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Original Article

Investigation of patho-bacteriological, serum bio-hematological, oxidative stress and antioxidant biomarkers due to pneumonic pasteurellosis caused by *Pasteurella multocida* in cattle

Fayyaz Ahmad[1], Muhammad Shahid Mahmood[1*], Riaz Hussain[2*], Sajjad Ur Rahman[1], Rao Zahid Abbas[3]
[1]Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan
[2]Department of Pathology, Faculty of Veterinary and Animal Sciences, Islamia University of Bahawalpur, Pakistan
[3]Department of Parasitology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan

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**Corresponding author email:*
dr.riaz.hussain@iub.edu.pk

Abstract

Hemorrhagic septicemia (HS) caused by *Pasteurella multocida* (*P. multocida)* is one of the leading causes of bovine respiratory problems in dairy animals. Therefore, identification of reliable and useful biomarkers of naturally occurring HS disease is of vital important for early diagnosis and monitoring of efficacy of treatment. Therefore, the current study was conducted to estimate the status of hematology, serum biochemistry, oxidative stress and antioxidant enzymes in dairy cattle (52) in and around district Bahawalpur. Deep nasal swabs and jugular blood was collected from normal and morbid animals showing signs of respiratory distress for molecular characterization of *P. multocida*. Different visceral tissues were obtained from animals died of respiratory signs for histopathological investigations. Results revealed significantly ($P < 0.05$) increased quantity of oxidative stress biomarkers while different antioxidant enzymes decreased significantly in erythrocyte of infected animals. Results on hematology revealed significantly increased total leukocyte counts and neutrophil counts while significantly decreased hemoglobin, hematocrit, monocyte, lymphocyte and erythrocyte counts. Results on serum biomarkers showed significantly ($P < 0.05$) increased quantity of different serum profile in *P. multocida* positive cattle. Grossly, lungs were congested, hyperemic and consolidated. Frothy exudate was observed in trachea of *P. multocida* infected cattle. Results on microscopic observation showed different pathological lesions in lungs, liver, heart and kidneys of *P. multocida* confirmed cases. Based on the results of our study, it can be suggested that continuous monitoring of disease is necessary to lower the prevalence of infectious agent. It is also suggested that blood biochemistry, oxidative stress and antioxidant oxidant enzymes are useful and reliable tools to clarify the pathogenesis of disease for proper therapeutics.

Keywords: Hemorrhagic septicemia (HS), *Pasteurella multocida*, Hemato-biochemistry, Oxidative stress, Antioxidant enzyme, Histopathology

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Introduction

Livestock production in Pakistan plays crucial role for the life of people and national economy (Khan et al., 2021). In Pakistan, approximately 8.8 million human populations are involved in rearing dairy animals particularly buffaloes and cattle (Ullah et al., 2021). Pakistan is ranked at number 4th on the basis of total milk production (59.759 million tons) in the world with an estimated head of 40 million buffaloes, 47.8 million cattle, 30.9 million sheep, 76.1 million goats and about 1.1 million of camels are directly involved in the supply of milk (Khan et al., 2011; Hussain et al., 2017; Hussain et al., 2018; Hussain et al., 2020). In southern Punjab Pakistan, the Cholistani cattle, camels, buffaloes, sheep and goats are considered as the major meat and milk production sources. However, the dairy animals encounter different bacterial diseases (Hussain et al., 2016; Anwar et al., 2022; Du et al., 2022), viral diseases (Jalees et al., 2022), parasitic diseases (Bhutta et al., 2022; Rabah et al., 2022) and various zoonotic infections (Rabah et al., 2022) during different livestock operation systems and different environmental conditions (sub-tropical and tropical). Livestock industry is facing huge economic losses due to infectious and non-infectious diseases such as foot and mouth disease, hemorrhagic septicemia, black quarter, tuberculosis and mastitis. Among these diseases one of the most important diseases is hemorrhagic septicemia (HS) which is caused by *P. multocida. P. multocida* is a non-motile, gram negative and facultative anaerobic bacterium which is divided into different serotypes on the basis of capsular and lipopolysaccharide (LPS) antigens (Siddaramappa, 2021; Smith et al., 2021). Among these serotypes, HS is mainly caused by serotype B2 and E2 (Kang et al., 2019; Muenthaisong et al., 2021; Abodalal and Ismail, 2023). HS causes high economic losses as compare to foot and mouth disease (FMD) due to its high mortality (Cuevas et al., 2020), which in fact results in reduced productivity of meat and milk. Economic losses due to HS are greater than 2.17 billion rupees in Punjab. Increased prevalence of this disease results in culling of animals from the dairy herds. The percentage prevalence of HS in Bahawalpur, Multan and DG khan districts was recorded as 25.5%, 17.51% and 3.5% respectively. Diagnosis of the disease at early stages is difficult which makes it more complicated and organism tend to establish in later phase and thus led to heavy economic losses in terms of low production and mortality (Kang et al., 2019). For the early diagnosis of the disease, acute phase proteins (APPs) are helpful. Initial screening is carried out through isolation and estimation of some specific acute phase proteins. Morbidity, case fatality ratio and mortality due to HS are 12.56%, 40.44% and 22.44% in buffaloes. HS is considered as a list B disease according to OIE and cause 100% mortality in Africa and Asia (Cuevas et al., 2020).

Different earlier studies have reported that physiological stress contributes important and necessary role to induce bovine respiratory infections (Crosby et al., 2022; Sayed et al., 2023) which can overcome the immune responses of the animals ultimately leading to rapid proliferation and replication of infectious agents in the nasopharynx. *Pasteurella multocida* bacteria includes wide range of its hosts and causes severe respiratory lesions particularly in lungs (Alarawi and Saeed, 2021). The most commonly observed lesions due to HS due to *P. multocida* bacteria including necrosis, degeneration, hemorrhages and inflammatory cells, presence of hemorrhages in lungs, kidneys and liver of the rats have been observed (Chung et al., 2017). At histological observations, presence of hemorrhages, degeneration, and severe inflammatory cells in the buffalo calves (Abubakar et al., 2013) receiving the *P. multocida* through the intramuscular route have examined. Moreover, calf getting the *P. multocida* through the intratracheal route showed more severe lesions in respiratory tract but the calves that receive the causative agent of hemorrhagic septicemia through the oral route, exhibit very severe lesions in the digestive tract (Chung et al., 2016). Although a lot of published research is available for *P. multocida*, but scarce information is documented for its pathogenicity and its effects on histopathological, hematological, immunological and biochemical parameters in Cholistani cattle reared in desert conditions. In published literature, no information is available regarding the status of oxidative stress and antioxidant enzymes in erythrocyte of cattle infected with *P. multocida*. In order to overcome this gap, the current study aims for the isolation, identification, molecular characterization of *P. multocida* and its effects on the hematology, oxidant-antioxidant biomarkers and serum biochemistry in healthy and infected cattle.

Material and Methods

Study animals

The present study was conducted on cattle housed at harsh desert conditions of Cholistan. Thirty healthy

cattle were selected as a control from the desert of Cholistan to obtain normal data for various parameters while 22 clinical cases of cattle infected with hemorrhagic septicemia disease were included in this study. The diseased cattle were subjected to detail clinical examinations and were provided with proper treatments. Blood samples were taken for the serum biochemistry analysis and to study the different hematological parameters. Blood samples (cardiac blood), nasal swabs and various tissues were collected for the isolation, identification and molecular characterization and confirmation of the etiological agent of hemorrhagic septicemia disease. Despite proper treatment, management and care diseased cattle did not recover and died within 24 h after the onset of clinical signs and were subjected to detail postmortem examination.

Culturing and confirmation of bacterial agent

Sterile cotton swabs were used to collect the deep nasal discharges from the healthy and morbid cattle showing the clinical signs and symptoms of the respiratory distress. All the swab samples were inoculated on brain heart infusion broth and incubated at 37°C for 24 h for the culturing of bacterium. The growth from the broth was streaked on the brain heart infusion agar and then on 5% sheep blood. Culture was further inoculated on different selective and specific media like MacConkey agar and blood agar to observe the morphology and hemolytic properties of infectious agent.

Identification of isolates

Initially the infectious agent was identified on the basis of colony size, morphology, colony characteristics and hemolysis pattern. Different biochemical tests such as indole production, nitrate reduction, Voges Proskauer test, H_2S production, citrate utilization, methyl red, urease activity and production of acid from dulcitol, galactose, lactose, sucrose, glucose, mannitol, maltose, mannose and sorbitol, sugars were conducted for identification of isolates (Pillai et al., 2013). Finally, confirmation of the bacterial agent was done by using previously specified forward and reverse primers for DNA amplification of *P. multocida* such as KMT1 T7 5-′ATCCGCTATTTACCCAGTGG-3′ and KMT1 SP6-R 5′- GCTGTAAACGAACTCGCCAC -3′ were used for all the serotypes of *P. multocida*. For the HS specific serotype specified forward and reverse primers KTT 72- F 5´-AGGCTCGTTTGGATTATGAAG-3 and KTSP 61-R 5´- ATCCGCTAACACACTCTC-3´. DNA amplification procedure including initial denaturation (94 °C for 5 minutes), primers annealing (55 °C for 57s), extension (55 °C for 1 minute), denaturation (94 °C for 1 minute), final extension (55 °C for 10 minute), and staining was carried according to the earlier protocols (Jogi et al., 2020; Narcana et al., 2020).

Hematological analyses

Blood samples were collected from the heathy, and diseased cattle from the jugular vein under aseptic conditions in vacutainers with and without anticoagulants (EDTA; 1.00mg/ml). Serum was separated from the blood samples by following the procedure as described (Degla et al., 2022) and stored at -20°C. Different hematological parameters such as white blood cell counts, differential leukocyte counts, and erythrocyte counts and hemoglobin concentration were determined.

Serum biochemical parameters

Serum was separated from blood collected without any anticoagulant on ice for different serum biochemical parameters such as urea (Cat # 996060), total protein, alanine aminotransferase (ALT; Cat # 30254), aspartate aminotransferase (AST; Cat # 30243), alkaline phosphatase (ALP; Cat # 30134), creatinine Cat # 99108, creatinine phosphokinase and lactate dehydrogenase (LDH; Cat # 21213; Analytical, Germany) determined from the serum using chemistry analyzer (Model: Rx Monza # 328-15-1001 according to previous techniques (Hussain et al., 2022). All the analytical grades chemicals used in this study were purchased from Merck (Germany) and Sigma Aldrich (St. Louis Missouri, USA).

Oxidant and antioxidant assessment

Oxidant-antioxidant biomarkers were investigated in term of recording of the concentration of malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO) and superoxide dismutase (SOD) in erythrocyte of infected and healthy cattle (Hussain et al., 2022).

The blood was centrifuged to obtain the red blood cells from it. The supernatant was discarded, and packed cells were washed with normal saline solution thrice to determine the various antioxidant enzymes in erythrocyte. The washed RBCs were hemolyzed by adding ice-cold water 9 ml to make the 10% red blood cells hemolysate. Antioxidant status were determined

in RBCs hemolysate by adding thiobarbituric acid by following the previous method (Esmaeilnejad et al., 2018) which result in the formation of colored complex. Lipid peroxidation in hemolysate of erythrocyte was determined as MDA nm/g of hemoglobin. The concentrations of catalase, SOD and reduced glutathione quantity was measured in the erythrocytes hemolysate by following the previous method (Hussain et al., 2022).

Macroscopic examination

Postmortem of the cattle that died due to HS disease during the study period was conducted soon after the death of the animals to observe various gross pathological lesions on the various organs.

Microscopic examination

For histopathological lesions, various tissues were obtained from the different visceral organs including lungs, liver, heart, spleen, intestine, and trachea. Tissues collected from the various organs were fixed in 10% neutral buffered formalin. Tissues fixed in the formalin were processed further to observe the histopathological lesions by following the routinely used procedures like dehydrations, embedding, sectioning, mounting and staining. Thick sections of the tissues, 4-5 µm were cut and stained by the Hematoxylin and Eosin staining techniques.

Statistical analysis

The data obtained in current study were expressed as means ± SE. The overall comparison of data on blood and serum biochemistry were compared using t test. The comparisons of data on hematological, oxidant and antioxidant parameters on the basis of different scores were conducted using one-way analysis of variance (ANOVA; SPSS statistical software program) and Duncan test. $P < 0.05$ was adjusted as statistically significant.

Results

Confirmation and identification of *P. multocida*

P. multocida bacterium was identified on the basis of round, sticky, mucoid, white in color, slightly elevated colonies 2-3mm diameters, non-hemolytic and shiny colonies on blood agar. Absence of growth was observed on the *MacConkey agar and SS agar*. Upon Gram staining, reddish-pink coccobacilli were observed suggestive of presence of gram-negative organism. Bipolar nature of the organism was confirmed with Giemsa staining. Different biochemical tests including triple sugar iron (TSI), indole, oxidase, catalase, nitrate reduction, urease was positive for the specific pathogen and citrate utilization test was negative. Finally, the bacterial agent (Figure 1) was confirmed using molecular techniques (Jogi et al., 2020; Narcana et al., 2020).

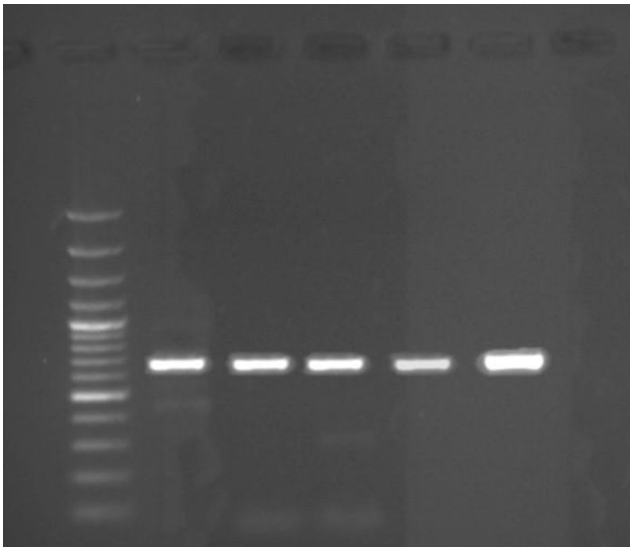

Figure-1. Photograph showing confirmation of *P. multocida* infection using specific primers (KTT 72 and KTSP 61) in cattle died of pneumonic pasteurellosis. DNA Marker =100 bp; Positive samples (PCR product approximately 620 bp). Ethidium bromide stain

Clinical signs observed in infected cattle

The scoring of various clinical ailments is indicated in table 1. Cattle suffering from the hemorrhagic septicemia were dull, depressed and exhibited increased body temperature. Bilateral copious mucopurulent nasal discharge, repeated spontaneous cough, bilateral extensive ocular discharge was observed in the cattle. At the terminal stage of the disease, open mouth breathing, typical respiratory sounds (crackles and wheezes), and hyperpnoea, anorexia, dyspnea, tachycardia, pyrexia, tachypnea, abdominal breathing, mild submandibular edema, recumbence, and subnormal temperature were most frequently observed. Rumen motility was also reduced in the affected animals.

Table-1. Different clinical signs and scoring parameters for selection of cattle infected with respiratory disease

Parameters	Clinical score			
	0	1	2	3
Rectal temperature (°C)	37.8–38.1	38.2-38.9	39.0-39.5	≥ 39.6
Nasal discharge	Normal	unilateral cloudy discharge	Bilateral excessive mucoid	bilateral Copious mucopurulent
Cough	Normal	Single cough	Repeated coughs	Repeated spontaneous coughs
Ocular discharge	Normal	Mild ocular discharge	Moderate amount of bilateral discharge	Bilateral extensive ocular discharge
Salivation	Normal	Mild without froth	Moderate with moderate foamy froth	Severe frothy discharge
Respiratory Sound	Normal	Normal	Mild sneezing and cough	Mild sneezing and cough
Submandibular edema	Normal	Normal	Normal	Mild

Gross pathological lesions

Postmortem examination of cattle died of hemorrhagic septicemia revealed submandibular edema, brisket edema, degeneration of masseter muscle, hydropericardium, hydroperitonium, lymph adenopathy, acute fibrino-hemorrhagic bronchitis, acute rhinitis, acute fibrinous pleuropneumonia, fibrino-hemorrhagic hepatitis, acute colitis, proctitis and muco-hemorrhagic enteritis. Trachea was red in color and contained exudate. Tracheal mucosa was reddened with sloughing of epithelium and had frothy straw-colored fluid.

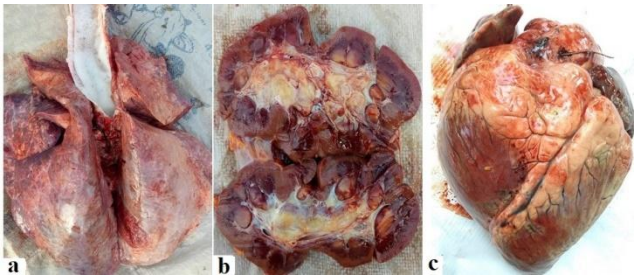

Figure-2. Photograph showing congestion, red hepatisation, edema in lungs and presence of frothy exudate in trachea, congestion, dark black color of kidneys and congestion, petechial myocarditis in cattle died of *P. multocida* infection.

Lungs were remarkably congested, discolored look like liver in consistency, consolidated, hemorrhages and thickening of alveolar septa were seen. Lungs were consolidated and firm in consistency, ribs impressions were present on the surface of lungs, petechial hemorrhages were seen on both right and left cranial lobes of lungs. Fibrinous broncho- pneumonia was observed in lungs of morbid cattle (Figure 2a). The kidneys of infected cattle were severely congested, dark in color, hyperemic, edematous and hemorrhagic (Figure 2b). The livers of morbid cattle indicated congestion, petechial hemorrhages and consolidation. The peripheral lymph nodes were severely congested in cattle showing signs of disease. Hydrothorax, carditis, pericarditis, hydropericardium containing the straw-colored fluid and pericarditis, hyperemic and congested heart (Figure 2c) and endocarditis were observed in cattle infected with hemorrhagic septicemia.

Histological changes

Histologically, lungs were congested; severe bronchopneumonia and infiltration of inflammatory cells in the alveolar septa were observed. Inter alveolar septa of lungs were significantly thickened with mononuclear cellular infiltration, necrotized, hyperplastic bronchiolar epithelium. Necrosis and hyperplasia were present in bronchiolar epithelium due to thickening of the alveolar walls by proliferation of alveolar cell. Infiltration of mononuclear inflammatory cells like lymphocytes and macrophages, monocytes and neutrophils were seen in the bronchiolar epitheliums. Infiltration of polymorph nuclear neutrophils, lymphocytes and macrophages was observed lungs. All the alveoli and bronchi were filled with edema fluid, neutrophils and macrophages (Figure 3a). The small intestines were hemorrhagic, disruption of villi and lining of epitheliums. Severe enteritis, degeneration of villi, congested mucosa of intestine, necrosis in epithelial cells of villi, necrosis and sloughing of epithelium of villi were observed in intestine of infected cattle. Microscopic observation of kidneys of various cattle died of pneumonia pasteurellosis revealed hemorrhages, degeneration and necrosis of renal tubules and tubular epithelium (Figure 3b). Protein rich fluids was observed in live sections of infected cattle. Different sections of liver of various cattle exhibited necrosis of hepatocyte, hemorrhages,

edema and infiltration of inflammatory cells (Figure 3c). Extensive hemorrhages were seen in the spleen of cattle died with respiratory illness. On microscopic observation, the various sections of heart of infected cattle indicated necrosis of cardia myofibers, edema, and congestion (Figure 3d).

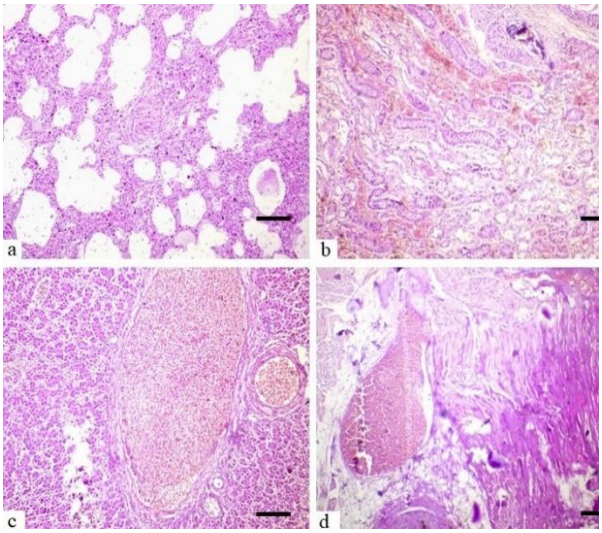

Figure-3. Photomicrograph showing extensive mononuclear cells infiltrations in alveoli, emphysema, interstitial pneumonia, congestion, fibrinous exudate and hemorrhages in lungs

(a), Glomerular hemorrhages, severe necrosis, degeneration of renal tubules, sloughing of epithelium of renal tubules, congestion, atrophy of glomeruli, hemorrhages, increased urinary spaces in kidneys (b), Necrosis of hepatocytes, fatty change, congestion and hemorrhages in liver (c) and Neutrophilic infiltration, edema, congestion, hemorrhages, coagulative necrosis, degeneration of cardiac cells and severe myocarditis in heart (d) of *P. multocida* positive cattle. H&E stain; 400X.

Hematological examination

The results on comparison of different hematological parameters of healthy and *P. multocida* affected cattle on the basis of clinical scoring and overall comparison (Figure 4) showed striking variation in different hematological parameters. The values of erythrocyte cells and concentration of hemoglobin were significantly reduced in infected cases. Significantly reduced values of monocytes, lymphocytes, and hematocrit were recorded as compared to healthy animals. The values of WBC count and neutrophils were increased significantly in the cattle showing the signs of hemorrhagic septicemia than the healthy animals. Hematocrit (%) was significantly less in cattle with respiratory disease from the healthy cattle.

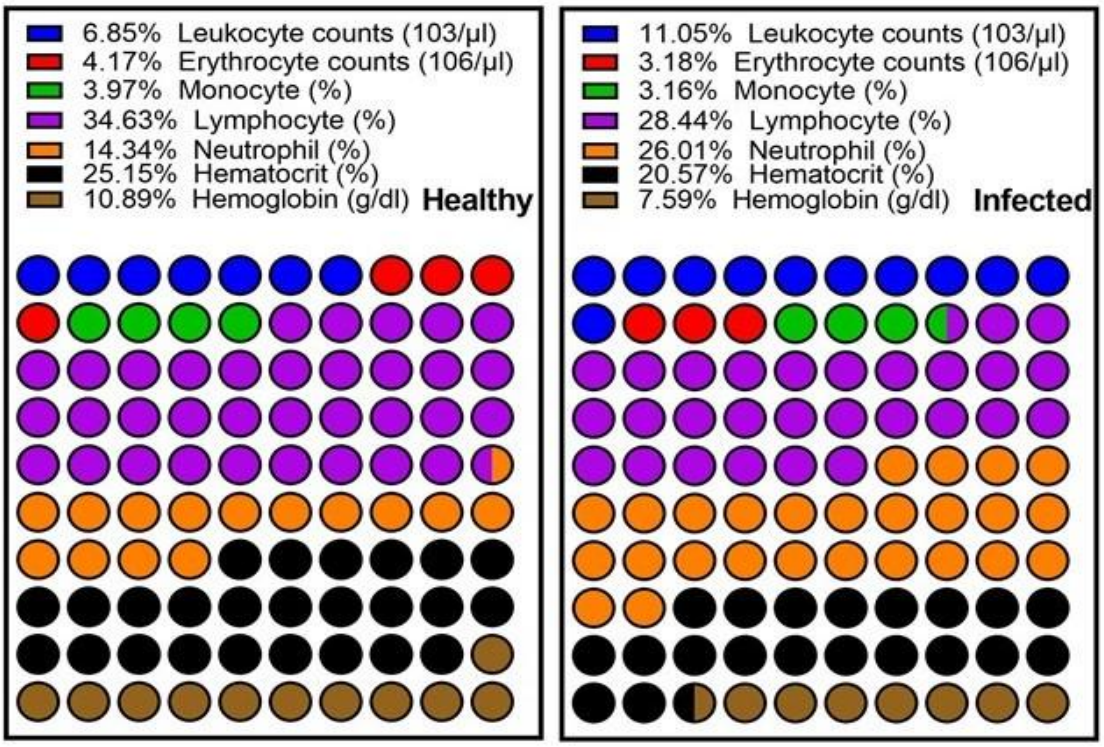

Figure-4. Photograph showing comparison of mean (±SE) profile of different hematological parameters of infected and healthy cattle

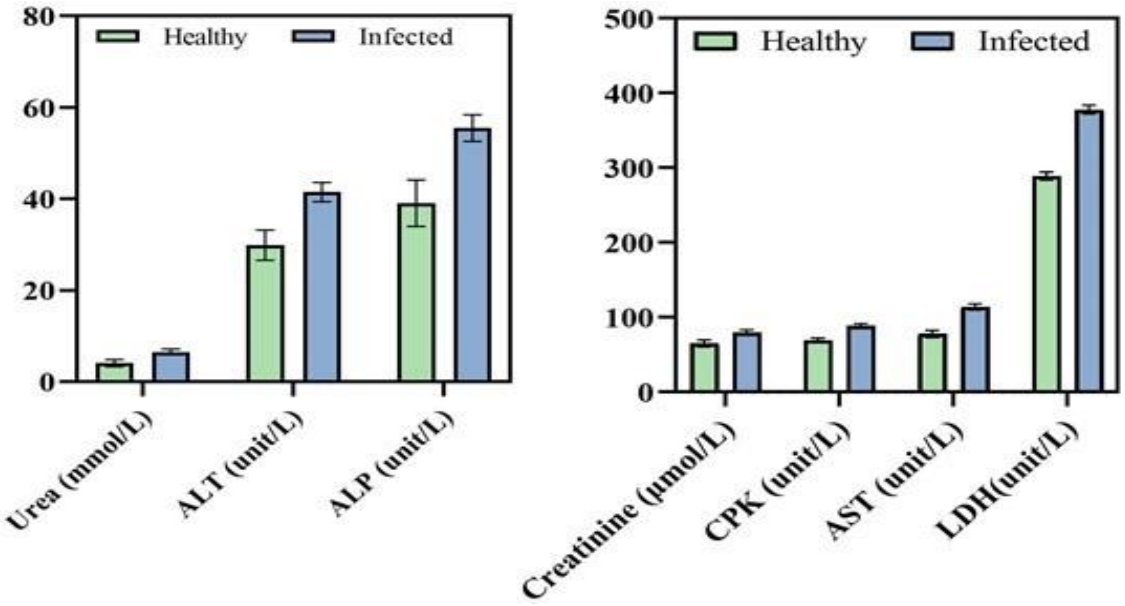

Figure-5. Photograph showing comparison of serum biochemistry parameters (Mean ± SD) of healthy and infected cattle ($p < 0.001$)

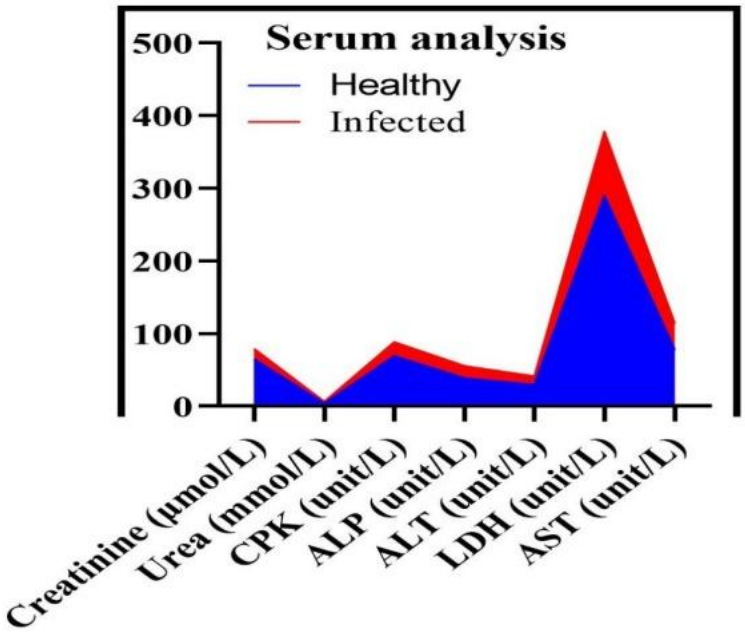

Figure-6. Photograph showing comparison of serum biochemistry parameters (Mean ± SD) of healthy and infected cattle ($p < 0.001$)

Oxidant-antioxidant status

Comparison of mean (±SE) profile of serum total proteins and blood hemoglobin concentrations reduced significantly in cattle infected with hemorrhagic septicemia. The results on various oxidative stress and antioxidant enzymes in erythrocyte of infected and healthy cattle indicate that concentration of MDA and nitric oxide is significantly increased in hemorrhagic septicemia affected cattle (Figure 7-8). The quantity of superoxide dismutase, reduced glutathione, catalase concentrations was significantly decreased in the cattle suffering from the hemorrhagic septicemia.

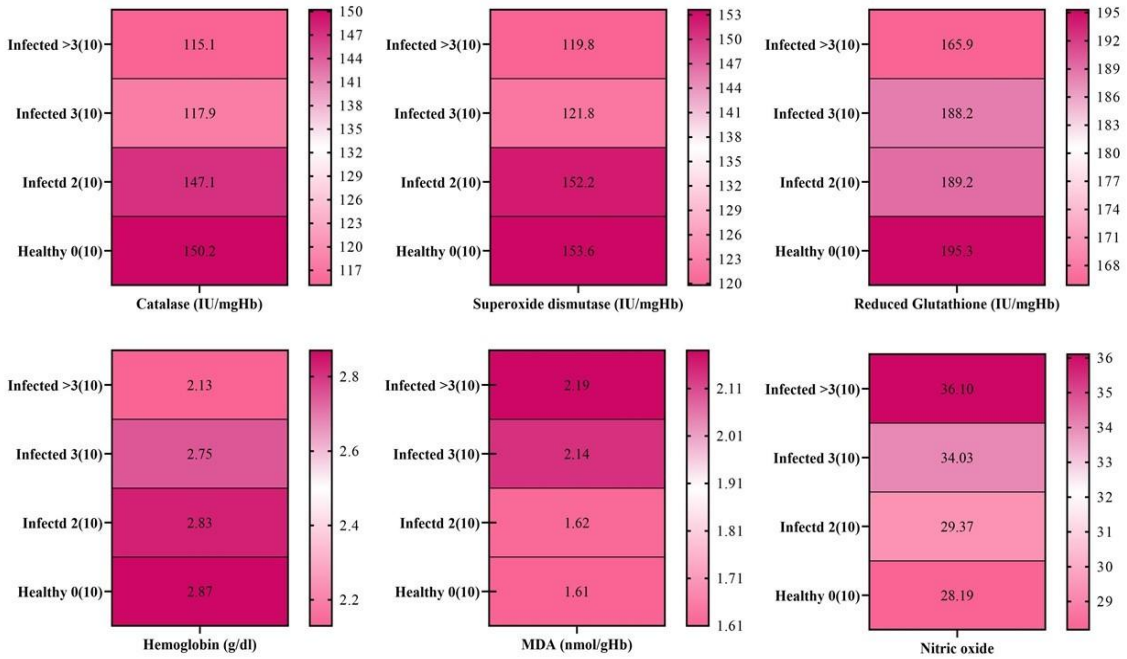

Figure-7. Photograph showing comparison of oxidative stress and antioxidant biomarkers in erythrocyte of infected and healthy cattle.

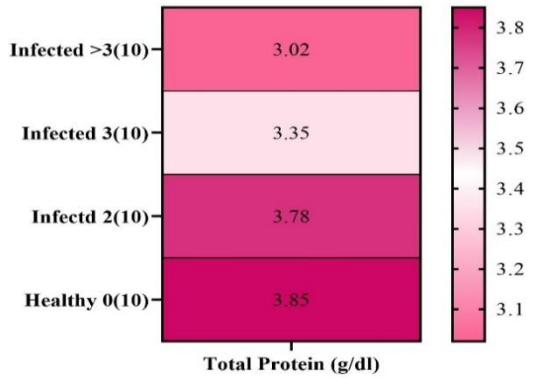

Figure-8. Photograph showing comparison of mean profile of serum total proteins content in infected and healthy cattle

Discussion

There are several predisposing factors including abrupt change in climate, intensive management, weaning, dehorning, transportation and failure of transfer of passive immunity are related to the respiratory infections (Hussain et al., 2017; Al-Shammari and Sadoon, 2022; Crosby et al., 2022). It is recorded that Investigation of different plasma proteins is crucial because these proteins perform different functions like construction of other tissue proteins, movement of biologically active substances that involve in specific and nonspecific immunity (Hussain et al., 2019; Ksenofontova et al., 2022). Determination and monitoring of different stress factors like physiological stress in different tissues and biochemical processes in body of animals is of vital importance to reduce the incidence of various disorders and disease outbreaks (Siddique et al., 2022; Abodalal and Ismail, 2023). Animals suffering from the hemorrhagic septicemia showed high rise in body temperature for 3 days in chronic form of HS and temperature becomes consistently subnormal ranging from the 100-102°F then recumbence that lead to death of animals as previously (Patel et al., 2016). Rumen motility was also decreased in diseased cattle but the mucus membranes were reddish and congested as already reported (Durrani et al., 2013; Griffin et al., 2010). Clinical signs of HS disease observed in infected cattle included pyrexia, salivation, hypoxia, coughing, anorexia lacrimation, tachycardia, depression, sub mandibular edema, brisket edema, and dyspnea. All these clinical signs are reported in the previous study due to pasteurellosis (Khan et al., 2011; Abubakar et al., 2013). Various clinical signs like pyrexia, coughing, sneezing, respiratory distress, hypoxia leading to sudden death have been observed in ovine and caprine suffering from the pasteurellosis (Rad et al., 2011). It has been recorded that severity of lesions induced in various visceral organs depend

upon the pathogenesis and the amount of toxin produced by the *P. multocida* (Marchart et al., 2003; Sebbar et al., 2021). Acute pleuropneumonia, tachycardia, pleurisy and hydrothorax occur when *P. multocida* enters in cattle via oral route while submandibular edema and brisket edema extending towards forelegs have been observed due to *P. multocida* through the tracheal routes. Cattle suffering from the HS showed significant increase in body temperature as reported in earlier studies (Abubakar and Zamri- Saad, 2011). Consolidation of lungs pneumonia, necrotic and fibrinous lungs in infected cattle can be related to deposition of fibrin in right cranial lobes at the contact surface of lobes (Chung et al., 2016). Various lesions in lungs including pneumonia, congestion and pleural adhesions are important features to know the severity and pathogenicity of respiratory passages (Abdel-Rassol et al., 2022).

Grass pathological lesions observed in cattle died of hemorrhagic septicemia included severe hemorrhages and congestion in respiratory and gastrointestinal tract along with submandibular and brisket edema as reported in earlier studies (Khan et al., 2011; McFadden et al., 2011; Abubakar et al., 2013). Hemorrhages in the liver, congested peripheral lymph nodes, hydrothorax and hydro pericardium containing the straw-colored fluid were the notable ailments due to hemorrhagic septicemia (Puspitasari et al., 2018). The macroscopic changes at necropsy level like presence of fibrin in the form of thin layer in lungs bronchi were filled with exudate and congested liver with several necrotic yellowish foci might be due to release of endotoxins (Sadeghian et al., 2011). RBC, PCV and Hb were significantly lower in infected cattle as compared to normal animals as already reported in different studies (Allam et al., 2021; Mudgal et al., 2018). Total leukocyte counts and neutrophils count suggestive of inflammatory response leading to acute neutrophilia in the body increased in all infected cattle (Hussaini et al., 2013; Sayed et al., 2023). It has been recorded that the tumor necrotic factor an inflammatory mediator transfer ferritin towards the macrophages receptors and promote storage of iron in the phagocytic stem cells leading to iron deficiency in erythrocyte precursor causing anemia. WBCs are considered as first line of defense in case of bacterial infections. In our study, increased number of neutrophils while decreased values of monocytes, and lymphocytes in infected cattle might be due to disorders in transport of oxygen carrying potential of erythrocytes leading induction injurious stimuli in multiple visceral tissues. Similarly, decrease in values of lymphocytes cells in the cattle infected with hemorrhagic septicemia (Allam et al., 2021). Previously decreased values of platelet count have been recorded was due to exposure to *P. multocida* (Roland et al., 2014). Serum albumin and total proteins were decreased in hemorrhagic septicemia infected animals. Urea and creatinine concentration were increased in cattle with HS disease and our findings are in line to previous published report (Lang et al., 2018). Increased quantity of serum creatinine and urea are indicator of dehydration and certain problems in kidneys (Kamal, 2010). Similarly, increased concentration of urea and creatinine in the serum of rabbits infected with virulent strain of *P. multocida* has also been reported (Hashem et al., 2018). Increased level of liver enzymes such as ALT and AST in the cattle infected with HS in our study could be due to degeneration and necrosis of liver caused by the toxins produced by the *P. multocida*. The significantly higher serum biochemical parameters such as including alanine aminotransferase, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alkaline phosphates, creatinine phosphokinase, urea and creatinine have been also reported (Hussain et al., 2022; Mahmood et al., 2022). The increased serum biochemical parameters can be related to lungs, liver, kidneys, cardiac and bronchial damage. The pathological alterations in cattle in our study might be due to physiological stress that plays necessary role to induce bovine respiratory infections (Mahmood et al., 2017; Crosby et al., 2022; Sayed et al., 2023) and can overcome the immune responses of the animals. Concentration of MDA and nitric oxide biomarkers of oxidative stress increased significantly in cattle infected with hemorrhagic septicemia. As the oxidative stress is an imbalance between free radicles and antioxidant enzymes. The increased amount of oxidative stress biomarkers might be due over production of free radicals and depletion of various antioxidant enzymes in cattle resulting in severe damage to red blood cells (Allam et al., 2021; Hussain et al., 2022). It has also been reported that this increase in MDA was due to cellular lipid peroxidation and due to lower values of GSH and SOD during preventing the cells against oxidative stress. Superoxide dismutase, reduced glutathione, catalase concentrations were significantly decreased in the cattle suffering from the hemorrhagic septicemia.

Previously similar findings have been recorded due to hemorrhagic septicemia (El-Deeb et al., 2019). NO concentration was increased in HS infected cattle and play important role in reducing the harmful inflammation by increasing the host ability to interact with pathogen (Sheridan et al., 2016). Blood parameters including the total proteins and hemoglobin were significantly reduced in the diseased cattle showing the typical signs of poor immune response and induction of physiological stress on blood forming tissues (Sebbar et al., 2021; Hussain et al., 2022).

Conclusion

This study indicates that cattle suffering from hemorrhagic septicemia were infected with *P. multocida* confirmed by biochemical and molecular techniques. They showed significant changes in hematological parameters, serum biochemistry and oxidant-antioxidant status which are useful tool for studying the pathogenesis of HS disease and improve diagnosis, prognosis of infection caused by *P. multocida*.

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Contribution of Authors

Ahmad F: Collected the data and executed the tests and contributed in manuscript editing.
Mahmood MS & Hussain R: Designed the research work and analyzed all the data and contributed in manuscript editing.
Rahman U & Abbas RZ: Contributed in manuscript editing and critique.
All the authors critically discussed and finally approved the contents of the manuscript.

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