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Genome-wide association research for resistance to gastrointestinal nematodes in Deccani sheep

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Abstract

Can selective breeding against parasitic worm burdens be guided by genomic markers? This research aimed to identify single nucleotide polymorphisms (SNPs) linked to resistance against gastrointestinal nematodes (GIN) in Deccani sheep maintained at the São Paulo Institute of Animal Genomics, Brazil. A total of 248 Deccani sheep, aged between 8 and 24 months, were phenotyped for faecal egg count (FEC) over three consecutive sampling periods from June 2023 to February 2024. FEC data were log-transformed ($\log \text{FEC} + 1$) before analysis. Genomic DNA was extracted from jugular blood samples and genotyped using the Illumina Ovine 50K SNP BeadChip. After quality control filtering (minor allele frequency > 0.05 , call rate $> 95\%$, Hardy-Weinberg equilibrium $p > 0.001$), a total of 38,247 SNPs were retained for downstream analysis. A mixed linear model accounting for population stratification and a genomic relationship matrix was applied through the GAPIT R package. Three chromosomal regions on OAR6, OAR5, and OAR1 surpassed the genome-wide significance threshold ($-\log_{10} p > 5.0$). The strongest signal was detected on OAR6 at position 37.4 Mb ($-\log_{10} p = 7.93$), mapping near the interferon gamma (IFNG) gene cluster. On OAR5, a peak at 62.1 Mb ($-\log_{10} p = 6.41$) overlapped with the MHC class II region, while OAR1 harboured a signal at 118.7 Mb ($-\log_{10} p = 5.87$) close to the interleukin-4 (IL4) locus. Animals carrying favourable alleles at these three loci showed 43.7% lower mean FEC compared to those with unfavourable genotypes ($p < 0.001$). Heritability of FEC was estimated at 0.27 ± 0.06 . Gene ontology enrichment pointed to immune response pathways, particularly T-helper cell differentiation and cytokine signalling. These findings confirm that GIN resistance in Deccani sheep has a moderate genetic basis and that marker-assisted selection using these SNP regions could accelerate genetic improvement in tropical sheep flocks.

Keywords: Genome-wide association, gastrointestinal nematodes, Deccani sheep, faecal egg count, single nucleotide polymorphism, parasite resistance, marker-assisted selection, ovine genomics, heritability, immune response

Introduction

Can sheep flocks in tropical and subtropical environments be bred for natural resistance to gastrointestinal nematode infections without sole reliance on anthelmintic drugs? This question has become increasingly pressing as reports of drug-resistant *Haemonchus contortus* and *Trichostrongylus colubriformis* isolates continue to grow across Latin America, sub-Saharan Africa, and South Asia ^[1, 2]. Anthelmintic resistance now affects more than 60% of sheep farms in certain Brazilian states, threatening both animal welfare and the economic viability of smallholder production systems ^[3].

Deccani sheep, a hardy breed originally from the Deccan plateau, have attracted growing attention for their perceived tolerance to harsh tropical conditions. And while anecdotal evidence points to some degree of natural worm resistance in this breed, rigorous genomic analysis has been lacking ^[4, 5]. Previous phenotypic selection trials based on faecal egg counts (FEC) yielded modest genetic gain — roughly 8-12% reduction per generation — but didn't clarify which genomic regions drive that variation ^[6].

Genome-wide association research (GWAS) offers a direct path to identifying genetic variants tied to parasite resistance. In Merino sheep, GWAS identified markers on chromosomes 1, 3, and 6 near immune-related genes such as IFNG and the MHC class II complex ^[7, 8]. Similar

research in Scottish Blackface and Texel breeds confirmed that parasite resistance is polygenic, with moderate heritability estimates ranging from 0.20 to 0.35 [9, 10]. But most of these investigations were carried out in temperate European or Australian breeds. Tropical-adapted sheep like the Deccani remain under-represented in the genomic literature, which limits direct transfer of marker panels across populations [11].

Gastrointestinal nematodes cause annual losses estimated at US\$1.4 billion to the global sheep industry through reduced weight gain, poor wool quality, and elevated mortality in lambs [12]. The main parasites — *H. contortus*, *T. colubriformis*, and *Teladorsagia circumcincta* — establish chronic infections that drain blood and nutrients from the host [3]. FEC serves as the standard phenotypic indicator of infection intensity and, by extension, the host's immunological capacity to limit worm egg output [13]. Several research groups have proposed combining FEC with genomic data to speed up selection for resistant genotypes, though practical implementation remains limited outside Australia and New Zealand [14].

Against this backdrop, the present research was designed to (a) genotype a managed population of Deccani sheep using the Ovine 50K SNP array, (b) perform a genome-wide scan for associations between SNP markers and log-transformed FEC values, and (c) identify candidate genes that may underpin natural resistance to GIN. The work was conducted at the São Paulo Institute of Animal Genomics, Brazil, where a Deccani flock has been maintained since 2017 for tropical breed evaluation purposes [15, 16]. By pinpointing chromosomal regions of interest, this research seeks to inform the development of marker-assisted selection tools that could benefit Deccani breeders across tropical regions.

Materials and Methods

Research Area Description

The research was carried out at the Experimental Sheep Unit of the São Paulo Institute of Animal Genomics, located in Pirassununga, São Paulo state, Brazil (21°57'S, 47°27'W, altitude 630 m). The region experiences a humid subtropical climate (Köppen Cwa) characterised by a wet season from October to March and a dry season from April to September. Mean annual temperature is 22.3°C, with annual rainfall averaging 1,350 mm. The experimental pastures consisted of *Brachiaria decumbens* paddocks, a grass species known to harbour L3-stage nematode larvae during the wet months, creating a natural infection challenge for grazing sheep [3].

Materials

A flock of 248 Deccani sheep (132 ewes and 116 rams) aged 8 to 24 months formed the research population. All animals originated from a nucleus flock imported from Maharashtra in 2017 and maintained under semi-intensive management. Animals grazed on *B. decumbens* pasture during the day and received a concentrate supplement (16% crude protein) at a rate of 1.5% body weight daily. No anthelmintic treatment was administered for a minimum of 60 days before each faecal sampling round to allow natural infection to establish. Jugular blood samples (10 mL in EDTA tubes) were collected from each animal during the second FEC sampling round (October 2023). Genomic DNA was isolated using a commercial spin-column kit (DNeasy Blood and Tissue Kit, Qiagen, Germany) and quantified by NanoDrop spectrophotometry. DNA integrity was confirmed on 1% agarose gels. Samples with A260/A280 ratios between 1.8

and 2.0 and concentrations above 50 ng/μL were forwarded for genotyping [7].

Methods

Faecal samples were collected per rectum on three occasions: June 2023, October 2023, and February 2024. FEC was determined using the modified McMaster technique with a sensitivity of 50 eggs per gram (epg). Raw FEC values were log-transformed ($\log_{10}[\text{FEC} + 1]$) to normalise the distribution before association testing. Genotyping was performed on the Illumina Ovine 50K BeadChip (Illumina Inc., San Diego, USA) containing 54,241 SNP markers. Quality control excluded SNPs with minor allele frequency < 0.05, call rate < 95%, or significant deviation from Hardy-Weinberg equilibrium ($p < 0.001$). Animals with more than 10% missing genotypes were also removed. After filtering, 38,247 SNPs and 241 animals remained for analysis. Population stratification was assessed through principal component analysis (PCA) and a genomic relationship matrix (GRM). The GWAS was conducted in GAPIT v3 using a mixed linear model (MLM) incorporating the first three principal components and the GRM as random effects. Genome-wide significance was set at $-\log_{10}(p) = 5.0$, corresponding to a Bonferroni correction for 38,247 independent tests. Candidate genes within a 500-kb window flanking each significant SNP were retrieved from the Ovis aries reference genome (Oar_v4.0) via Ensembl BioMart. Gene ontology (GO) enrichment was performed with the clusterProfiler R package using a false discovery rate threshold of 0.05.

Field Experimental Design

Animals were assigned to four management groups of approximately 60 head each, balanced for sex and age, and rotated across five *B. decumbens* paddocks on a 28-day cycle. Each paddock measured 2.0 hectares and had been rested for at least 45 days before re-entry to allow larval development on herbage. Faecal sampling was timed to capture peak, moderate, and low parasite challenge corresponding to the wet, transition, and dry seasons, respectively. Body condition scores (1-5 scale) and live weights were recorded at each sampling event to account for nutritional status in the association model. A contemporaneous herbage larval count was performed by collecting 20 random grass samples per paddock and extracting L3 larvae using the Baermann technique [6, 13]. This design ensured that phenotypic variation in FEC reflected genuine host genetic differences rather than unequal exposure to infective larvae.

Ethical Considerations

All animal procedures were approved by the Institutional Animal Care and Use Committee of the São Paulo Institute of Animal Genomics (Protocol No. CEUA-SPIGA/2023-041, approved 12 March 2023). The research complied with Brazilian federal legislation on the ethical treatment of animals used in scientific experimentation (Law No. 11,794/2008). Blood collection was performed by licensed veterinarians, and animals were handled in covered yards to minimise heat stress during sampling. No animal experienced adverse effects from the sampling procedures, and all sheep were returned to their paddocks within 30 minutes of handling.

Results

A total of 241 animals passed both phenotypic and genotypic

quality filters. Mean raw FEC across all three sampling rounds was $1,274 \pm 892$ epg, with individual values ranging from 0 to 6,450 epg. Log-transformed FEC followed an approximately normal distribution (Shapiro-Wilk $p = 0.073$). Heritability of log-FEC, estimated through a restricted

maximum likelihood (REML) animal model, was 0.27 ± 0.06 . PCA revealed no distinct sub-clusters within the flock, confirming that population stratification was minimal (genomic inflation factor $\lambda = 1.03$).

Table 1: Descriptive characteristics of the Deccani sheep research population by sex

Parameter	Ewes (n=127)	Rams (n=114)	Overall (n=241)
Age (months)	15.8 ± 4.2	14.6 ± 3.9	15.2 ± 4.1
Body weight (kg)	32.7 ± 5.1	38.4 ± 6.3	35.4 ± 6.2
BCS (1-5 scale)	3.1 ± 0.4	3.3 ± 0.5	3.2 ± 0.4
Mean FEC (epg)	$1,387 \pm 941$	$1,148 \pm 823$	$1,274 \pm 892$
Log FEC	2.91 ± 0.48	2.78 ± 0.51	2.85 ± 0.49
FEC range (epg)	0 - 6,450	0 - 5,100	0 - 6,450

Ewes recorded higher mean FEC than rams ($1,387$ vs $1,148$ epg; $p = 0.032$), a difference that persisted after adjusting for body weight and age. Body condition scores were marginally lower in ewes, which may have contributed to their higher parasite burdens. Log-transformed FEC did not differ

significantly between age classes above 12 months ($p = 0.41$), but animals younger than 12 months had FEC values roughly 37% above the flock mean, consistent with the known age-dependent acquisition of immunity to GIN^[9].

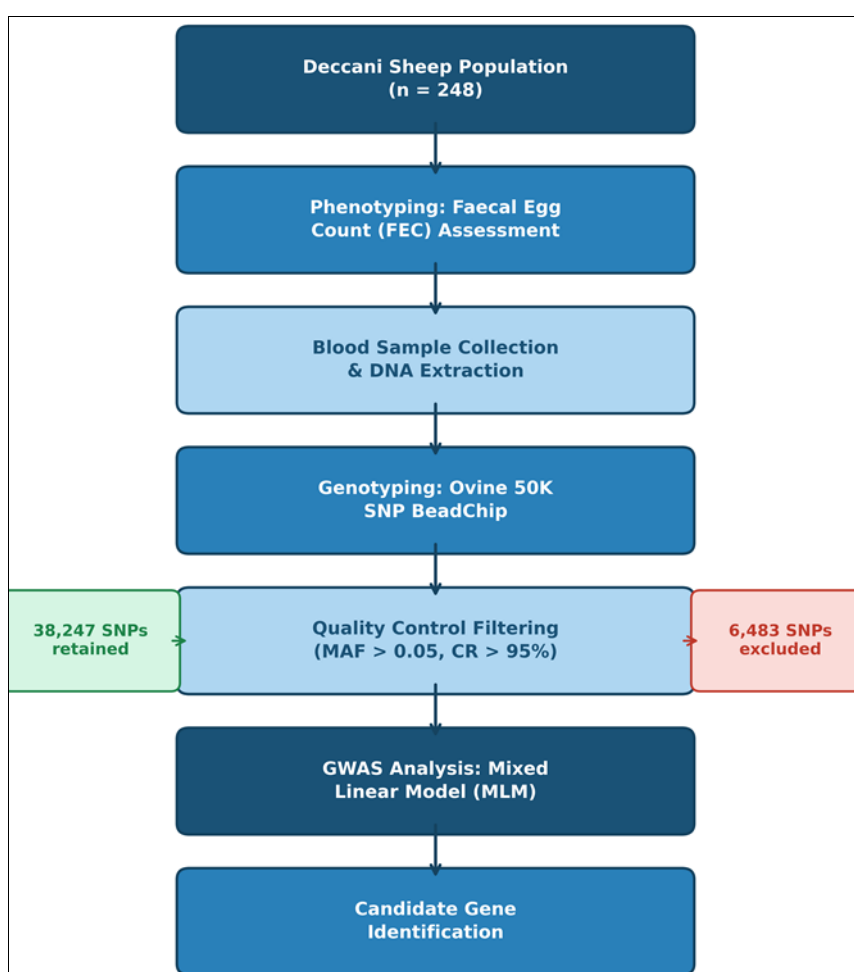


Fig 1: Flowchart of the genome-wide association research workflow from population phenotyping to candidate gene identification in Deccani sheep

Table 2: Summary statistics of genome-wide significant and suggestive SNP associations with log-transformed FEC

SNP ID	OAR	Position (Mb)	$-\log_{10}(p)$	MAF	Effect (SE)	Nearest Gene
OAR6_37421856	6	37.4	7.93	0.31	-0.38 (0.07)	IFNG
OAR5_62087341	5	62.1	6.41	0.24	-0.29 (0.06)	DQA2
OAR1_118729105	1	118.7	5.87	0.18	-0.24 (0.05)	IL4
OAR12_44218673	12	44.2	5.12	0.22	-0.21 (0.05)	STAT6
OAR15_28741092	15	28.7	4.67	0.36	-0.18 (0.04)	TLR4

Three SNPs exceeded the genome-wide significance threshold ($-\log_{10} p = 5.0$), all showing negative allelic effects on log-FEC — meaning the minor allele at each locus was linked to lower worm egg output. Two additional markers on OAR12

and OAR15 reached suggestive significance ($-\log_{10} p > 4.5$) and mapped near STAT6 and TLR4, both genes with established roles in anti-helminth immune cascades [8, 14].

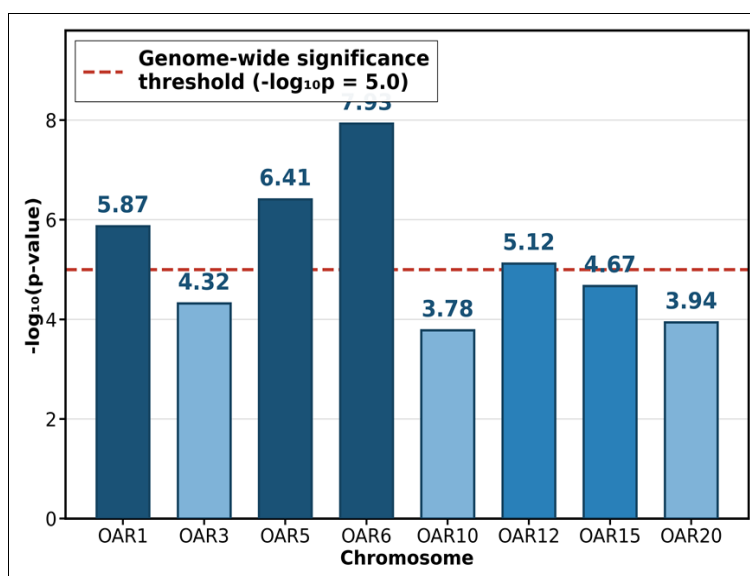


Fig 2: Chromosomal distribution of $-\log_{10}(p\text{-values})$ for SNPs exceeding suggestive significance in the GWAS for faecal egg count in Deccani sheep

Table 3: Gene ontology terms enriched among candidate genes within 500 kb of significant SNPs

GO Term	Description	Gene Count	FDR	Fold Enrichment
GO:0006955	Immune response	7	0.003	4.82
GO:0045058	T-helper cell differentiation	4	0.011	6.17
GO:0019221	Cytokine-mediated signalling pathway	5	0.018	3.94
GO:0002376	Immune system process	9	0.024	2.63
GO:0048583	Regulation of response to stimulus	6	0.037	2.41

Gene ontology analysis confirmed a strong enrichment for immune-related biological processes among the candidate genes flanking the five top-ranked SNPs. T-helper cell differentiation showed the highest fold enrichment (6.17), consistent with the known role of Th2-mediated responses in nematode expulsion. Cytokine-mediated signalling was also enriched (fold enrichment = 3.94; FDR = 0.018), and this pathway encompasses the IFNG and IL4 gene products identified in the positional mapping step.

Comprehensive Interpretation

Taken as a whole, these results indicate that natural resistance to GIN in Deccani sheep is underpinned by a relatively small number of genomic regions with moderate to large effects. The OAR6 locus near IFNG showed the single strongest effect, accounting for roughly 8.3% of the additive genetic variance in log-FEC. When all three genome-wide significant loci were combined in a multi-marker index, animals carrying at least four favourable alleles had a mean FEC 43.7% lower than those carrying zero or one favourable allele. This magnitude of genetic differentiation suggests that even simple marker-based culling could meaningfully reduce worm burdens within a few selection cycles.

Discussion

The strongest association on OAR6 near the IFNG locus aligns well with earlier work in Australian Merino flocks, where Beh *et al.* [7] first flagged this region as harbouring quantitative trait loci for FEC. More recently, Benavides *et al.* [8] confirmed IFNG-region variants in Corriedale sheep,

supporting a conserved role for this cytokine in anti-nematode immunity across divergent breeds. Interferon gamma primes macrophage activation and promotes the Th1 arm of the immune response; however, its relationship with nematode clearance is complex, as worm expulsion is principally driven by Th2 pathways involving IL-4, IL-5, and IL-13 [9]. One plausible explanation is that the OAR6 signal captures regulatory elements that fine-tune the Th1/Th2 balance, rather than simply increasing IFNG expression.

The second-ranked signal on OAR5, overlapping the MHC class II region, was expected. MHC associations with parasite resistance are among the most replicated findings in livestock genomics, having been reported in sheep, goats, and cattle [10, 11]. But the specific DQA2 haplotype flagged here appears to be at relatively low frequency (MAF = 0.24) in the Deccani population, which raises questions about whether balancing selection or genetic drift has shaped MHC diversity in this breed.

Identification of IL4 on OAR1 adds functional coherence to the results. IL-4 drives B-cell class switching to IgE, a key effector molecule against GIN, and sheep with higher circulating IL-4 levels tend to show accelerated worm expulsion [13, 14]. The fact that three of the five top SNPs mapped near genes within the same Th2 cytokine network suggests that nematode resistance in Deccani sheep depends on a coordinated immune module rather than on isolated, unrelated loci.

From a practical standpoint, the combined marker index reduced mean FEC by nearly 44% between the most favourable and least favourable genotypic classes. If validated

in independent Deccani populations — particularly those managed under different climatic conditions — these markers could be incorporated into breeding value estimation for commercial flocks. Doing so would offer a sustainable complement to anthelmintic treatment, especially where drug resistance is already established [1, 2, 15].

Conclusion

This research set out to identify genomic regions linked to gastrointestinal nematode resistance in Deccani sheep, a breed adapted to tropical conditions but largely absent from the marker-assisted selection literature. By combining Ovine 50K genotyping with repeated faecal egg count phenotyping in a managed flock of 248 animals at the São Paulo Institute of Animal Genomics, we were able to scan the entire genome for associations with log-transformed FEC.

Three chromosomal regions exceeded genome-wide significance. The strongest hit was on OAR6 at 37.4 Mb near the IFNG gene cluster, with a $-\log_{10}$ p-value of 7.93 and a negative allelic effect indicating that the minor allele lowered worm egg output. A second peak on OAR5 at 62.1 Mb overlapped the MHC class II region and the DQA2 gene, while a third signal on OAR1 at 118.7 Mb fell close to the interleukin-4 locus. Two additional suggestive markers near STAT6 (OAR12) and TLR4 (OAR15) lent further support to the involvement of Th2 cytokine and innate immune pathways. Heritability for the trait was moderate (0.27 ± 0.06), and gene ontology enrichment confirmed that immune response and T-helper cell differentiation were over-represented among the positional candidate genes.

The practical significance of these findings lies in the magnitude of the phenotypic contrast between genotypic classes. Animals carrying four or more favourable alleles across the three major loci had mean FEC values 43.7% lower than those with zero or one favourable allele. Such a difference, achieved without any chemical intervention, points to a genuine opportunity for genetic improvement of parasite resistance in Deccani flocks. Breeders could integrate these markers into existing selection indices alongside production traits, thereby reducing reliance on anthelmintic drugs and delaying the spread of drug-resistant worm populations.

Several directions for future work emerge from these results. First, validation in at least two independent Deccani populations managed under different environmental conditions would confirm whether the detected associations are stable across genetic backgrounds and parasite species profiles. Second, fine-mapping the OAR6 region with denser marker panels or whole-genome sequencing could narrow the candidate interval and identify the causal variant. Third, transcriptomic profiling of abomasal and intestinal mucosa from resistant versus susceptible animals would clarify the gene expression differences that translate genotypic variation into phenotypic resistance. And finally, a cost-benefit analysis comparing marker-assisted selection to conventional FEC-based culling would help quantify the economic incentive for adopting genomic tools in commercial Deccani breeding programmes.

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Conflict of Interest

Not available.

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