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Oxidative Stress in Goats with *Peste des Petits* Ruminants (PPR)

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ABSTRACT

Background: Goats in India are endangered by various disease outbreaks. A limited number of disease conditions in goats have been investigated with regard to the effect of oxidative stress. Oxidative stress is an active field of research implicated in numerous disease processes including sepsis, enteritis, pneumonia, mastitis, joint diseases *etc*. The present work was undertaken to study the oxidative stress contributing pathological changes in naturally occurring *Peste des petits* ruminant virus (PPRV) in goats of Jabalpur region.

Methods: A total of 30 positive PPR samples (19 plasma and 11 lung tissues) were processed for oxidative stress assessment by oxidative stress markers malondialdehyde (MDA) and glutathione (GSH). The oxidative stress was revealed among 30 PPR positive goats when compared with 10 healthy animals. Necropsy examination was conducted to observed gross pathology and lung tissues were collected for histopathological examination.

Result: Oxidative stress was observed with decreased GSH and increased MDA concentration among PPRV positive goats in plasma and in lung tissues respectively. Nineteen PPR positive goats revealed different degree of clinical signs and were devided into three groups *viz* group I mild, group II moderate and group III severe. Grossly, eleven positive goat carcasses revealed pneumonic lesions. Microscopically, lung sections revealed broncho-interstitial pneumonia. These findings are suggestive of oxidative stress induces pathological changes and lead to dehydration and death of goats.

Key words: Goat, Glutathione, Malondialdehyde, Oxidative stress, PPR.

INTRODUCTION

Goats in India are endangered by various disease outbreaks. A limited number of disease conditions in goats have been investigated with regard to the effect of oxidative stress. Oxidative stress is an active field of research implicated in numerous disease processes including sepsis, enteritis, pneumonia, mastitis, joint diseases *etc* (Manat *et al.* 2017). The oxidative stress arises from an imbalance between oxidants and antioxidants in response to excessive generation of free radicals or slow elimination of free radicals by antioxidants. During viral infection, oxidative stress *i.e.* oxidative distress may result from either or both of (i) increased replication and/or pathogenesis of virus causing over production of reactive oxygen species (ROS) and (2) failure of normal defence mechanisms, leading to decreased elimination of ROS (Baruchel and Wainberg, 1992). Peterhans (1997) demonstrated that a virus could generate ROS from phagocytes. Disturbance in the intracellular redox environment induces cell apoptosis, senescence and disrupted differentiation. Thus, oxidative stress can cause damage to all molecular targets *viz* nucleic acid, lipid and protein. Since, increased lipid peroxidation (LPO) in blood cannot be excreted into the urine and they remain circulate in the blood stream until they are decomposed by enzymatic reactions such as the glutathione peroxidise-glutathione reductase enzymes. Likewise when virus proliferates at tissue site increased level of LPO occur (Thimmulappa *et al.,* 2019).

Thus keeping the aforesaid facts in mind, reliable methods were used in the present paper to determine lipid peroxidation (Malondialdehyde-MDA) and antioxidant (Glutathione- GSH) levels in plasma and lung tissues to find out the extent of oxidative stress in PPR affected goats. Pathological changes in lungs of PPR affected goats were also used to correlate with oxidative stress.

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MATERIALS AND METHODS

The present research work was conducted for a period of one year from June 2018 to May 2019, at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur (M.P.) region to determine the oxidative stress and its relation with pathology of lungs of PPR positive goats.

Preparation of tissue homogenates

All 11 PPR positive goats lung tissue samples were processed for demonstration of oxidative stress separated in two parts. One gram (g) of tissue samples were homogenized in 09 mL assay buffer (phosphate buffer saline -PBS, 50 mM pH 7.4) store on ice to estimate GSH levels. And 01g of tissue sample was homogenized in 09 mL of potassium phosphate buffer (10 mM pH7.4) for estimation of MDA levels. Each tissue homogenate was centrifuged at 10,000 revolutions per minute (rpm) for 15 minutes (min) in cold centrifuge and the resultant supernatant was used for estimation of GSH and MDA (Noeman *et al*., 2011).

Preparation of plasma samples

All 19 PPR positive goats plasma samples were processed for demonstration of oxidative stress separated in two parts for estimation of GSH and MDA respectively. Anticoagulant-treated blood was centrifuged at 5000 for 05 min at 4°C. The top plasma layer was transferred to a new tube (Chauhan, 2006).

Determination of malondialdehyde (MDA)

Membrane peroxidative changes in tissue homogenate were determined in terms of MDA production by the method as described by Shafiq-U-Rehman (1984). One mL of tissue homogenate was incubated at 37 ±0.5°C for 02 hours (h) in a centrifuging tube. To each sample 01 mL of 10 per cent trichloroacetic acid (TCA - weight/ volume - w/v) was added. The reacted mixture was centrifuged at 2000 rpm after thorough mannual mixing for 10 min then 01 mL of supernatant with 01 mL of 0.67 per cent of thiobarbituric acid (TBA-w/v) was added and placed in boiling water. Blank solution was prepared by adding the entire reagent except tissue homogenate. The absorbance was measured at 535 nanometers (nm).

Lipid peroxidative changes in plasma were determined in terms of MDA production by the method described by Yagi (1987). Twenty microliters (µL) of plasma was mixed with 4.0 mL of N/12 H_2SO_4 and 0.5 mL of 10 per cent phosphotungstic acid (PTA) and allowed to stand at room temperature for 5 min, then the mixture was centrifuged at 3000 rpm for l0 min. The supernatant was discarded and the sediment was mixed with 02 mL of N/12 H_2SO_4 and 0.3 mL of 10 per cent PTA. The mixture was centrifuged at 3000 rpm for l0 min. Sediment was suspended in 4.0 mL of distilled water and 1.0 mL of TBA reagent (a mixture of equal volumes of 0.67% TBA aqueous solution and glacial acetic acid) was added for reaction. Then the mixture was heated at 95°C for 60 min in an oil bath. After cooling with tap water, 05 mL of n-butanol was added and the mixture is hand shaken vigorously, then centrifugation at 3000 rpm for 15 min, the n-butanol layer was taken for fluorometric measurement at 553 nm with excitation at 515 nm.

Interpretation

Mean optical density (O.D) was calculated by using the following formula. The amount of LPO expressed as nanomole (nM) of MDA.

$$\frac{\text{OD of test}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture} \times 10^9 \times \text{DF time of incubation (2 hours)}}{\text{Volume of sample taken}}$$

DF-Dilution factor.

EC-Molar extinction coefficient (EC) of MDA-TBA complex *i.e*. 1.56×10^8 /M/cm.

Determination of glutathione (GSH)

GSH was estimated by the 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) method as suggested by Prins and Loos (1969). Tissue homogenate (0.2 mL) was added to 04 mL of 0.08 NH_2SO_4 and mixed and placed at room temperature for 10 min then 0.5 mL of tungstate solution was added to clear the brown haemolysate in a centrifuging tube. Later the tube was stoppered and the mixture was hand shaken vigorously for 05 min. The stopper was removed and suspension was allowed to stand at room temperature for 05 min in order to avoid air bubble formation on the top of supernatant. Then the suspension was centrifuged for 15 min at 2000 rpm at room temperature. After centrifugation, 0.2 mL of DTNB reagent was added and mixed well. Within a minute, absorbance was measured at 412 nm against blank in which 02 mL of distilled water was substituted for the supernatant.

GSH was estimated in prepared plasma sample by the DTNB method as suggested by Prins and Loos (1969). One-half volume of 0.6 per cent sulfosalicylic acid (SSA) was added to plasma sample and centrifuged at 8000 g for 10 min at 4°C. The supernatant was transferred into a new test tube and 0.5 mL of tungstate solution was added. The tube was stoppered and the mixture was shaken vigorously for 05 min. The stopper was removed and suspension was allowed to stand for 05 min in order to avoid air bubble formation on the top of supernatant. The suspension was then centrifuged for 15 min at 3000 rpm at room temperature. Later 02 mL supernatant was mixed with 2.5 mL tris buffer and 0.2 mL of DTNB reagent within a minute the absorbance was measured at 412 nm against standard blank.

Interpretation

The GSH concentration was calculated from the standard curve at the end point of the method (Fig 1).

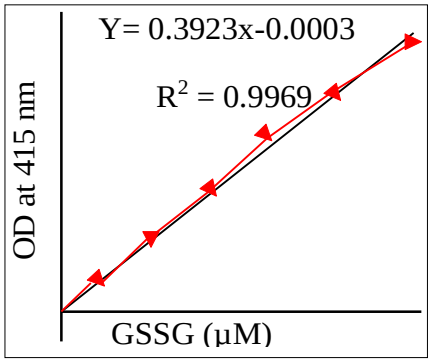


Fig 1: Standard curve by end point method for GSH estimation.

Pathological studies

PPR positive goats were examined for gross (stomatitis-pneumo-enteritis) lesions. The cases were graded as mild, moderate and severe based on modified score card proposed by Pope *et al.* (2013) (Table 1).

Lung tissue sample from PPRV positive goats were fixed in 10 per cent neutral buffered formalin (10% NBF) were processed by paraffin embedding techniques. Five micron thick sections were made and stained with hematoxylin-eosin (H and E) (Gridley, 1960) stain.

Statistical analysis

Obtained data is tabulated and subjected for two-sample t-test as described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The present work was conducted to determine the oxidative stress contributing to pathological changes in lungs in naturally occurring PPR infection in goats of Jabalpur region or area.

Oxidative stress in plasma of PPRV positive goats

The levels of MDA in plasma of apparently healthy and PPRV positive goats was determined as 2.73±0.87 and 34.71±0.66 (nmol/mL), whereas, GSH levels in healthy and PPRV positive goats was determined as 130±17.16 and 51.8±6.47 (µmol/L) respectively (Table 2). Significant difference in MDA and GSH concentration was observed between PPRV positive and healthy goats plasma samples.

Similar to our findings Nisbet *et al*. (2007) reported significantly (P<0.01) lower GSH and significantly higher MDA levels in sera samples of sheep with PPRV than the controls (1.93±0.11 nmol/mL vs. 1.49±0.06 nmol/mL). An imbalance between oxidant-antioxidant activities in pathogenesis of PPRV in goat has been reported by Kataria and Kataria (2012). Kumar *et al.* (2018) measured higher levels of pro-oxidant MDA obtained from lipid peroxidation along with lower levels of anti-oxidants and opined that is an indication higher oxidative stress in goats which are affected with PPRV. In the present study a significant increase in MDA concentration in plasma of PPRV positive goats indicating the host defense system is drastically affected by oxidative stress.

Oxidative stress in PPRV positive lung tissues

The level of MDA in healthy and PPRV positive goats was denoted as 46.33±18 and 688.59±18 (nmol/g) respectively. Whereas, level of GSH in healthy and PPRV positive goats were measured as 11.20±1.71 and 03.62±0.97 (µmol/g) respectively (Table 3). Significant differences in MDA and GSH concentration were observed between lung tissues of PPRV positive and healthy goats.

The data pertaining to GSH and MDA levels in plasma and lungs tissues of PPRV affected goats is scarce. However, similar observation were reported in lung tissues of other ribonucleic acid (RNA) viral infections in goats by Beck *et al.* (2001) and Ko *et al.* (2005). The increased MDA and decreased GSH levels may further corroborate with acute inflammatory reactions, expansive irreversable cellular injury and membrane lipid peroxidation induced by PPRV in lower respiratory tract results in broncho-interstitial pneumonia in goats.

Table 1: Clinical score and sample collection from goats.

Clinical score	Clinical symptoms
I	Mild temperature. watery mucoid oculo-nasal discharge, reddened eyes and mild conjunctivitis and congested nasal mucosa.
II	Foetid watery diarrhea, fever. Severe conjunctivitis severely congested/oedematous nasal mucosa and coughing. Erosive necrotic lesions on buccal cavity.
III	Muco-haemorrhagic diarrhea, marked dyspnoea, coughing and shivering. Purulent oculo-nasal discharge and severe conjunctivitis; severe erosive ulcerative necrotic nasal cavity. Oedematous lips, bran like deposition on tongue.

Table 2: Oxidative stress in blood plasma of PPRV positive goats.

Group	N	MDA (nmol/ml)		GSH (µmol/L)	
		Mean±SE	SD	Mean±SE	SD
Group I (PPRV positive)	19	34.71±0.66	02.87	51.8±6.47	28.20
Group II (Healthy control)	05	02.73±0.87	01.94	130±17.16	38.37

**P<0.01.

Table 3: Oxidative stress in lung tissue of PPRV positive goats.

Group	N	MDA (nmol/g)		GSH (µmol/g)	
		Mean±SE	SD	Mean±SE	SD
Group I (PPRV positive)	11	688.59±18	59.69	03.62±0.97	03.21
Group II (Healthy control)	05	46.33±18	40.24	11.20±1.71	03.82

**P<0.01.

Pathology of PPRV positive goats

Nineteen goats infected with PPRV was assessed clinically by clinical score I, II and III as shown in Table 4. All the clinical signs observed are in agreement with the findings of Zahur *et al.* (2011). Similar clinical signs were also observed by Bamouh *et al.* (2019) in experimentally infected goats with PPRV. Other findings in the present study such as high fever, lesions in mouth, oral and nasal congestion, respiratory signs and diarrhea leading to death of the goats were also reported by Patel *et al.* (2015). In addition to the aforesaid findings Patel *et al.* (2017) recorded oral lesions on the lower gum, dental pad, hard palate, inner side of the cheek, dorsum of the cheek and commissurs of the mouth. During investigation typical clinical signs and symptoms of PPRV infection were observed in goats under clinical score III.

During necropsy of 11 PPRV positive goat carcasses, most of them (07) were found to be dehydrated, emaciated with sunken eyes and rough body coat (Fig 2), but some (04) goats were in fair body condition is an indication for an early infection. Hindquarters of 07 goats were soiled with soft, watery greenish excreta. Mucopurulent nasal discharge were observed in 04 goats, 02 goats showed dried-up discharges on eyes and nose. Crusts were present around the nostrils and on muzzle in 05 goats. All necropsied goats revealed pneumonic lesions. In most of the carcasses, lesions appeared as dark red to purple colour and firm in consistency. The most commonly affected lung lobes are cranial and cardiac lobes which revealed congestion and red hepatization (consolidation) (Fig 3). Kumar *et al.* (2013) also reported prominent lesions in PPRV infected goats includes, congestion, consolidation, changes in colour of lungs and sometimes, frothy mucous is observed in cut surface of lungs on squeezing, antero-ventral areas of right lungs are frequently involved; anterior and cardiac lobes of lungs become dark red or purple, firm to touch. Varying degree of congestion, haemorrhages and consolidation were also observed in right lobe of lungs, red hepatization and emphysema was reported by Patel *et al.* (2017).

Table 4: Clinical assessment of PPRV in goats.

Clinical score		I	II	III	Total
PPRV	N	02	05	12	19
Positive	%	10.52	26.31	63.15	100



Fig 2: PPRV positive 8 month's goat carcass dehydrated and rough body coat.



Fig 3: Lung from PPRV positive goat showing areas of red hepatisation on.

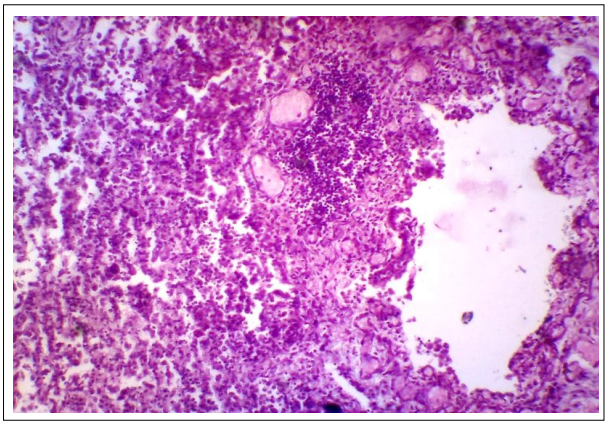


Fig 4: Photomicrography of PPRV positive goat lung tissue showing denudation of bronchiolar epithelium and cellular infiltration. H & E X 100.

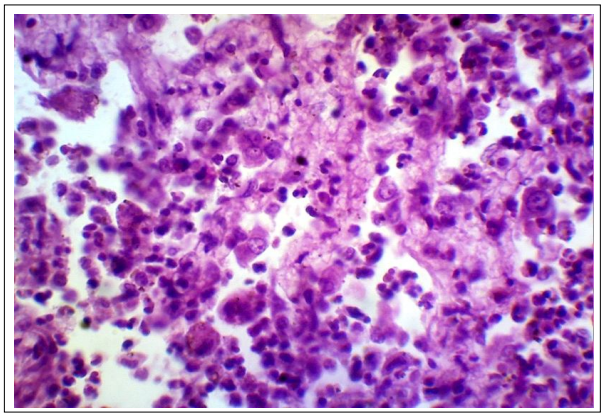


Fig 5: Photomicrography of PPRV positive goat lung tissue showing thickened interalveolar septa and cellular infiltration. H & E X 400.

Microscopic changes in lung sections of PPRV positive goats revealed broncho-interstitial pneumonia (Fig 4). Distended alveolar lumen filled with macrophages, lymphocytes, syncytia and giant cells were found in present study which is similar to earlier reports of Brown *et al.* (1991) and Islam *et al.* (2001). Thickening of inter-alveolar septa was observed which is composed of inflammatory cells *viz.,* neurtophils, lymphocytes and macrophages (Fig 5). Desquamation, thickening and hyperplasia of bronchiolar epithelium with necrotic cellular exudates consisting of neutrophils and macrophages in the lumen were also found. These observation are similar to the findings of Muse *et al.* (2012). Mahajan *et al.* (2017) reported interstitial pneumonia with lympho-mononuclear cell infiltration in lungs. Manimaran *et al.* (2017) reported extensive serofibrinous bronchointestitial pneumonia, characterized by diffuse infiltration of large number of lymphocytes, macrophages with numerous syncytia containing up to 06-08 nuclei.

CONCLUSION

The present study revealed a significant of oxidative stress in naturally occurring PPR viral disease in goats of Jabalpur region. Which are positively correlated with clinical signs, gross lesions and histopathological lesions of lungs which in turn lead to dyspnoea, dehydration and death (3D) of goats.

Conflict of interest

All authors declare that they have no conflict of interest.

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