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IL-1β Immune Response Gene Has Role Against Nematode *Haemonchus contortus*

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KR conducted the experimental work, collected samples, performed acquisition of data, drafted the article. AP conceptualized and designed the work, conducted research, analysed and interpreted the data, and drafted the article. S Banerjee has conducted the experimental work. S Batabyal conceptualized the work, guided through the biochemical analysis. NK worked on manuscript writing.

Key words
IL-1beta, *Haemonchus controtus*, Differential mRNA expression analysis, Molecular docking, Molecular phylogeny

ABSTRACT

Sheep are economically important livestock in India, but their productivity is severely affected by parasitic infections such as *Haemonchus contortus*. This study focused on characterizing the IL-1β gene in sheep and evaluating its role in immune response during parasitic infection. The cloned IL-1β gene was 978 bp, encoding a 266 amino acid protein with typical structural motifs identified through bioinformatics analysis. Protein interaction and pathway analysis via STRING and KEGG revealed IL-1β's involvement in key immune pathways alongside TNF-α, MYD88, and NF-κB. Differential gene expression studies showed significant upregulation of IL-1β, IL-10, CD14, TLR-4, and TNF-α in infected animals, while IL-6 was downregulated. These changes suggest an activated inflammatory response with regulatory feedback via IL-10. Hematological analysis indicated anemia, eosinophilia, and neutrophilia in diseased sheep, reflecting systemic inflammation. Biochemical findings, including reduced protein levels and elevated liver enzymes, suggest tissue damage and metabolic stress due to parasitism. Overall, IL-1β emerges as a central mediator of the ovine immune response, potentially useful as a biomarker for infection and a target for therapeutic or vaccine strategies. Insights into its gene regulation and interaction networks may support breeding programs focused on enhancing disease resistance in sheep populations.

INTRODUCTION

Interleukin-1β (IL-1β), a potent pro-inflammatory cytokine that is crucial for host-defense responses to infection and injury, is a member of the 11 IL-1 family. It is produced by activation of macrophages as a proprotein which is proteolytically processed to its active form by caspase. This cytokine is an important mediator of the inflammatory response and is involved in a variety of cellular activities including proliferation, differentiation, and apoptosis (Vaure and Liu, 2014). It works together with tumor necrosis factor-α. The recruitment of mast cells and eosinophils to the abomasal mucosa in response to *Haemonchus contortus* or other larvae may induce local cytokine production. A wide variety of cytokines both cell types can produce, including those typical of TH1 and TH2 cells. Eosinophils and mast cells can produce TH2 cytokines (e.g. IL-4, IL-13, and IL-10) and TH1cytokines (e.g., IL-12, IL-16, and IFN-γ), cytokines influencing cell growth and recruitment (e.g., IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor, a cytokine involved in inflammation and tissue repair (e.g., IL-1, IL-6, IL-8, transforming growth factor β, and TNF-α) (Behm and Ovington, 2000; Henz *et al*., 2001). The presence of a large number of these cells in resistant animals during infection may be the reason for a mixed TH1-TH2 response. The network of cytokines and signalling pathways appears to be involved in the development of TH2-mediated resistance to intestinal nematode infection. The known cytokine members of this network currently include IL-4, IL-13, IL-9, IL-10, TNF-and IL-1 (Else *et al*., 1994). Expulsion of the gastrointestinal nematode *Trichuris*

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muris is mediated by TH2- type response, involving IL-4, IL-9, and IL-13. Observe that TH2 response-associated resistance is dependent on the presence of IL-1a and IL-1b (Helmby and Grencis, 2004).

In studies, it was reported that the ovine inflammatory response associated with ID and footrot is also reflected in the increased expression of the pro-inflammatory cytokine *IL-1β*. Expression of *TLR2, TLR4,* and *IL-1β* was dependent on the disease status of the feet, not the animal. Some studies showed a significant association between the high expression of IL1β and high *D. nodosus* load in footrot samples (Maboni *et al*., 2017). Similarly in small ruminant lenti virus infection expression of IL-1α, IL-1β, and IL-6 on the transcript and protein levels are decreased, and no expression of IFN-γ and TNF-α on the protein level (despite the presence of their transcripts) in the whole blood of animals (Jarczak, 2014).

Genome sequencing increases our capacity to enhance our understanding of the evolutionary histories of related species. The present genome of each species has developed over millions of years as a result of mutation and natural selection. Cloning and sequencing the *IL-1β* gene in sheep (*Ovis aries*), a common household ruminant, is the aim of this study. Additionally, it emphasizes bioinformatics methods to identify and contrast the structural and functional domains of peptides produced from CD14 as well as investigate the domains 3D structures. The *IL-1β* gene's differential mRNA expression profile in healthy and sick (*Haemonchus*-infected) sheep was carried out to support the aforementioned findings.

MATERIALS AND METHODS

Sample collection and RNA isolation

Sheep liver tissue (1 g) was obtained from a slaughterhouse run by the Kolkata Municipality Corporation. For the collection of samples, adult males (n=6) between the ages of 1 and 1.5 years were considered. Liver tissue was placed in a vial and soaked in Trizol before being transferred to the laboratory on ice for RNA isolation. Total RNA was extracted using the TRIzol extraction technique (Life Technologies, USA), as per standard protocol, and was then used to synthesize cDNA (Pal and Chatterjee, 2017; Pal *et al*., 2011). Ethical approval was not required because the samples were collected from the slaughterhouse. The concentration of cDNA was calculated, and samples with concentrations of more than 1.2 mg/ml were considered for further investigation. Tissue from abomassum was obtained from both healthy and *H. contortus*-infected sheep to conduct quantitative PCR expression profiling. Both the tip and the middle of the abomassum were used to collect tissue. During the ΔΔCT (DDCT) computation (used in qPCR analysis), gene expression from the tip of the abomassum was used as a control.

cDNA synthesis and PCR amplification of the IL-1β gene

The 20 µl reaction mixture comprising of 5 µg of total RNA, 0.5 µg of oligo dT primer (16–18mer), 40U of ribonuclease inhibitor, 1000M of dNTP mix, 10mM of DTT, and 5U of MuMLV reverse transcriptase in an appropriate buffer consisted 20 µL of the reaction mixture was incubated for 1 h at 37°C. By heating the mixture unliganded at 70 °C for 10 min and then chilled on ice. Following that, PCR was used to confirm the cDNA's integrity. Nanodrop was used to determine the concentration of cDNA. To amplify the full-length open reading frame (ORF) of the *CD14* gene sequence in sheep, a pair of ovine CD14 primers was constructed using DNASTAR software (Hitachi Miraibio Inc., USA).

F= 5′ CGTCTTCCTGGGACATTTTC 3′ and
R=5′ GTCTGAGGATGGGCTCTGGG 3′,

Reaction mixture (25µL) comprised of 80–100ng cDNA, 3.0µL 10X PCR assay buffer, 0.5µL of 10mM dNTP, 1U Taq DNA polymerase, 60 ng of each primer, and 2mM $MgCl_2$. Initial denaturation at 94°C for 3 min, subsequent denaturation at 94°C for 30sec, annealing at 61 °C for 35 sec, and extension at 72°C for 3 min were done for 35 cycles followed by a final extension at 72 °C for 10 min in a thermocycler (PTC-200, MJ Research, USA).

cDNA cloning and sequencing

A 1% agarose gel electrophoresis was used to examine the amplified product of the ovine *IL-1β* gene. A Gel extraction kit was used to purify the components from the gel (Qiagen GmbH, Hilden, Germany). Cloning was done with the pGEM-T simple cloning vector (Promega, Madison, WI, USA). Then, 10 L of the ligated product was well mixed with 200L competent cells, and heat shock was delivered in a water bath at 42 °C for 45 sec. Following that, the cells were placed on cooled ice for 5 min before being introduced to the SOC medium. The pellet from the bacterial culture was centrifuged and plated on an LB agar plate with ampicillin (100 mg/mL) added to the agar plate at 1:1000, IPTG (200 mg/mL), and X-Gal (20 mg/mL) for blue-white screening. A small-scale alkaline lysis approach was used to isolate plasmids from overnight-grown cultures, as described before (Sambrook *et al*., 2001). PCR using CD14 primers and restriction enzyme digestion were used to characterize recombinant plasmids. IL-1β gene segments produced by the enzyme EcoRI (MBI Fermentas, USA) were introduced into a recombinant plasmid, which was sequenced in an automated sequencer using the dideoxy chain termination technique using T7 and SP6

primers (ABI prism, Chromous Biotech, Bangalore). The nucleotide sequence so obtained was analyzed for protein translation, sequence alignments, and contigs comparisons by DNASTAR Version 4.0, Inc., USA.

Study of predicted ruminant IL-1β protein using bioinformatics tools

Edit sequence (Lasergene Software, DNASTAR) was used to generate the predicted peptide sequence of the IL-1β gene of CB cattle, which was subsequently aligned with the IL-1β gene peptide of other livestock and avian using Lasergene Software's Megalign sequence Program (DNASTAR). Prediction of the signal peptide of the IL-1β gene was conducted using the software (Signal P 3.0 Sewer-prediction results, Technical University of Denmark). The signal peptide is required for a cell to translocate a protein to the cellular membrane, and the signal peptide is eventually cleaved to yield a mature protein. Using the program, the existence and location of the *IL-1β* gene's signal peptide were predicted (Signal P 3.0 Sewer-prediction results, Technical University of Denmark). Disulfide bonds are required for protein folding and stability. The biologically active part of the protein is its three-dimensional structure. Di-sulfide bonds were predicted using suitable software (http://bioinformatics.bc.edu/clotelab/DiANNA/). Protein sequence-level analysis was employed (http://www.expasy.org./tools/blast/) for the assessment of the position of GPI anchor, and N-linked glycosylation sites. The molecule's N-linked glycosylation determines whether it is membranous or soluble. In the case of membrane protein, an N acetylation site was discovered (MNSLFT), whereas the GPI anchor is responsible for anchoring. The presence of a C-mannosylation site has been discovered, and it plays a crucial biological function in signalling and cell-to-cell adhesion (Loke *et al*., 2016). O-linked glycosylation sites were detected using NetOGlyc 3.1 server (http://www.expassy.org/), whereas the domain linker site and protein phosphorylation site, were assessed through NetNGlyc 1.0 software (http://www.expassy.org/). Prediction of alpha-helix and beta-sheet using Secondary Structure Predictions (Phyre 2).

Three-dimensional structure prediction and model quality assessment

A molecular visualization tool as PyMOL (http://www.pymol.org/) was employed for model generation and visualization of the three-dimensional structure of ovine *IL-1β* gene.

Protein-protein interaction network depiction

To understand the protein interaction network of IL-1β protein, we performed a search in the STRING 9.1 database**.** The functional interaction was assessed with a confidence score. Interactions with scores < 0.3, scores ranging from 0.3 to 0.7, and scores >0.7 are classified as low, medium, and high confidence, respectively. Also, we executed a KEGG analysis which depicted the functional association between the *IL-1β* gene and other related proteins.

Differential mRNA expression profiling for IL-1β *gene with respect to healthy and diseased sheep*

The sheep population under the current study was divided into two groups, diseased (infected with parasitic infection of *H. contortus*) and healthy (no infection), as assessed through egg per gram count.

Animals, faecal sample collection and détermination of FEC

We collected 60 sheep samples at random from the LFC farm, WBUAFS, and Mohanpur campus. The samples were taken before the regular deworming treatment and were suspected of having pre-existing gastrointestinal parasites because they had not been dewormed in the previous three months. During grazing, the animals were thought to have been exposed to a natural virus. The sheep's faeces were extensively analyzed using the salt flotation method, and the faecal egg count was tested for each sample. Based on statistical analysis, samples were collected from two groups, designated as healthy (Mean ± SD). Sheep were sold and slaughtered regularly for the mutton-producing unit. For the case study, a total of 12 tissue samples were tested, 6 with high FEC (grouped as diseased) and 6 with low FEC (grouped as healthy). Abomasum, rumen, small intestine, caecum, liver, and lymph node tissue samples were taken.

Hematological analysis

A blood sample was collected aseptically from the jugular vein of sheep in separate sterile vials with anticoagulants in the morning h between 8 a.m. to 10 a.m. 5ml sample was used to isolate DNA and stored at -20 °C until used for analysis, 2-3 ml blood for hematological parameters, 5 ml blood for serum without anticoagulant was collected centrifuged (10 min at 1000 rpm) and preserved at –20 °C until analysis.

The hematological parameters like total erythrocyte count (TEC), total leucocytes count (TLC) haemoglobin concentration (Hb), packed cell volume (PCV), total leukocyte count (TLC), and differential leukocyte count (DLC) were studied by standard methods (Jain, 1993).

Biochemical analysis

The serum biochemical parameters, studied in the experiments were liver function test, total protein, albumin,

globulin, albumin: globulin, alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, indirect bilirubin, and direct bilirubin, and kidney function test, glucose, uric acid, urea, and blood unea nitrogen (BUN) by using a semi-auto biochemistry analyzer (Span diagnostic Ltd.) with standard kits (Trans Asia Bio-Medicals Ltd., Solan, HP, India). The methodology used for the estimation of total protein, albumin, total and direct bilirubin, ALT, ALP, glucose, creatinine, urea, and uric acid were the biuret method, bromocresol green (BCG) method, 2- 4- DNPH method, modified kind and king's method, GOD/POD method, modified Jaffe's Kinetic method. GLDH-urease method and trinder peroxidise method, respectively.

Real-time PCR (RT-PCR)

An equal quantity of cDNA as quantified through Nanodrop was used in each reaction of a 96-well optical plate of the ABI 7500 system. Each reaction consisted of a 1ng cDNA template, 10 µl of 2X SYBR Green PCR Master Mix, 20pMol each of forward and reverse primers, and make up the final volume of up to 20 µl with nuclease-free water. Each sample was run in triplicate. Analysis of real-time PCR (qRT-PCR) was performed by the delta-delta-Ct (ΔΔCt) method, Ct denotes the threshold value. The list of primers used for the QPCR study has been listed below with an annealing temperature of 60 °C.

IL-1β primer:

F-CGTCTTCCTGGGACATTTTC,

R-GTCTGAGGATGGGCTCTGGG

18S rRNA primer:

F-TCCAGCCTTCCTTCCTGGGCAT,

R-GGACAGCACCGTGTTGGCGTAGA.

RESULTS

Molecular characterization of IL-1β *gene in sheep*

Sheep IL-1β gene cDNA was sequenced and derived amino acid was predicted. The sheep IL-1β gene was reported to be 978 bp with the derived peptide sequence being 266 aa. The IL-1β protein sizes of the different species vary between 267 aa in cattle, and dogs and 270 aa in humans.

Figure 1 illustrates the results of secondary structure and disordered protein prediction which showed that 13% of the protein was an alpha helix, 45% was a beta-strand, and 31% was a disordered protein. Figure 2 illustrates the helical shape of the *IL-1β* gene, which is represented as the structure of the *IL-1β* gene using Pymol.

Fig. 1. Secondary structure of *IL-1β* gene and disordered protein.

Fig. 2. 3D structure for IL-1β was predicted through Pymol with secondary structure, depicted as helix as cyan, loop as pink, sheet as red.

Protein-protein interaction network depiction and estimation of biological function

Figure 3 shows, using String analysis, how IL-1β interacts with other proteins in sheep. The related proteins revealed are IL-1A interleukin-1 alpha (produced by activated macrophages, IL-1 stimulates thymocyte proliferation), IL-1R1 (interleukin-1 receptor type 1 precursor), IL-1R2 (interleukin-1 receptor type 2 precursor), MYD88 (myeloid differentiation primary response protein MyD88; adapter protein involved with the toll-like receptor and TIR domain) IL-1RAP (interleukin-1 receptor accessory protein precursor), TNF (tumor necrosis factor, membrane form Intracellular domain 1 intracellular, NFKB1 (nuclear factor NF-kappa-B p105 subunit), IL α 18 (interleukin-18; augments natural killer cell activity in spleen cells and stimulates interferon gamma), TRAF6

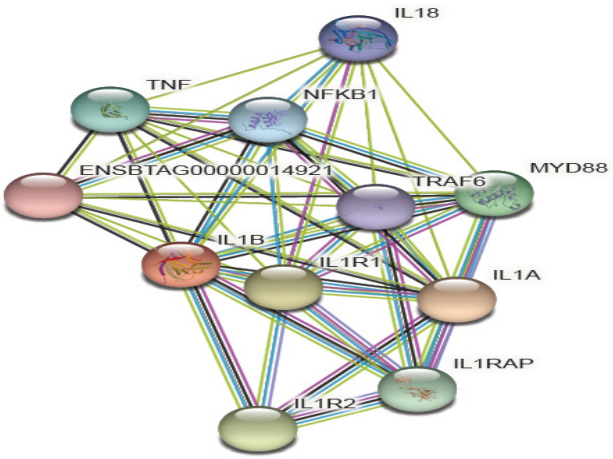

Fig. 3. Protein-Protein interaction network of *IL-1β* gene.

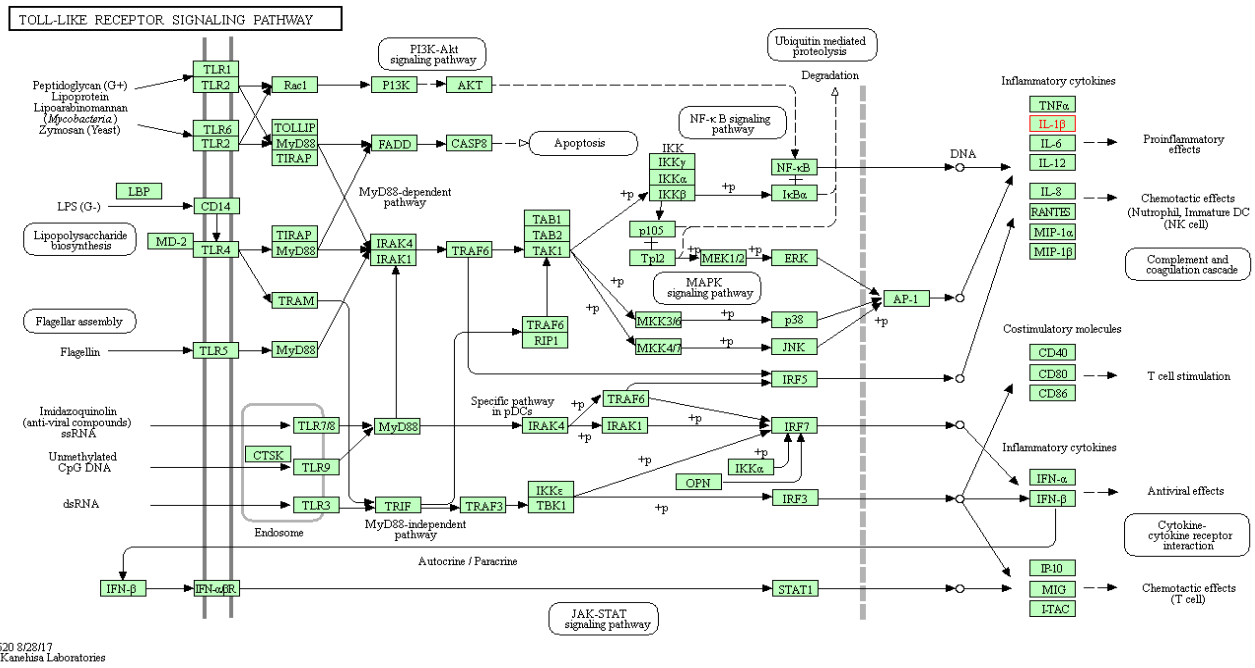

Fig. 4. Pathway for parasitic resistance of IL-1β.

(TNF receptor-associated factor 6; E3 ubiquitin ligase that, together with UBE2N and UBE2V1, all these proteins together play important role in interleukin-6 significantly pathway.

KEGG analysis

IL1-β acts through various biochemical pathways. In the current study, immunity against parasitic infection is important, hence the pathway for parasitic resistance has been depicted in Figure 4.

IL-1β *gene mRNA expression*

The differential mRNA expression profile of Garole sheep immune response genes with respect to disease and healthy conditions of parasitic infestation (*H. contortus)* is presented in Figure 5. The differential mRNA expression level of immune-responsive genes such as IL-1β, IL-6, IL-10, CD14, TLR-4, and TNF-α in healthy and diseased sheep, play a major role in immune regulation against parasitic infection (*H. contortus*).

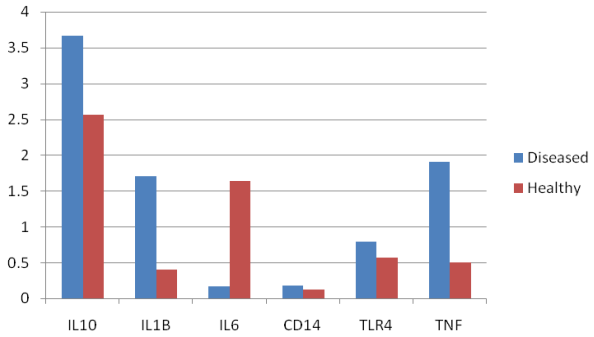

Fig. 5. Differential mRNA expression profile of sheep immune response genes with respect to diseased and healthy conditions of parasitic infestation (*H. controtus).*

Haematological and biochemical parameters with respect to healthy and infected sheep

The haematological parameters were assessed concerning diseased (*H. contortus* infected) vs. healthy sheep (Table I). In healthy and diseased sheep, respectively, the mean haemoglobin values were determined to be 10.37±0.18 and 7.96±0.19 g/dl, as indicated in Table I. In healthy and diseased sheep, respectively, the mean ESR values were 0.90±0.04 and 1.00±0.00 mm (Table I). While mean PCV values of 36.25±0.64 and 27.84±0.67% were observed in healthy and diseased sheep, respectively (Table I). In the current investigation, diseased sheep had significantly lower PCV levels than healthy sheep. Neutrophilia and eosinophilia may be responsible for the diseased sheep's higher total leukocyte count than the healthy ones.

Table I. Hematological parameters in diseased and healthy sheep.

Parameter	Healthy	Diseased
Hb (g/dl)	10.37 ± 0.19^{a}	7.96 ± 0.19^{b}
ESR (mm)	0.90±0.04	1.00±0.00
PCV (%)	36.25 ± 0.64^{a}	27.84 ± 0.67^{b}
TEC (mm^3)	$4.95\pm0.08^{a}x10^{6}$	3.9 ± 0.05^{b} x10^{6}
TLC (mm^3)	6587.50 ± 210.81^{b}	9200 ± 159.23^{a}
Neutrophil (%)	30.25 ± 0.59^{b}	53.50 ± 1.00^{a}
Eosonophil (%)	2.12 ± 0.44^{b}	16.62 ± 0.49^{a}
Basophil (%)	0.00	0.00
Lymphocyte (%)	60.87 ± 0.29^{a}	29.12 ± 0.29^{b}
Monocyte (%)	1.87±0.29	1.37±0.18

The values that have superscripts showed significant differences between groups. The superscript 'a' was showing high mean value than 'b'. Significant at ($p \leq 0.05$).

Table II. Liver function tests and kidney function tests in diseased and healthy sheep.

Parameters	Healthy	Diseased
Liver function tests		
Total protein (g/dl)	7.77 ± 0.03^{a}	6.11 ± 0.02^{b}
Albumin (g/dl)	2.80 ± 0.03^{a}	1.92 ± 0.04^{b}
Globulin(g/dl)	4.97 ± 0.06^{b}	4.18 ± 0.06^{a}
Albumin: Globulin	0.58 ± 0.01^{a}	0.46 ± 0.01^{b}
SGPT (IU/L)	32.12 ± 1.0^{b}	39.75 ± 1.4^{a}
SGOT (IU/L)	68.87 ± 2.7^{b}	86.87 ± 2.6^{a}
Alkaline phosphatase (IU/L)	64.87 ± 2.09^{b}	85.25 ± 3.89^{a}
Total bilirubin (mg/dl)	0.37 ± 0.03^{b}	0.67 ± 0.07^{a}
Direct bilirubin (mg/dl)	0.16 ± 0.01^{b}	0.30 ± 0.02^{a}
Indirect bilirubin (mg/dl)	0.21 ± 0.05^{b}	0.37 ± 0.09014^{a}
Kidney function tests		
Glucose (mg/dl)	68.62 ± 2.33^{a}	55.50 ± 1.21^{b}
Creatinine (mg/dl)	1.20 ± 0.02^{b}	1.71 ± 0.05^{a}
Uric acid (mg/dl)	0.76 ± 0.04^{b}	0.89 ± 0.01^{a}
Urea (mg/dl)	36.12 ± 1.56^{b}	48.37 ± 1.2^{a}
BUN (mg/dl)	16.87 ± 0.95^{b}	23.62 ± 0.73^{a}

The Mean values that have a superscript, was showing significant differences between groups. The superscript 'a' was showing high mean value than 'b'. Significant at ($p \leq 0.05$).
SGOT, Serum glutamic oxaloacetic transaminase; SGPT, Serum glutamate pyruvate transaminase, BUN, blood urea nitrogen.

Overall, significant differences were observed in Hb, PCV, TEC, TLC, neutrophil, eosinophil, and lymphocyte.

Better Hb, PCV, and TEC was observed in healthy sheep in comparison to that infected. However, total leucocyte count was pronounced in infected sheep. A marked significant increase in eosinophil count was observed in infected sheep. A similarly pronounced increase was observed in neutrophils and lymphocytes, indicative of involvement of immune responsiveness.

The comparative biochemical parameters are presented in Table II. Overall, the total protein, albumin, globulin, and albumin: globulin ratio was observed to be better in healthy sheep. Other reports indicate more in infected sheep (SGPT SGOT, alkaline phosphatase, total bilirubin, direct bilirubin, indirect bilirubin).

DISCUSSION

In India, livestock plays an important role in socio-economic development. Sheep is an important livestock species. In India, sheep contribute 12.7% of the total livestock population out of which 1.65% population of sheep from West Bengal (19th Livestock Census, 2014). Sheep can be reared for wool, meat, milk, carpet, and manure production. Its maintenance cost is low, no damage to trees, and its converter of waste feeds into profitable products and plays an important role in the livelihood of small farmers and landless labor. The major reason for the low productivity of sheep is inadequate grazing, and infectious and mainly parasitic diseases resulted in high economic loss. Indigenous sheep are mostly resistant to commonly occurring diseases of small ruminants. Some immune responsive genes may be responsible for disease resistant such as:-IL-β, IL-6, IL-10, TNF-α, CD-14, and TLR-4. It is of utmost necessity to explore the genes conferring immunity to the sheep. In this present study, the objective was to characterize the immune response genes in Indigenous sheep of West Bengal, the differential mRNA expression level of immune response genes in healthy and disease sheep (*H. contortus* infected sheep), and to study parasitological, hematological, and biochemical parameters.

Molecular characterization of the *IL-1β* gene was reported to be 978 bp with the derived peptide sequence being 266 aa. The signal peptide was predicted from 1-51 aa in sheep. In IL-1β of sheep, 30 sites were identified for O-linked glycosylation sites. N acetylation site was detected (MKSCTQ). The c-mannosylation site was observed to start from the 203 amino acid position. The predicted total no of cysteines 6 and number of bonds 2, cysteines form disulphide bonds at positions 82-91 and 126-154. The secondary structure prediction of IL-1β showed 13 % alpha-helical structure and 45% beta-strand. Disordered proteins were 31%. The differential mRNA expression level of immune response genes such as IL-1β, IL-6, TNF-α, Cd14, IL-10, and TLR-4 in healthy and diseased sheep (*H. contortus*). A present study revealed enhanced mRNA expression of the *IL-1β* gene, *TNF-α* gene, *Cd14* gene, *IL-10* gene, *TLR-4* and decreased expression of *IL-6* gene in disease sheep and decreased mRNA expression of *1β* gene, *TNF-α* gene, *Cd14* gene, *IL-10* gene, TLR-4 and increased mRNA expression of *IL-6* gene in healthy sheep.

In our current study, the mRNA expression level of the *IL-1β* gene in diseased sheep was high as compared to healthy as shown in Figure 5. IL-1β is a potent pro-inflammatory cytokine that is crucial for host-defence responses to infection and injury, it is a member of the 11 IL-1 family, and it is produced by activation of macrophages as a proprotein which is proteolytically processed to its active form, this cytokine is an important mediator of the inflammatory response and is involved in a variety of cellular activities including proliferation, differentiation, and apoptosis (Dinarello, 1996).

In the present study increased expression of IL-1β in disease was observed as compared to healthy sheep. Increased expression of IL-1β in disease sheep is in accordance with the finding observed in *Teladorsagia circumcincta* infected lamb, which showed increased expression of IL-1β, TNFα, and IFN-γ at 3-day post-infection (Hassan *et al*., 2011). Similarly, some more studies reported that upregulated expression of inflammatory cytokines such as TNF-α, IL-1β, and MIP-1α in fundic and pyloric abomasa after 7 days of post-infection and also concluded that increased expression of IL-1β associated with parasite expulsion (Li *et al*., 2007).

However, it was demonstrated that IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice were not able to induce TH2 immune response in *Trichuris muris* infection and concluded that IL-1β plays a very important role in producing efficient TH2 immune response against the parasite (Helmby and Grenics, 2004). Similarly, IL-1β can induce TH1, TH2, and TH17 respectively as reported**.** In gastrointestinal nematode infection, the intestinal epithelial cells respond to infection by releasing innate cytokines, such as IL-1β and thymic stromal lymphopoietin (TSLP) as these cytokines play a major role in the eradication of gastrointestinal nematodes.

Hence, IL-1β overexpressed in our present study maybe it is responsible for inducing IL-10, as it is observed that IL-1β is a pro-inflammatory cytokine, which stimulates IL- 10 production which plays a protective role in the reperfusion injury model (Souza *et al*., 2003). In studies, it was demonstrated that in *H.controtus* infection, elevated expression of IL-10, FCεR1A and early expression of TH1 and TH2 cells are involved in the immune regulation against nematode infection (Estrada-

Reyes *et al*., 2017). IL-10 produced from TH2 type cells is responsible for stimulating B cell differentiation, production of different immunoglobulins such as IgE, IgG1, IgG4, and IgA, mastocytosis, eosinophil activation, and function (Janeway *et al*., 2004). Activated eosinophils are responsible for expelling nematodes.

In the current investigation, a diseased sheep's haemoglobin level was significantly lower than that of a healthy sheep ($p \leq 0.05$). The current finding was consistent with previously reported results (Prakash and Bano, 2009; Shekh *et al*., 2018). The L4 larvae and adult worms drain blood from the animal's abomasum, causing anaemia and edoema, which may be the cause of the value decline. According to reports, *H. contortus* infection causes an average blood loss of 0.05 ml per day per worm (Urquhart *et al*., 2000). Non-significant differences between diseased and healthy sheep are revealed by the analysis of variance for ESR ($p \leq 0.05$). ESR did not significantly alter in the current research. The current findings of PCV were consistent with the previous findings (Shekh *et al*., 2018). The drop in PCV levels in infected sheep may be caused by bleeding from the abomasa as a result of parasite-induced wounds and by a delay between blood loss and the activation of the erythropoietic system to make up for blood loss (Dargie and Allonby, 1975). In the current study, a drop in TEC values ($p \leq 0.05$) in infected sheep may be caused by bleeding from the abdomen due to wounds created by the *H. contortus*, as well as by a delay between blood loss and the activation of the erythropoietic system to make up for blood loss (Dargie and Allonby, 1975). In comparison to healthy sheep, a significant rise in neutrophil counts was seen in diseased sheep. As the worm burden grows, neutrophil levels rise. It is recognized as a significant chemotaxis parasiticide effector and as a marker of efficient host defense against helminthiasis (Okoye *et al*., 2013). When compared to healthy sheep, a substantial rise in eosinophil count was found. An essential sign of helminth infection is an increase in blood eosinophils. Eosinophils and antibodies work together to combat parasites and assist the host get rid of them. Significantly fewer lymphocytes were seen in the diseased sheep in the current investigation. The reduction in lymphocytes may be brought on by the infiltration of these cells into other organs and the killing of lymphocytes in lymphoid organs (Sandhu *et al*., 1998). Similar results were observed in a study of sheep helminth infection (Sheikh *et al*., 2018; Okoye *et al*., 2013). Between healthy and diseased sheep, there were no discernible differences in basophil values, however, there were non-significant differences in monocyte values.

When compared to healthy sheep, there was a significant decrease in total protein levels. The current findings are consistent with those obtained in goats by Purohit *et al*. (2003). Since *H. contortus* is a blood-sucking parasite and damages intestinal mucosa, which results in inappropriate digestion and poor protein absorption, the lower amount of total protein in the infected animal may be caused by increased serum leakage (Radostits *et al*., 1994). Additionally, it was observed that an animal infected with *Haemonchus* had a mean daily feces clearance of 210–340 ml/day (Dargie and Allonby, 1975). Between healthy and diseased sheep in the current investigation, albumin levels were significantly reduced, as shown in Table II. A higher value was observed in healthy sheep than in diseased sheep. The current findings were consistent with those of Arora *et al*. (2003). Hypoalbuminemia may result from the selective loss of albumin that is smaller in size and more osmotically sensitive to fluid flow. It may also result from enhanced albumin catabolism and protein malabsorption owing to the damaged intestinal mucosa (Catchpole and Gregory, 1985).

In the current investigation, it was shown that diseased sheep had much lower levels of globulin than healthy sheep did. According to Dhanlakshmi *et al*. (2002), the major cause of hypoproteinaemia may be inappetence, which leads to a decrease in dietary protein intake and plasma losses from damaged intestinal mucosa (Purohit *et al*., 2003). In healthy and diseased sheep, there are appreciable variations in the A: G ratio ($p \leq 0.05$). In the current investigation, diseased sheep had significantly lower levels of the A/G ratio than healthy sheep. The current findings were consistent with those of Maiti *et al*. (1997). According to research, changes in albumin and globulin levels led to a decrease in the A/G ratio. In the current investigation, diseased sheep had significantly higher levels of total bilirubin than healthy sheep. The current findings were consistent with those of Zaki *et al*. (2003). Infected animals had much higher levels of direct bilirubin than healthy sheep. As demonstrated, there is a non-significant difference in indirect bilirubin levels between healthy and ill sheep (Table II). The current findings are consistent with Zaki *et al*. (2003). Hepatocyte injury and intestinal pathology might both contribute to an increase in bilirubin. Infected animals had significantly higher levels of SGPT and SGOT than healthy sheep. The current finding was consistent with Zaki *et al*. (2003). According to Sharma and Joshi (2001), in sheep infected with *H. contortus*, the large elevations in SGPT and SGOT levels may be caused by increased membrane permeability and abnormalities in hepatic function. When compared to healthy sheep, diseased animals had a much higher level of alkaline phosphatase. An increase in the alkaline phosphatase value may be caused by damaged intestinal mucosal cells as a result of parasitic pathogenesis in

GIT disorders because alkaline phosphatase is widely distributed throughout the body and is concentrated in bone, intestinal mucosa, renal tubules cells, liver, and placenta.

The glucose level in diseased sheep was much lower in our study than in healthy sheep, which was consistent with (Kumar *et al*., 2005) who found low glucose levels in goats infected with *Haemonchus* species. Significantly lower food intake and poor nutrient digestion brought on by parasite illnesses' gastrointestinal problems may also contribute to significant reductions in glucose levels (Hayat *et al*., 1996). Reduced bloodstream absorption and quick digestion and uptake of soluble fats and carbohydrates from the stomach by parasites. Diseased sheep show a significant increase in serum urea and uric acid levels. Impaired regulation of renal tubular transport may be the cause of the increased value of these two measures in sick sheep compared to healthy sheep (Hiranyachattada *et al*., 2000). Significant increases in serum BUN and urea levels in sick sheep compared to healthy sheep may be caused by liver damage from parasite infection and decreased control of renal tubular transport (Hiranyachattada *et al*., 2000).

CONCLUSIONS

In conclusion, the present study describes the molecular characterization of IL-1β in sheep and provides fundamental information necessary to progress the study of functional immune responses in this animal, which may be useful for understanding the antibacterial and antiparasitic immunity in sheep, allowing the immune response of these sheep to be studied in greater detail and provides the potential to use them to manipulate the immune response as recombinant proteins. The potential now exists for the production of IL-1β to test their usefulness in vaccine studies and to look more generally at their role in the immune response. Differential mRNA expression levels of immune response genes in healthy and diseased sheep described that differentially expressed genes such as IL-1 beta, IL-6, IL-10, TNF-α, CD14, and TLR4 have a major role in homeostasis maintenance and immune responses. Changes occurring in the sheep T lymphocytes, help to understand the general description of nematode immunity at the cellular level. These immune-responsive genes obtained here provide new research directions for the study of long-term resistance to *H. contortus* infection in sheep.

DECLARATIONS

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IRB approval

The research protocol was reviewed and approved by the Institutional Animal Ethics Committee of the West Bengal University of Animal and Fishery Sciences, Kolkata.

Ethical statement

The animal study was reviewed and approved by Institutional Animals Ethics Committee ,West Bengal University of Animal and Fishery Sciences.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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