Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2024 March Vol 6 Issue 1: 10-16

Original Research

Investigation of oxidative stress profile and pathomorphology of lungs in goats sick of pneumonia-enteritis complex

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ABSTRACT

Peste des Petits Ruminants (PPR), which causes pneumonia-enteritis complex, is an acute, highly contagious disease causing immunosuppression and high mortality in sheep and goats. Epidemiology, clinical signs and pathologic findings of the disease have been studied, but information on the oxidative stress profile is scanty. This study investigated the oxidative stress profile and the pulmonary histopathology of PPR infected goats. Twenty male goats were used for this study. The unvaccinated goats were naturally exposed to PPR according to environmental and animal market conditions consistent with routine PPR virus exposure in endemic areas. Following confirmation of PPR in the goats, their blood and serum samples were collected and evaluated for biomarkers of oxidative stress such as superoxide dismutase, malondialdehyde and catalase, while the neutrophil: lymphocyte ratio (NLR) and serum electrolytes were used to assess physiologic stress. Four goats were euthanized before onset of the clinical signs and every three days following clinical manifestations. Severity of histopathology observed in the lungs were scored and used to categorize the goats into four groups as; normal, mild, moderate and severe. Results showed that oxidative stress increased in PPR affected goats from moderate and severe pulmonary lesions as the disease progressed. On the basis of pulmonary histopathology, malondialdehyde was a better indicator of oxidative damage than superoxide dismutase and catalase. The study also indicated that NLR and Ca:P are reliable indicators of generalised physiologic stress in goats naturally affected by PPR.

Keywords: Oxidative stress, malondialdehyde, superoxide dismutase, pneumonia, enteritis.

INTRODUCTION

Small ruminants (sheep and goats) have been referred to as the 'poor man's cow', being more affordable than large ruminants (cattle). Over the years, sheep and goats contributed significantly to the protein requirement of Nigerians and to income of small farmers in Africa and Asia. *Peste des petits ruminants* (PPR) is a contagious viral disease of small ruminants (Radostits *et al.*, 2006, Kumar *et al.*, 2014) clinically characterized by pyrexia, conjunctivitis, oculo-nasal discharges, necrotizing and erosive stomatitis, diarrhoea (Taylor *et al.*, 1984) and particularly by bronchopneumonia (Balamurugan *et. al.*, 2014) and followed usually by death (Gargadennec *et al.*, 1942). The causative virus is transmitted through close contact with infected animals or fomites (Radostits *et al.*, 2006, Kumar *et al.*, 2014) and is present in large amounts in all body excretions and secretions of infected animals. The risk of outbreaks in flocks is greatly increased when new stock are introduced, or when animals are returned unsold from livestock markets, as goats brought from the markets have been known to harbour and transmit the virus having mingled with infected goats or carriers at the goat market (Radostits *et al.*, 2006).

According to a 2013 FAO estimate, the morbidity, mortality, production losses and treatment costs of PPR altogether was the cause of an economic loss of $2,972.5 million/year during 2012-2017 in South Asian region among which, in India alone, it would be $2569.00 million/year (Felix, 2013). Thus control of this disease for poverty alleviation is considered a priority (Perry, 2002; Diallo, 2006).

Understanding the role oxidative stress plays in disease has garnered research interest in recent years. As the search for an effective treatment and control of PPR continues, more understanding of the pathophysiology of the disease remains crucial. Very little is known of role of oxidative stress in the disease processes of PPR. Thus this study sought to evaluate

http://doi.org/10.54328/covm.josvas.2023.153

oxidative stress and patho-morphologic changes in lungs of goats sick of natural PPR infection.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Twenty male goats, of about eight months of age and sourced from a local market in Umuahia, Abia State were used for the study. The goats had been routinely exposed to environmental and animal market conditions consistent with regular PPR virus exposure in endemic areas and were not vaccinated against PPR. They were housed in pairs in fly-proof animal house under ambient conditions at the Department of Veterinary Pathology Animal Research housing facilities, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU). They were dewormed and provided with food and clean drinking water throughout duration of the study. Ethical approval was obtained from the University Ethical Committee (*MOUAU/CVM/202325*).

Animals were allowed to acclimatize and clinical parameters, including rectal temperature, respiratory rates, nature of mucus membranes, etc. were monitored twice daily and the process continued through the pre-clinical and throughout the clinical stages of the disease. Blood samples were collected from the animals for haematology and serum biochemical analyses every three days.

Four goats were euthanized before the onset of clinical signs and at three-day intervals following clinical manifestations.

Confirmation of *Peste des Petits Ruminants* was based on the established history and epidemiology (PPR is endemic in Nigeria/West Africa), typical pneumoenteritis complex clinical signs (conjunctivitis, serous to mucous oculo-nasal discharges, pyrexia, respiratory distress, fetid diarrhoea, dehydration and emaciation etc.), characteristic lesions (necrotic ulcerations in the oral cavity), gross and microscopic post-mortem findings (typically including bronchopneumonia, haemorrhagic enteritis with the characteristic "zebra stripping"), haematologic laboratory findings (including lymphopenia) and the careful elimination of differential diagnoses (FAO, 1999; Kataria & Kataria, 2012).

STUDY DESIGN

Because of the affinity of PPR virus for the lungs (Balamurugan *et. al*., 2014), scoring of pulmonary lesions was used to group the goats according to stages/severity of lesions observed.

HISTOPATHOLOGY

Lung tissue samples were collected and fixed in 10 % phosphate-buffered formal saline for at least 48 hours. Thereafter, they were dehydrated in ascending concentrations of alcohol, cleared in xylene for 1 hour 30 min, and embedded in paraffin wax. Sections 5 μm thick were cut and mounted on slides, stained with hematoxylin, counterstained with eosin (H&E stains) and viewed under light microscopy.

Group 1 = Normal (no pneumonic lesions)

Group 2 = Mild (thickening of the pulmonary interstitium)

Group 3 = Moderate (congestion, interstitial pneumonia, consolidations, distended septa)

Group 4 = Severe (purulent/supurative bronchopneumonia)

DATA COLLECTION

OXIDATIVE STRESS ASSAY

Superoxide dismutase (SOD) and Catalase were determined by the Hydroxylamine method (Weydert & Cullen, 2010). Malondialdehyde (MDA) was determined by the modified thiobarbituric acid method (Draper & Hadley, 1990).

HAEMATOLOGY

Total White Blood Cell (tWBC) was determined using the haemocytometer method by Schalm *et al.* (1975). The differential WBC count was determined using the Leishman technique (Schalm *et al*., 1975).

SERUM ELECTROLYTE CONCENTRATION

Calcium assay was done using the Ortho- cresolphthalein direct method (Endres & Rude, 2008). Inorganic Phosphorus assay was done using the Fiske-SubbaRow method (Endres & Rude, 2008), sodium assay, using the Dimethyl sulfoxide colorimetric method (Scott *et al*., 2008), chloride assay, using the Mercuric thiocyanate single reagent method (Scott *et al*. 2008). Potassium test was determined by the Turbidimetric Tetraphenylborate method (Scott *et al*. 2008).

SERUM MARKERS OF KIDNEY FUNCTION

Urea assay was determined by the modified Berthelot reaction method (Lamb & Price, 2008) while creatinine assay was determined by the modified Jaffe method (Lamb & Price, 2008).

SERUM PROTEIN AND ENZYME ASSAY

Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) activity levels were determined as described by Colville (2002) while total protein and total bilirubin were determined as described by Johnson (2008) and Higgins *et al*. (2008) respectively.

DATA ANALYSIS

The data were analysed using Student t-test and One Way Analysis of Variance test statistic. The variant means were separated using LSD and values of $P \leq 0.05$ were considered significant. The results were presented as means ± SD in Tables and Graphs. Statistical analysis was performed using

Statistical Package for the Social Sciences software® version 20.

RESULTS

Evaluation for oxidative stress biomarkers in natural *Peste des petits ruminants* infection in goats were done according to the severity of microscopic lesions of pneumonia. The pulmonary lesions, as categorised by specific histomorphologic findings, are presented in Plates I - IV.

SERUM CONCENTRATION OF SUPEROXIDE DISMUTASE, CATALASE AND MALONDIALDEHYDE

The results of the serum oxidative stress markers and the neutrophil to lymphocyte ratio of goats naturally-infected with *Peste des petits ruminants* according to the severity of microscopic lung lesions is presented in Figs I - IX.

There was no significant ($p>0.05$) difference in the serum concentration of superoxide dismutase biomarker across all

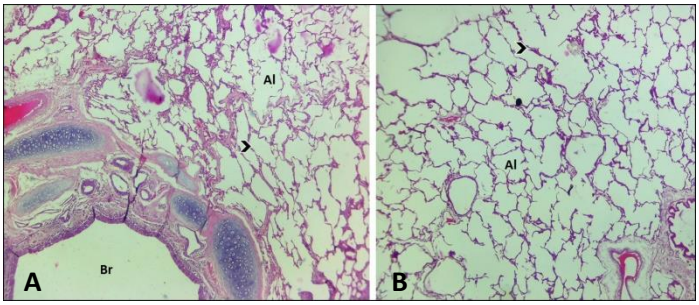


Plate I: Group I goats (Non-clinical group) had relatively normal lung histomorphology without any distinct pneumonic lesions with the alveoli (Al) and bronchi (Br) clear and patent and the interalveolar septa (arrow heads) intact (*H&E X40*).

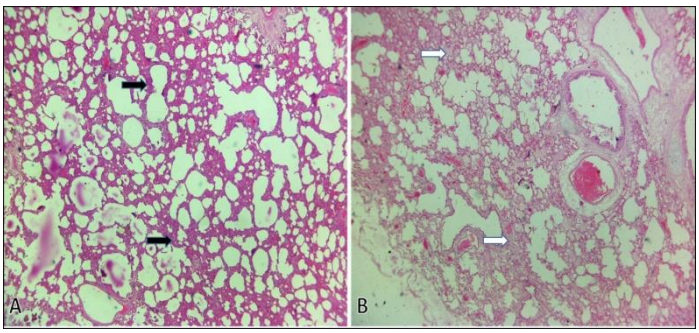


Plate II: Group II goats had mild pulmonary lesions characterised by the initial thickening of the pulmonary interstitium (black arrow) sometimes accompanied by interstitial oedema (white arrow) (*H&E X40*).

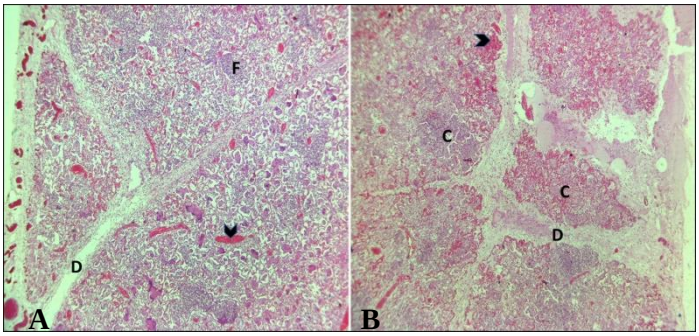


Plate III: Group III goats were considered as having moderate lesions; characterised by pulmonary congestion, inflammation of the lung tissue, consolidations and distended pulmonary septa. (*H&E X40*)

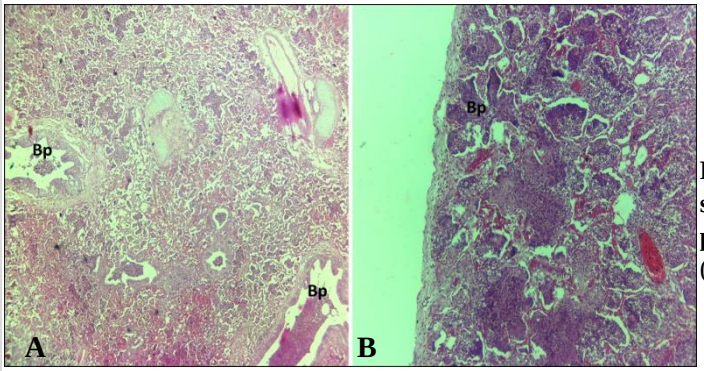


Plate IV: Group IV goats were considered to have severe pulmonary lesions; characterised by severe purulent/supurative bronchopneumonia (Bp). (*H&E X40*).

Table I: Blood neutrophil and lymphocyte counts of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

	Pre/Ctr	Mild	Moderate	Severe
Neutrophil	38.17±0.33	34.60±3.44	49.88±5.20	46.36±4.20
Lymphocyte	61.33±0.73	63.80±3.08	49.00±5.24	52.86±4.19

There was no significant (p>0.05) difference in the neutrophil and lymphocyte counts when histomorphologically affected groups were compared to group 1

the groups (Fig I). There was also no significant (p>0.05) difference in the serum concentration of catalase biomarker across the groups (Fig II). However, the evaluation for malondialdehyde, showed a significant (p<0.05) increase in the groups showing moderate (Gp 3) and severe (Gp 4) microscopic pulmonary lesions when compared to the histomorphologically normal group 1 (Fig III).

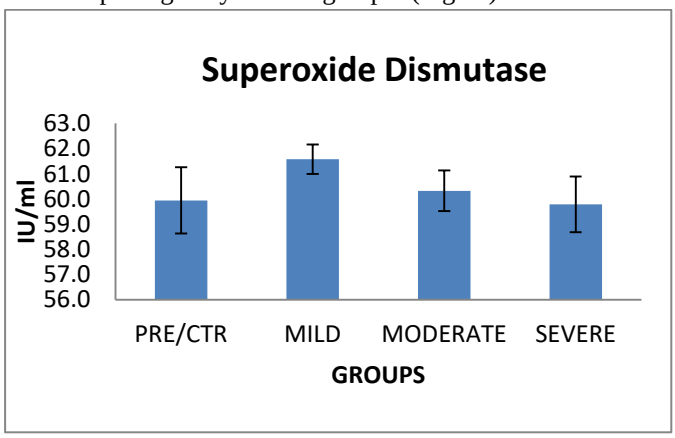


Fig. I: Serum concentration of superoxide dismutase biomarker of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

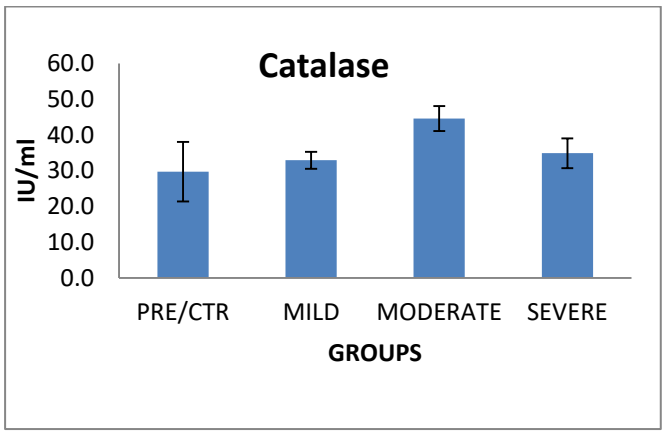
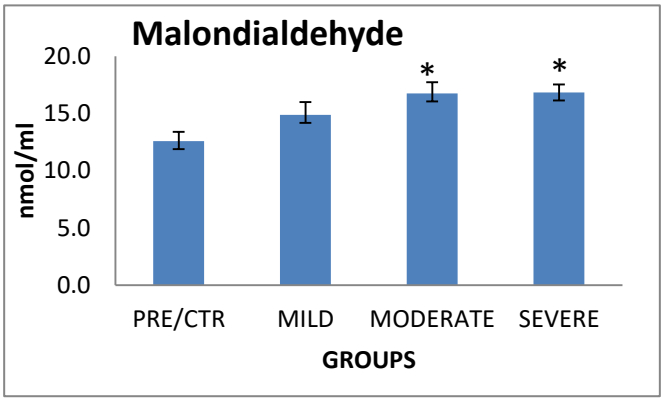


Fig. II: Serum concentration of catalase biomarker of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

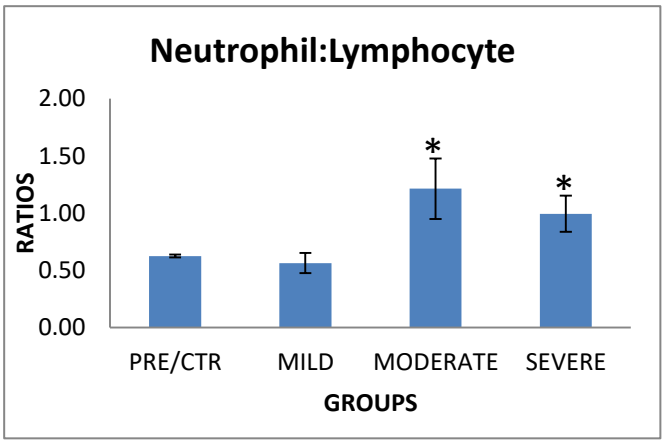
NEUTROPHIL TO LYMPHOCYTE RATIO

The neutrophil to lymphocyte counts as presented in table I shows that there was no significant (P>0.05) difference in the neutrophil and lymphocyte counts across all groups however Fig IV shows that there was a significant (P≤0.05) increase in the neutrophil to lymphocyte ratio between all histomorphologically affected groups and their histomorphologically normal counterpart (Gp 1)



**Values significantly (p < 0.05) different from those of the control*

Fig. III: Serum concentration of malondialdehyde biomarker of goats sick of pneumonia-enteritis complex at varying stages of pneumonia



**Values significantly (p < 0.05) different from those of the control*

Fig. IV: Blood neutrophil to lymphocyte ratio of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

Table II: Serum electrolyte concentration of goats of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

ELECTROLYTES	PRE/CTR	MILD	MODERATE	SEVERE
Ca	9.00±0.20	9.06±0.10	9.18±0.14	8.77±0.15
P	10.60±2.32	8.83±0.92	7.78±0.54	5.25±0.51*
Na	123.56±2.03	133.11±5.01	124.70±0.62	120.48±2.19
Cl	93.66±3.27	96.54±2,08	89.05±2.28	89.24±2.99
K	1.39±0.30	2.00±0.15	1.76±0.30	1.76±0.21

****Values significantly (p < 0.05) different from those of the control***

Table III: Serum enzyme concentration of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

ENZYMES	PRE/CTR	MILD	MODERATE	SEVERE
AST	50.60±6.44	72.51±15.61	72.07±18.81	61.28±8.15
ALT	12.00±1.47	10.00±0.44*	9.88±0.23*	9.38±0.11*
ALP	42.24±13.25	29.02±1.75	33.44±3.90	29.64±2.92
TP	58.18±4.36	62.28±2.88	60.79±1.01	55.95±2.49
Albumin	38.04±3.38	38.16±0.29	38.55±0.79	37.00±1.39
Total Bilirubin	0.22±0.03	0.24±0.02	0.28±0.02	0.25±0.02
Urea	44.95±1.47	46.63±3.77	52.67±3.60	44.29±2.56
Serum Creatinine	1.01±0.16	1.03±0.08	1.22±0.21	1.14±0.06

**Values significantly (p < 0.05) different from those of the control*

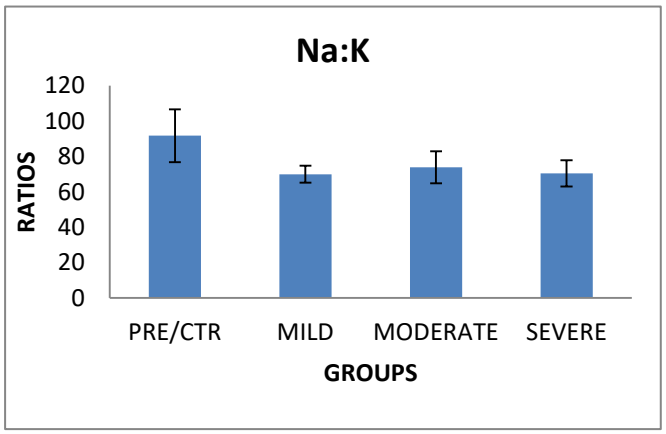


Fig. V: Serum sodium to potassium ratio of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

DISCUSSION

This work investigated the oxidative stress patterns in the pathomorphologic changes of pneumonic lungs of goats that may have been caused by natural *Peste des Petits Ruminants* infection.

PPR could be diagnosed on the basis of epidemiology, known clinical manifestations, characteristic pathologic lesions, post-mortem findings or laboratory tests and by discountenancing the differential diagnoses (FAO, 1999; Kataria & Kataria, 2012). In this study, differential diagnoses of PPR which include Foot and mouth disease, bluetongue, caprine pox, contagious ecthyma, pneumonic pasteurellosis and caprine pleuropneumonia, were ruled out based on absence of characteristic pathologies of each of the diseases as described by Santhamani *et. al.,* (2016). Prominent lesions observed in this study were; consolidations of t lungs, with the antero-ventral areas of the right lung particularly affected. Pulmonary histology revealed bronchopneumonia and congested alveolar borders which have been reported as characteristic histo-pathological changes of PPR in goats (Kumar *et al*., 2002; Balamurugan *et. al*., 2014)

In the digestive tract, the posterior part of colon and rectum showed discontinuous streaks of congestion on the mucosal folds commonly referred to as "Zebra markings" and typical of PPR (Balamurugan *et. al*., 2014).

A significant increase in malondialdehyde in the groups which lungs showed severe microscopic pulmonary lesions (groups 3 and 4) despite no significant increase in serum levels of superoxide dismutase (SOD) and catalase, is still an indication that there was oxidative damage following PPR disease in the goats. Apparently, the oxidant malondialdehyde was a more accurate indicator of oxidative stress than the antioxidants, SOD and catalase.

Malondialdehyde is an oxidative aldehyde by-product of lipid peroxidation produced by the action of reactive oxygen species (ROS) on tissues, whereas SOD and catalase are immune-mediated antioxidants produced to antagonise ROS. Thus, theoretically, a 'direct' evaluation of oxidative tissue damage through evaluating oxidants like malondialdehyde, may be more reliable in measuring oxidative damage that

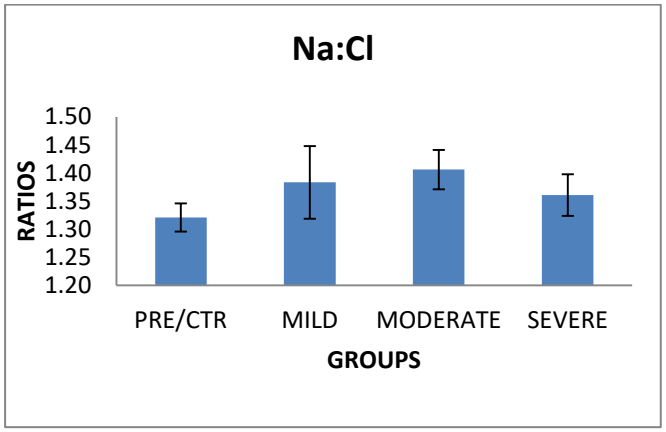


Fig. VI: Serum sodium to chloride ratio of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

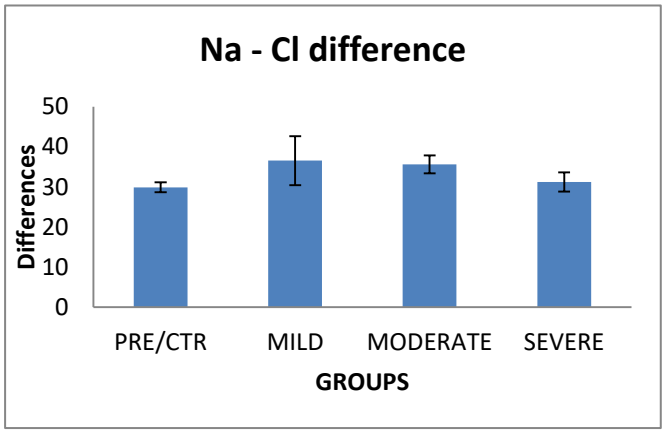
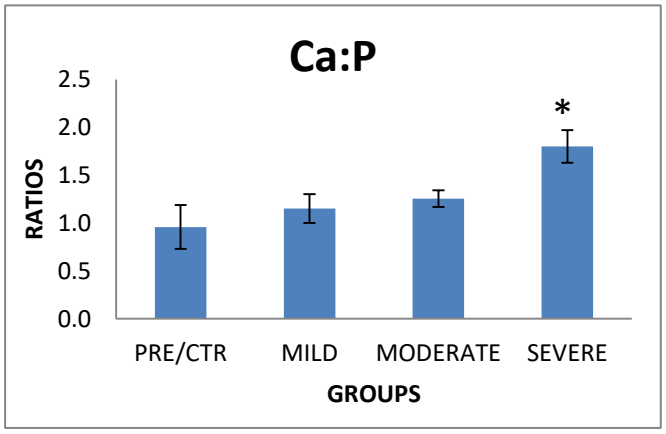


Fig. VII: Serum sodium to chloride difference of goats sick of pneumonia-enteritis complex at varying stages of pneumonia



**Values significantly ($p < 0.05$) different from those of the control*

Fig. VIII: Serum calcium to phosphorus ratio of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

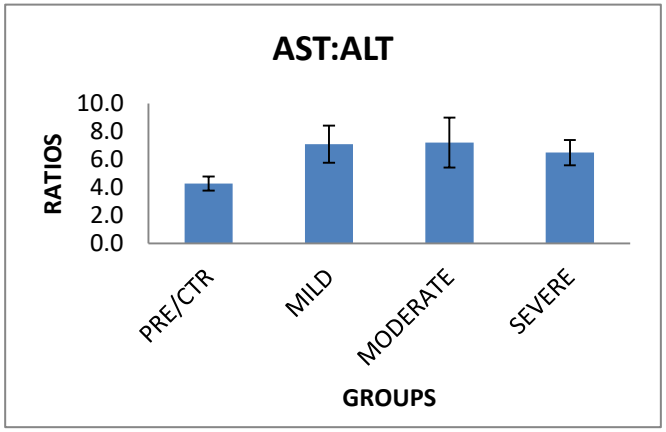


Fig. IX: Serum AST to ALT ratio of goats sick of pneumonia-enteritis complex at varying stages of pneumonia

'indirect' measurements of antioxidants since the latter may be dependent on the ability of the immune system to mount defensive response to oxidative stress. This is especially evident in cases of severe or later stages of PPR. In fact, an initial increase in SOD and catalase at the early stages of microscopic pulmonary lesions, though not significant, was followed by a decrease in the serum levels of these antioxidants at the later stages of the disease, apparently following severely diminished immune response. Significant increases in the NLR at the later stages of the disease also confirmed the existence of severe physiologic stress and of systemic inflammation (Zahorec, 2021), whereas single considerations of neutrophil and lymphocyte counts appear not to be reliable indicators. Significantly high NLR ratio has been consistently associated with poor prognosis in other diseases.

The significant decrease in phosphorus at the terminal stages of the condition, a consistent finding with the investigations done in this work, and also of sodium, again represents possible muscular degeneration and impaired kidney function respectively. The significantly increased serum calcium to phosphorus ratio (Ca:P) at the terminal stage of the disease process indicated a possible hyperparathyroidism (Sharma *et. al*., 2023) in PPR affected animals with severe histopathologic changes.

CONCLUSION

This work has shown that oxidative stress in PPR affected goats occurs alongside severe pulmonary lesions, in accordance with severity of the disease process. Additionally, on the basis of pulmonary histopathology, of the three endogenous oxidative biomarkers studied in this work, malondialdehyde appeared to be the most reliable for monitoring oxidative stress in goats. This could be attributable to malondialdehyde being an oxidant and a direct

evidence of oxidative damage than the antioxidants SOD and catalase, which are both host immune dependent. Also, production of aldehyde lipid oxidative by-products may be a greater source of oxidative stress than hydrogen peroxide free radicals in pneumonic goats. Neutrophil to lymphocyte ratio (NLR) and calcium to phosphorus ratio (Ca:P) are also reliable indicators of oxidative/physiologic stress in pneumonic goats.

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