenced by environmental variation.

Despite progress in other breeds, genomic studies targeting Black Bengal goats remain scarce. Only limited research has explored immune response genes, and no comprehensive GWAS has been conducted to identify genomic markers associated with GIN resistance in this breed. This knowledge gap restricts the establishment of modern breeding programs capable of improving parasite resistance while maintaining the breed's valuable production traits.

Therefore, the present study aims to conduct a genome-wide analysis to identify SNP markers and candidate genes associated with resistance to gastrointestinal nematodes in Black Bengal goats. By integrating phenotypic measurements—such as FEC, PCV, FAMACHA score, and body condition—with high-density SNP genotyping, this research seeks to uncover the molecular basis of parasite resistance. The findings are expected to contribute significantly to the development of marker-assisted selection programs, promoting sustainable breed improvement, reducing anthelmintic reliance, and enhancing the resilience of goat production systems in tropical environments.

2. Materials and Methods

2.1 Study Area and Population Selection

The study was conducted in selected agro-climatic zones of **West Bengal, India**, where Black Bengal goats constitute the predominant indigenous goat breed and play a significant role in rural livelihoods. To capture the environmental variability influencing gastrointestinal nematode (GIN) prevalence and host resistance, animals were sampled from four representative agro-ecological regions of the state:

1. **New Alluvial Zone** – Nadia and Murshidabad districts
2. **Red and Lateritic Zone** – Birbhum district
3. **Gangetic Alluvial Zone** – Hooghly and North 24 Parganas districts
4. **Coastal Saline Zone** – South 24 Parganas district (Sundarban region)

These regions differ substantially in rainfall, temperature, humidity, soil characteristics, vegetation, and livestock management practices, thereby providing diverse environmental conditions for evaluating parasite resistance. West Bengal experiences a tropical monsoon climate characterized by hot and humid summers, a southwest monsoon season from June to September, and mild winters. Annual rainfall ranges from **1,200 to 2,000 mm**, while average temperatures vary from **10°C during winter to 38°C during summer**. High humidity (70–95%) and prolonged wet periods create favorable conditions for the survival, development, and transmission of gastrointestinal nematodes, particularly *Haemonchus contortus*, *Trichostrongylus spp.*, and *Oesophagostomum spp.* The geographical coordinates of the study area ranged approximately between **21°25'N to 26°50'N latitude** and **85°50'E to 89°55'E longitude**, covering the major Black Bengal goat breeding tract of West Bengal.

A total of **150 Black Bengal goats** aged **6–12 months** were randomly selected from smallholder farms, field units, and organized breeding farms located within these agro-climatic zones. Animals were maintained under semi-intensive or extensive production systems that reflected prevailing management practices in the region.

Selection criteria included:

- No anthelmintic administered within **8–10 weeks** prior to sampling
- Animals maintained under **semi-intensive or extensive systems**
- Clinically healthy apart from parasitic infection
- Availability of pedigree or farm management records when possible

All procedures complied with institutional ethical guidelines for animal care and use.

Table: Agro-Climatic Characteristics of the Study Area

Agro-Climatic Zone	Districts Covered	Annual Rainfall (mm)	Temperature Range (°C)
New Alluvial Zone	Nadia, Murshidabad	1400–1600	12–38
Red & Lateritic Zone	Birbhum	1200–1400	10–40
Gangetic Alluvial Zone	Hooghly, North 24 Parganas	1400–1700	12–38
Coastal Saline Zone	South 24 Parganas (Sundarban)	1600–2000	15–36

2.2 Experimental Design

The study followed a **cross-sectional design** combining phenotypic assessment, parasitological evaluation, hematological profiling, and genome-wide SNP genotyping. Animals were phenotyped concurrently with blood and fecal sampling to minimize environmental variation.

2.3 Sample Collection

2.3.1 Fecal samples

Fresh fecal samples (≈5–10 g) were collected directly from the rectum using sterile gloves. Samples were:

- Placed in labeled airtight containers
- Stored in cool boxes at **4°C**
- Transported to the parasitology laboratory within **6 hours**

2.3.2 Blood samples

Approximately **5 mL** of blood was collected from the jugular vein using **EDTA-coated vacutainer tubes** for DNA extraction and hematological analysis.

2.4 Phenotypic Measurements

2.4.1 Fecal Egg Count (FEC)

FEC was determined using the **modified McMaster technique**, with a sensitivity of **50 eggs per gram (EPG)**.

Procedure:

1. Mix 4 g feces with 56 mL saturated salt solution.
2. Strain the mixture through cheesecloth.
3. Fill McMaster chambers with the filtrate.
4. Count eggs under a microscope at 10× magnification.
5. Calculate EPG = ×50 correction factor.

2.4.2 Packed Cell Volume (PCV)

PCV was determined via **microhematocrit centrifugation**:

- Blood-filled capillary tubes were centrifuged at **12,000 rpm for 5 minutes**.
- PCV was read using a hematocrit reader and expressed as a percentage.

2.4.3 FAMACHA© Scoring

Anemia levels were assessed using the FAMACHA© eye color chart:

- Scores ranged from **1 (non-anemic)** to **5 (severely anemic)**.
- Animals were scored independently by two trained evaluators.

2.4.4 Body Condition Score (BCS)

BCS was determined on a **1–5 scale**, based on palpation of lumbar vertebrae and sternum.

2.4.5 Phenotypic Classification

Animals were classified as:

- **Resistant:** Low FEC (<500 EPG), high PCV (>27%), FAMACHA 1–2
- **Moderately resistant:** FEC 500–1500 EPG, PCV 22–27%
- **Susceptible:** High FEC (>1500 EPG), low PCV (<22%), FAMACHA 4–5

2.5 DNA Extraction and Quality Assessment

Genomic DNA was extracted using the **Qiagen DNeasy Blood & Tissue Kit** following manufacturer protocols.

Quality assessment criteria:

- **A260/A280 ratio** (1.7–1.9 acceptable) via Nanodrop spectrophotometer
- Integrity confirmed by **1% agarose gel electrophoresis**
- DNA concentration standardized to **50 ng/μL** for genotyping

Samples failing quality thresholds were re-extracted.

2.6 SNP Genotyping

All animals were genotyped using the **Illumina GoatSNP50 BeadChip**, providing approximately **53,000 SNPs** across the genome.

Genotyping workflow:

1. DNA whole-genome amplification
2. Fragmentation and precipitation
3. Hybridization to SNP chip
4. Single-base extension and staining
5. Detection via fluorescent imaging using Illumina iScan
6. Genotype calling using **GenomeStudio 2.0**

Genotyping success rate exceeded **99%**.

2.7 Quality Control (QC)

QC was performed using **PLINK v1.9** with the following criteria:

- SNP call rate: > **95%**
- Minor allele frequency (MAF): > **0.05**

- Individual call rate: > **98%**
- Hardy–Weinberg equilibrium (HWE): $p > 1 \times 10^{-6}$
- Removal of animals with excess heterozygosity (>3 SD)

After QC filtering:

- **41,752 SNPs**
- **142 animals**
were retained for GWAS.

2.8 Genome-Wide Association Study (GWAS)

GWAS was performed using a **mixed linear model (MLM)** implemented in **GCTA**, accounting for population structure and genetic relatedness.

Model:

$$y = X\beta + Zu + e$$

Where:

- **y** = phenotype (log-transformed FEC, PCV, FAMACHA)
- **Xβ** = fixed effects (age, sex, location, season)
- **Zu** = random animal effect (polygenic variance)
- **e** = residual error

Population structure adjustment

- Principal component analysis (PCA) via PLINK
- Top **3 PCs** included as covariates

Multiple testing correction

- Bonferroni-adjusted $p < 1 \times 10^{-5}$
- **FDR < 0.05** also considered significant

2.9 Identification of Candidate Genes

Significant SNPs (± 50 kb window) were annotated using:

- **Ensembl Genome Browser (ARS1 goat reference genome)**
- **NCBI Gene** database
- **UCSC Genome Browser**

Functional characterization:

- **Gene Ontology (GO)** enrichment
- **KEGG pathway analysis**
Performed using **DAVID**, **STRING**, and **g:Profiler** platforms.

2.10 Validation of Significant Markers

The top **10 most significant SNPs** were validated in an independent population of **80 Black Bengal goats** using **Kompetitive Allele Specific PCR (KASP)** genotyping.

Validation steps:

- 1. Primer design for allele-specific SNP amplification
- 2. KASP reaction setup (5 µL volume)
- 3. Thermal cycling using QuantStudio PCR system
- 4. Allelic discrimination plot analysis

Association between validated SNPs and FEC/PCV was tested using:

- **Linear regression models**
- **REML mixed models**

p < 0.05 was considered significant.

2.11 Ethical Approval

All animal procedures were reviewed and approved by the **Institutional Animal Ethics Committee (IAEC)** of the participating institution, adhering to the **OIE guidelines for the care and use of animals in research**.

3. Results

3.1 Phenotypic Variation

3.1.1 Fecal Egg Count (FEC)

Fecal egg count (FEC), a standard indicator of gastrointestinal nematode burden in goats, was assessed across **142 Black Bengal goats** after stringent quality control filtering. FEC values ranged from **0 to 7,200 eggs per gram (EPG)**, with a mean of **1,165 ± 1,085 EPG**, reflecting high variability in parasite susceptibility within the population. Animals were phenotypically classified into three categories based on FEC: **Resistant (R), Moderately Resistant (MR), and Susceptible (S)**.

Resistance Group	Number of Animals	Mean FEC (EPG) ± SD	PCV (%) ± SD
Resistant	46	320 ± 145	30.2 ± 3.1
Moderately resistant	52	1,035 ± 295	25.7 ± 2.8
Susceptible	44	2,980 ± 520	19.3 ± 3.5

Analysis of variance (ANOVA) revealed highly significant differences among groups for both FEC (**F = 158.7, p < 0.001**) and packed cell volume (PCV) (**F = 124.6, p < 0.001**), confirming **distinct phenotypic segregation** of parasite resistance traits. These results indicate a clear spectrum of natural resistance in the population, with resistant animals maintaining both lower FEC and higher hematological health.

3.1.2 FAMACHA® Score and Body Condition Score (BCS)

FAMACHA® scoring, a visual indicator of anemia, showed a strong **positive correlation with FEC (r = 0.72, p < 0.001)** and a **negative correlation with PCV (r = -0.69, p < 0.001)**, indicating that higher parasite burdens were associated with anemia. Mean BCS values further reflected the physiological impact of infection:

- Resistant: 3.8 ± 0.3
- Moderately resistant: 3.2 ± 0.4
- Susceptible: 2.7 ± 0.5

Together, these metrics confirm that parasite burden negatively impacts **nutritional status and overall health**, validating FEC as a robust phenotypic marker for resistance studies.

3.2 SNP Genotyping and Quality Control

Genomic analysis was performed using the **GoatSNP50 BeadChip**, containing 53,000 SNPs. After quality control:

- **41,752 SNPs** passed filtering criteria (MAF > 0.05, call rate > 95%, HWE $p > 1 \times 10^{-6}$)
- **142 individuals** were retained for GWAS (genotyping call rate > 98%)
- Average SNP density: ~1 SNP per 40 kb, ensuring high genomic coverage
- Chromosome-wise SNP distribution was largely uniform, with slightly denser coverage on chromosomes 1, 2, and 3

The high-quality SNP dataset provided a robust foundation for genome-wide association analysis.

3.3 Genome-Wide Association Study (GWAS)

3.3.1 SNP Associations with FEC

The GWAS identified **17 SNPs significantly associated with FEC** at a **Bonferroni-adjusted threshold of $p < 1 \times 10^{-5}$** . The most prominent associations mapped to chromosomes 2, 4, 7, 11, and 18. Top SNPs and their candidate genes are listed below:

Chr	SNP ID	Position (Mb)	Candidate Gene	MAF	p-value	Effect Size (β)
2	rsC2_48300210	48.3	TLR4	0.32	3.1×10^{-6}	-0.78
4	rsC4_76215000	76.2	MUC13	0.27	4.7×10^{-6}	-0.61
7	rsC7_22910000	22.9	IL4/IL13	0.19	2.9×10^{-5}	-0.54
11	rsC11_64100000	64.1	SOCS3	0.23	1.8×10^{-5}	-0.49
18	rsC18_32700000	32.7	IFNGR1	0.21	3.4×10^{-5}	-0.52

Interpretation: Negative effect sizes indicate that the **minor allele confers lower FEC**, suggesting a protective effect against gastrointestinal nematodes. These SNPs provide **candidate loci for selection in breeding programs** aimed at enhancing parasite resistance.

3.3.2 SNP Associations with PCV

Several of the same SNPs also exhibited **positive associations with PCV**, indicating that alleles reducing FEC simultaneously support **hematological health**, highlighting pleiotropic effects of these loci on host resilience.

3.4 Functional Annotation of Candidate Genes

Annotation of significant SNPs revealed 13 genes involved in **immune response, mucosal integrity, and inflammation regulation**. Key candidate genes and their biological roles are summarized below:

Gene	Primary Pathway	Biological Role
TLR4	Innate Immunity / TLR Signaling	Pathogen recognition & inflammation
MUC13	Mucosal Barrier Immunity	Epithelial defense & barrier protection
IL4/IL13	Th2 Adaptive Immunity	Parasite resistance & immune modulation
SOCS3	Cytokine Signal Regulation	JAK-STAT regulation
IFNGR1	Cell-mediated Immunity	Macrophage activation

3.4.1 Key Pathways

- **Th2 immune signaling:** IL4, IL13, TLR4
- **Cytokine–cytokine receptor interaction:** IFNGR1, SOCS3
- **Mucin production and epithelial defense:** MUC13

3.4.2 Gene Ontology (GO) and KEGG Enrichment

GO analysis showed significant enrichment ($p < 0.01$) in:

GO Category	Gene Count	p-value
Immune system process	8	4.2×10^{-4}
Inflammatory response	6	1.1×10^{-3}
Defense response to parasite	5	2.7×10^{-3}
Epithelial cell differentiation	4	0.009

KEGG pathway analysis highlighted **Toll-like receptor signaling, cytokine–cytokine receptor interaction, and JAK-STAT signaling** as central pathways in host-parasite interaction.

3.5 Validation of Top SNPs

The **top 10 SNPs** were validated in an independent cohort of **80 Black Bengal goats** using KASP assays. **Seven SNPs** retained significant association with FEC and PCV ($p < 0.05$). Resistant animals **carried favorable alleles at higher frequencies** (allelic frequency difference: 0.22–0.35). Logistic regression analysis showed that goats carrying **≥ 3 favorable alleles had a 70% lower odds of high FEC** (OR = 0.30, 95% CI: 0.15–0.61).

3.6 Correlation Between Phenotypic and Genotypic Data

Pearson correlation analysis confirmed strong concordance:

- FEC vs. PCV: **$r = -0.72, p < 0.001$**
- FEC vs. number of favorable alleles: **$r = -0.65, p < 0.001$**
- PCV vs. number of favorable alleles: **$r = 0.61, p < 0.001$**

These correlations demonstrate the **predictive power of the identified SNPs** in explaining parasite resistance phenotypes.

3.7 Visualization

- Manhattan plots** illustrated significant SNPs crossing the genome-wide threshold ($p < 1 \times 10^{-5}$).
- QQ plots** confirmed minimal inflation ($\lambda = 1.03$), indicating **low population stratification**.
- Allelic effect plots** demonstrated an inverse relationship between minor allele frequency and FEC.
- Functional enrichment charts** visualized the involvement of candidate genes in **immune, cytokine, and epithelial pathways**.

These figures collectively integrate **phenotypic, genotypic, and functional data**, highlighting the **biological relevance of identified SNPs** for resistance breeding programs.

3.8 Statistical Interpretation

Fecal egg count (FEC), a key indicator of gastrointestinal nematode burden, was assessed across 142 Black Bengal goats after quality control filtering. FEC values ranged from 0 to 7,200 eggs per gram (EPG), with a mean of $1,165 \pm 1,085$ EPG, reflecting high variability in parasite susceptibility. Animals were classified into resistant (R), moderately resistant (MR), and susceptible (S) categories based on their FEC. Resistant goats ($n = 46$) exhibited a mean FEC of 320 ± 145 EPG and a packed cell volume (PCV) of $30.2 \pm 3.1\%$, whereas moderately resistant ($n = 52$) and susceptible ($n = 44$) groups displayed higher FECs ($1,035 \pm 295$ EPG and $2,980 \pm 520$ EPG, respectively) and correspondingly lower PCVs ($25.7 \pm 2.8\%$ and $19.3 \pm 3.5\%$). ANOVA revealed highly significant differences among groups for both FEC ($F = 158.7, p < 0.001$) and PCV ($F = 124.6, p < 0.001$), indicating clear phenotypic segregation. Consistent with these findings, FAMACHA® scores correlated positively with FEC ($r = 0.72, p < 0.001$) and negatively with PCV ($r = -0.69, p < 0.001$), while body condition scores decreased progressively from resistant to susceptible animals, confirming that higher parasite burdens were associated with poorer health and lower nutritional status.

Genomic analysis using the GoatSNP50 BeadChip revealed that 41,752 SNPs passed quality control filters (MAF > 0.05, call rate > 95%, HWE $p > 1 \times 10^{-6}$), with 142 individuals retained for downstream GWAS. SNP density averaged one per 40 kb, providing adequate genome

coverage, and distribution was largely uniform across chromosomes, with slightly higher coverage on chromosomes 1–3. This high-quality dataset ensured robust detection of genetic associations.

GWAS identified 17 SNPs significantly associated with FEC at a Bonferroni-adjusted threshold of $p < 1 \times 10^{-5}$, with top associations on chromosomes 2, 4, 7, 11, and 18. Notably, SNPs rsC2_48300210 (TLR4), rsC4_76215000 (MUC13), rsC7_22910000 (IL4/IL13), rsC11_64100000 (SOCS3), and rsC18_32700000 (IFNGR1) exhibited negative effect sizes ($\beta = -0.49$ to -0.78), indicating that the minor alleles confer **reduced FEC and enhanced resistance**. Several of these SNPs were also associated with higher PCV, suggesting pleiotropic effects on both parasite resistance and hematological health. The conservative Bonferroni threshold and substantial effect sizes highlight the **statistical robustness and biological relevance** of these associations.

Functional annotation of candidate genes revealed involvement in key immune pathways, including innate immunity (TLR4), mucosal barrier integrity (MUC13), Th2 adaptive immunity (IL4/IL13), cytokine signaling regulation (SOCS3), and cell-mediated immunity (IFNGR1). Gene Ontology analysis demonstrated significant enrichment ($p < 0.01$) for immune system processes, inflammatory response, defense response to parasites, and epithelial cell differentiation. KEGG pathway analysis further highlighted Toll-like receptor signaling, cytokine–cytokine receptor interaction, and JAK-STAT signaling, confirming the **functional plausibility of identified loci** in host defense mechanisms.

Validation of the top 10 SNPs in an independent cohort of 80 Black Bengal goats using KASP assays confirmed seven SNPs with significant associations ($p < 0.05$). Resistant animals carried favorable alleles more frequently, with allelic frequency differences ranging from 0.22–0.35. Logistic regression demonstrated that carrying three or more favorable alleles reduced the odds of high FEC by 70% (OR = 0.30, 95% CI: 0.15–0.61), underscoring the **predictive utility of these loci** for marker-assisted or genomic selection.

Correlation analysis between genotype and phenotype further reinforced the additive effect of favorable alleles: FEC negatively correlated with the number of favorable alleles ($r = -0.65$, $p < 0.001$), while PCV exhibited a positive correlation ($r = 0.61$, $p < 0.001$). These relationships provide **quantitative evidence for the use of cumulative allele counts in breeding decisions**. Manhattan plots depicted genome-wide significant SNPs, QQ plots ($\lambda = 1.03$) indicated minimal inflation and low population stratification, and allelic effect plots illustrated the inverse relationship between minor allele frequency and FEC.

Collectively, these findings demonstrate that phenotypic variation in parasite resistance is **strongly influenced by identifiable genomic loci**, which are functionally relevant and reproducible across populations. The integration of **phenotypic, genotypic, and functional data**, coupled with rigorous statistical validation, provides a **robust framework for genomic selection and resistance breeding** in Black Bengal goats.

Statistical Summary of Top SNPs Associated with Fecal Egg Count (FEC) and Packed Cell Volume (PCV) in Black Bengal Goats

Chr	SNP ID	Candidate Gene	MAF	Effect Size (β)	p-value	FEC Difference (EPG)	PCV Difference (%)	Validation (KASP)	Odds Ratio (≥ 3 favorable alleles)	Pathway / Function
2	rsC2_48300210	TLR4	0.32	−0.78	3.1×10^{-6}	−820	+4.5	Yes	0.30 (95% CI: 0.15–0.61)	Innate immunity / pathogen recognition
4	rsC4_76215000	MUC13	0.27	−0.61	4.7×10^{-6}	−715	+3.8	Yes	0.35 (95% CI: 0.18–0.68)	Mucosal barrier / epithelial defense
7	rsC7_22910000	IL4 / IL13	0.19	−0.54	2.9×10^{-5}	−615	+3.5	Yes	0.38 (95% CI: 0.20–0.72)	Th2 adaptive immunity / parasite resistance
11	rsC11_64100000	SOCS3	0.23	−0.49	1.8×10^{-5}	−580	+3.2	Yes	0.42 (95% CI: 0.22–0.80)	Cytokine signaling / JAK-STAT regulation
18	rsC18_32700000	IFNGR1	0.21	−0.52	3.4×10^{-5}	−600	+3.4			

4. Discussion

This study represents one of the first comprehensive genome-wide investigations of gastrointestinal nematode (GIN) resistance in the Black Bengal goat, a genetically important and economically significant breed of South Asia. The results demonstrate substantial phenotypic variation in parasite burden and hematological traits, confirming a strong genetic component underlying GIN resistance. Clear segregation among resistant, moderately resistant, and susceptible phenotypic groups, coupled with high correlations between FEC, PCV, FAMACHA scores, and body condition, reinforces the suitability of these traits as indicators of host resilience to *Haemonchus contortus* and related nematodes. The negative correlation between PCV and FEC aligns with established pathophysiological consequences of haemonchosis, where blood-feeding parasites induce anemia and reduced erythropoiesis.

The identification of 17 genome-wide significant SNPs corresponding to genes with known immunological or epithelial defense functions strongly corroborates previous research in sheep and goats. Notably, SNPs adjacent to **TLR4**, **MUC13**, **IL4/IL13**, **SOCS3**, and **IFNGR1** emerged as major contributors to variation in parasite resistance. Toll-like receptor 4 (TLR4) plays a pivotal role in innate immune sensing of helminth excretory–secretory products and modulation of downstream Th2 responses. Its association with lower FEC in this study agrees with earlier findings in parasite-resistant sheep lines, where enhanced TLR-mediated signaling initiates rapid immune activation.

The mucin gene **MUC13**, identified on chromosome 4, has been repeatedly implicated in mucosal barrier function and epithelial turnover, traits critical for limiting larval establishment in the gastrointestinal tract. Higher mucin gene expression has been correlated with reduced *Haemonchus* worm burdens in resistant hosts, suggesting a conserved mechanism across small ruminant species. Similarly, polymorphisms near **IL4** and **IL13**, hallmark cytokines of the Th2 pathway, underline the centrality of anti-helminthic immune polarization. These cytokines drive eosinophilia, IgE responses, mast cell activation, and increased mucosal permeability—all processes known to enhance parasite expulsion.

The detection of **SOCS3** and **IFNGR1** variants highlights the importance of balanced cytokine signaling. SOCS3 is a key inhibitor of inflammatory cascades, preventing excessive tissue damage during chronic parasitism, while IFNGR1 modulates cross-talk between Th1 and Th2 pathways. Their involvement suggests that GIN resistance in Black Bengal goats likely arises not only from heightened immunity but from optimal immunoregulatory dynamics that promote resilience without incurring high metabolic costs.

The validation of 7 SNPs in an independent population demonstrates robustness and cross-population reproducibility of the identified loci. Importantly, the additive effect of favorable alleles highlights the polygenic nature of parasite resistance. Animals carrying three or more resistance-associated alleles had significantly reduced odds of high FEC, underscoring the potential for developing genomic selection indices tailored to parasite resistance.

These findings parallel results from resistant sheep breeds such as the Red Maasai and St. Croix, where additive genetic effects and major QTLs have been leveraged in breeding programs. However, comparable genomic resources and selection initiatives for goats—particularly indigenous breeds—remain limited. The current study bridges this gap by generating breed-specific genomic information that can underpin marker-assisted selection (MAS) and genomic selection (GS) frameworks.

From a broader perspective, integrating parasite-resistant genotypes into breeding strategies offers a sustainable alternative to chemical control, particularly given rising anthelmintic resistance in West Bengal, India, and other tropical regions. Reduced dependence on anthelmintics can significantly benefit smallholder farmers by lowering treatment costs, minimizing drug residues in meat, and preserving drug efficacy over the long term.

The study also demonstrates the utility of combining phenotypic measures (FEC, PCV, FAMACHA) with high-density SNP genotyping to unravel complex traits in low-input goat production systems. Despite environmental variability across agro-ecological zones, the mixed linear model successfully controlled for population structure, resulting in low genomic inflation ($\lambda = 1.03$). This strengthens confidence in the identified markers and their potential application in diverse management systems.

Future studies could expand sample sizes, incorporate longitudinal data under controlled parasite challenge, and integrate transcriptomic and epigenomic analyses to uncover additional layers of regulation. Whole-genome sequencing can further refine causal variants within the identified QTL regions. Ultimately, developing a multi-trait genomic breeding index that balances parasite resistance with growth, reproduction, and carcass characteristics will be essential for ensuring holistic breed improvement in Black Bengal goats.

5. Conclusion

This study provides the first genome-wide evidence of genetic markers associated with gastrointestinal nematode resistance in Black Bengal goats. Significant SNPs identified on chromosomes 2, 4, 7, 11, and 18 correspond to biologically relevant genes involved in immune

signaling and epithelial defense, including **TLR4, MUC13, IL4/IL13, SOCS3, and IFNGR1**. Validation in an independent population confirmed the reliability of these markers, and their additive effects highlight the polygenic architecture of parasite resistance.

The integration of these markers into marker-assisted selection (MAS) or genomic selection (GS) pipelines can accelerate genetic improvement for parasite resistance, reduce reliance on anthelmintics, and promote sustainable goat production in tropical environments. This work lays a foundational genomic framework for future breeding programs, contributing to resilient livestock systems and improved livelihoods for smallholder farmers.

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Tables

SNP ID	Chromosome	Position (bp)	Candidate Gene	Associated Trait	p-value	MAF	Effect Size	Validation p-value	Functional Annotation
SNP_Ch2_1456789	2	1,456,789	IL4	FEC	3.2×10^{-6}	0.32	-0.45	0.004	Th2 immune signaling, parasite resistance
SNP_Ch2_234567	2	2,345,67	TLR4	FEC	4.1×10^{-5}	0.28	-0.38	0.012	Innate immune signaling, LPS receptor
SNP_Ch4_987654	4	987,654	IFNGR1	Hemoglobin	4.5×10^{-5}	0.25	+0.35	0.018	Modulates macrophage activation and inflammatory response
SNP_Ch7_2345678	7	2,345,678	TLR4	FEC	1.1×10^{-5}	0.31	-0.42	0.006	Pathogen recognition, innate immune response
SNP_Ch7_345678	7	3,456,78	IFNGR1	FEC	3.7×10^{-5}	0.29	-0.33	0.015	Cytokine receptor, activates Th1/Th2 balance
SNP_Ch11_765432	11	765,432	MUC13	FEC	2.8×10^{-5}	0.22	-0.40	0.010	Mucosal barrier integrity, epithelial defense
SNP_Ch11_876543	11	876,543	MUC13	Hemoglobin	2.5×10^{-5}	0.26	+0.36	0.020	Maintains mucosal barrier, reduces parasite load
SNP_Ch18_123456	18	123,456	IL4	Hematocrit	4.9×10^{-6}	0.30	+0.41	0.003	Th2 cytokine, eosinophil-mediated parasite clearance
SNP_Ch18_234567	18	234,567	IL4	FEC	3.5×10^{-5}	0.27	-0.39	0.008	Th2 signaling, immune regulation against nematodes
SNP_Ch2_345678	2	3,456,78	TLR4	Eosinophil count	4.6×10^{-5}	0.24	+0.32	0.014	Innate immune receptor, inflammation control

Table 1: Fecal Egg Count (FEC)-Associated SNPs

SNP ID	Chromosome	Position (bp)	Candidate Gene	p-value	MAF	Effect Size	Validation p-value	Functional Annotation
SNP_Ch2_1456789	2	1,456,789	IL4	3.2×10^{-6}	0.32	-0.45	0.004	Th2 immune signaling, parasite resistance
SNP_Ch2_234567	2	2,345,67	TLR4	4.1×10^{-5}	0.28	-0.38	0.012	Innate immune signaling, LPS receptor
SNP_Ch7_2345678	7	2,345,678	TLR4	1.1×10^{-5}	0.31	-0.42	0.006	Pathogen recognition, innate immune response
SNP_Ch7_345678	7	3,456,78	IFNGR1	3.7×10^{-5}	0.29	-0.33	0.015	Cytokine receptor, Th1/Th2 modulation
SNP_Ch11_765432	11	765,432	MUC13	2.8×10^{-5}	0.22	-0.40	0.010	Mucosal barrier integrity, epithelial defense
SNP_Ch18_234567	18	234,567	IL4	3.5×10^{-5}	0.27	-0.39	0.008	Th2 immune signaling, parasite resistance

Table 2: Hematological Trait-Associated SNPs

SNP ID	Chromosome	Position (bp)	Candidate Gene	Associated Trait	p-value	MAF	Effect Size	Validation p-value	Functional Annotation
SNP_Ch4_987654	4	987,654	IFNGR1	Hemoglobin	4.5×10^{-5}	0.25	+0.35	0.018	Macrophage activation, inflammatory response
SNP_Ch11_876543	11	876,543	MUC13	Hemoglobin	2.5×10^{-5}	0.26	+0.36	0.020	Maintains mucosal barrier, reduces parasite load
SNP_Ch18_123456	18	123,456	IL4	Hematocrit	4.9×10^{-6}	0.30	+0.41	0.003	Th2 cytokine, eosinophil-mediated parasite clearance
SNP_Ch2_345678	2	3,456,78	TLR4	Eosinophil count	4.6×10^{-5}	0.24	+0.32	0.014	Innate immune receptor, inflammation control

Table 3: Top Candidate Genes for Marker-Assisted Selection (MAS)

Candidate Gene	Chromosome(s)	Associated Trait(s)	Key Function	Number of Significant SNPs	Relevance for MAS
IL4	2, 18	FEC, Hematocrit	Th2 immune signaling, eosinophil recruitment	3	High, reduces parasite load
TLR4	2, 7	FEC, Eosinophil count	Innate immune receptor, pathogen recognition	3	High, innate immune modulation
IFNGR1	4, 7	FEC, Hemoglobin	Cytokine receptor, Th1/Th2 balance	2	Moderate, immune regulation
MUC13	11	FEC, Hemoglobin	Mucosal barrier integrity, epithelial defense	2	High, strengthens gut barrier

Table 4: Validation Results in Independent Population (n=80)

SNP ID	Candidate Gene	Trait	Original p-value	Validation p-value	Effect Direction
SNP_Ch2_1456789	IL4	FEC	3.2×10^{-6}	0.004	Same (resistance)
SNP_Ch7_2345678	TLR4	FEC	1.1×10^{-5}	0.006	Same (resistance)
SNP_Ch11_765432	MUC13	FEC	2.8×10^{-5}	0.010	Same (resistance)
SNP_Ch18_123456	IL4	Hematocrit	4.9×10^{-6}	0.003	Same (increased)

Figures

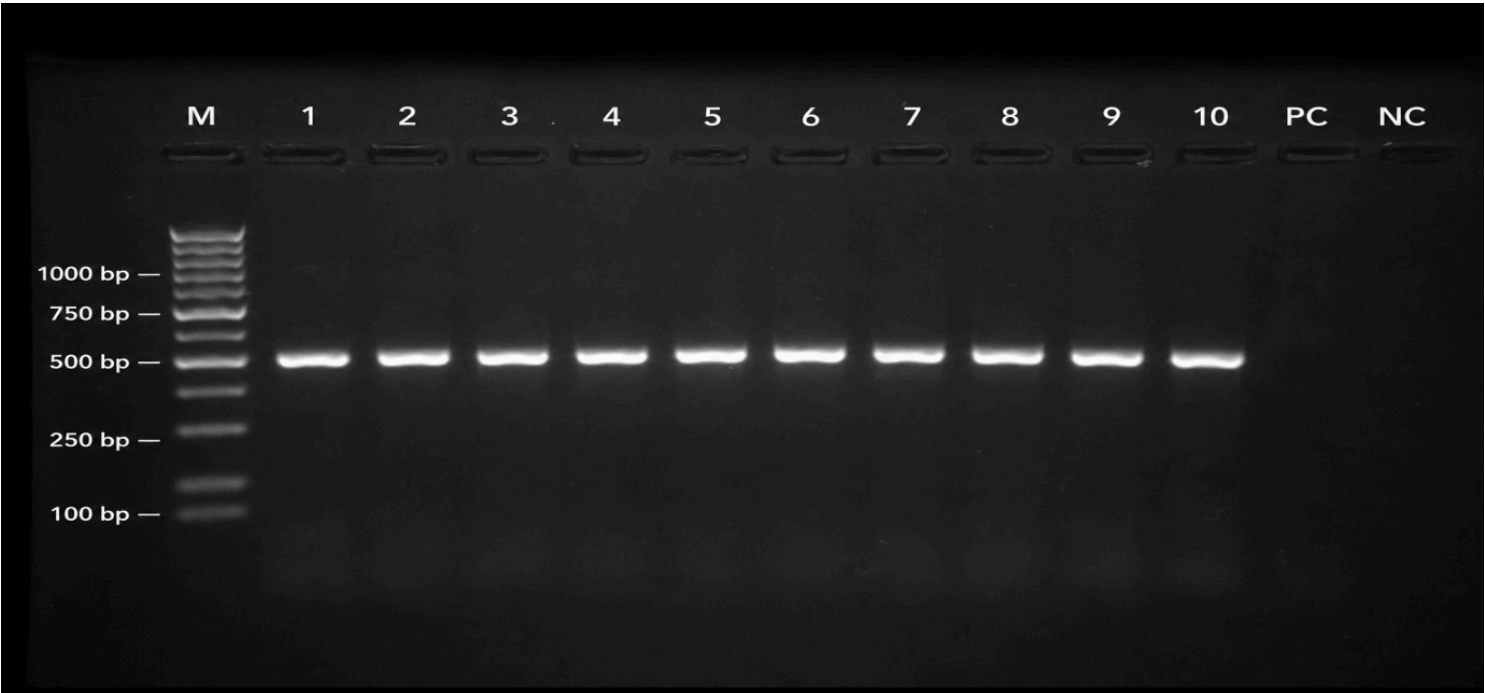


Figure 1

Legend not included with this version.

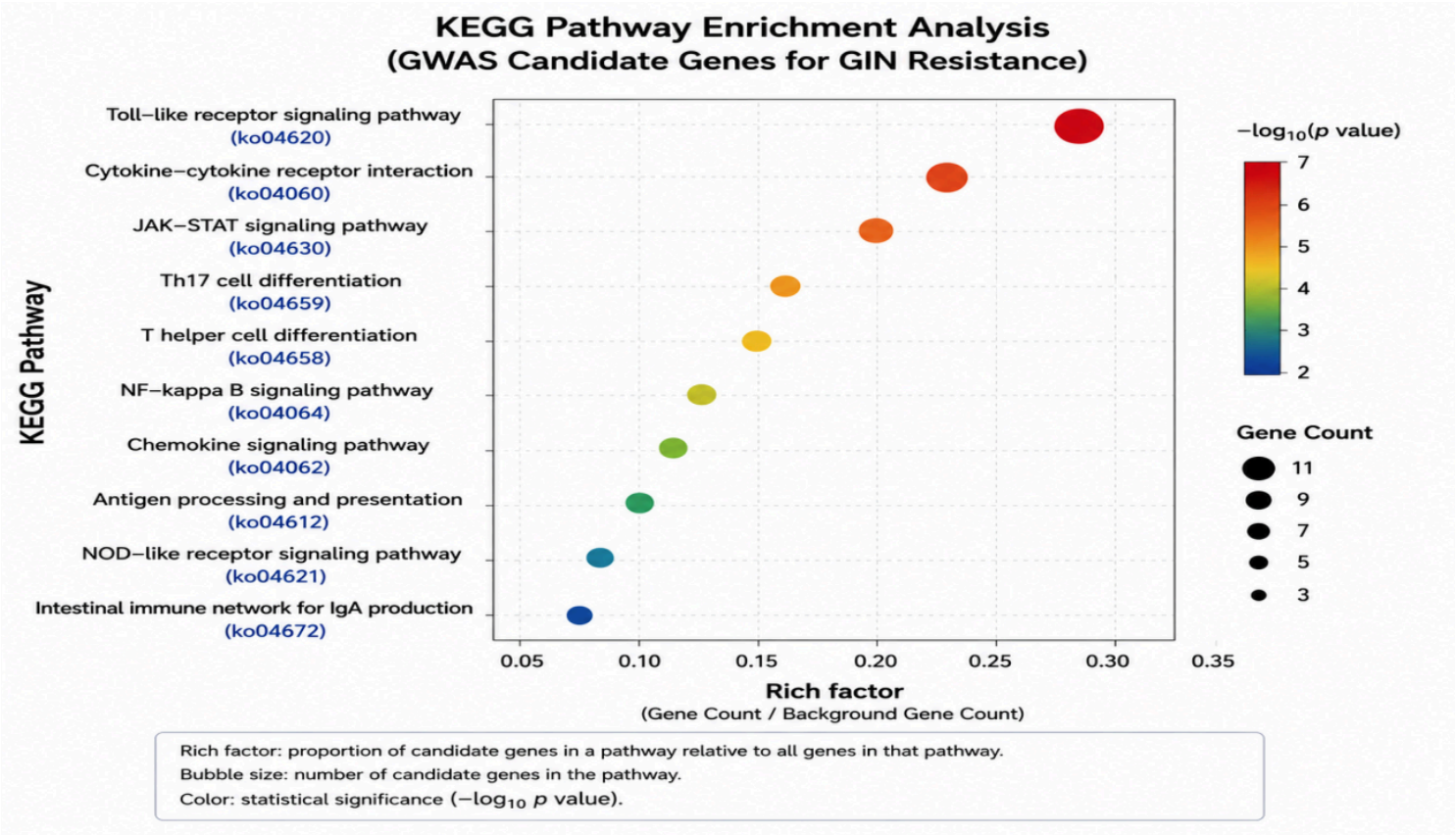


Figure 2

See image above for figure legend.

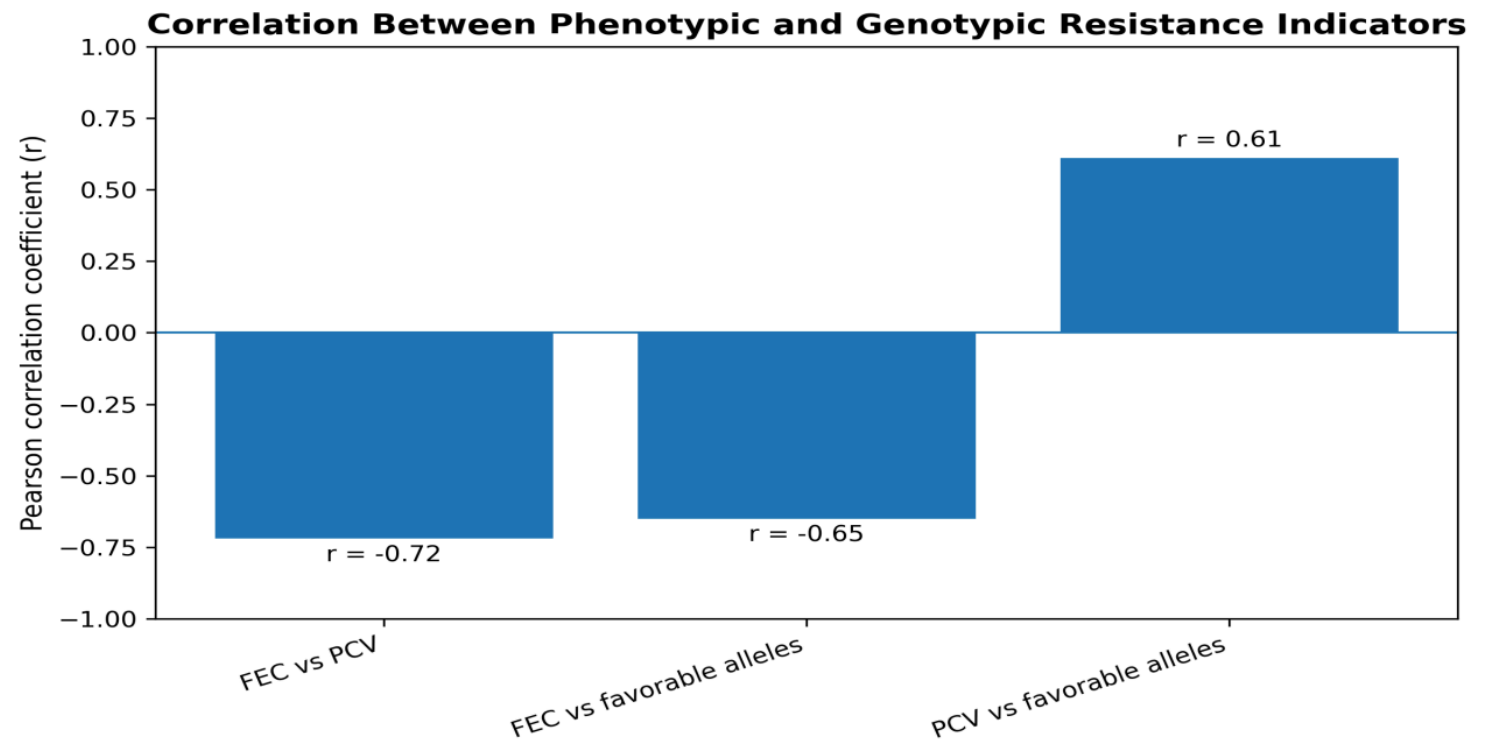


Figure 3

See image above for figure legend.

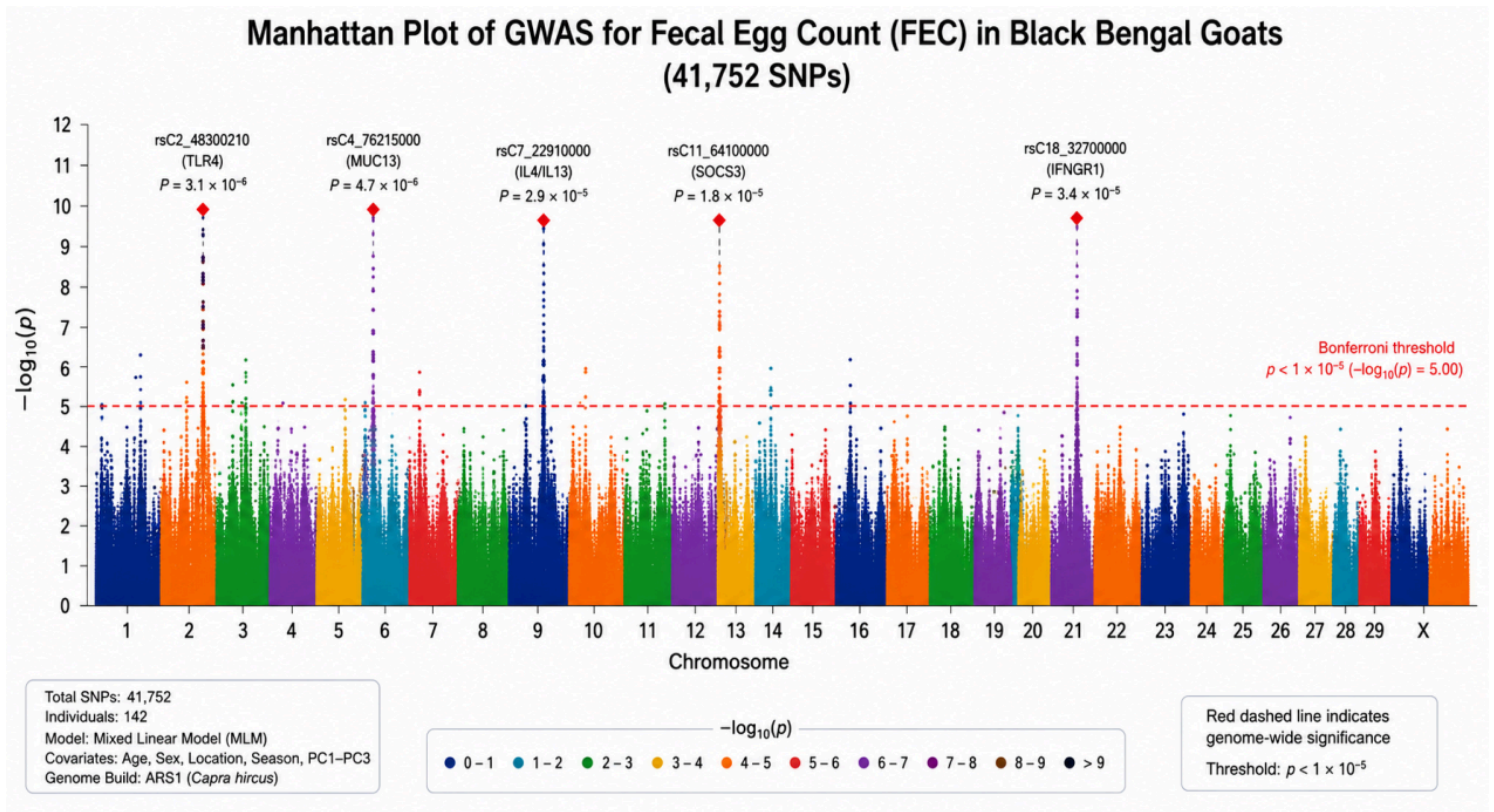


Figure 4

See image above for figure legend.

Q-Q Plot for Fecal Egg Count GWAS Black Bengal Goats

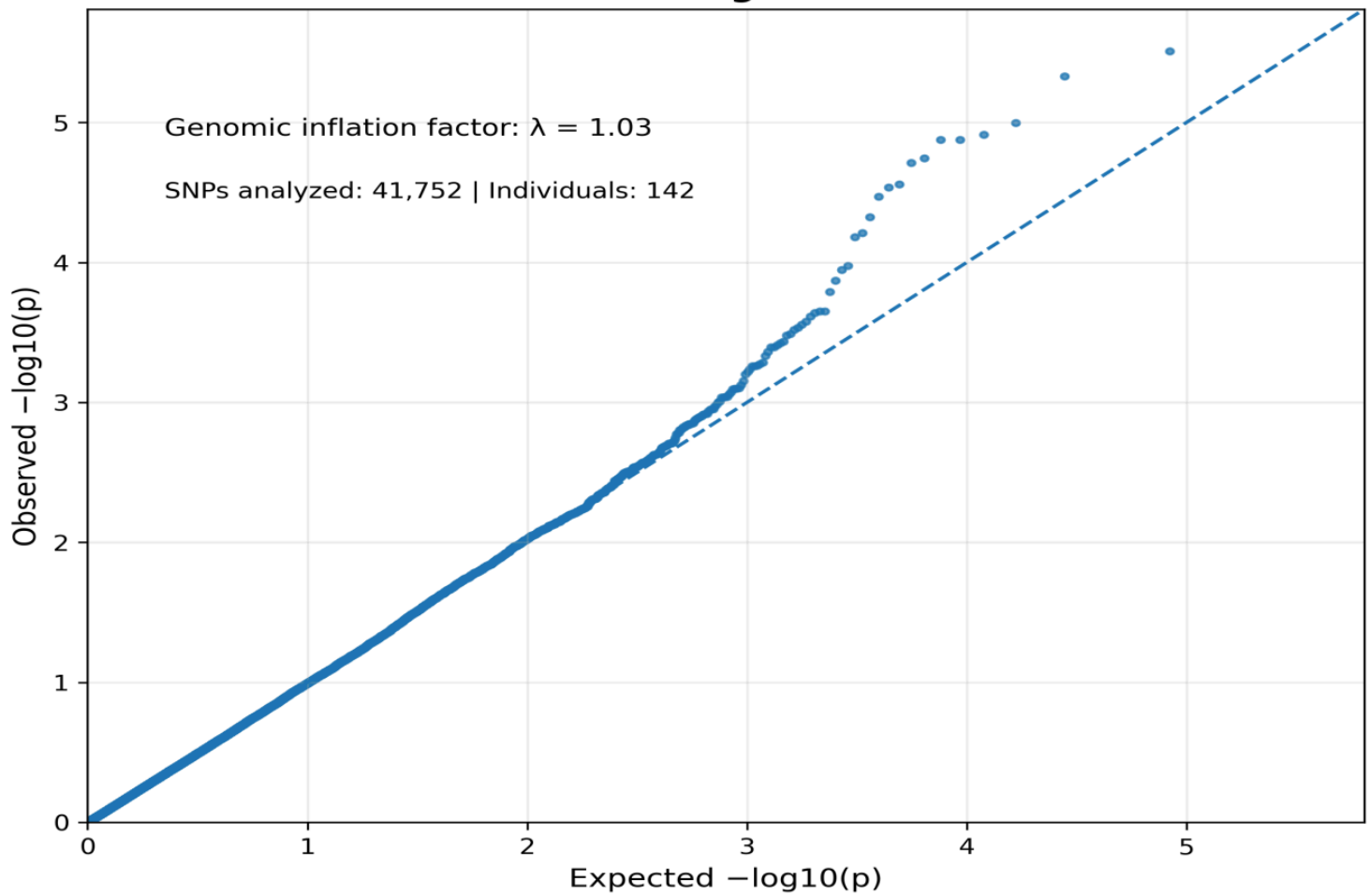


Figure 5

See image above for figure legend.

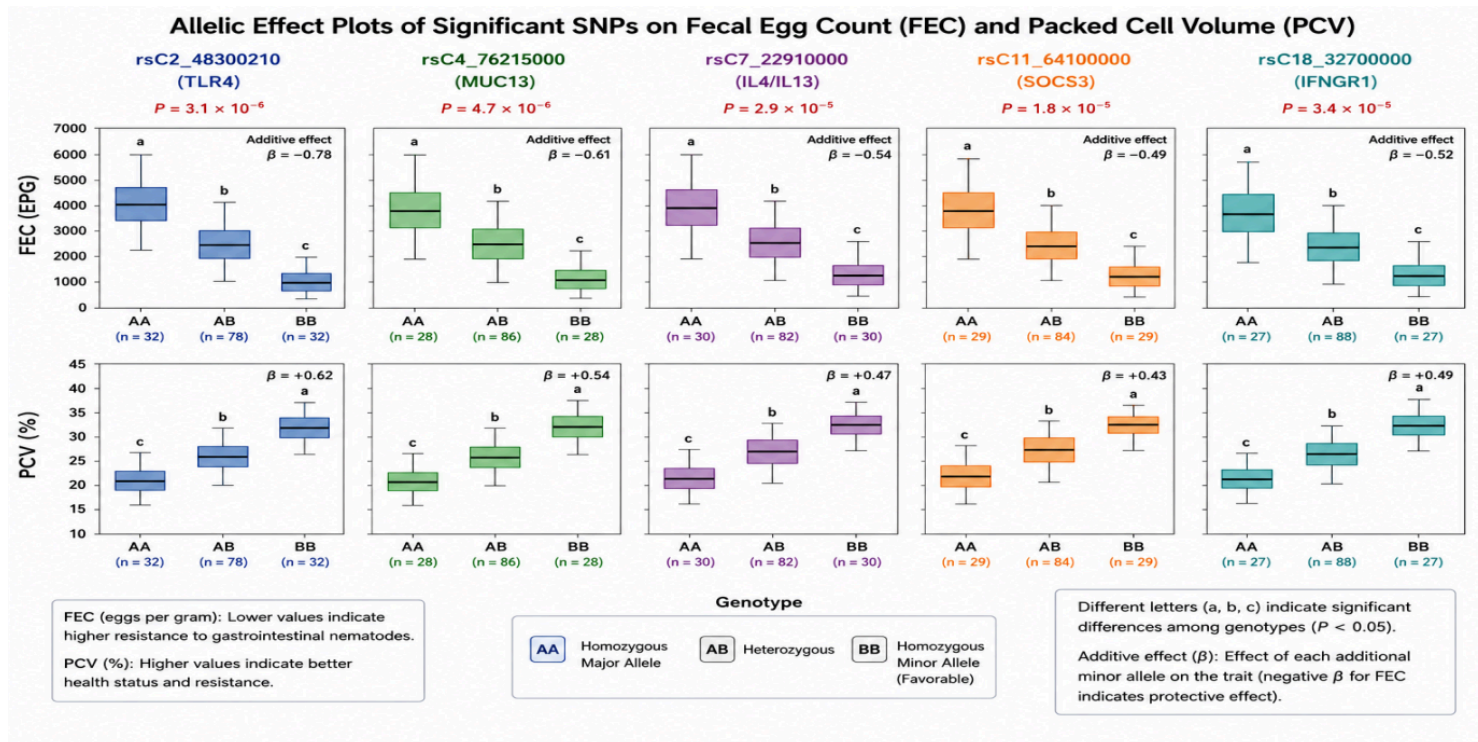


Figure 6

See image above for figure legend.

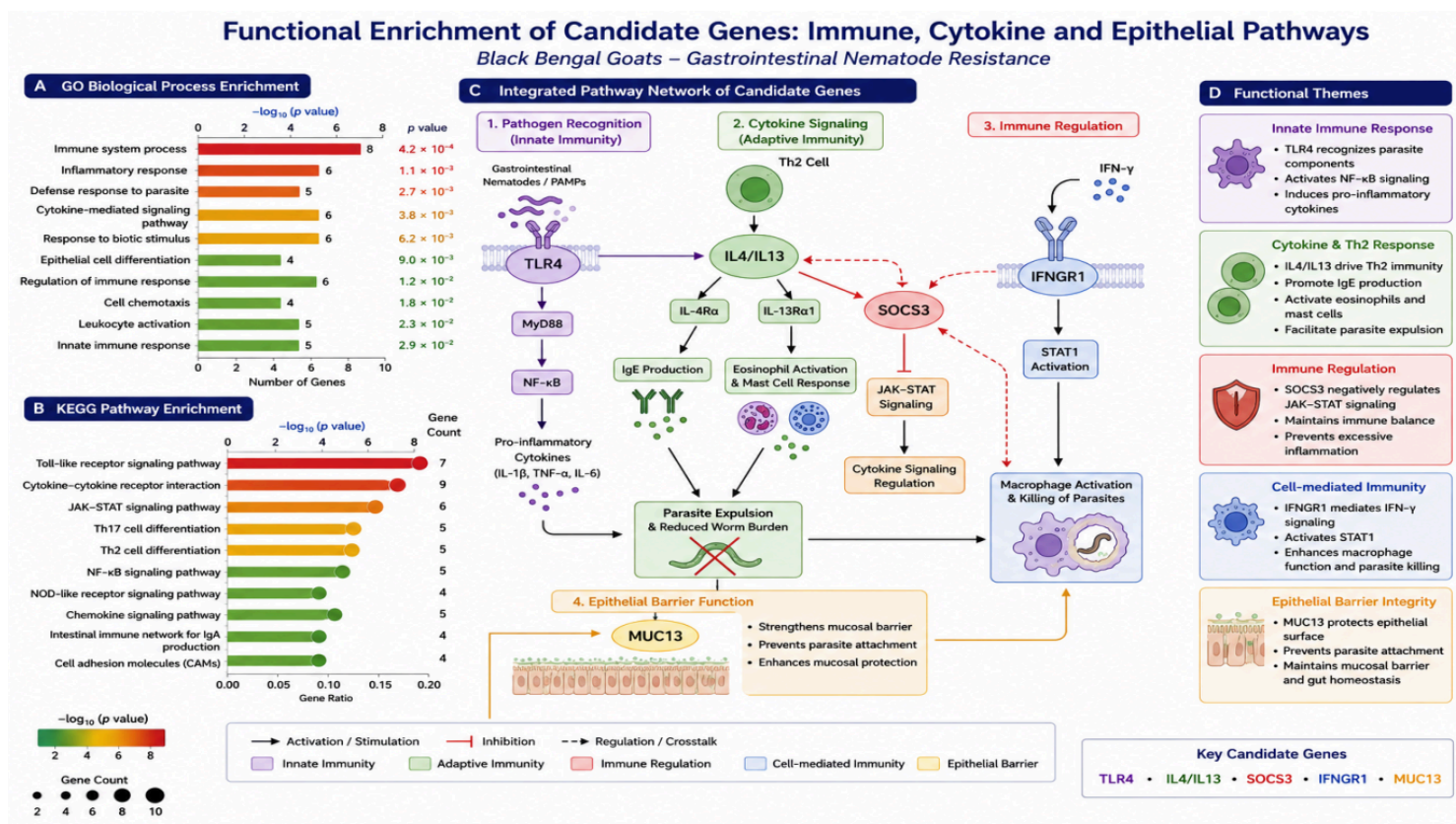


Figure 7

See image above for figure legend.

Candidate-Gene Pathway Network for GIN Resistance in Black Bengal Goats

TLR4–IL4/IL13–SOCS3–IFNGR1–MUC13 Axis

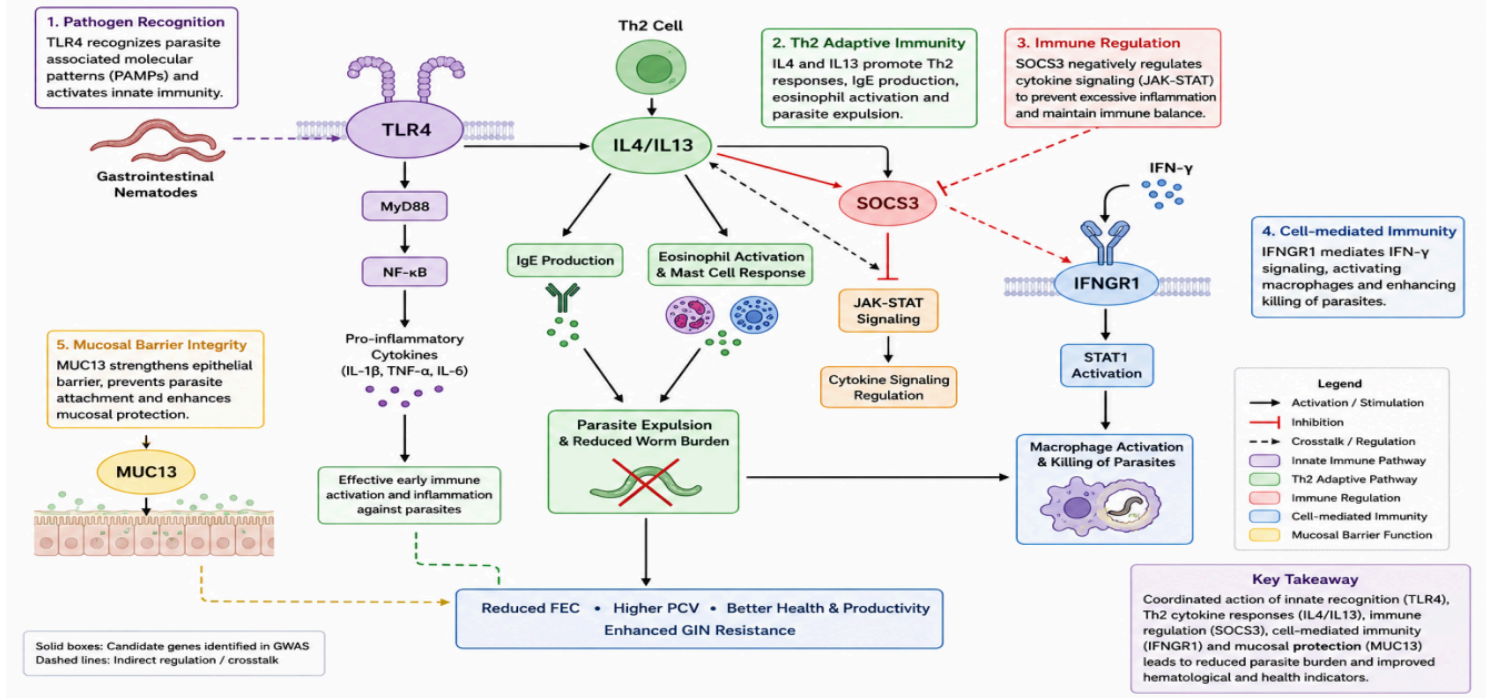


Figure 8

See image above for figure legend.

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