

## Haematobiochemical Profiles and Oxidative Stress Parameters in Pigs (*Sus scrofa domestica*) with *Mycoplasma hyopneumoniae* infection

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<https://doi.org/10.21608/svu.2025.373517.1380>

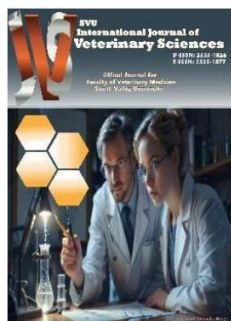
Submit Date: 07/04/2025; Revise Date: 04/06/2025; Accept Date: 16/06/2025

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### ABSTRACT

*Mycoplasma hyopneumoniae* significantly impacts pig health and livelihoods. In this study, we evaluated the effects of *Mycoplasma hyopneumoniae* infection on haematological, biochemical, and oxidative stress parameters in pigs. Clinical infection was confirmed using a *Mycoplasma*-specific antibody test kit. *Mycoplasma hyopneumoniae* antibodies were detected in twelve infected pigs and compared to eight healthy controls. Using standard assays, blood samples were analyzed for haematological, biochemical, and oxidative stress markers. No significant differences ( $P > 0.05$ ) were observed in haematobiochemical parameters between infected and healthy pigs. However, oxidative stress markers such as malondialdehyde (MDA), hydrogen peroxide ( $H_2O_2$ ), and nitric oxide were significantly elevated in infected pigs. Enzymatic antioxidants, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST), along with non-enzymatic antioxidants like reduced glutathione (GSH), were significantly decreased. Oxidative stress markers, unlike haematobiochemical parameters, are crucial in the pathogenesis of *Mycoplasma hyopneumoniae* exposure in pigs and could serve as supportive indicators in evaluating host response during infection.

**Keywords:** *Mycoplasma hyopneumoniae*, Pigs, Oxidative Stress, Haematobiochemical Parameters.



## INTRODUCTION

The Nigerian growing pork industry, with an average pig population exceeding 8 million and production of over 309,000 tonnes (FAOSTAT, 2023; Adesehinwa et al., 2024), remains vulnerable to disease outbreaks despite considerable growth (Abiola et al., 2015). Globally, the porcine respiratory disease complex (PRDC), including *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) and Porcine Circovirus, is a leading contributor to morbidity and mortality rates among pigs. In the United States, respiratory disease has been identified as the leading cause of mortality in nursery and grower-finisher units (National Animal Health Monitoring System, 1996). Limited data exist on enzootic pneumonia in Nigeria. However, global research shows its endemicity in most swine-raising countries, with 38% to 100% herd prevalence (Simionatto et al., 2013) and widespread implications for pig health, especially in high-density farming environments (Kuberka et al., 2024).

Enzootic pneumonia is a chronic respiratory disease of pigs caused by the bacteria *Mycoplasma hyopneumoniae* (Balestrin et al., 2022; Garza-Moreno et al., 2018), characterized by chronic pneumonia, non-productive dry cough, and growth retardation, thereby affecting the production performance (Sonaglio et al., 2022). Enzootic pneumonia spreads through direct animal contact and aerosols. The proximity of vulnerable farms to infected farms

has been demonstrated to be a significant risk factor for mycoplasma outbreaks (Bargen et al., 2004).

The pathogenesis of enzootic pneumonia caused by *M. hyopneumoniae* is still unclear. Nevertheless, it involves a complex interplay between the bacterial effects and host responses. Colonization by *M. hyopneumoniae* mostly affects ciliated epithelial cells from the trachea to the bronchioles. This results in cilia loss and disruption of epithelial cells, making the host more vulnerable to secondary bacterial and viral infections (Zimmer et al., 2020).

Enzootic pneumonia in pigs typically has a gradual progression, a chronic nature, a subtle beginning, and a high correlation with other diseases. Some aspects of the lungs are affected by bilateral foci of bronchopneumonia, and gross lesions are apparent following the onset of the disease (Hattab et al., 2023).

Together, vaccination and antimicrobial use form a complementary approach to controlling *M. hyopneumoniae* in pig herds. Treatment with antimicrobials is effective and can reduce the burden of infection and impacts of disease (Thacker et al., 2006), although antimicrobials cannot stop pigs from getting infected with the bacteria. To enhance disease control, commercial bacterins, primarily inactivated bacterins, have demonstrated efficacy in reducing clinical signs, lung lesions, and economic losses (Maes et al., 2008). Additionally, several experimental vaccines,

including subunit, live attenuated, and DNA-based and live attenuated formulations, are under development to improve immunogenicity and cross-protection, though none are yet commercially available (Tao et al., 2019; Maes et al., 2021). While these vaccines do not confer sterilizing immunity, they are most effective when integrated with good biosecurity and management practices. Improved management, housing, and biosecurity are crucial for the control of *M. hyopneumoniae* infections in pig herds (Maes et al., 2008).

Assessment of haematobiochemical and oxidative stress parameters is crucial for evaluating the physiological status of animals, aids in the diagnosis of diseases, and helps determine the severity of infections (Banwo et al., 2024). Given the link between oxidative stress and pneumonia-causing infections in pigs (Lykkesfeldt and Svendsen, 2007), identifying key oxidative stress biomarkers, in addition to pathogen elimination, can inform improved therapeutic strategies using antioxidant interventions to mitigate free radical damage and improve clinical outcomes (Banwo et al., 2024). Haematobiochemical parameters such as white blood cell counts, liver enzymes, and protein levels are essential in understanding the broader physiological impact of enzootic pneumonia. Oxidative stress which can result in cellular damage, impaired immune response and exacerbate disease progression, can be evaluated by measuring malondialdehyde (MDA) level,

superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities. Changes in these parameters can indicate immune system activation, inflammation, and potential organ stress.

In the present study, we evaluated the impact of *Mycoplasma hyopneumoniae* on haematobiochemical and oxidative stress parameters in commercial pigs.

## MATERIALS AND METHODS

### Study area

The study was conducted at two different locations in Oyo State, Nigeria. The selected sites included a farm cluster in Ologuneru, within the Ido Local Government Area, and the University of Ibadan Teaching and Research Farm in the Ibadan North Local Government Area.

### Animal sampling

Two Duroc herds with suspected outbreaks of enzootic pneumonia and no vaccination history were selected for sampling. Pigs with clinical respiratory signs, including persistent dry, non-productive cough, lethargy, elevated body temperatures, and reduced appetite, were examined. Eight healthy pigs from a separate farm, without clinical signs or any disease conditions or infections that could act as confounding factors, served as the control group. All pigs were of the Duroc breed and within the same age range of three to eight months, ensuring uniformity in breed and age to minimize variability and enhance the reliability

of the comparisons. Efforts were made to select farms with broadly similar feeding regimens and intensive housing systems to reduce management-related variation.

Blood samples were aseptically collected via the anterior vena cava into vacutainer tubes with EDTA for haematology and vacutainer tubes without additives. Clotted blood samples were centrifuged at 4000 revolutions per minute for 10 minutes. Clear serum was separated and stored at -20 °C until analysis.



**Fig. 1:** *M. hyopneumoniae* rapid antibody test kit

### Haematological assay

Haematological analysis in Table 1 included the assessment of haemoglobin concentration using Drabkin's solution, where 0.02 mL of blood was mixed with 5 mL of the reagent and read at 540 nm using a spectrophotometer. Red blood cell (RBC) counts were performed using a hemocytometer, with cells counted under a microscope at 10x magnification. White blood cell (WBC) counts followed a similar procedure, focusing on the four corners of the haemocytometer. Differential leukocyte counts were determined using Giemsa-stained blood smears, and 100 cells were enumerated to

determine the proportions of different leukocytes. Platelet counts were conducted by diluting blood with 1% ammonium oxalate and counting cells using a Neubauer counting chamber. Packed cell volume (PCV) was measured by centrifuging blood in microhaematocrit tubes and reading the results using a microhaematocrit reader (Thrall and Weiser, 2002).

### Serum biochemical analysis

Biochemical analysis involved measuring electrolytes using flame emission photometry, while ion-selective electrodes (ISEs) were used for bicarbonate analysis. Total protein was estimated using the Biuret method, with optical density measured at 540 nm. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by incubating samples with buffered substrates and measuring optical density at 505 nm. Following the manufacturer's protocols, Bilirubin, blood urea nitrogen (BUN), and creatinine were analyzed using the commercial Randox® assay kit (Randox Laboratories, Ltd., UK) (Banwo et al., 2024).

Oxidative stress markers were assessed using standard biochemical assays. GSH levels in serum were evaluated with the adapted DTNB (5,5'-dithiobis (2-nitrobenzoic acid) method following Amar et al. (2019) with some modifications. Glutathione peroxidase (GPx) activity was measured by the method of Kendall et al. (2017). Glutathione-S-transferase (GST)

was estimated by following Moatamedi et al (2014) using 1-chloro-2,4-dinitrobenzene as substrate. Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich (1972) with slight modification by Oyagbemi et al., (2015). Myeloperoxidase (MPO) activity was determined by the method of Pulli et al., (2013). Quantification of MDA, a marker of lipid peroxidation, is achieved using the thiobarbituric acid reactive substances (TBARS) assay, following a modified protocol by Varshney and Kale (1990). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) generation was estimated as described by Wolff (1994). Total Protein (TP) was estimated by the method described by Zheng et al. (2017), and Vitamin C concentration was estimated as described by Angirekula et al. (2018). Nitric Oxide was measured as described by Olaleye et al. (2007).

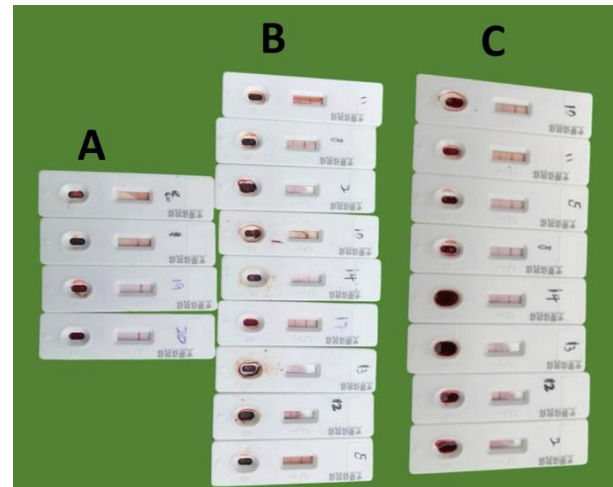
### Statistical Analysis

All statistical analyses were conducted using GraphPad Prism 6.0 software. Results obtained were expressed as mean and standard deviation (SD). A confidence level of 95% and p-values < 0.05 were considered statistically significant (Banwo et al., 2024). Comparisons between means were performed using unpaired t-tests.

### RESULTS

Confirmatory tests using a *Mycoplasma hyopneumoniae* rapid antibody test kit (Beijing Dayoutailai Biotech Co., Ltd) were used to detect the presence of *Mycoplasma hyopneumoniae* antibodies in twelve pigs. Eight

healthy pigs from a separate farm tested negative and served as the negative control.



**Fig. 2:** Showing field results using *M. hyopneumoniae* antibody test kit, (A) tested negative and served as a control, while (B) and (C) tested positive.

Haematological analysis of the infected and control pigs revealed no statistically significant differences across key parameters (Table 1). Packed Cell Volume (PCV), Red Blood Cell (RBC) count, Hemoglobin (Hb) concentration, and White Blood Cell (WBC) count remained within reference ranges for both groups. For instance, the mean PCV for infected pigs was  $46.25 \pm 5.48\%$ , compared to  $45.13 \pm 4.16\%$  in the control group. Similarly, RBC counts were  $10.21 \pm 2.20 \times 10^6$  cells/ $\mu$ L in infected pigs and  $14.77 \pm 5.90 \times 10^6$  cells/ $\mu$ L in controls. Hemoglobin concentrations also showed no significant variation. These findings suggest that haematological parameters alone may not serve as reliable diagnostic indicators for *M. hyopneumoniae* infections, corroborating earlier studies that have reported similar outcomes.

**Table 1:** Effect of *Mycoplasma hyopneumoniae* on the haematological parameters (Mean±SD) of pigs

Parameters	<i>Mycoplasma hyopneumoniae</i> positive (n = 12)	Healthy control (n = 8)
PCV (%)	46.25±5.48	45.13±4.16
RBC (x10 <sup>6</sup> /μL)	10.21±2.20	14.77±15.90
Hb (g/dL)	15±1.70	14.3±1.30
MCV (fL)	46±4.12	43.70±14.12
MCH (pg)	15±1.41	13.90±4.51
MCHC (g/dl)	32±0.73	31.87±0.71
WBC (x10 <sup>9</sup> /L)	6.97 ±235.80	6.26±223.98
Neutrophils (x10 <sup>9</sup> /L)	4.64±1.61	4.11±1.49
Lymphocytes (x10 <sup>9</sup> /L)	2.25±9.98	2.13±8.77
Eosinophils (x10 <sup>9</sup> /L)	0.47±0.64	28.43±41.80
Monocyte (x10 <sup>9</sup> /L)	0.09±0.32	0±0
Platelets (x10 <sup>9</sup> /L)	510.583±201.311	445.250±78.682

Biochemical parameters in Table 2 further supported the limited diagnostic utility of routine blood markers. No significant deviations were observed in glucose, sodium, potassium, or chloride levels between infected and control pigs. For example, glucose levels averaged  $61 \pm 6.57$  mg/dL in infected pigs and  $64 \pm 9.75$  mg/dL in controls, while sodium levels were  $141.08 \pm 2.02$  mmol/L and  $141.5 \pm 2.51$  mmol/L, respectively. Liver function markers, including Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), also showed no significant differences. These results indicate that biochemical markers, much like haematological ones, provide limited insight into the presence or progression of enzootic pneumonia.

In contrast, oxidative stress markers (Table 3) revealed substantial disruptions in infected pigs, demonstrating their potential role in the pathogenesis of the disease. Malondialdehyde (MDA), a marker for lipid peroxidation, was significantly elevated in infected pigs ( $7.05 \pm 1.08$  nmol/mL) compared to controls ( $5.49 \pm 0.33$  nmol/mL). Similarly, nitric oxide (NO) levels were markedly higher in the infected group, with values of  $2.25 \pm 0.26$  μmol/mL versus  $1.56 \pm 0.44$  μmol/mL in controls. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) levels also showed an upward trend, though not statistically significant. These elevated markers indicate increased oxidative damage in infected pigs, suggesting a heightened state of cellular stress and inflammation.

**Table 2:** Shows the effects of *Mycoplasma hyopneumoniae* on the serum biochemistry of pigs

Parameters	<i>Mycoplasma hyopneumoniae</i> positive (n=12)	Healthy control (n=8)	P-value
Total Protein (g/dL)	6.97±0.22	6.82±0.24	0.0851
Albumin (g/dL)	3.88±0.20	3.78±0.28	0.8856
AST (IU/L)	16±1.91	15.63±2.33	0.8191
ALT (IU/L)	12.67±1.83	13±2.39	0.6246
ALP (IU/L)	60.50±7.39	60.50±8.80	0.6445
Total bilirubin (mg/dL)	0.68±0.23	0.75±0.26	0.7521
Conjugated bilirubin (mg/dL)	0.34±0.1	0.35±0.20	0.5847
Total cholesterol (mg/dL)	126.83±31.35	128.63±18.06	0.2369
Triglyceride (mg/dL)	35.08±12.74	17.75±9.68	0.2660
HDL (mg/dL)	22.25±8.34	23.88±6.47	0.2221
LDL (mg/dL)	66.67±33.16	65.13±24.43	0.2206
Cholesterol (mg/dL)	15.58±3.65	13.50±1.41	0.0817
Creatinine (mg/dL)	0.90±0.15	0.98±0.25	0.8298
Glucose (mg/dL)	61±6.57	64±59.75	0.6516
Sodium (mmol/L)	141.08±2.02	141.5±2.51	0.2439
Potassium (mmol/L)	4.05±0.24	4.11±0.27	0.2547
Chloride (mmol/L)	107.5±3.37	107.5±3.77	1.000
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	22.00±1.41	22±22.13	0.7717
BUN (mg/dL)	45.67±8.47	49.63±9.66	0.9073

AST= aspartate transaminase, ALT= alanine transaminase, ALP= alkaline phosphatase, BUN= blood urea nitrogen, HDL= high-density lipoprotein, LDL= low-density lipoprotein. Group comparison shows no statistically significant differences between groups (all  $p > 0.05$ ).

This study also noted significant reductions in enzymatic antioxidants, which further highlight the oxidative imbalance. Superoxide Dismutase (SOD) levels in infected pigs were significantly lower at  $8.44 \pm 1.62$  U/mg compared to  $16.15 \pm 4.30$  U/mg in controls. Similar trends were observed for Glutathione Peroxidase (GPx) and Glutathione-S-Transferase (GST), which decreased to  $24.48 \pm 3.27$  U/mg and  $72.90 \pm 9.29$  U/mg, respectively, in infected pigs. These

enzymes are critical in mitigating oxidative damage, and their depletion suggests that infected pigs experience considerable oxidative stress, likely contributing to disease progression. While haematological and biochemical parameters showed no significant changes, the marked disruption in oxidative stress markers may reflect underlying physiological disturbances associated with exposure to *Mycoplasma hyopneumoniae*. These findings

suggest that oxidative stress evaluation could complement serological data in understanding the host response to infection. This underscores the potential value of incorporating oxidative stress assessments into routine diagnostic

protocols and supports the continued implementation of biosecurity measures to limit the transmission of *M. hyopneumoniae* in commercial pig farms.

**Table 3:** Effects of *hyopneumoniae* on markers of oxidative stress parameters (Mean±SD) of infected and healthy pigs *Mycoplasma*

Parameters	<i>Mycoplasma hyopneumoniae</i> positive	Healthy control
MDA	7.05±1.08**	5.49±0.33
H <sub>2</sub> O <sub>2</sub>	42.40±7.10	37.35±2.84
GPx	24.48±3.27***	35.78±2.01
GSH	112.16±6.27***	165.34±13.89
NO	2.25±0.26***	1.56±0.44
Vit C	2.06±0.05	2.10±0.20
SOD	8.44±1.62***	16.15±4.3
GST	72.90±9.29***	93.80±12.43

Malondialdehyde (MDA), Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), Glutathione Peroxidase (GPx) and Glutathione-S-Transferase (GST), \* indicate significant difference along the same row (\* = P<0.05; \*\* = P<0.01; \*\*\* = P<0.001).

## DISCUSSION

This study investigated the impact of *Mycoplasma hyopneumoniae* infection on haematological, biochemical, and oxidative stress parameters in commercial pigs from two farms in Ibadan, Nigeria. Our findings provide preliminary insight into the physiological responses associated with exposure to *M. hyopneumoniae* in pigs, based on serological evidence.

The haematological analysis revealed no statistically significant differences between infected and healthy pigs across key parameters such as packed cell volume (PCV), red blood cell (RBC) count, and haemoglobin (Hb) concentration. These findings align with

previous studies (Thacker and Minion, 2012), which reported minimal haematological changes in pigs with *M. hyopneumoniae* infection. However, they contrast with Tazayan et al., (2021), who observed erythropenia, leukopenia, lymphopenia and increased blood sedimentation rate (BSR) in infected pigs. The discrepancies may be attributed to differences in disease severity, sample size, or the presence of co-infections. Haematological changes may become more pronounced in younger piglets, more vulnerable to systemic impacts.

Similarly, biochemical parameters such as glucose, sodium, potassium, and liver function markers (ALT, AST) did not show significant differences between infected and control pigs.



These findings contrast with Tazayan et al. (2021), who reported hypoproteinemia, hypoalbuminemia, and elevated hepatic enzyme levels in infected pigs. The absence of significant biochemical alterations in this study suggests that routine biochemical markers may have limited diagnostic value for enzootic pneumonia, especially in its early stages. However, in severe or prolonged cases, metabolic disruptions may become more evident.

Unlike haematological and biochemical parameters, oxidative stress markers revealed significant disruptions in infected pigs, indicating an oxidative imbalance that may play a critical role in disease progression. Elevated malondialdehyde (MDA) and nitric oxide (NO) levels, along with significant reductions in antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST), suggest heightened oxidative stress in *M. hyopneumoniae*-infected pigs, which may be due to prolonged utilization of the enzyme to combat reactive oxygen species (ROS) during *M. hyopneumoniae* infection, which demonstrated that *M. hyopneumoniae* infection induces oxidative stress, leading to tissue damage and disease exacerbation.

The elevated MDA levels observed in this study, a marker of lipid peroxidation, are consistent with findings by Wang (2006), who highlighted the role of oxidative damage in worsening

respiratory pathology. Similarly, the depletion of antioxidant enzymes supports the hypothesis that oxidative stress is a major contributor to disease severity.

Altered levels of antioxidant enzymes, both elevated and reduced, have been reported in various diseases because of increased ROS production, driven by enzyme regulation or their utilization to neutralise ROS (Mates 2000; Valko et al., 2007). In the present study, the significant reductions in antioxidant enzymes align with the report of Štukelj et al. (2013), where GPx activity was significantly lower in porcine reproductive and respiratory syndrome (PRRS) positive pigs than in a corresponding group of PRRS-negative pigs.

The significant increase in total protein in *M. hyopneumoniae*-infected pigs compared to healthy controls suggests a strong association with disease progression. This finding aligns with Štukelj et al. (2013), who reported similar trends in pigs suffering from porcine reproductive and respiratory syndrome.

These findings suggest that oxidative stress markers could serve as more reliable indicators of *M. hyopneumoniae* infections in pigs than conventional haematological and biochemical parameters.

Strengthening farm-level biosecurity measures, such as enhanced sanitation, isolation of infected animals, and vaccination programs, could help reduce the prevalence of *M. hyopneumoniae* infections in commercial pig farms.

## CONCLUSION

Our research shows the limited diagnostic value of haematological and biochemical parameters in detecting *M. hyopneumoniae* infections, particularly in early-stage cases. However, the significant alterations in oxidative stress markers suggest a possible role of oxidative imbalance in the host response to infection. These markers may offer complementary value in understanding disease pathophysiology. These findings underscore the need to incorporate oxidative stress assessments as adjunct tools into the routine diagnostic evaluation of *M. hyopneumoniae* exposure in pigs.

## RECOMMENDATION

Future research should focus on elucidating the molecular mechanisms underlying oxidative stress in enzootic pneumonia, exploring the potential of antioxidant therapies as adjunctive treatments, and developing more targeted diagnostic approach that integrate the early detection of oxidative stress markers in *M. hyopneumoniae* in pigs. Addressing these areas will enhance disease management strategies and improve the health and productivity of commercial pig farms.

## CONFLICT OF INTEREST

The authors declare that no conflicts of interest exist.

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