Research Article

Ultrasonography and Serum Biochemical Analysis in Clinical and Subclinical Pneumonia in Pre-Weaned Buffalo Calves (*Bubalus bubalis*)

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Abstract | Bovine respiratory disease (BRD) is a primary cause of morbidity and mortality in calves. Early diagnosis of BRD in young calves particularly the subclinical cases is very important. Therefore, this study aims to record the lung ultrasonographic and serum biochemical changes accompany the clinical and subclinical BRD in calves. A total number of 40 buffalo calves were clinically examined by the usual physical examination methods. Thorax ultrasonography on both sides was applied, and blood samples were collected for serum biochemical analysis. After clinical examination calves were grouped into diseased (n=15) and apparently healthy (n=25), but after TUS buffalo calves were regrouped into healthy (n=18), subclinical (n=7), and clinically diseased (n=15) the ultrasound examination of lungs revealed different levels of lung affections varying from mild comet tail to pulmonary hyperechogenicity and pleural irregularity. The levels of serum interleukin-6, haptoglobin, and malondialdehyde were similar in both the healthy group (35.74 pg/ml, 237.30 ng/ml, and 56.77 ng/ml, respectively) and the subclinical group (34.71 pg/ml, 241.23 ng/ml, and 60.35 ng/ml, respectively). However, these parameters were markedly elevated in the clinical group (83.46 pg/ml, 543.77 ng/ml, and 130.31 ng/ml, respectively). Additionally, the albumin level in the clinical group (3.60 g/dL) was significantly lower than those in the healthy (4.22 g/dL) and subclinical groups (4.00 g/dL). The activities of GGT and ALP in the clinical group (68.20 U/L and 190.40 U/L, respectively) were substantially higher than in the healthy group (12.94 U/L and 142.38 U/L, respectively) and the subclinical group (15.88 U/L and 127.19 U/L, respectively). Routine clinical examination aided with thoracic ultrasonographic scanning and serum biochemical analysis for buffalo calves could facilitate the early detection of subclinical pneumonia, therapy helping farmers to avoid adverse consequences and minimize the economic losses.

Keywords | Thoracic ultrasonography, Interleukin-6, oxidative stress, Haptoglobin, Subclinical pneumonia, Buffalo calves

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INTRODUCTION

Bovine respiratory disease (BRD) is one of the most economically significant diseases affecting the cattle industry worldwide because of its substantial impact on animal health, welfare, productivity, and treatment costs. In addition to causing considerable economic losses, BRD adversely affects animal welfare and remains a leading cause of mortality in dairy heifers in the United States. It has also been identified as the second most common cause of death in pre-weaned dairy calves (USDA, 2007; USDA, 2008). Pulmonary tissue damage associated with BRD markedly impairs animal performance and productivity. Weekly monitoring studies have demonstrated that lung consolidation significantly reduces average daily gain in pre-weaned dairy calves (Sáadatnia *et al*., 2023). In feedlot cattle, morbidity rates may exceed 70%, while mortality rates can range from 5% to 70%, depending on disease etiology and management practices (Edwards, 2010; Griffin *et al*., 2010).

Although BRD has been extensively investigated in cattle, relatively few studies have addressed its prevalence in buffaloes. Earlier reports indicated that BRD prevalence is higher in young buffaloes (1–4 years old) compared with adults (Robin, 1974; Makhdoomi *et al*., 2013). More recently, Jaibhaye *et al*. (2022) reported a BRD prevalence of 4.6% in Indian buffaloes. Respiratory diseases are commonly associated with various physiological and biochemical alterations, including changes in circulating biomarkers. Disturbances in internal homeostasis contribute to the development of clinical respiratory manifestations and inflammatory responses.

Several clinical scoring systems have been developed to improve BRD diagnosis under field conditions (Theurer *et al*., 2013). Respiratory scoring (RS) systems, including the California and Wisconsin methods, rely on the evaluation of objective clinical signs such as nasal discharge, coughing, rectal temperature, ocular discharge, and abnormal respiratory sounds (Love *et al*., 2014). However, subclinical pneumonia may occur in the absence of obvious clinical signs despite having potential long-term consequences comparable to clinical pneumonia, including reduced growth performance, decreased milk production, and lower carcass yield. Furthermore, buffaloes often exhibit mild or subclinical manifestations of disease, which may complicate early diagnosis (Martínez-Burnes *et al*., 2024), for example Reddy *et al*. (2018) reported that four buffalos suffering from mild pneumonia died during treatment. This might be due to long-standing subclinical pneumonia

Recently, thoracic ultrasonography (TUS) has emerged as a valuable on-farm, non-invasive diagnostic tool capable of providing accurate information regarding lung consolidation and the progression of pulmonary lesions. Early detection of BRD is essential for limiting disease progression and minimizing economic losses (Li *et al*., 2022). Therefore, identifying reliable biomarkers for naturally occurring BRD, particularly in mild and moderate cases, is of considerable importance for reducing disease incidence and recurrence (Joshi *et al*., 2018). Alterations in plasma biochemical parameters may provide useful information for the early diagnosis of BRD, assessment of disease severity, and evaluation of the inflammatory response associated with pneumonia (Kumar *et al*., 2018). Consequently, integrating respiratory scoring systems, biochemical analyses, and thoracic ultrasonography may represent a comprehensive and effective approach for the early detection of BRD, particularly during the introduction of new animals into farms (Jourquin *et al*., 2023).

The present study aimed to describe the dynamics of lung consolidation during the early stages of pneumonia using thoracic ultrasonography and to evaluate the associated serum biochemical alterations in clinical and subclinical cases of BRD in pre-weaned buffalo calves (*Bubalus bubalis*) compared with clinically healthy controls.

MATERIALS AND METHODS

Animals and housing

A total of 40 buffalo calves ranged between 2-8 weeks of age, were from the animal farm of the Faculty of Veterinary Medicine, Suez Canal University. The calves were housed in an outdoor yard during daylight and transferred to closed pens at sunset. Calves were suckling from foster mothers twice daily and beside the calf starter meal.

Clinical examination

Clinical examination of the animals was conducted. Calves suspected to have BRD. All clinical signs, rectal temperature, mucous membrane and thorax auscultation by stethoscope were recorded.

Ultrasonographic examination

Ultrasonographic examination of thoracic region from the right and left sides was conducted in all animals using a 4.5–7.5MHz linear ultrasound for calves, EXGAo, Noveko International Inc, ECM, Echo Control Medical, France). The protocol of TUS examination followed Ollivett and Buczinski (2016). The probe was moved from the caudodorsal thorax to the cranioventral starting from the 10th to 6th intercostal spaces ICS to examen the caudal lobes of right and left lungs. Then on the right thorax the probe was at the 5th ICS to examine the middle lobe till reaches the base of the heart until the internal thoracic artery and vein were seen to examine the cranial and caudal aspects of the cranial lobe. While on the left side the probe was placed on the 5-4th ICS, the progressed till reaching the heart to examine the caudal and cranial aspects of the cranial lobe of the left lung respectively. The TUS scoring followed the ordinal 6-point TUS scoring method (Table 1).

Table 1: The ordinal 6-point thoracic ultrasound (TUS) scoring method and the TUS2 and TUS3 criteria, and their definitions.

Thoracic ultrasound score	TUS2	TUS3	Definition
0	-	-	Normal aerated lung
1	-	-	Diffuse comet tails
2	+	-	Lobular pneumonia: Discrete areas of consolidation ≥ 1 cm^2 within an otherwise aerated lung
3	+	+	Lobar pneumonia: consolidation of 1 entire lung lobe
4	+	+	Lobar pneumonia: consolidation of 2 entire lung lob
5	+	+	Lobar pneumonia: consolidation of ≥3 entire lung lobes

Blood sampling

A blood sample was drawn from the jugular vein of each calf in a plain tube. After allowing the blood to clot, it was centrifuged at 1000 ×g for 10 minutes. The serum was then separated and kept at -20°C for biochemical analysis.

Serum biochemical assay

The kits used to assess alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein, and albumin were obtained from CliniChem (Budapest, Hungary). Bovine ELISA kits from Sunlong Biotech. (China) were used to measure the haptoglobin and malondialdehyde (MDA) levels. Fine Test (China) provided the bovine ELISA kits used to detect interleukin-6 (IL-6) and superoxide dismutase (SOD). All the serum biochemical parameters were measured using the standard procedures outlined in the manufacturer's booklet. Serum globulin was determined by subtracting albumin from total protein, and the albumin-to-globulin (A/G) ratio was determined by diving albumin by globulin (Kaneko *et al*., 2008).

Statistics

Data were analyzed using SPSS version 25 (IBM Corp., Armonk, NY). Normality of the data was assessed using the Shapiro-Wilk test. Homogeneity of variance was evaluated using Levene's test. For ALP which satisfying the assumption of normality and homogeneity of variance ($p > 0.05$), a standard One-Way ANOVA was conducted followed by Tukey's HSD post-hoc test was applied for pairwise comparisons. However, because most variables exhibited significant heteroscedasticity ($p < .05$ in Levene's test), Welch's ANOVA was employed as the parametric test for these markers. Where Welch's ANOVA yielded significant results, post-hoc pairwise comparisons were conducted using the Games-Howell procedure, which does not assume equal variances. For variables failing the normality assumption (GGT and Albumin), the Kruskal-Wallis H test was utilized, followed by Dunn-Bonferroni post-hoc analysis. Effect sizes were reported according to the statistical test. For parametric data analyzed via Welch's ANOVA, Omega squared (ω^2) was calculated. For non-parametric data analyzed via the Kruskal-Wallis test, Epsilon squared (ε^2) was used. While Partial Eta Squared (η_p^2) is reported in standard ANOVA. Results were considered statistically significant at $p < 0.05$.

RESULTS

Clinical presentation

Clinical examination revealed that normal healthy animals which did not show any of the clinical respiratory signs and no abnormal lung sounds (n=25, 62.5%), six calve (n=6, 15%) showed mild symptoms like slight unilateral nasal discharge and the temperature didn't exceed 38.5 °C, two calves had (n=2, 5%) bilateral mucoid nasal discharge, mild ocular discharge and the body temperature was (39.5 °C). There was only one severe case of a calf having offensive odor yellow colored diarrhea and the body temperature was 40°C (n=1, 2.5%). On auscultation of trachea and lungs sounds on both sides, there were crackling and some wheezes lung sounds in the diseased animals in addition to rattling tracheal and another 6 calves (15%) had crackles without respiratory clinical signs.

Ultrasonographic findings

Normal lungs ultrasonography showed neither consolidation nor pleural effusion in 18 normal calves (45%) (Figure 1). Twenty-two calves (55%) showed different pulmonary lesions on TUS, that number is subdivided as follows: fifteen calves showed comet tails artifacts in the form of bright echogenic bands (hyperechogenic areas). The comet tail artifacts varied from single to multiple comet tails > 0.5 -1cm especially in the cranioventral lobes were fanning out of the pleural line to the edge of the image scored as TUS1 (Figures 2 and 3). Four cases of pulmonary consolidation > 1.5cm width appeared on sonography, three of them expressed consolidation on the right lung and one case on the left lung. Some pulmonary consolidations appeared as hypoechoic structure surrounded by hyperechoic wall (Figure 3). Another form of consolidation where the bronchial walls appeared hyperechogenic with acoustic shadowing or comet tail (Figure 4) scored as TUS2 or lobular pneumonia. No pleural effusion was recorded, but there were irregularity and thickening of pleural membranes and both layers seemed slightly hyperechogenic in three calves (Figure 5). The subclinical cases were 5 casesTUS1 (as diffused comet tails vs 2 cases of TUS2 as comet tails with consolidation ≥ 1 cm.

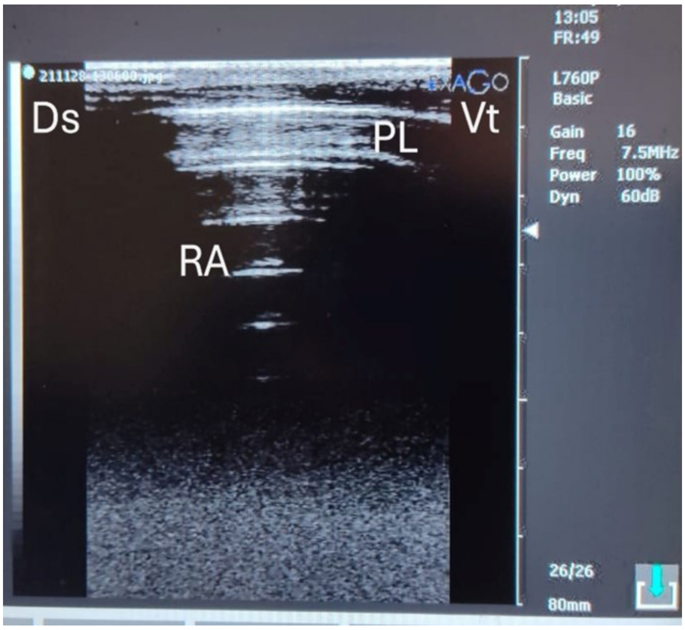

Figure 1: Ultrasonogram of the surface of a normal lung in pre-weaned calf. The echogenic line represented pleural line (PL) as well as echogenic lines running parallel to the pulmonary surface referred as reverberation artifacts (RA). Ds= Dorsal; Vt= Ventral.

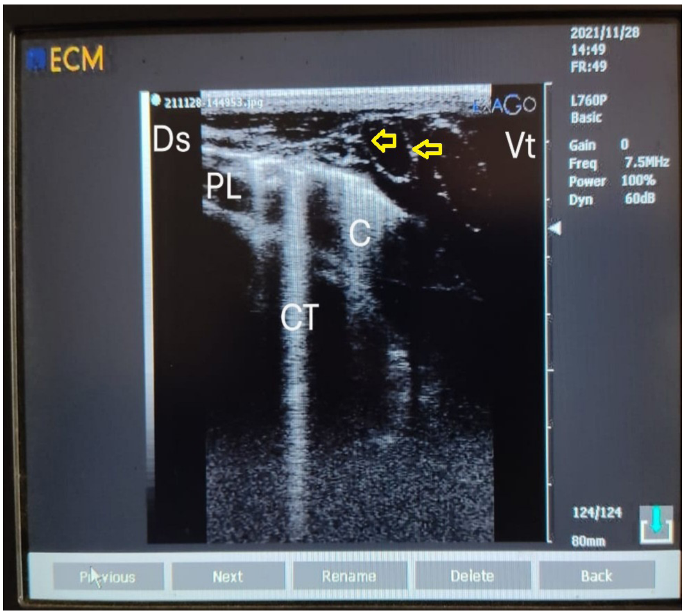

Figure 2: Thoracic ultrasonographic image of calves by examination of lung showed comet tail with reverberation artifact in caudal aspect of cranial lobe of right lung linear probe placed on 1–2 intercostal space Ds=Dorsal, Vt=Ventral, PL=Pleural line and CT= comet tail and C= consolidation, arrows= thoracic blood vessels.

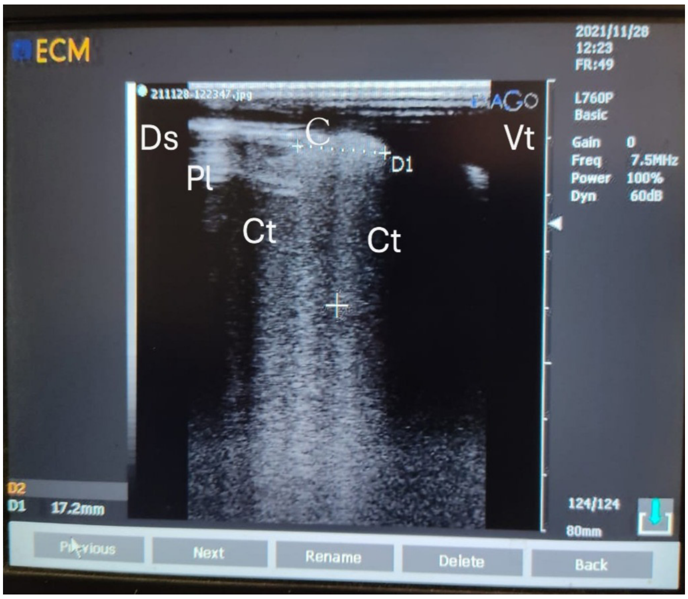

Figure 3: Thoracic ultrasonographic image of calves by examination of lung showed reverberation artifact in caudal aspect of cranial lobe of right lung linear probe placed on 3–4 intercostal space Ds=Dorsal, Vt=Ventral, PL=Pleural line, C= comet tails and CO= consolidation.

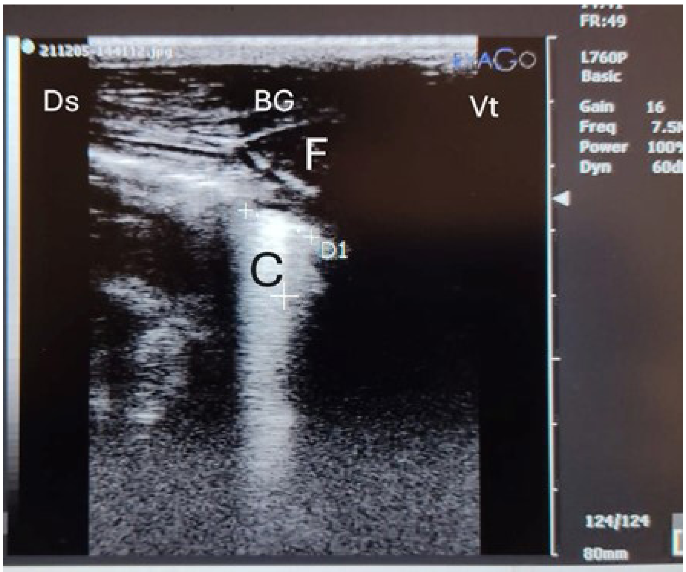

Figure 4: Ultrasonogram in a4 weeks old calf with bronchopneumonia (caudal aspect of cranial lobe) imaged from the upper third of the thorax in the left 5th ICS by using 7.5 MHz linear array transducer. It showed consolidated lung vs. mirror-image. Consolidation of lungs= C. Air bronchograms (BG) appeared as hyperechoic reflective bands within consolidated pulmonary lobe with comet-tail artifacts (CT) and hyperechoic spots that may be filled with fluid= (F). PL=Pleural line; Ds=Dorsal; Vt=Ventral.

Serum biochemical analysis

The levels of IL-6, total protein, and haptoglobin revealed a highly significant increase ($p < 0.001$), while the albumin showed a highly significant drop ($p < 0.001$) in the clinical

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group in comparison to the healthy and subclinical groups. These parameters did not show significant changes between the healthy and the subclinical groups. The globulin level and A/G ratio showed significant differences among the three groups. The clinical group had the highest globulin level, followed by the subclinical group, then the healthy group. In the meantime, the healthy group had the highest A/G ratio, followed by the subclinical group, and the clinical group had the lowest. However, the GGT and ALP activities showed highly significant elevations ($p < 0.001$) in the clinical group compared to the healthy and subclinical groups. These liver enzymes did not show a significant difference between the healthy and the subclinical groups. The SOD activity exhibited a highly significant decrease ($p < 0.001$), although the MDA level revealed a highly significant increase in the clinical group compared to those of the healthy and subclinical groups (Table 2). There were no significant variations in SOD activity or MDA levels between the healthy and subclinical groups.

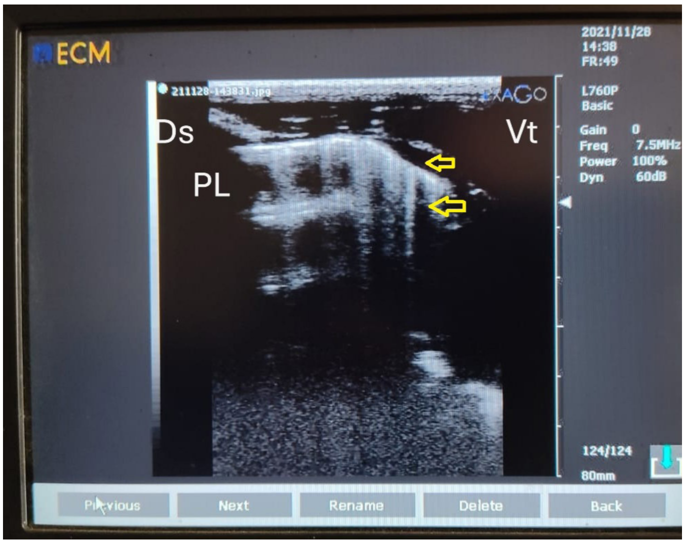

Figure 5: Thoracic ultrasound of subclinical bovine respiratory disease-affected calves. Examination of cranial aspect of cranial lung lobe of the right lung revealed arrows = pleural irregularity.

DISCUSSION

According to Buczinski *et al.* (2025), calves with clinical signs and lung lesions are considered as having clinical pneumonia; calves with clinical signs and no lung anomalies are considered as calves with upper respiratory tract infection; calves without clinical signs but lung lesions are considered as subclinical pneumonia; and calves with no abnormal signs and normal lung findings are considered as healthy calves. After the clinical examination of the forty buffalo calves in the current study the percentage of apparently healthy animals 62.5% and after the TUS, the percentage of affected animals with lung consolidations and pulmonary affections increased while the percentage of healthy calves decreased. These results verify the importance of combination between the clinical signs and physical and ultrasonographic examination. Thoracic auscultation is of limited value (Buczinski *et al.*, 2014) and it requires a skillful practitioner and quite environment for careful listening and lung sounds differentiation. Concerning buffalos which are reported to be very tolerant to express any symptoms of diseases (Mahmmod, 2014) due to their anatomical, physiological characteristics, and the rough environment where they are usually raised, water buffaloes are assumed to be more resistant to certain diseases and show minor clinical signs than cattle (Cuevas-Gómez *et al.*, 1997; Martínez-Burnes *et al.*, 2024). In fact, there is a weak to mild positive correlation between the clinical signs score and the TUS in dairy cattle and buffalo calves (Hussein *et al.*, 2018). Also, Rabeling *et al.* (1998) detected subclinical lung lesions by ultrasonography as atypical consolidation when examined 24 clinically normal calves. Healthy calves had normal lungs which couldn't be imaged by ultrasonography due to air content except for the appearance of reverberation artifacts that refer to the pleura (visceral and parietal layers). The sliding movement of the pleural lines were detected ultrasonographical as smooth hyperechoic lines during respiration. Ultrasonography of lungs revealed different pathological conditions within the lungs starting from a single comet tail to multiple comet tails. The presence of comet-tail artifacts on thoracic ultrasonography (TUS) is indicative of increased fluid accumulation, inflammation, and cellular infiltration within the pulmonary parenchyma. These pathological changes enhance the propagation and reflection of ultrasound waves, resulting in the generation of comet-tail artifacts. Clinically, the detection of comet tails is highly relevant because it reflects a variety of pulmonary and cardiogenic disorders. These artifacts are commonly associated with interstitial syndrome, cardiogenic pulmonary edema. According to Hussein *et al.* (2018) in buffalo calves the comet tail reflects sever congestion in lungs. The hypoechogenic areas surrounded by hyperechogenic walls of the bronchogram which indicates lung consolidation usually result from accumulation of inflammatory cells and cellular debris that displace the air from the lung tissue of clinical and subclinical cases of bronchopneumonia (Caswell and Williams, 2007). While the hypoechoic areas result from accumulation of fluids in the alveoli. Pleural irregularity means disruption of the pleural line continuity by comet tails or areas of consolidation. Some studies consider pleural irregularity as indication of infection or pneumonia in cattle (Omar *et al.*, 2024) and in buffalo calves (Al-Noaemy and Al-Iraqi 2023).

Cytokines play a crucial part in the inflammatory and immunological reactions that occur during pulmonary defense mechanisms (Rodríguez et al., 2015). In early

Table 2: Serum biochemical parameters in healthy, subclinical, and clinical groups of buffalo calves.

Parameters	Groups	Mean	95% confidence interval for mean	Statistic (F or H)	df	Effect Size
IL6 (pg/ml)	Healthy	35.74 ± 1.38[b]	(32.82-38.66)	54.237	2, 23.45	0.038 (ω^2)
	Subclinical	34.71 ± 0.79[b]	(32.78-36.64)			
	Clinical	83.46 ± 4.55[a]	(73.71-93.22)			
Total protein (g/dl)	Healthy	7.07± 0.09[b]	(6.89-7.25)	34.280	2, 17.19	0.031 (ω^2)
	Subclinical	7.27 ± 0.09[b]	(7.06-7.50)			
	Clinical	7.84 ± 0.05[a]	(7.73-7.95)			
Albumin (g/dl)	Healthy	4.22 ± 0.07[a]	(4.07-4.37)	25.704	2	0.485 (ε^2)
	Subclinical	4.00 ± 0.09[a]	(3.76-4.23)			
	Clinical	3.60 ± 0.03[b]	(3.52-3.67)			
Globulin (g/dl)	Healthy	2.84 ± 0.07[c]	(2.70-2.98)	209.946	2, 16.25	0.048 (ω^2)
	Subclinical	3.28 ± 0.07[b]	(3.10-3.45)			
	Clinical	4.28 ± 0.03[a]	(4.20-4.36)			
A/G ratio	Healthy	1.49 ± 0.03[a]	(1.44-1.55)	304.511	2, 13.06	0.048 (ω^2)
	Subclinical	1.22 ± 0.05[b]	(1.11-1.34)			
	Clinical	0.84 ± 0.01[c]	(0.82-0.86)			
Haptoglobin (ng/ml)	Healthy	237.30 ± 6.79[b]	(222.97-251.63)	59.188	2, 22.35	0.040 (ω^2)
	Subclinical	241.23 ± 5.68[b]	(227.33-255.14)			
	Clinical	543.77 ± 27.22[a]	(485.38-602.16)			
GGT (U/L)	Healthy	12.94 ± 0.39[b]	(12.10-13.78)	31.218	2	0.568 (ε^2)
	Subclinical	15.88 ± 0.55[b]	(14.54-17.22)			
	Clinical	68.20 ± 3.27[a]	(61.19-75.21)			
ALP (U/L)	Healthy	142.38 ± 3.11[b]	(135.81-148.94)	61.692	2,37	0.769 (η_p^2)
	Subclinical	127.19 ± 6.91[b]	(110.29-144.10)			
	Clinical	190.40 ± 3.82[a]	(182.21-198.59)			
SOD (ng/ml)	Healthy	1.31 ± 0.03[a]	(1.24-1.37)	85.009	2, 22.59	0.043 (ω^2)
	Subclinical	1.28 ± 0.02[a]	(1.22-1.34)			
	Clinical	0.79 ± 0.03[b]	(0.73-0.87)			
MDA (ng/ml)	Healthy	56.77 ± 3.32[b]	(49.77-63.77)	124.959	2, 22.29	0.046 (ω^2)
	Subclinical	60.35 ± 0.87[b]	(58.22-62.48)			
	Clinical	130.31 ± 4.30[a]	(121.08-139.54)			

Mean values ± standard error. F, F-statistic; H, Kruskal-Wallis H-statistic; df, Degree of freedom; Statistically significant differences ($p < 0.001$) are denoted by superscript letters (a, b, c) where groups sharing the same letter do not differ significantly based on the post-hoc analysis. IL-6: interleukin-6, A/G ratio: albumin/globulin ratio, GGT: gamma-glutamyl transferase, ALP: alkaline phosphatase, SOD: superoxide dismutase, MDA: malondialdehyde.

stage of pneumonia, alveolar macrophages are stimulated and release pro-inflammatory cytokines such as IL-6 (El-Sayed *et al*., 2025). IL-6 triggers lung dendritic cells, alveolar macrophages, endothelial cells, and epithelial cells, to produce chemokines that attract neutrophils and monocytes to the affected area (Ackermann *et al*., 2010). Moreover, IL-6 is essential for promoting the production of acute-phase proteins in the liver, which are critical during acute inflammation (McGill and Sacco, 2020). Our results showed that serum IL-6 level in the clinical group was significantly increased. These findings are in line with Kabu *et al*. (2016) who reported that IL-6 level was detected to be higher in the calves with clinical pneumonia than in the control group due to the existing inflammation.

In our study, the clinically diseased calves had hyperproteinemia and hyperglobulinemia. Hyperproteinemia is usually related to infection and inflammation due to increased synthesis of acute phase proteins and immunoglobulins (Ramadan *et al*., 2019). A notable decrease in serum albumin level was detected in the clinical group. Albumin is the main negatively reacting acute phase protein, and it's concentration decreases when infection and inflammation are present (Eckersall and Bell, 2010). This may be caused by the liver, which reduces albumin synthesis while increasing the production of acute phase proteins (Basbug *et al*., 2016). We also found a significant decrease in A/G values in the clinical group mainly due to the increased immunoglobulin synthesis (Ramadan *et al*., 2019). Haptoglobin is a primary acute phase protein and a preferred indicator in respiratory diseases, triggered by a pulmonary infection and inflammation (Khalphallah *et al*., 2022; Schrödl *et al*., 2016). The clinical group in our investigation showed a substantial increase in serum haptoglobin. This result was consistent with prior research showing that cattle with respiratory disorders had serum haptoglobin levels above reference levels (Jain *et al*., 2011).

The clinical group showed a significantly higher GGT and ALP activities. This could be due to the systemic hypoxia, which triggers liver damage and increase the liver enzymes (Jaramillo *et al*., 2022). Another research reported a substantial rise in liver enzymes in BRD affected calves (Ramadan *et al*., 2019), which may be related to potential hepatic dysfunction caused by an inflammatory response in pneumonic calves (Almujalli *et al*., 2015).

The MDA level of the clinical group was significantly increased in our investigation. A prior study found a notable rise in lipid peroxidation products including MDA, which is an indication of oxidative stress and a crucial component of the severe inflammation that occurs in the lungs of calves with BRD. When an infection of the respiratory tract and inflammation, oxidative stress may result from phagocytic cells at the infection site producing excessive amounts of reactive oxygen species (ROS) to eliminate harmful microorganisms (Joshi *et al*., 2018). One of the main endogenous antioxidant enzymes is SOD. In our study, SOD activity significantly reduced in the clinical group. Our findings are consistent with another study that found that the SOD activity of BRD-stressed calves was much lower than that of healthy calves (Joshi *et al*., 2018). This may be attributed to its wide usage in ROS neutralization (Panciera and Confer, 2010).

CONCLUSION

Clinical signs and laboratory analyses remain essential components of disease diagnosis; nevertheless, their diagnostic value may be limited in detecting subclinical respiratory conditions such as subclinical pneumonia. Thoracic ultrasonography, as a non-invasive and real-time imaging modality, provides additional diagnostic information that improves the detection of pulmonary lesions not evident during routine clinical examinations. Therefore, the incorporation of TUS into bovine health assessment protocols can facilitate earlier diagnosis, optimize treatment decisions, and improve overall herd health and management strategies

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NOVALITY STATMENT

This study provides novel evidence about the ultrasonographic findings when examining pre-weaned buffalo calves and measured the serum biochemistry. Unlike other studies that evaluate the ultrasonography in clinically diseased cattle suffering from BRD, the present study focuses on the buffalo calves which is a species with little studies and references. We screened pre-weaned buffalo calves living in the same farm and having the same environmental conditions by physical examination, thoracic ultrasonography, and measured haptoglobin and interleukin- 6 and MDA in buffalo calve. Our findings revealed the ability of buffalo calves to hide the clinical signs of pneumonia even if the ultrasonography revealed some pulmonary lesions. Also, the subclinical cases do not vary significantly in the blood biochemistry from the healthy calves.

AUTHORS CONTRIBUTION

WIM did the clinical examination, ultrasonographic examination, collected the blood samples, and wrote the manuscript. SKA performed the statistical data analysis and wrote the manuscript. NSA conducted the laboratory work for biochemical analysis, the statistical data analysis, and wrote the manuscript.

Ethical approval

The experimental protocol followed the international guidelines and was approved by the Faculty of Veterinary Medicine Ethics Committee, Suez Canal University (SCU-VET-AREC-R-2026009).

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ackermann MR, Derscheid R, Roth JA (2010). Innate immunology of bovine respiratory disease. Vet. Clin. N. Am. Food Anim. Pract., 26(2): 215-228. https://doi.org/10.1016/j.cvfa.2010.03.001

Almujalli A, El-Deeb W, Eljalii E, Fouda T, AlBlwy M (2015). Clinical, biochemical and bacteriological investigation of pneumonia in calves with special reference to alpha-1-acid glycoprotein response. Int. J. Vet. Health Sci. Res., 3(5): 60-63.

Al-Noaemy MT, Al-Iraqi OM (2023). Detection of lung lesions by ultrasonography technique in buffalo calves Egypt. J. Vet. Sci., 54(2): 165-171. https://doi.org/10.21608/ejvs.2022.159888.1392

Basbug O, Ağaoğlu ZT, Tuzcu N, Coskun A, Aydogdu U, Yigin A (2016). Tumour necrosis factor-alpha, haptoglobin, serum amyloid A and neopterin levels in cattle with lumpy skin disease. Kafkas. Univ. Vet. Fak. Derg., 22(3): 417-424.

Buczinski S, Forté G, Francoz D, Bélanger AM (2014). Comparison of thoracic auscultation, clinical score, and ultrasonography as indicators of bovine respiratory disease in preweaned dairy calves. J. Vet. Intern. Med., 28(1): 234-242. https://doi.org/10.1111/jvim.12251

Buczinski S, Ollivett TL, Pardon B (2025). Invited review: Lung ultrasonography-improving our understanding and management of respiratory disease in young calves. J. Dairy Sci., 108(12): 12903-12923. https://doi.org/10.3168/jds.2025-26764

Caswell J, Williams K (2007). Respiratory System In: Maxie MG, editor. Jubb, Kennedy & Palmer's Pathology of Domestic Animals. 5th ed Philadelphia: Elsevier Ltd., pp. 591-593.

Cuevas-Gómez I, McGee M, Sánchez JM, O'Riordan E, Byrne N, McDaneld T, Earley B (2021). Association between clinical respiratory signs, lung lesions detected by thoracic ultrasonography and growth performance in pre-weaned dairy calves. Ir.Vet. J., 74(1): 7. https://doi.org/10.1186/s13620-021-00187-1

Eckersall PD, Bell R (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Vet. J., 185(1): 23-27. https://doi.org/10.1016/j.tvjl.2010.04.009

Edwards TA (2010). Control methods for bovine respiratory disease for feedlot cattle. Vet. Clin. North. Am. Food. Anim. Pract., 26(2): 273-284. https://doi.org/10.1016/j.cvfa.2010.03.005

El-Sayed A, Eissa A, Ebrahim D, Ateya A, Gadalla H, Alharbi HM, Alwutayd KM, Babaker MA, Aly Elzeer A (2025). Variants in nucleotide sequences, gene expression; and hematological, immune, and antioxidant biomarkers linked to pneumonia risk in holstein calves. Vet. Sci., 12(7): 620. https://doi.org/10.3390/vetsci12070620

Griffin D, Chengappa MM, Kuszak J, McVey DS (2010). Bacterial pathogens of the bovine respiratory disease complex. Vet. Clin. North. Am. Food Anim. Pract., 26(2): 381-394. https://doi.org/10.1016/j.cvfa.2010.04.004

Hussein HA, Binici C, Staufenbiel R (2018). Comparative evaluation of ultrasonography with clinical respiratory score in diagnosis and prognosis of respiratory diseases in weaned dairy buffalo and cattle calves. J. Anim. Sci. Technol., 60: 29. https://doi.org/10.1186/s40781-018-0187-3

Jaibhaye C, Bhikane A, Masare P, Bhonsle A (2022). Epidemiological, clinico-diagnostic and therapeutic aspects of bovine respiratory disease in Indian buffaloes. Buffalo Bull., 41(4): 683. https://doi.org/10.56825/bufbu.2022.4143484

Jain S, Gautam V, Naseem S (2011). Acute-phase proteins: As diagnostic tool. J. Pharm. Bioallied Sci., 3(1): 118-127. https://doi.org/10.4103/0975-7406.76489

Jaramillo C, Renaud DL, Arroyo LG, Kenney DG, Gamsjaeger L, Gomez DE (2022). Serum haptoglobin concentration and liver enzyme activity as indicators of systemic inflammatory response syndrome and survival of sick calves. J. Vet. Intern. Med., 36(2): 812-819. https://doi.org/10.1111/jvim.16357

Joshi V, Gupta V K, Bhanuprakash A G, Mandal R S K, Dimri U, Ajith Y (2018). Haptoglobin and serum amyloid A as putative biomarker candidates of naturally occurring bovine respiratory disease in dairy calves. Microb. Pathog., 116: 33-37. https://doi.org/10.1016/j.micpath.2018.01.001

Jourquin S, Lowie T, Debruyne F, Chantillon L, Vereecke N, Boyen F, Boone R, Bokma J, Pardon B (2023). Dynamics of subclinical pneumonia in male dairy calves in relation to antimicrobial therapy and production outcomes. J. Dairy Sci., 106(1): 676-689. https://doi.org/10.3168/jds.2022-22212

Kabu M, Elitok B, Kucukkurt I (2016). Detection of serum amyloid-A concentration in the calf clinically diagnosed with pneumonia, enteritis and pneumoenteritis. Microbiol. Cienc. Rural., 46(2): 293-299. https://doi.org/10.1590/0103-8478cr20150571

Kaneko JJ, Harvey JW, Bruss ML (2008). Clinical biochemistry of domestic animals (Vol. 6th edition): Academic press. https://doi.org/10.1016/B978-0-12-370491-7.00020-9

Khalphallah A, Elsayed HK, Mottelib AA, Mohammed MG (2022). Ultrasonography as a differential diagnostic tool of bovine respiratory tract disorders with reference to serum haptoglobin and lipid profiles changes. J. Adv. Vet. Res., 12(2): 153-165.

Kumar P, Jain V, Kumar T, Kumar V, Rana Y (2018). Clinical and haematobiochemical studies on respiratory disease in buffaloes. Int. J. Livest. Res., 8(8): 178–184. https://doi.org/10.5455/ijlr.20171210043959

Li J, Zhu Y, Shoemake B, Liu B, Adkins P, Wallace L (2022). A systematic review of the utility of biomarkers as aids in the early diagnosis and outcome prediction of bovine respiratory disease complex in feedlot cattle. J. Vet. Diagn. Invest., 34(4): 577-586. https://doi.org/10.1177/10406387221081232

Love WJ, Lehenbauer TW, Kass PH, Van Eenennaam AL, Aly SS (2014). Development of a novel clinical scoring system for on-farm diagnosis of bovine respiratory disease in pre-weaned dairy calves. Peer J., 2: e238. https://doi.org/10.7717/peerj.238

Mahmmod Y (2014). Natural babesia bovis infection in water buffaloes (*Bubalus bubalis*) and crossbred cattle under field conditions in Egypt: A preliminary study. J. Arthropod. Borne Dis., 8(1): 1-9.

Makhdoomi DM, Gazi M, Tufani DN, Zahid U (2013). Therapeutic management of respiratory infections in large and small ruminants- A clinical study of 96 animals. Intas polivet., 14, (1): 88-92.

Martínez-Burnes J, Barrios-García H, Carvajal-de la Fuente V, Corona-González B, Obregón Alvarez D, Romero-Salas D (2024). Viral diseases in water buffalo (*Bubalus bubalis*): New insights and perspectives. Animals (Basel). 14(6): 845. https://doi.org/10.3390/ani14060845

McGill JL and Sacco RE (2020). The Immunology of Bovine Respiratory Disease: Recent Advancements. Vet. Clin. North Am. Food Anim. Pract., 36 (2): 333-348. https://doi.

org/10.1016/j.cvfa.2020.03.002

Ollivett TL, Buczinski S (2016). On-farm use of ultrasonography for bovine respiratory disease. Vet. Clin. North Am. Food Anim. Pract., 32(1): 19-35. https://doi.org/10.1016/j.cvfa.2015.09.001

Omar I, Abdelghany AH, El-Attar HE, Abdel-Raoof Y, Ghanem MM (2024). Diagnostic utility of ultrasonographic and clinical assessment of bovine respiratory disease in feedlot calves. Benha Vet. Med. J., 47(2): 103-106. https://doi.org/10.21608/bvmj.2024.336267.1891

Panciera RJ, Confer AW (2010). Pathogenesis and pathology of bovine pneumonia. Vet. Clin. North Am. Food Anim. Pract., 26(2): 191-214. https://doi.org/10.1016/j.cvfa.2010.04.001

Rabeling B, Rehage J, Döpfer D, Scholz H (1998). Ultrasonographic findings in calves with respiratory disease. Vet. Rec., 143(17): 468-471. https://doi.org/10.1136/vr.143.17.468

Ramadan M, Ghanem M, El-Attar HE, Abdel-Raoof Y (2019). Evaluation of clinical and hematobiochemical alterations in naturally occurring bovine respiratory disease in feedlot cattle calves in Egypt. Benha Vet. Med. J., 36(2): 305-313. https://doi.org/10.21608/bvmj.2019.16753.1088

Reddy PS, Rao VV, Rani NL, Subramanyam KV (2018). Therapeutic studies on bacterial pneumonia in buffaloes. Buff. Bull., 37(3): 343-359.

Robin L (1974). Bovine respiratory disease: Its radiological features. Vet. Radiol., 15(1): 41-47. https://doi.org/10.1111/j.1740-8261.1974.tb00669.x

Rodríguez F, González JF, Arbelo M, Zucca D, Fernández A (2015). Cytokine expression in lungs of calves spontaneously infected with Mycoplasma bovis. Vet. Res. Commun., 39(1): 69-72. https://doi.org/10.1007/s11259-014-9620-3

Sáadatnia A, Mohammadi GR, Azizzadeh M, Mirshahi A, Mohieddini AA, Buczinski S (2023). Effect of ultrasonographic lung consolidation on health and growth in dairy calves: A longitudinal study. J. Dairy Sci., 106(11): 8047-8059. https://doi.org/10.3168/jds.2023-23296

Schrödl W, Büchler R, Wendler S, Reinhold P, Muckova P, Reindl J, Rhode H (2016). Acute phase proteins as promising biomarkers: Perspectives and limitations for human and veterinary medicine. Proteom. Clin. Appl., 10(11): 1077-1092. https://doi.org/10.1002/prca.201600028

Theurer M E, Anderson DE, White BJ, Miesner MD, Mosier DA, Coetzee JF, Lakritz J, Amrine DE (2013). Effect of Mannheimia haemolytica pneumonia on behavior and physiologic responses of calves during high ambient environmental temperatures. J. Anim. Sci., 91(8): 3917-3929. https://doi.org/10.2527/jas.2012-5823

USDA USDoA (2007). Heifer calf health and management practices on U.S. Dairy Operations, 2007. In APHIS, ed. Dairy 2007: USDA-APHIS-VS, CEAH.

USDA USDoA (2008). Part II: Changes in the U.S. Dairy Cattle Industry, 1991–2007, In: NAHMS,ed. Dairy 2007. Fort Collins, CO: USDA-APHIS-VS, CEAH.