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ORIGINAL ARTICLE

Hematological and serum biochemical parameters and profiling of cytokine genes in lumpy skin disease in Vrindavani cattle

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Abstract

Lumpy skin disease (LSD) is a notifiable re-emerging transboundary viral disease of bovines that inflicts heavy losses in affected livestock farms. Genetic variations contribute substantially to the inter-individual differences in the immune response against disease agents. The present study aimed to evaluate the genetic basis of differential immune response in Vrindavani cattle by comparing the hematological, biochemical and cytokine genes' expression profiles of LSD-affected and unaffected animals. After 21 days of the outbreak at the farm, the animals were grouped as affected (those who developed symptoms) and unaffected/healthy (those who did not). Standard hematological and biochemical parameters were evaluated in both the groups. Expression profiling of important *Th1* (IL2, INFG and GMCSF) and *Th2* (IL4, IL6 and IL10) cytokines was also performed via a relative quantification approach using real-time PCR. Erythrogram and leucogram analyses revealed significant differences in total leucocyte count (TLC: 14.18 ± 0.74 versus 11.38 ± 0.68 x10^3/μL), hemoglobin (Hb: 8.66 ± 0.42 versus 10.84 ± 0.17 g%) and percentage of neutrophils (46.40 ± 1.98 versus 35.40 ± 2.11%), lymphocytes (49.40 ± 1.99 versus 62.40 ± 1.86) and monocytes (4.20 ± 0.37 versus 2.40 ± 0.40) between the affected and healthy animals, respectively. The production of liver enzymes (SGOT and SGPT) was significantly higher in affected animals (74.18 ± 4.76 and 59.51 ± 2.75) when compared to the healthy counterparts (65.95 ± 9.18 and 39.21 ± 3.31). The expression profiling of *Th1* and *Th2* cytokines revealed significant differences between the two groups, except IL10. The expression of IL2, GMCSF and IL6 were upregulated in healthy animals while that of INFG, IL4 and IL10 were upregulated in LSD-affected animals. The highest abundance was observed for IL2 transcripts in healthy animals among all assessed cytokines with $\log_2$fold change of 1.61 as compared to affected counterparts. Overall, the immune response in healthy animals (after exposure to LSD virus) was predominated by the expression of *Th1* cell proliferation and there was an increased production of pro-inflammatory cytokines as compared to the affected counterparts. The results revealed that the effective immune response to LSD in cattle consists of changes in hematological and biochemical parameters and altered expression profile of cytokines with enhanced phagocytosis and lymphocyte recruitment. Furthermore, optimal expression of *Th1* cytokines is required for maintaining optimal health against infectious insult with LSD virus in cattle.

Keywords Cytokines · Immune response · LSD · Variation · Vrindavani

Introduction

India is bestowed with rich livestock inventory and abundant biodiversity of flora and fauna (Jacob et al. 2020) with more than 200 breeds of livestock registered with its nodal agency, i.e., National Bureau of Animal Genetic Resources (NBAGR). India is home to more than 302 million bovines (including 192.49 million cattle and 109.85 million buffaloes). However, the optimal animal productivity and farmer profitability are hampered by many factors including the increase in incidence of disease outbreaks (Perry and Grace

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2009). The recent disease outbreaks that occurred in Indian livestock included foot-and-mouth disease (2016) and African swine fever (2020) among others. These dreadful infectious diseases create havoc and severely hamper the economics of livestock production. Mostly, these diseases are highly infectious and affect livestock populations across state and national borders. Lumpy skin disease (LSD), caused by pathogenic organisms of *Capripox* genus, is one such dreadful re-emerging transboundary viral disease that is highly infectious and pathogenic to cattle and buffaloes (Kumar et al. 2021). It causes significant economic losses to the livestock industry (Hamdi et al. 2021).

LSD is classed as a notifiable disease by the World Organization for Animal Health (WOAH/OIE). Sheep pox and goat pox viruses are reported to exhibit antigenic similarity (~96%) and serological indistinctness with LSD virus (Tulman et al. 2002; Milovanović et al. 2019). The transmission of LSD across animals occurs mainly through blood-sucking arthropod vectors including stable flies and ticks (Hamdi et al. 2021). Direct contact between infected and susceptible animals is not considered a significant source of LSD infection (Neamat-Allah 2015). The morbidity rate in cattle and buffaloes is reported to be moderate (~20%) while the mortality varies from low to moderate levels (1–5%) with estimates quite higher in naïve populations exposed to LSD virus outbreaks (Arjkumpa et al. 2021). The economic losses incurred due to the incidence of LSD in any population are attributable to altered productivity and reduced reproduction performance (LSD causes abortion and sterility in males and females, respectively), damaged hides along with spendings on therapeutics and prophylaxis (Sudhakar et al. 2020). It has significant impact on animal product trade and food security aspects too (El-Mandrawy et al. 2018). Keeping in view the recent rapid transmission of LSD into naive areas and its serious consequences in terms of economic losses, it is important to assess various aspects of the disease to gain better insights into the pathogenesis, diagnosis and planning control strategies through efficient prophylactic and therapeutic measures (Jalali et al. 2017).

The outcome of any pathogenic insult of an individual is dependent on several factors that are grouped under three categories under the ambit of the epidemiological triad, i.e., host, pathogen and environment (Hueffer et al. 2011). The intricate interactions among various aspects of these agents decide the outcome of any pathogenic insult, i.e., whether an individual will develop disease or be resilient/tolerant to its effects. Similarly, the outcome of LSD infection varies depending on the immune and vaccination status of the animal, virus strain involved and prevalence of vectors in the environment (Sudhakar et al. 2020; Hamdi et al. 2021). Individuals' genetic makeup contributes significantly to the varied immune response against the same virus strain and environmental distribution of vectors in the microenvironment. Upon exposure of the population to pathogenic attack, certain individuals develop severe form of the disease while others remain tolerant and exhibit minor symptoms only. It is important to explore these individual variations so that genetic mechanisms involved in the varied immune response against a particular infection are better understood. Studying the genetic basis of disease resilience is highly challenging due to various factors that include ethical concerns, negative correlation with (re)production traits, sporadic occurrence of diseases and their low heritability (Gunia et al. 2018). However, upon natural outbreak and near-uniform exposure of animals to pathogenic agents, the genetic basis of immune response can be well understood.

LSD is spreading rapidly to naïve regions and populations across the globe. The immune response mechanisms against LSD infection in natural hosts are poorly understood. A thorough understanding of genetic mechanisms driving the optimal immune response against the pathogenic agent helps in designing meticulous disease control programs and the development of effective vaccines. India is home to 302 million bovines which are susceptible to disease outbreaks including LSD. Several crossbred strains have been developed across India that has helped it in reaching and retaining the topmost milk-producing nation status across the globe. However, these populations too remain susceptible to disease attacks. Vrindavani is a synthetic high-milch cattle population with inheritance from indigenous (Hariana) and exotic (Holstein Friesian, Jersey and Brown Swiss) breeds (Ahmad et al. 2020). Vrindavani cattle are known for high production and reproduction performance under tropical climatic conditions of India (Singh et al. 2011). Recently, LSD has affected animal populations in various Indian states including Odisha, Gujarat, Rajasthan, Punjab and Himachal Pradesh. It is important to understand the variation of immune response of cattle populations and understand its genetic basis to the finest detail. Deep insights into the host immune response to LSD infection, such as cytokines related to inflammatory processes, could be helpful as biomarkers to differentiate between the infected and tolerant/resilient animals. Keeping in view the above points, the present study was planned to study the hematology, serum biochemistry and expression profile of inflammatory cytokine genes of *Th1* and *Th2* cell lineages in Vrindavani animals after their exposure to the same pathogenic insult (LSD pathogen) under similar environmental conditions.

Material and methods

Population and sample collection

The present experiment was performed, during the LSD outbreak in India, on Vrindavani cattle population maintained

under organized conditions of nutrition, healthcare and management. The farm is located around 172 m above mean sea level with longitude and latitude coordinates of 79.44° E and 28.39° N, respectively. The animals experience tropical climatic conditions in upper Gangetic plains of India. The region shares land boundaries with several neighboring states including Uttarakhand, Bihar, Jharkhand, Chattisgarh, Madhya Pradesh, Rajasthan and Haryana along different directions. Besides, it also shares its boundary with one neighboring nation, i.e., Nepal. The farm is completely closed for the introduction of germplasm from external sources and follows artificial insemination for the propagation of gene pool across generations. Animals suffered with febrile conditions and nodular lesions, especially on neck area, resembling that of LSD. All the animals maintained in the farm did not develop these symptoms and some of them even maintained their (re)production performance. The vaccination status of animals against LSD on this farm was negative. The animals were routinely vaccinated for other diseases like brucellosis, foot-and-mouth disease and others.

Blood samples (5 mL each) were collected from affected and healthy animals under aseptic conditions via jugular venipuncture in sterile vacutainers containing no anticoagulant (for serum separation) and ethylenediamine tetraacetate (EDTA) as anticoagulant for isolation of nucleic acid material. Animals were classed as affected and healthy (unaffected) based on appearance of clinical symptoms on passing of 21 days after the detection of first case in the farm. Clinical symptoms that pointed toward the occurrence of LSD included appearance of nodular skin lesions along with pyrexia and decreased performance in affected animals. Tolerant healthy animals did not show any such symptoms or pyrexia. The whole blood samples were used for the assessment of hematological parameters in animals of both groups. Serum samples were obtained from whole blood through centrifugation method (3000 rpm for 10 min). The serum samples were stored at refrigeration temperature until they were processed for serum biochemical parameters. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples using the Ficoll-Histopaque density centrifugation method following the standard methodology. Subsequently, RNA was isolated from PBMCs using traditional TRIzol method (Rio et al. 2010). The RNA was converted to cDNA for further processing using commercial kit method. Concurrently, the clinical data on affected and resilient animals were also collected by qualified veterinarian stationed at the farm.

Hematological profile and serum biochemical analyses

The hematological profile studied in susceptible affected and unaffected healthy animals included hemoglobin (Hb), total leucocyte count (TLC), total erythrocyte count (TEC), packed cell volume (PCV), platelet count and percentages of neutrophil, lymphocyte and monocyte cells. Various serum biochemical parameters assessed for healthy and LSD-affected animals included blood urea nitrogen (BUN), creatinine, SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvate transaminase), total protein, albumin (A) and globulin (G) concentrations and A:G ratio, total bilirubin and direct bilirubin. The calcium and phosphorus content in serum were also assessed for affected and unaffected animals. Hematological analyses were done with automated hemanalyzer (URIT 3000 Vetplus, Chine) while the serum biochemical analyses were carried out with automated serum biochemistry analyzer (Chem 5X, Erba Mannheim biochemical analyzer) using commercially available kits.

Table 1 Details of mRNA primers used for studying expression patterns of cytokine genes

S. No.	Gene	Primer sequence	Product length
1	IL2	**F:** GCCCAAGGTTAACGCTACAG **R:** GGTTCAGGTTTTTGCTTGGA	104 bp
2	IL4	**F:** CCACACGTGCTTGAACAAAT **R:** TCAGCGTACTTGTGCTCGTC	107 bp
3	IL6	**F:** GCCTGAGAGCTATTCGGATG **R:** TAAGTTGTGTGCCCAGTGGA	104 bp
4	IL10	**F:** TGTTGACCCAGTCTCTGCTG **R:** TTCACGTGCTCCTTGATGTC	136 bp
5	GMCSF	**F:** AGAAGTGAAGCAGGCCAAAC **R:** TCCCTCCAGTGTGAAGATCC	108 bp
6	INFG	**F:** GGCTTTTGGGTTTTTCTGGT **R:** AGGCCCACCCTTAGCTACAT	110 bp
7	GAPDH	**F:** TGACCCCTTCATTGACCTTC **R:** GATCTCGCTCCTGGAAGATG	143 bp

IL2: interleukin 2; *IL4:* interleukin 4; *IL6:* interleukin 6; *IL10:* interleukin 10; *GMCSF:* granulocyte–macrophage colony-stimulating factor; *INFG:* interferon gamma; *GAPDH:* glyceraldehyde 3-phosphate dehydrogenase; *F:* forward primer sequence; *R:* reverse primer sequence; *bp:* base pair

Expression profile of cytokine genes

A total of six candidate cytokine genes from *Th1* and *Th2* cell lineages were selected for relative quantification using real-time PCR amplification. Primers were designed using Primer3Plus program and sequence information from NCBI (Table 1). Real-time PCR amplification was performed in five biological replicates each of two groups with AriaMx 3000P instrument (Agilent Technologies, CA, USA) using the Brilliant-III ultra-Fast SYBR Green qPCR master mix (Agilent Technologies, CA, USA). Each sample was run in triplicate in 20 µL reaction mixture consisting of cDNA (1 µL), Brillant SYBR Green master mix (10 µL), 1 µL each of forward and reverse primers with final volume made up with nuclease-free water. A generalized qPCR protocol was followed with an initial denaturation at 95 °C for 3 min followed by 40 cycles of denaturation at 95 °C for 5 s and a primer-specific annealing temperature step for 10 s. A no-template control (NTC) reaction was set up for each primer to monitor for the sample contamination and primer–dimer formation during amplification. Glyceraldehyde phosphate dehydrogenase (GAPDH) served as an endogenous control while control group served as the calibrator group.

Statistical analysis

The results from hematological, biochemical and gene expression profiling analyses were statistically analyzed using independent-samples *t* test in GraphPad Prism software. The threshold cycle (Ct) of a specific gene for each animal was taken as a mean of technical triplicates used in the experiment which was subsequently normalized using the mean of Ct estimates for threshold gene (endogenous control), i.e., GAPDH. The relative fold change in the mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) and log2 transformation was used to represent and compare fold changes for analysis. All data are expressed as mean ± standard error.

Results and discussion

Lumpy skin disease is an important transboundary viral disease of bovines, with significant socio-economic impact, that is rapidly spreading across naive (previously unaffected) regions of the globe. Genetic variations contribute substantially to the inter-individual differences in the immune response against disease agents within an animal population (Turner et al. 2011; Anacleto et al. 2019). Understanding the genetic basis of variable response, within individuals of a population, is destined to help take better decisions with regard to breeding of these animals and planning timely therapeutic interventions. The natural outbreak of LSD in animals and assessment of immune response provides better opportunity to gain deeper insights into the genetic basis of disease resilience in natural hosts (bovines for LSD) under near-uniform environmental conditions. Assessment of different aspects of an immune response against pathogenic agent using unnatural hosts and in vitro models is reported to be different from results under in vivo models using natural hosts (Urbina et al. 2003; Boraschi et al. 2021). The imperfect correlation between studies involving in vivo and in vitro models is due to host–pathogen interactions and the microenvironment within the host, which are missing under in vitro models (Rojas-Caraballo et al. 2014). Therefore, studies on various aspects of immune response in natural hosts gain immense significance. In the present study, we aimed to evaluate the differences in various hematological and biochemical parameters along with the expression profile of important *Th1* and *Th2* cytokines between LSD-infected and healthy cattle. Elucidating the hematological, biochemical and expression profile of cytokine genes provides an opportunity to better understand the immune functioning and response of animals against infectious pathogens.

The outbreak of disease in the population under the present experiment was confirmed based on clinical presentation of animals with high fever (pyrexia) and appearance of skin nodules. The appearance and thickness of skin nodules varied across affected animals; the number of nodules ranged from few to hundreds in affected animals while the nodules were absent in healthy animals. At few instances, the nodules coalesced in affected animals with serous or purulent discharge. Furthermore, the affected animals showed edema of the ventral part of abdomen along with palpable swollen lymph nodes. The appearance of clinical symptoms was in accordance with earlier scientific reports about the clinical presentation of the disease (Badr et al. 2022; Milovanović et al. 2019).

The present experiment tried to integrate the hematological, biochemical and immunological (both cell-mediated and humoral) components of tolerant and susceptible animals post-LSD outbreak. Innate immunity, though non-specific, forms the first line of defense in biological organisms and involves the complex functioning of many cell types (Romo et al. 2016). It helps in stimulating the cascades to effectively mount the immune response against infectious pathogens. Polymorphonuclear leucocytes (neutrophils) and monocytes play an important role in an effective immune response against pathogens. These cells are characteristic of inflammatory response and are involved in stimulating the release of cytokines, chemokines and other immunomodulatory molecules which are directly related to effective immune response and help control the spread and pathogenesis of microbial infection leading to the clinical form of disease (Chen et al. 2018). Erythrogram results revealed significant

Table 2 Average hematological parameters of susceptible (affected) and tolerant (unaffected) animals

S. No.	Parameter	LSD-affected	Unaffected post-LSD exposure
1	Hb g%*	8.66 ± 0.42	10.84 ± 0.17
2	PCV %	34.36 ± 1.84	34.65 ± 0.15
3	TLC × 10^3/μL*	14.18 ± 0.74	11.38 ± 0.68
4	TEC × 10^6/μL	7.02 ± 0.41	7.84 ± 0.17
5	Neutrophil (%)*	46.40 ± 1.98	35.40 ± 2.11
6	Lymphocyte (%)*	49.40 ± 1.99	62.40 ± 1.86
7	Monocyte (%)*	4.20 ± 0.37	2.40 ± 0.40

The estimates marked with asterisk (*) differed significantly at 5% level of significance ($p < 0.05$) between the two groups, i.e., LSD-affected ad LSD unaffected/healthy

Hb: hemoglobin; *PCV:* packed cell volume; *TLC:* total leucocyte count; *TEC:* total erythrocyte count

Table 3 Average serum biochemical and mineral parameters of susceptible (affected) and tolerant (unaffected) animals

S. No.	Parameter	LSD-affected	Unaffected post-LSD exposure
1	Total Protein	5.63 ± 0.86	5.93 ± 0.35
2	Albumin*	1.95 ± 0.07	2.91 ± 0.11
3	Globulin	3.69 ± 0.86	3.02 ± 0.39
4	A:G ratio	0.66	0.153
5	Total Bilirubin	0.21 ± 0.03	0.24 ± 0.02
6	Direct Bilirubin	0.37 ± 0.13	0.26 ± 0.05
7	BUN	12.57 ± 0.80	12.00 ± 0.44
8	Creatinine	1.04 ± 0.05	1.07 ± 0.07
9	SGOT*	74.18 ± 4.76	59.51 ± 2.75
10	SGPT*	65.95 ± 9.18	39.21 ± 3.31
11	Calcium	8.70 ± 0.48	9.19 ± 0.34
12	Phosphorus	8.57 ± 0.68	7.81 ± 0.17

The estimates marked with asterisk (*) differed significantly at 5% level of significance ($p < 0.05$) between the two groups, i.e., LSD-affected ad LSD unaffected/healthy

A: albumin; *G:* globulin; *BUN:* blood urea nitrogen; *SGOT:* serum glutamic oxaloacetate transaminase; *SGPT:* serum glutamic pyruvate transaminase

differences ($p < 0.05$) in total leucocyte count (TLC), hemoglobin (Hb) and percentage of neutrophils, lymphocyte and monocytes between the affected and healthy groups (Table 2). The TLC and the percentage of neutrophils and monocytes were higher in affected animals as compared to the healthy ones. These results are indicative of enhanced phagocytosis and lymphocyte recruitment in LSD-affected animals. Similar to our findings, El-Mandrawy et al. (2018) reported significant differences in TEC and hemoglobin levels of LSD-infected animals when compared to healthy ones. However, the lymphocyte count was higher in healthy animals (62.40%) as compared to LSD-affected counterparts (49.40%). The results were in accordance with Neamat-Allah (2015) who reported lower estimates on erythrogram analysis in affected animals, mainly due to hemolytic anemia. The altered erythrogram and leucogram profiles in affected animals has been attributed to the effects of viremia and corticosteroid production in affected animals as part of the host response to infection (Ismail and Yousseff 2006). Altered erythrocyte counts are also attributed to dehydration status of affected animals and absolute erythrocytosis (El-Mandrawy et al. 2018). Altered leucocyte count, being lower in affected animals, is also attributable to the increased demand and migration of immunologically important cells to tissues (Jalali et al. 2017). Similarly, on serum biochemical analysis, significant differences ($p < 0.05$) between healthy and LSD-affected animals were observed for AST (SGPT), ALT (SGOT) and albumin levels (Table 3). The concentration of AST and ALT liver enzymes was higher in LSD-affected animals (65.95 and 74.18, respectively) than healthy counterparts (39.21 and 59.51, respectively). These changes were indicative of altered liver metabolism due to an inflammatory state or hepatic damage due to viremia (Sevik et al. 2016). Similar to our findings, El-Mandrawy et al. (2018) reported significant differences in blood albumin levels between LSD-affected and healthy animals. The serum levels of bilirubin and creatinine were not significantly different between LSD-infected and control (healthy resilient) animals. An altered serum biochemical profile has been attributed to distorted liver function leading to decreased protein synthesis and increased catabolic rate in affected animals (El-Mandrawy et al. 2018). Altered production of liver enzymes in LSD-affected animals is, therefore, attributable to muscle damage occurring due to diseased pathogenesis.

Several studies have reported the involvement of both components of the immune system, i.e., cellular (cell-mediated) and humoral (antibody-mediated) for effective response against pox viral infections including LSD (Babiuk et al. 2009; Abdelwahab et al. 2016; Milovanović et al. 2019). *Th1* cytokines are mainly responsible for cellular immune response, involved mainly in the inhibition of macrophage function and stimulation of immunoglobulin production from B-cells. On the other hand, the *Th2* cells are involved in functioning of the humoral immune response and are responsible for the proliferation of B-cells and production of antibodies (Skapenko et al. 2005; Raphael et al. 2015; Li et al. 2019). In order to gain maximum insights into the differential immune response of tolerant and susceptible individuals of the same population, three cytokines each from *Th1* and *Th2* immune systems were selected. In the present study, cytokines from *Th1* (IL2, INFG and GMCSF) and *Th2* (IL4, IL6 and IL10) cell lineages were selected to assess their real-time expression in healthy and LSD-affected animals after the outbreak in the farm. These cytokines play an important

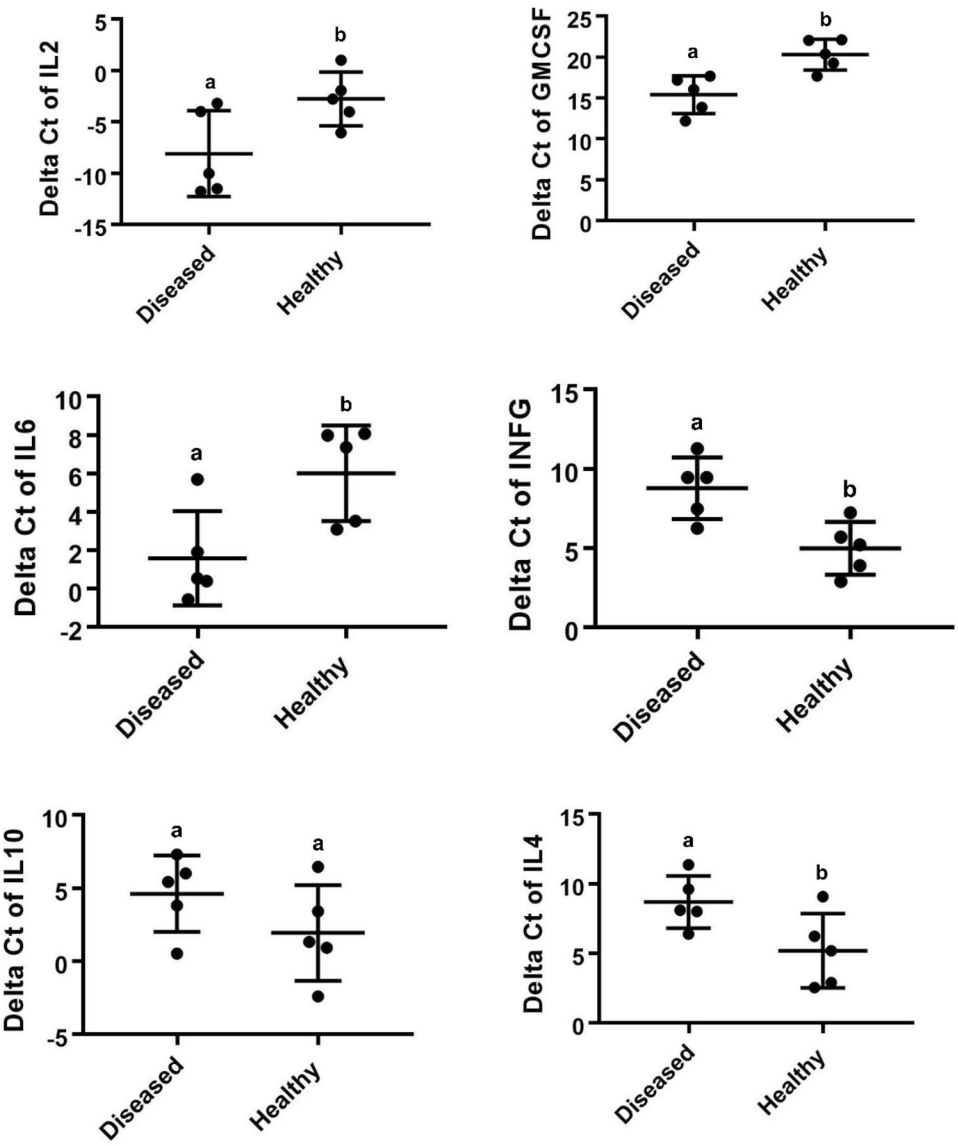

Fig. 1 Dot plot showing the dispersion of ΔCt value of the *Th1* and *Th2* cytokines in LSD-affected (diseased) and tolerant/healthy animals. The expression level of IL2, GMCSF, INFG, IL6 and IL4 cytokines differed significantly between the healthy and diseased groups at 5% level of significance. Groups with different superscripts differed significantly from each other for a specific cytokine

role in mounting an effective innate and adaptive immune response in terms of the humoral and cellular components against external pathogens. The fold change of IL2, GMCSF, INFG, IL6 and IL4 were significantly different between healthy and susceptible affected counterparts (Fig. 1). Highest upregulation with a $\log_2$fold change of 1.61 was revealed for IL2 in healthy animals when compared to the affected ones. IL2 is a pro-inflammatory cytokine that has been reported to be a key regulator of immune response by regulating the growth and differentiation of T-lymphocytes (D'Souza et al. 2002; Liao et al. 2013; Ross and Cantrell 2018). Its functioning is modulated by boosting the natural killer cell activity, enhancing immunoglobulin formation and maintaining the homeostatic proliferation of various immune cells (Malek 2003; Wang et al. 2018). The increased IL2 production in healthy animals was indicative of the potent inflammatory response developed in animals against LSD infection. Similar to IL2, GMCSF showed significant upregulation ($p < 0.05$) in healthy animals when compared to the affected ones. The primary role of GMCSF is to help in growth and differentiation of specialized immune cells, i.e., granulocytes and macrophages (Bhattacharya et al. 2015; Lotfi et al. 2019). Furthermore, it modulates the activity of dendritic cells and helps in enhancing the functioning of T-cells (Shi et al. 2006). GMCSF has been reported to promote inflammatory reaction in animals by eliciting of production of other pro-inflammatory cytokines. It is also involved in inhibition of the secretion of anti-inflammatory cytokines from specialized immune cells, especially of myeloid lineage (Hamilton 2020; Petrina et al. 2021) and plasminogen-dependent fibrinolysis (Bhattacharya et al. 2015). GMCSF plays an important role in countering the

pathogenic insult of animals with the LSD virus, especially during the early stages of infection by promoting the production of pro-inflammatory cytokines and inhibiting the secretion of anti-inflammatory biomolecules. Similarly, the expression of IL6 was significantly upregulated ($p < 0.05$) in healthy animals when compared to susceptible ones with a $\log_2$fold change of 1.33. Several studies have reported the essential role of IL6 in mounting of an effective immune response against viral pathogens (Velazquez-Salinas et al. 2019), especially during early infection (Vitenberga-Verza et al. 2022). IL6 is a pleiotropic molecule that is produced from multiple cell lineages and is involved in stimulating the cascades related to JAK/STAT3 pathway that subsequently leads to the activation of several genes associated with immunomodulation involving both pro- and anti-inflammatory chemokines (Morris et al. 2018; Yang et al. 2021). In the present study, the upregulation of IL6 transcripts in healthy animals is indicative of its immunomodulatory role in mounting of the effective immune response against LSD virus attack in cattle.

On the other hand, the transcripts of INFG were significantly downregulated ($p < 0.05$) in healthy animals when compared to LSD-affected counterparts. INFG is considered a component of *Th1*-driven immune reaction with pro-inflammatory properties (Berger 2000; Kak et al. 2018). INFG has been reported to produce its immunomodulatory effects through excessive tissue damage, necrosis and increased inflammatory response (Karki et al. 2021). It has also been reported to contribute to disease pathology. In LSD-affected animals, increased production of INFG could have been responsible for the exhibition of exaggerated symptoms that resulted from increased necrosis and tissue damage. It is considered a double-edged sword wherein a balance has to be maintained between mounting an effective immune response to control infection and limiting the advancement of disease pathology (Kak et al. 2018). INFG has also been reported to modulate a range of functions including activation of immune cells and stimulation of immunoglobulin release in viral functions (Chesler and Reiss 2002).

Non-significant differences were revealed between healthy and LSD-affected animals with reduced (downregulated) expression of IL10 transcripts in healthy resilient animals as compared to susceptible counterparts maintained under the same environmental conditions. The expression of IL10 has been reported to inhibit the production of various cytokines from different types of immune cells. The downregulated expression of IL10 in healthy animals was in line with findings of elevated secretion of pro-inflammatory cytokines. IL10 has been reported to impede pathogen clearance and worsen the disease pathology (Couper et al. 2008). Increased production of IL10 in LSD-affected animals was responsible for increased tissue damage as evident from the symptoms and their prolonged immunopathology. Furthermore, the profiling of IL4 transcripts revealed significant differences between healthy and LSD-affected animals. Its expression was downregulated in healthy animals as compared to the diseased ones. The expression of IL4 transcripts is involved in the promotion of *Th2*-driven immune response along with lymphocyte development and leucocyte survival in various pathophysiological states (Gadani et al. 2012). It is also involved in the inhibition of *Th1*-driven cellular response and reduced production of proinflammatory cytokines. It is a potent regulator and plays a central role in shaping the immune response against disease-causing pathogens (Chen et al. 2011). It has also been reported to play an important role in tissue repair and homeostasis via the activation of alternative macrophage activation pathways (Gadani et al. 2012). In LSD-affected animals, IL4 could be involved in tissue repair once the disease has set in. The higher expression of IL4 transcripts in LSD-affected animals was in line with the reports that it affects disease pathogenesis leading to delayed virus clearance in many diseases (Moran et al. 1996; Chen et al. 2011). The results of hematological and biochemical profiles should ideally be inferred in totality and not in isolation. The hematological, biochemical and cytokine profiles are important parts of the inflammatory process which helps in maintaining the effective homeostatic static inside living organisms, especially against disease-causing pathogens. Overall, the changes in profiles are intricately related to each other involving a complex cascade of events (Jalali et al. 2017).

The results of the present study form a base to better understand the mechanisms that are responsible for the differential clinical course of the pathogenic agent in individuals of a single population. These results help gain better insights into the genetic mechanisms of disease resistance via the immunogenetics approach. The findings of the present study will be helpful in the early detection of LSD-affected animals via the hematological and biochemical analysis which may, in turn, also assist in stratifying the tolerant and susceptible animals. The findings from the relative cytokine expression will be useful for the identification and stratification of the two groups. Furthermore, the relative cytokine profile of tolerant and susceptible animals may be exploited in vaccine development against LSD disease in natural hosts, besides using the information from experiments on laboratory animals and in vitro studies.

Overall, the results revealed that the effective immune response to LSD in cattle consists of changes in hematological, biochemical and expression profile of cytokines with enhanced phagocytosis and lymphocyte recruitment. The immune response in healthy animals (after exposure to the LSD virus) was predominated by the expression of enhanced *Th1* cell response and increased production of pro-inflammatory cytokines when compared to LSD-affected counterparts.

The highest abundance was observed for IL2 transcripts in healthy animals among all assessed cytokines. Furthermore, optimal expression of *Th1* cytokines is required for maintaining optimal health against infectious insult with LSD virus in cattle. The effective immune response against pathogen insult to animals involves an intricate pattern of expression of cytokines, chemokines and other immunomodulatory biomolecules.

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Data availability statement The data supporting the findings of this study are presented in various tables and figues and is available within the article.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

Ethical approval All protocols and experimentation in the present study were approved by institute animal ethic committee (IAEC) of ICAR-Indian Veterinary Research Institute in line with the national standards. The animals were maintained under standard managemental support and welfare conditions.

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