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Dr. Vinod Kumar RC

Ph.D. Scholar, Department of
Animal Genetics and Breeding,
Veterinary College, KVAFSU,
Bidar Karnataka, India

Dr. Nagaraja R

Dean, Veterinary College,
KVAFSU, Karnataka, India

Dr. Manjunatha Reddy GB

Senior Scientist, ICAR-
National Institute of
Veterinary Epidemiology and
Disease Informatics,
Yelahanka, Bengaluru,
Karnataka, India

Dr. Naveen Kumar S

Professor and Head,
Department of Animal
Genetics and Breeding,
Veterinary College, KVAFSU,
Bidar, Karnataka, India

Dr. Yathish HM

Senior Scientist, Animal
Genetics and Breeding, ICAR-
National Research Centre on
Mithun, Medziphema,
Nagaland, India

Dr. Ramesh PT

Registrar, KVAFSU, Bidar,
Karnataka, India

Dr. Narasimha Murthy

Associate Professor,
Department of Veterinary
Gynaecology and Obstetrics,
Veterinary College, KVAFSU,
Bidar, Karnataka, India

Corresponding Author:**Dr. Nagaraja R**

Dean, Veterinary College,
KVAFSU, Karnataka, India

Genetic polymorphism in *IL10* exon 1 and their association with antibody response following lumpy skin disease vaccination in Hallikar and crossbred Holstein Friesian cattle

Vinod Kumar RC, Nagaraja R, Manjunatha Reddy GB, Naveen Kumar S, Yathish HM, Ramesh PT and Narasimha Murthy

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Abstract

Interleukin-10 (*IL10*) is a major anti-inflammatory cytokine that limits excessive immune activation while preserving immune homeostasis. Genetic variation in *IL10* may therefore contribute to inter animal differences in vaccine-induced immunity. The present study investigated polymorphisms in *IL10* exon 1 and their association with antibody response following Lumpy skin disease vaccination in Hallikar and Crossbred Holstein Friesian cattle. A total of 154 cattle (86 Hallikar and 68 Crossbred Holstein Friesian) were screened, and 100 seronegative animals (50 per breed) with indirect ELISA percentage positivity (PPV) <35 and Serum Neutralization Test (SNT) titre <1:8 were enrolled. Animals were vaccinated with the live attenuated Goatpox vaccine (Uttarkashi strain). Serum samples collected on day 0 and 28 days post-vaccination were evaluated by indirect ELISA and SNT. Based on the quartile distribution of fold change in log₂-transformed SNT titres, animals were classified as higher and lower antibody responders. A total of 48 animals (12 higher and 12 lower responders from each breed) were selected for molecular analysis. *IL10* exon 1 was amplified using newly designed primers, followed by bidirectional Sanger sequencing. Consensus sequences were generated using CLC Genomics Workbench and aligned with the bovine reference sequence using CLUSTAL W in BioEdit (version 7.2.5). *IL10* exon 1 was completely conserved in Hallikar cattle with no SNPs, insertions or deletions. In CB-HF cattle, a G→A transition was detected at nucleotide position 207. Although the A allele occurred exclusively among higher responders, the association was not significant by two-tailed Fisher's exact test ($P = 0.1092$). These findings indicate limited variation in the analysed *IL10* exon 1 region and do not establish this locus as a determinant of vaccine induced antibody response. Larger, genetically diverse populations and functional analysis of regulatory, receptor and expression level variation are required before *IL10*-related variants can be considered for marker-assisted selection

Keywords: *IL10*, Lumpy skin disease, vaccination, antibody response, single nucleotide polymorphism

Introduction

Lumpy skin disease (LSD) is an economically important transboundary viral disease of cattle caused by lumpy skin disease virus (LSDV), a member of the genus *Capripoxvirus* within the family *Poxviridae*. The disease may occur in acute or subacute forms and is characterized by fever, anorexia, and distinctive cutaneous nodules, typically 2-5 cm in diameter, which may also involve the mucous membranes of the nostrils and mouth, as well as the udder, rectum, and genitalia (Moda *et al.*, 2024; Manjunatha Reddy *et al.*, 2025) ^[1, 2]. LSDV is primarily transmitted mechanically by blood-feeding arthropod vectors, including biting flies, mosquitoes, and ticks. The disease has emerged as a major threat to cattle production, causing substantial morbidity, mortality, and production losses. Milk yield losses associated with LSD vary considerably across regions, with estimated losses of approximately 8-90 litres per affected animal (Naidu *et al.*, 2025) ^[3]. In recent years, LSD outbreaks in India have resulted in severe economic losses, estimated at approximately USD 2.2 billion (Manjunatha Reddy *et al.*, 2023; Tadakod S *et al.*, 2026) ^[4, 5]. Vaccination remains the principal strategy for the prevention and control of LSD, particularly in endemic and high-risk regions (Tuppurainen *et al.*, 2017; Reddy GB *et al.*, 2026) ^[6, 7].

Despite vaccination, considerable animal-to-animal variation may occur in the magnitude of the antibody response. This variability can be influenced by several factors, including breed, age, sex, nutritional status, physiological condition, management practices, and genetic variation in immune response genes (Meeusen *et al.*, 2007^[8]; Glass *et al.*, 2012^[9]; Bayyappa *et al.*, 2025)^[10]. Genetic variation in candidate genes involved in pathogen recognition, antigen presentation, and cytokine-mediated immune regulation may therefore contribute to differences in vaccine responsiveness among cattle (Leach *et al.*, 2010^[11]; Glass *et al.*, 2012^[9]).

Interleukin-10 (IL-10) is a pleiotropic cytokine with potent anti-inflammatory and immunoregulatory activity. It is produced by regulatory T cells, helper T-cell subsets, macrophages, dendritic cells, monocytes, B cells and other leukocytes. IL-10 limits excessive production of pro-inflammatory cytokines, reduces antigen-presenting-cell activation and contributes to the resolution of inflammation thereby preventing immunopathology while maintaining tissue homeostasis (Saraiva and O'Garra, 2010)^[12]. IL-10 regulates the interface between innate activation and adaptive immunity, variation in the *IL10* signalling pathway may influence vaccine responsiveness. Changes in coding sequence may alter cytokine structure or stability whereas promoter and regulatory variants may influence transcription. In cattle, polymorphisms in *IL10*, *IL10RA* and *IL10RB* have been investigated in relation to somatic cell score, mastitis and *Mycobacterium avium* subsp. *paratuberculosis* infection (Verschoor *et al.*, 2009^[13]; Verschoor *et al.*, 2010^[14]). These studies indicate that *IL10*-pathway diversity may contribute to disease-related phenotypes, although effects can depend on the genomic region, population and phenotype examined.

The present study selected *IL10* as a candidate immune response gene because its anti-inflammatory function could influence the magnitude and duration of antibody production after LSD vaccination. However, information on *IL10* sequence variation associated with antibody response to LSD vaccination in indigenous Hallikar and CB-HF cattle is scarce. The objective of the present analysis was therefore to characterize *IL10* exon 1 in higher and lower antibody responders of both breeds and to test whether identified nucleotide variants were associated with vaccine-induced humoral response.

Materials and Methods

Experimental animals and pre-vaccination management

A total of 154 cattle, comprising 86 Hallikar and 68 Crossbred Holstein Friesian (CB-HF) cattle were identified from different villages of Bagepalli and Shidlaghatta taluks of Chikkaballapur District, Karnataka. Animals were selected based on dentition, and only cattle possessing a minimum of one pair of permanent incisor teeth were considered for screening. The age of each animal was recorded in years based on the incisor eruption pattern. Before vaccination, all the identified animals were dewormed with fenbendazole bolus 1.5g and were

supplemented with a trace mineral bolus to minimize the possible confounding effects of parasitic infestation and mineral deficiency on vaccine-induced immune responses.

Animal selection and vaccination

Before enrolment, all identified animals were screened for pre-existing antibodies against LSDV using indirect enzyme-linked immunosorbent assay (iELISA) and Serum Neutralization Test (SNT). Animals exhibiting an iELISA percentage positivity value of less than 35 PPV and an SNT antibody titre of less than 1:8 were considered seronegative and included in the study. Based on these criteria, 50 Hallikar and 50 CB-HF cattle were enrolled in the study and vaccinated subcutaneously with 1 ml of live attenuated Goatpox vaccine (Uttarkashi strain; Hester Biosciences Ltd., India) containing not less than 1×10^3 TCID₅₀ per dose, as per the manufacturer's recommendations and the Government of India guidelines for heterologous vaccination against LSD.

Classification of antibody responder groups

Post-vaccination antibody responses were assessed at 28 days using iELISA and SNT. Since SNT is the reference assay for evaluating functional neutralizing antibodies, SNT titres were used for fold-change calculations and responder classification. Animals were categorized into higher and lower antibody responder groups based on the fold change in log₂-transformed SNT titres by using quartile based distribution. Animals with fold-change values $\leq$ Q1 (25th percentile) were designated as lower responders, those with values between Q1 and Q3 (25th-75th percentile; including Q2, the median) as average responders, and those with values $\geq$ Q3 (75th percentile) as higher responders. Only the higher and lower responder groups were selected for sequencing and subsequent SNP association analysis.

DNA isolation and quality assessment

Genomic DNA was isolated from EDTA-anticoagulated whole blood samples using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The concentration and purity of the extracted DNA were assessed using a UV spectrophotometer, and DNA integrity was confirmed by electrophoresis on 1.5% agarose gel. DNA samples of adequate quality and concentration were used for amplification of *IL10* exon 1.

Selection of the target region and primer design

IL10 exon 1 was selected as one of the candidate immune response gene regions for molecular characterization. The *Bos taurus* reference sequence was obtained from the Ensembl Genome Browser (*IL10* gene/transcript ID: ENSBTAT00000008190, *IL10* exon ID: ENSBTAE000000066213). Primers were designed using NCBI Primer-BLAST and synthesized by Eurofins Genomics India, Bengaluru, the details of the primers were represented in Table 1.

Table 1: Details of primer sequences used for amplification of bovine *IL10* exon 1

Gene	Exon	Primer	Sequence (5'-3')	Expected product size (bp)
<i>IL10</i>	Exon 1	Forward	AGCTGGAGAGGTCTAGGGAAG	367
		Reverse	AGAAAGTCTTCACCTTGCCGAA	

Primer reconstitution

Lyophilized primers were reconstituted in nuclease free water to obtain a stock concentration of 100 pmol/μl or 100 μM. A working concentration of 10 pmol/μl or 10 μM was prepared by mixing 10 μl of primer stock with 90 μl of nuclease-free water. Stock and working primer solutions were stored at -20 °C and 4 °C, respectively,

PCR reaction mixture

PCR amplification was carried out in a final volume of 25 μl using the following reaction composition as mentioned in the Table 2.

Table 2: Composition of PCR reaction mixture

Sl. No.	Component	Volume (μl)
1.	PCR Master Mix (2X)	12.5
2.	Template DNA	2.0
3.	Forward primer	1.0
4.	Reverse primer	1.0
5.	Nuclease-free water	8.5
	Total volume	25.0

PCR thermal cycling conditions

PCR amplification of *IL10* exon 1 was performed using the following standardized thermal profile, the details of the PCR thermal cycling conditions were mentioned in Table 3.

Table 3: PCR thermal cycling conditions used for amplification of bovine *IL10* exon 1

Sl. No.	Step	Temperature	Duration
1.	Initial denaturation	94 °C	5 min
2.	Denaturation	94 °C	40 sec
3.	Annealing	57.8 °C	30 sec
4.	Extension	72 °C	30 sec
5.	Step 2 to 4 were repeated 35 times		
6.	Final extension	72 °C	7 min
7.	Hold	4 °C	Until removal

The optimum annealing temperature for *IL10* exon 1 was determined as 57.8 °C based on the production of a sharp and distinct band of the expected size without visible non-specific amplification.

Agarose gel electrophoresis

PCR products were evaluated using 1.5 percent agarose gel electrophoresis in 1× TBE buffer. Electrophoresis was performed with a 100 bp DNA ladder at 80V for 60 minutes. Amplified products were visualized under ultraviolet illumination and documented using a gel documentation system.

Purification and Sanger sequencing

PCR amplified products selected from the higher and lower responder groups were purified and subjected to separate forward and reverse sequencing. Bidirectional sequencing was performed using the Sanger sequencing method.

Sequence editing and consensus sequence generation

Forward and reverse Sanger sequencing reads were quality checked and low quality bases at the 5' and 3' ends were trimmed using CLC Genomics Workbench (version 9.5.2). The reverse reads were reverse-complemented and assembled with the corresponding forward reads to generate consensus sequences.

Sequence alignment and SNP identification

The consensus sequences were aligned with the *Bos taurus* reference sequence using the CLUSTAL W algorithm implemented in BioEdit (version 7.2.5) to identify SNPs. Chromatograms corresponding to the identified polymorphic positions were manually inspected to confirm single peaks and overlapping heterozygous peaks using Chromas Lite (version 2.6.6).

Statistical analysis

The association between identified *IL10* SNPs and antibody response category was evaluated using the two-tailed Fisher's exact test. Contingency tables were constructed by comparing the distribution of reference and variant nucleotides between higher and lower antibody responder groups. The statistical analysis was performed using IBM SPSS Statistics. A P-value below 0.05 was considered statistically significant.

Results

Post vaccination humoral immune response

A marked increase in antibody titres was observed in both breeds at 28 days post-vaccination (dpv). The post-vaccination indirect ELISA PPV values, SNT titres, and geometric mean titres (GMT) are presented in Table 4.

Table 4: Post-vaccination humoral immune response in Hallikar and CB-HF cattle

Parameter	Hallikar (Mean ± SE)	CB-HF (Mean ± SE)
Indirect ELISA PPV (0 dpv)	20.67 ± 1.48	24.51 ± 1.43
Indirect ELISA PPV (28 dpv)	91.29 ± 3.08	85.75 ± 1.90
SNT titre (log ₂) (0 dpv)	1.98 ± 0.07	2.19 ± 0.07
SNT titre (log ₂) (28 dpv)	6.19 ± 0.08	5.91 ± 0.07
GMT (0 dpv)	3.94	4.56
GMT (28 dpv)	72.93	60.13

Values for indirect ELISA PPV and log₂-transformed SNT titres are presented as mean ± SE. Geometric mean titres are presented as absolute values.

Classification of antibody responder groups

The fold change in log₂-transformed SNT titres was calculated separately for Hallikar and Crossbred Holstein Friesian (CB-HF) cattle and classified using a quartile based distribution. In Hallikar cattle, the first quartile (Q1), median (Q2) and third quartile (Q3) values were 4.03, 4.04 and 5.03, respectively. Accordingly, animals with fold-change values ≤ 4.03 were classified as lower responders, those with values > 4.03 and < 5.03 were classified as average responders, and animals with fold-change values ≥ 5.03 were classified as higher responders.

In CB-HF cattle, the Q1, Q2 and Q3 values were 3.04, 4.03 and 4.04, respectively. Animals with fold-change values ≤ 3.04 were categorized as lower responders, those with values > 3.04 and < 4.04 were categorized as average responders, and animals with fold-change values ≥ 4.04 were categorized as higher responders. Based on this classification, 12 higher and 12 lower antibody responder animals from each breed were selected for bidirectional Sanger sequencing and subsequent SNP association analysis.

Genomic DNA isolation and quality assessment

High quality genomic DNA was successfully isolated from all selected animals. The DNA concentration ranged from 60 to 110 ng/μl, with a mean concentration of approximately 85 ± 12 ng/μl. The A260/A280 ratio ranged from 1.75 to 2.00, indicating good purity. Agarose gel electrophoresis showed intact high-molecular-weight

genomic DNA suitable for PCR amplification.

PCR amplification of *IL10* exon 1

PCR amplification of *IL10* exon 1 was successful in all selected animals. Agarose gel electrophoresis revealed distinct bands of the expected size without non-specific amplification or primer-dimer formation (Figure 1)

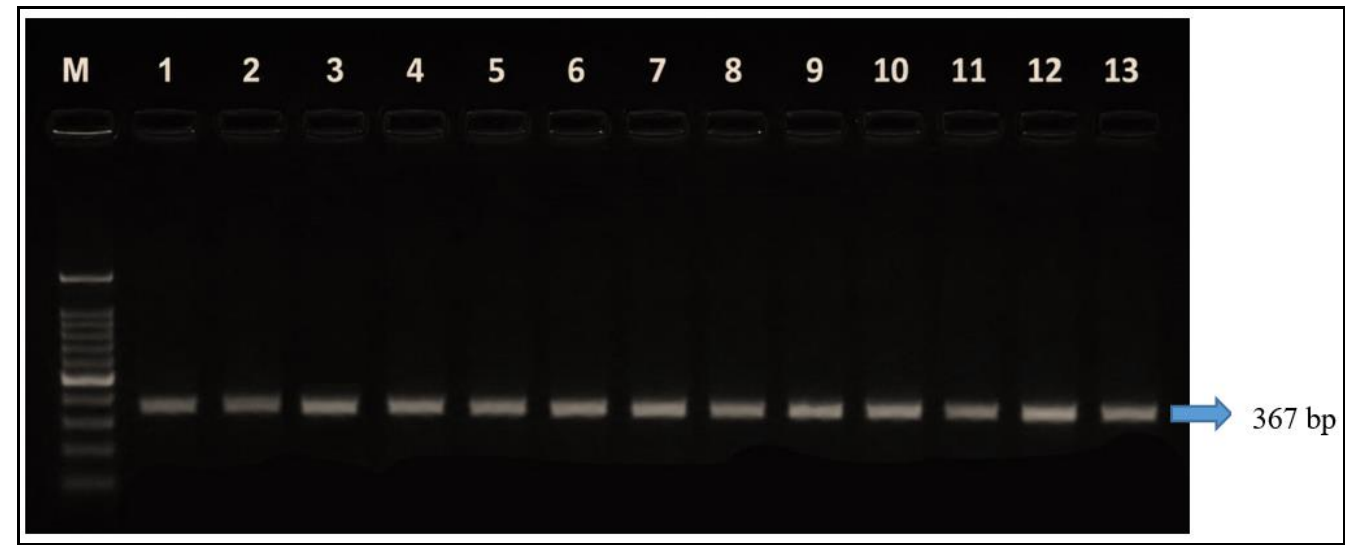


Fig 1: Agarose gel (1.5%) showing PCR amplified product of *IL10* (exon 1) gene. Lane M: Molecular marker (100 bp DNA ladder), Lanes 1, 2, 3, 4, 5, 6: PCR amplified product 367 bp (Hallikar) and Lane 7, 8, 9, 10, 11, 12 : PCR amplified product 367 bp (HF crossbred)

***IL10* exon 1 polymorphisms in Hallikar cattle**

Multiple sequence alignment of the *IL10* exon 1 consensus sequences obtained from Hallikar cattle showed complete conservation of the analysed region. No SNPs, insertions, or deletions were detected when compared with the bovine

reference sequence indicating the absence of genetic variation in the amplified region as showed in the Figure 2. Consequently, association analysis between *IL10* exon 1 polymorphisms and antibody response was not performed.

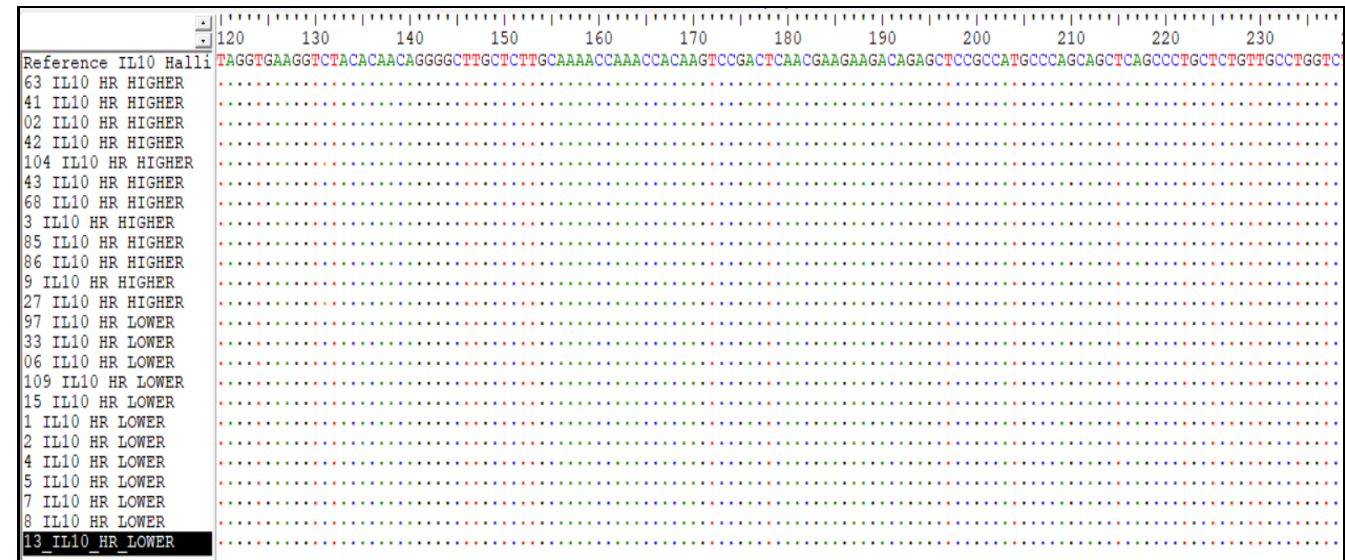
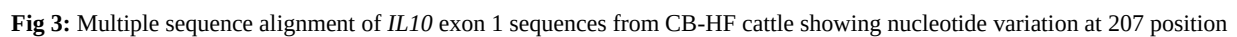


Fig 2: Multiple sequence alignment of the *IL10* exon 1 gene showing complete sequence conservation among Hallikar cattle

***IL10* exon 1 polymorphism in Crossbred Holstein Friesian cattle**

Sequence analysis of *IL10* exon 1 in CB-HF cattle identified a single nucleotide polymorphism at nucleotide position 207, where the reference nucleotide ‘G’ was substituted by ‘A’, representing a G→A transition mutation. The variant ‘A’ nucleotide was detected only among higher antibody

responder animals whereas, all lower responders retained the reference ‘G’ nucleotide. No additional sequence variations were identified within the amplified region. Representative multiple sequence alignment and chromatograms illustrating the detected polymorphisms are presented in Figures 3 and 4 respectively.



The distribution of *IL10* exon 1 SNP between higher and lower antibody responder groups was evaluated using the two-tailed Fisher's exact test. A single nucleotide polymorphism was identified at nucleotide position 207,

Table 5: Association of *IL10* exon 1 polymorphisms with antibody response in CB-HF cattle

Note: Values for higher and lower responder groups represent allele counts (2n = 24 alleles) obtained from 12 animals in each responder group. Statistical significance was evaluated using the two-tailed Fisher's exact test.

Discussion

The present study identified marked breed-specific differences in nucleotide diversity within the analysed *IL10* exon 1 fragment. Complete conservation in Hallikar cattle contrasted with a single G→A transition at position 207 in CB-HF cattle. IL-10 acts mainly as a negative regulator of inflammation. It suppresses excessive activation of macrophages and antigen-presenting cells and limits the production of inflammatory mediators, thereby reducing collateral tissue injury (Saraiva and O'Garra, 2010^[12]). During vaccination, this regulation may be beneficial by preventing immunopathology, but excessive IL-10 signalling can also restrict antigen presentation and effector-cell activation. Thus, the relationship between IL-10 and vaccine response is unlikely to be linear and may depend more strongly on expression kinetics, cellular source and receptor signalling than on a single coding-region variant. Previous bovine studies have reported greater diversity in regulatory and receptor components of the *IL10* pathway. Verschoor *et al.* (2009)^[13] identified *IL10* promoter and *IL10* receptor polymorphisms in dairy cattle and reported an association between *IL10RA* variation and somatic cell score whereas, the *IL10* promoter variants themselves were not significantly associated. Verschoor *et al.* (2010)^[14] further identified *IL10*-pathway variants in cattle evaluated for *Mycobacterium avium* subsp. *paratuberculosis* phenotypes. These findings support the possibility that receptor or regulatory variation may exert stronger phenotypic effects than variation in the limited exon 1 fragment studied in the present study.

The absence of polymorphism in Hallikar cattle suggests that the *IL10* exon 1 region is highly conserved in this indigenous breed. In contrast, a G>A polymorphism at nucleotide position 207 was identified in Crossbred Holstein Friesian cattle, likely reflecting their admixed genetic background. Although the 'A' allele was observed only among higher antibody responders, the association was not statistically significant, indicating that the identified variant was not associated with antibody response following LSD vaccination. The lack of significance may be attributed to the low allele frequency, limited sample size or absence of a functional effect. Therefore, further validation in larger cattle populations, together with functional characterization of the variant, is required to determine its biological significance and potential role in vaccine responsiveness.

Conclusions

IL10 exon 1 was completely conserved in Hallikar cattle whereas a single G→A transition at nucleotide position 207 was identified in CB-HF cattle. Although the variant A allele occurred exclusively in higher antibody responders, its association with LSD vaccine-induced antibody response was not significant ($P = 0.1092$). These findings indicate limited polymorphism within the analysed *IL10* exon 1 region and provide no evidence for a major influence on humoral vaccine responsiveness. Larger population studies incorporating broader genomic, expression and functional analyses are required to establish the potential relevance of *IL10* variants for marker-assisted selection.

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