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Hematologic, Histopathological, and Inflammatory Biomarkers associated with Bovine Respiratory Disease in Calves

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Abstract

Bovine respiratory disease (BRD) poses a significant health concern in cattle, particularly in calves, leading to substantial economic losses in the livestock industry. Early and accurate detection of BRD is crucial for effective management and treatment. Understanding the changes in these biomarkers between healthy and BRD-affected animals can provide valuable insights into the disease progression and pathophysiology. This study aimed to evaluate the diagnostic potential of hematologic, histopathological, and biochemical biomarkers for detecting bovine respiratory disease (BRD) in calves. Twenty-four calves, split evenly between healthy and BRD-affected, were analyzed for various markers. BRD-affected calves showed significantly higher levels of White blood cell counts, cytokines, acute phase proteins, immunoglobulins, and oxidative biomarkers, while demonstrating lower mean corpuscular volume (MCV), hemoglobin (HCT), albumin, and RBC values. Histopathological analysis revealed distinct findings in different BRD phases. The study suggests that elevated acute phase proteins, immunoglobulins, neutrophils, and specific WBC populations, along with decreased RBC count, could aid in diagnosing BRD in calves. Further research is recommended to compare these biomarkers with other respiratory disorders and explore BRD at different stages, including histopathological examination.

Keywords: Diagnostic potential, Bovine respiratory disease, acute phase protein, cytokines, immunoglobulin, oxidative biomarker.

Introduction

Bovine Respiratory Disease (BRD) is a major challenge for the global beef industry, causing substantial economic losses and animal welfare concerns [1]. This complex syndrome arises from a combination of host factors, environmental stressors, and microbial pathogens [2,3]. Environmental factors, such as transportation, handling, inadequate ventilation, and poor hygiene, contribute significantly to BRD outbreaks [4,5,6]. These stressors compromise animal health and increase susceptibility to a variety of pathogens, including bacteria, viruses, parasites, and fungi [7]. The clinical presentation of BRD is variable, ranging from mild to severe respiratory symptoms [8]. Early signs often include fever, depression, and reduced appetite, which may progress to more severe manifestations such as nasal and ocular discharge, dyspnea, and coughing [9]. Despite the significant impact of BRD, accurate and timely diagnosis remains a challenge due to the disease's nonspecific clinical signs and the lack of a gold standard diagnostic test [10].

Early and accurate diagnosis of BRD is crucial for implementing effective treatment strategies and preventing disease progression [11]. However, traditional diagnostic methods, relying primarily on clinical signs, often lead to delayed or inaccurate diagnoses. The lack of sensitive and specific diagnostic tests contributes to the overuse of antibiotics, exacerbating the issue of antimicrobial resistance [12]. To address these challenges, a comprehensive approach to BRD management is required. This includes implementing robust biosecurity measures to minimize pathogen exposure, optimizing animal husbandry practices to reduce stress, and developing effective vaccination strategies. Additionally, there is a critical need for innovative diagnostic tools that can accurately identify BRD cases at an early stage, enabling targeted treatment and reducing the use of antibiotics [13,14].

This study aims to contribute to the development of improved diagnostic strategies for BRD by investigating the diagnostic potential of hematologic,

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histopathological, and biochemical biomarkers for detecting bovine respiratory disease (BRD) in calves.

Material and Methods

Current study was approved by the Benha University, Faculty of Veterinary Medicine's Institutional Animal Care and Use Committee Research Ethics number (BUFVTM 00-00-24). The current study included twenty-four healthy and BRD-affected calves (12 each). Calves' blood and serum samples were obtained once the BRD condition was confirmed as negative or positive. calves were visually evaluated for the presence or absence of BRD symptoms. Calves with respiratory distress, inappetance, coughing, nasal or ocular discharge, depression, and other symptoms were classified as BRD-suspects and were further confirmed by the BRD scoring system. The calf's rectal temperature was taken whenever two or more of these clinical symptoms were seen. A calf having a score of five or higher on the clinical rating system [15] was deemed morbid and included in the study. Additionally, a thorough clinical examination that included thoracic auscultation, body temperature, pulse rate, and respiratory rate was performed on all calves [16].

Hematologic and biochemical indicators

A jugular vein puncture was used to obtain two blood samples from each calf [17]. The initial blood sample was drawn into a test tube that was labeled and contained 5 mg of k2EDTA at a concentration of 1 mg/1 ml of blood as an anticoagulant for the measurement of hematological parameters (PCV%, Hb content, and RBC count). After collecting the second blood sample without the use of an anticoagulant, it was clotted at room temperature for 20 minutes and centrifuged for 10 minutes at 3,000 rpm. The clear, non-hemolyzed serum samples were then separated and kept at -20°C until they were subjected to further biochemical examination [17].

Hematological analyzers were used to determine CBC variables such as Hct, hemoglobin concentration, mean corpuscular volume, and total WBC, lymphocyte, neutrophil, MID % (mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells), and RBC counts [18]. Albumin was measured calorimetrically using the dye-binding method with bromocresol green [19]. Commercial diagnostic kits were utilized to examine blood cytokines, albumin, immunoglobulin molecules, haptoglobin, serum amyloid A (SAA), C-reactive protein (CRP), and oxidative biomarker utilizing an automated biochemical analyzer and spectrophotometric technique [17,20].

Histopathology:

Postmortem examination of affected calves was performed and revealed Cranioventral reddening and firm to hard consolidation, interlobular edema, multiple abscesses and bronchopneumonia.

Tissue samples were taken from the lungs of dead calves (n=3) and promptly fixed in 10% neutral buffered formalin for 3 days. The specimens were then dehydrated with increasing grades of ethyl alcohol, cleaned in xylene, and embedded in paraffin. Using a rotatory microtome, five µm thick tissue paraffin slices were created, and H&E stain was applied [21]. A Nikon Eclipse E800 light microscope was utilized for analyzing the slices, and a digital camera was used to take photographs.

Statistical Analysis

One-way analysis of variance (ANOVA) was initially applied to the collected data using IBM's SPSS software (version 20, Chicago, IL, USA). The differences between the groups were then compared using Tukey's b multiple comparison tests [22], where significant differences were found at $P < 0.05$.

Results

BRD-affected calves had significantly higher levels of all estimated cytokines and acute phase proteins, including the serum amyloid A (SAA), C-reactive protein (CRP), and haptoglobin, when compared to healthy calves ($P < 0.05$), Table 1.

The oxidative biomarkers NO and TAC, and all estimated immunoglobulins (E, G, M, and A) were considerably greater in BRD-affected calves than in healthy calves ($P < 0.05$).

When compared to healthy calves (Table 3), BRD-affected calves exhibited significantly lower estimated RBC variables (RCB, HCT, and MCV) ($P < 0.05$). The white blood cell (WBC) count (Table 3), particularly Neutrophil and mid-size WBC population, significantly increased in BRD-affected calves than in healthy one, but Lymphocyte count and % were obviously decreased ($P < 0.05$).

The histopathological results of BRD-affected calves, including various stages of the illness, including acute and chronic bronchitis, acute and chronic bronchiolitis, bronchiolitis obliterans, necrotizing hemorrhagic pneumonia, suppurative pneumonia, fibrnous bronchopneumonia stage, pulmonary and alveolar oedema, and fibrinous pleurisy are shown in BRD affected calves (Fig. 1-7 and supplementary file 1).

Discussion

The purpose of this study was to investigate the potential associations between bovine respiratory disease (BRD) and hematologic indicators, plasma haptoglobin (Hp) and amyloid-A, immune system function biomarkers, and oxidative hemostasis. The current study compared BRD affected calves to healthy ones rather than related respiratory diseases. Thus, most estimated hematological indicators were either considerably raised or decreased in BRD calves, supporting them as a helpful diagnostic tool for detecting early BRD in calves. Nevertheless, to

verify their accuracy, including sensitivity and specificity for BRD syndrome identification, it is required to compare them with previous research that estimated same hematological indicators compared to similar respiratory diseases.

Prior analyses and reports have suggested that BRD is associated with systemic alterations that are visible in different bodily fluids, primarily blood. However, the complete blood cell (CBC) profile, which includes white (WBC) and red blood cells white blood cells (RBC), wasn't reliably distinguish BRD from other respiratory infections [23]. In comparison to healthy calves with high lymphocyte counts and percentages, BRD-affected calves had decreased numbers. Previous investigations also showed a strong negative correlation between BRD in pre-weaned calves and lymphocyte count, which is likely caused by viral infections that lower lymphocyte numbers [24,25]. Among the several studies on the diagnostic efficacy or association between bovine respiratory disease (BRD) and hematologic biomarkers in dairy calves, one recent study concluded that WBC change had a limited diagnostic accuracy for BRD identification [24]. The most dependable WBC marker linked to BRD was found to be basophil [24] and Eosinophils count [26], albeit it still lacked BRD diagnostic precision [23,24,26]. Even while hematological measures by themselves cannot provide a conclusive diagnosis of BRD, changes in these values can help with prognostication, diagnosis, and tracking the course of the disease [23,24,26].

Significantly elevated IL-6 levels in BRD-affected calves are due to fast and temporary production in response to lung infections and tissue damage. IL-6, like IL-1 and tumor necrosis factor (TNF), is a key proinflammatory cytokine that contributes to host defense by stimulating acute phase responses (APP production in the liver), hematopoiesis, and immune response activation lymphocyte differentiation [27,28]. Acute inflammatory cytokines activate surrounding cells, including epithelial cells and key innate immune effector cells such as vascular endothelium, neutrophils, alveolar and intravascular macrophages, dendritic cells, NK cells, NK T cells, eosinophils, and mast cells. Chemokines are released upon activation, causing neutrophils and monocytes to migrate into the afflicted area. Over time, lung dendritic cells (DCs), including plasmcytoid DCs, natural killer (NK) cells, NK T cells, alpha/beta and gamma/delta T cells, and B cells, are also drawn to the area. The different lung dendritic cells (DCs) and neutrophils interact with microbial pathogen-associated molecular patterns (PAMPs) in the context of adaptive immune responses. Following recognition of PAMPs by intravascular macrophages, alveolar macrophages, and epithelia, these cells actively produce inflammatory mediators including prostaglandins and leukotrienes, which stimulate endothelial cells to open gaps and permit the entry of serum factors into the lung, particularly through alveolar lumens [27]. In addition to a variety of molecules with immune-protective properties like complement, hydrolytic enzymes, IgG, IgM, IgA, and IgE, collectins, acute phase proteins, and others, this fluid physically contains diluted microbiological agents and poisons [27]. That could explain the elevated levels of IgG, IgM, and IgA in BRD-affected calves.

When pro-inflammatory cytokines, specifically interleukin 6 (IL-6), is produced in a local lesion during inflammation, it prompts the liver to produce various acute phase proteins such as serum amyloid A (SAA), C-reactive protein (CRP), and haptoglobin. A significantly greater concentration of serum haptoglobin and amyloid-A in BRD-affected calves compared to healthy ones supports earlier findings indicated a substantial positive link with calf BRD [29,30], and overall is a valuable diagnostic tool for diagnosing early pulmonary inflammation in calves [31]. Haptoglobin was regarded in earlier research as an indicator of inflammation and illness condition in calves [31,32]. Haptoglobin had scavenging and peroxidase ability to remove metabolites (such free hemoglobin) generated by cellular breakdown under pro-oxidative and pro-inflammatory stress, assisting in preventing oxidative tissue damage. Thus, in infected or stressed animals, the circulating levels of acute-phase proteins, such as SAA, haptoglobin, CRP and ceruloplasmin, increase by a factor of > 100, to restore homeostasis and inhibit microbe multiplication [26,33]. Furthermore, rather than allowing pathogens to use heme residues, haptoglobin assists in recycling them for use in subsequent metabolic processes [31].

The endogenous reaction to invadors is responsible for the increased levels of nitric oxide (NO) in BRD-affected calves compared to healthy ones. In biological systems, NO is a key free radical signaling molecule that is both lipophilic and hydrophilic. At low concentrations, NO can stimulate immune cell growth and activity, but at high concentrations, it covalently binds to proteins, lipids, and DNA to inhibit or kill target pathogens [34]. Multiple antimicrobial intermediates are formed when NO reacts spontaneously with oxygen or superoxide to produce reactive nitrogen and oxygen intermediates. The majority of NO's cytotoxic effects are caused by these reactive NO species, which disrupt DNA, limit enzyme function, and induce lipid peroxidation, causing oxidative and nitrosative damage [34,35].

The current lower RBCs, Hemoglobin, HCT, and MCV levels and BRD affected-calves is an intriguing finding. This conclusion is consistent with a previous publication that suggested additional research be done to investigate the lower MCV values in BRD

calves relative to their healthy counterparts [24]. While other studies suggested that RBC variables had poor accuracy and linkage [24], a previous Multivariable Logistic Regression investigation found that just two CBC variables—eosinophil and RBC counts—had significant connections with BRD in calves [26].

The gold standard for correctly identifying BRD in calves is postmortem examination in conjunction with diagnostic testing for BRD agents. Identifying the cause of death and the percentage of calves that pass away from unidentified respiratory disorders is another way that necropsy data analysis can be used to improve BRD detection. Using gross necropsy and histopathologic investigation, numerous studies have identified important respiratory infections [4,23,36]. However, depending only on autopsy results and pathogen isolation may not be sufficient for early detection and prevention of BRD due to the varied time interval between disease onset and mortality, which can vary from days to weeks. Necropsy is useful for diagnosing BRD, but it is not often used until an outbreak spreads to epidemic proportions within a herd [37].

Conclusion

In conclusion, calf populations affected by BRD may be correlated or diagnosed cumulatively based on the presence of low RBC variables concurrent with high levels of cytokines, acute phase proteins, immunoglobulins, albumin, oxidative biomarkers, neutrophils, and mid-size WBC population when compared to healthy calves. When the current study's findings were compared to those of previous similar research, they verified the diagnostic potential of various biomarkers, particularly acute-phase proteins such as SAA, haptoglobin, and CRP in BRD-affected calves. Furthermore, the current investigation may have suggested biomarkers that might be used to diagnose BRD in calves, including cytokines, immunoglobulins, NO, and RBC variables. Further research is necessary to confirm the accuracy of the present investigated biomarkers' diagnosis. This research should compare the biomarkers' trends in similar respiratory disorders as well as different BRD phases.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (BUFVTM 00-00-24).

TABLE 1. Comparing cytokines and acute phase proteins variables including serum amyloid A (SAA), C-reactive protein (CRP), and haptoglobin in healthy and BRD-affected calves, with data expressed as means ± SE.

Parameter	Healthy	Diseased	P-value[2]
Il6[1]	1.56±0.09^{B}	7.98±0.49^{A}	<0.001
Haptoglobin	13.83±0.36^{B}	19.43±0.19^{A}	<0.001
SAA[1]	0.84±0.02^{B}	1.33±0.04^{A}	<0.001
CRP[1]	4.41±0.17^{B}	23.53±1.42^{A}	<0.001

[1] IL-6, interleukin 6, CRP, C-reactive protein, SAA, serum amyloid A.
[2]The same superscript letters A and B inside the same row imply a non-significant difference (P>0.05) between the comparing means.

TABLE 2. Comparing immune-protective molecules, albumin, and oxidative biomarker in healthy and BRD-affected calves, with data expressed as means ± SE.

Parameter	Healthy	Diseased	P-value[2]
IgE[1]	8.08±0.22^{B}	19.15±0.52^{A2}	<0.001
IgG	16.47±0.1^{B}	17.54±0.15^{A}	<0.001
IgM	0.35±0.01^{B}	0.70±0.03^{A}	<0.001
IgA	2.30±0.07^{B}	4.09±0.13^{A}	<0.001
Albumin	2.91±0.06^{B}	2.34±0.07^{A}	<0.001
NO[1]	11.00±0.45^{B}	27.96±0.86^{A}	<0.001
TAC[1]	5.35±0.08^{B}	7.07±0.11^{A}	<0.001

[1] Ig, immunoglobulin, NO, nitric oxide, TAC, Total antioxidant capacity.
[2]The same superscript letters A and B inside the same row imply a non-significant difference (P>0.05) between the comparing means.

TABLE 3. Comparing hematological parameters in healthy and BRD-affected calves, with data expressed as means ± SE.

Parameter	Healthy	Diseased	P-value[2]
RBCs[1]	6.72±0.20^{A}	5.61±0.15^{B}	<0.001
Hemoglobin	10.67±0.23^{A}	9.34±0.22^{B}	<0.001
HCT	35.73±0.21^{A}	34.59±0.23^{B}	<0.001
MCV	35.00±0.15^{A}	32.25±0.26^{B}	<0.001
WBCs	9.58±0.11^{B}	12.81±0.19^{A2}	<0.001
Lymphocyte count	4.63±0.13^{A}	3.56±0.12^{B}	<0.001
lymphocyte %	48.33±1.47^{A}	27.80±1.12^{B}	<0.001
Neutrophil count	4.36±0.13^{B}	7.87±0.18^{A}	<0.001
Neutrophil %	38.13±4.64^{B}	61.33±1.04^{A}	<0.001
MID %[1]	1.04±0.05^{B}	1.58±0.06^{A2}	<0.001

RBC = red blood cell, HCT, Hematocrit, MCV, mean corpuscular volume.
[1]MID %, mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells
[2]The same superscript letters A and B inside the same row imply a non-significant difference (P>0.05) between the comparing means.

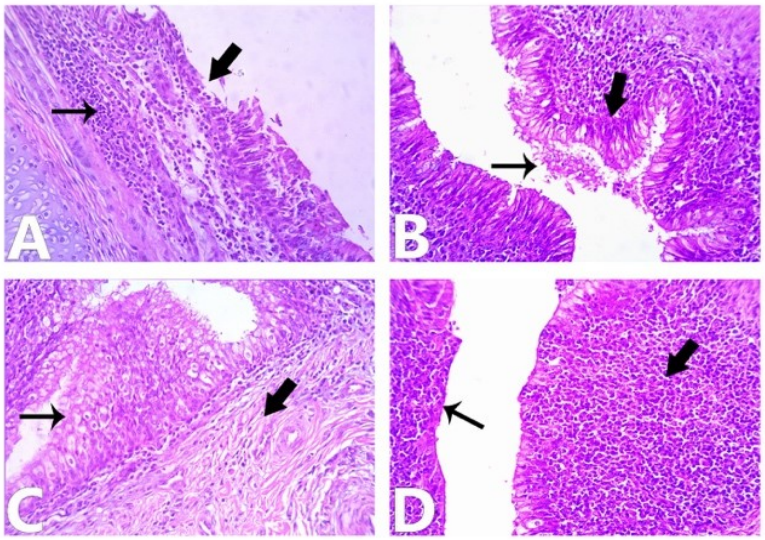

Fig.1. Representative photomicrographs of H&E-stained pulmonary sections showing acute bronchitis (&B) A- Partial desquamation of the bronchiolar epithelium (thick arrow) and inflammatory cellular infiltration of the lamina propria (thin arrow). B- Hyperplasia of the bronchial epithelial cells and goblet cells with cell debris in the lumen (thin arrow). Chronic bronchitis (C&D) C- Peribronchial fibroplasia (thick arrow) with hyperplasia of the bronchial epithelium and goblet cells (thin arrow). D- Desquamation of their epithelial cells (thin arrow) with peribronchial lymphoid hyperplasia (thick arrow) X200.

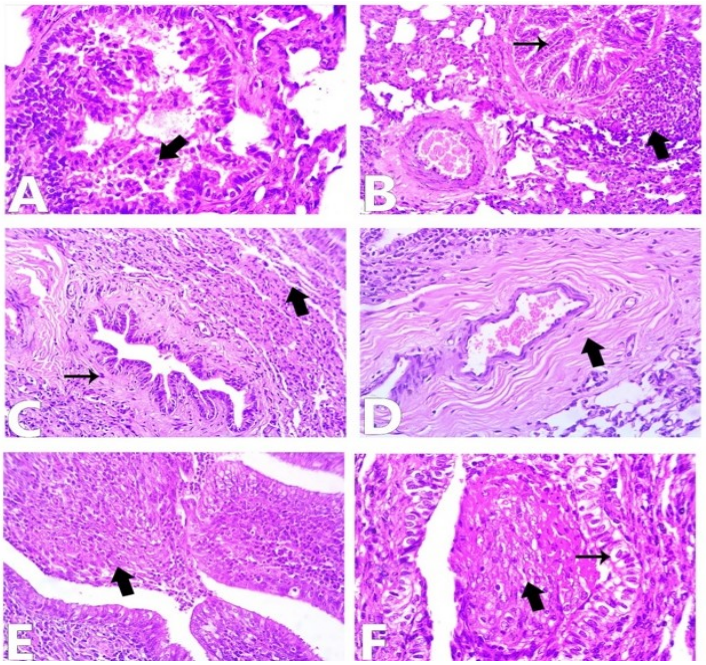

Fig. 2. Representative photomicrographs of H&E-stained pulmonary sections showing acute bronchiolitis (A&B) A- Extensive necrosis and exfoliation of the bronchiolar epithelial cells with intraluminal desquamated and necrotic epithelia (thick arrow) B- Hyperplasia of the bronchiolar epithelium with formation of finger like projections (thin arrow), narrowing of the lumen with peribronchial inflammatory cells infiltration (thick arrow) and congestion of the pulmonary vessel. Chronic bronchiolitis (C&D) C- Severe peribronchiolar fibrosis (thin arrow) with mild bronchiolar epithelial hyperplasia and atelectasis of the surrounding alveoli (thick arrow) D- Perivascular fibrosis (thick arrow). Obliterative bronchiolitis (E&F) E- Intraluminal inflammatory cells mixed with fibroblasts (thick arrow) and hyperplasia of the bronchiolar epithelium. F- Fibrinoid mass (thick arrow) infiltrated with desquamated epithelium and inflammatory cells within the bronchiolar lumen with severe degeneration of the lining epithelium (thin arrow) X200.

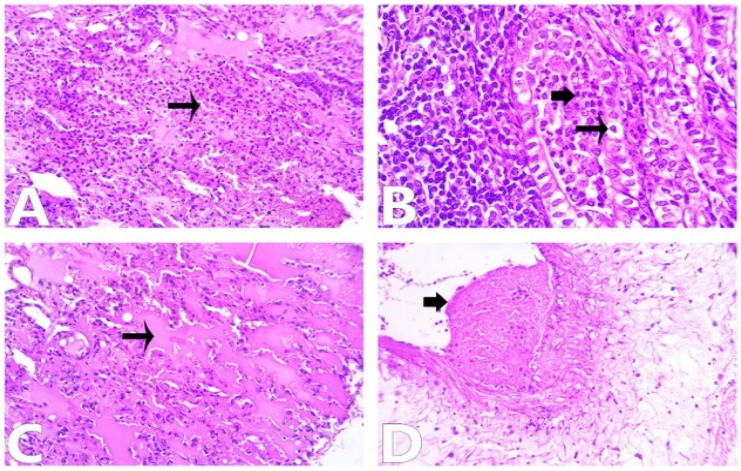

Fig. 3. Representative photomicrographs of H&E-stained pulmonary sections showing necrotizing hemorrhagic pneumonia A- Alveolar septal necrosis accompanied by extravasated erythrocytes and inflammatory cell infiltration of the alveoli (thin arrow) B- Vacuolation of the bronchial mucosal epithelium (thin arrow) with inflammatory cells (thick arrow) packed the lumen C- Diffuse alveolar edema (thin arrow) D- Thrombosis (thick arrow) and vasculitis of the pulmonary vessel X200.

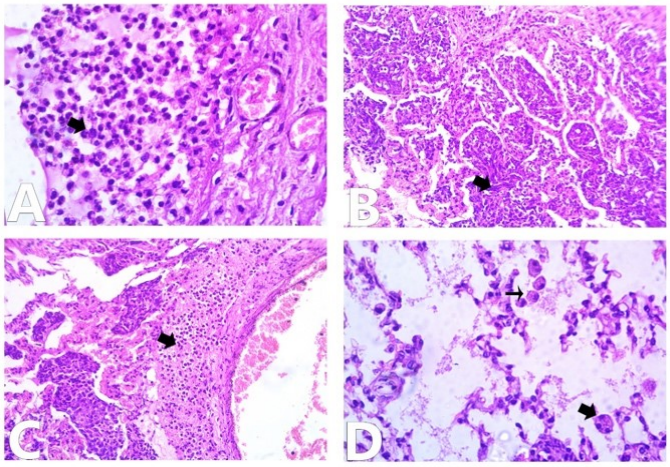

Fig. 4. Representative photomicrographs of H&E-stained pulmonary sections showing suppurative pneumonia A- live and dead neutrophils (thick arrow) with some mononuclear inflammatory cells within the lumens of the alveoli X400 B- Inflammatory cells with streaming appearance (thick arrow) of basophilic chromatin within the alveolar spaces X200 C- Vasculitis with necrosis and inflammatory cells (thick arrow) infiltrates in the walls of pulmonary vessel X200 D- Alveolar macrophages (thin arrow) and multinucleated giant cells (thick arrow) within the alveolar lumens X200.

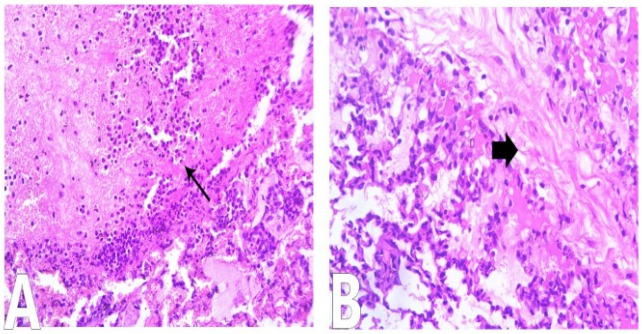

Fig. 5. Representative photomicrographs of H&E-stained pulmonary sections showing fibrinous pneumonia A- A mass of fibrin threads (thin arrow) intermixed with erythrocytes and inflammatory cells mostly neutrophils and mononuclear cells B- Thickened interlobular septa due to fibrin rich exudation (thick arrow) X200.

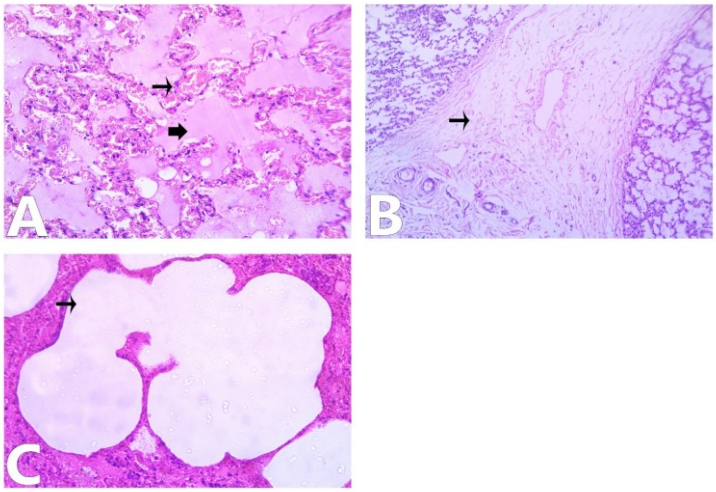

Fig. 6. Representative photomicrographs of H&E-stained pulmonary sections showing A-pulmonary alveolar edema (thick arrow) and congestion of the interalveolar capillaries (thin arrow). B- Edematous thickening of the interalveolar septa(thin arrow) C- Compensatory alveolar emphysema (thin arrow) X 200.

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المؤشرات الحيوية الدموية والهيستوباثولوجية والالتهابية المرتبطة بأمراض الجهاز التنفسي البقري في العجول

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الملخص

يشكل مرض الجهاز التنفسي البقري (BRD) مشكلة صحية كبيرة في الماشية، وخاصة في العجول، مما يؤدي إلى خسائر اقتصادية كبيرة في صناعة الثروة الحيوانية. يعد الكشف المبكر والدقيق عن مرض الجهاز التنفسي البقري أمرًا بالغ الأهمية للإدارة والعلاج الفعالين. إن فهم التغييرات في هذه المؤشرات الحيوية بين الحيوانات السليمة والمصابة بمرض الجهاز التنفسي البقري يمكن أن يوفر رؤى قيمة حول تطور المرض وعلم وظائف الأعضاء المرضية. تهدف هذه الدراسة إلى تقييم الإمكانات التشخيصية للمؤشرات الحيوية الدموية والنسيجية والكيميائية الحيوية للكشف عن مرض الجهاز التنفسي البقري (BRD) في العجول. تم تحليل أربعة وعشرين عجلاً، مقسمة بالتساوي بين الأصحاء والمصابين بمرض الجهاز التنفسي البقري، بحثًا عن علامات مختلفة. أظهرت العجول المصابة بمرض الجهاز التنفسي البقري مستويات أعلى بشكل ملحوظ من تعداد خلايا الدم البيضاء، والسيتوكينات، وبروتينات المرحلة الحادة، والغلوبولين المناعي، والمؤشرات الحيوية المؤكسدة، مع إظهار انخفاض متوسط حجم الكريات الدموية (MCV)، والهيموجلوبين (HCT)، والألبومين، وقيم خلايا الدم الحمراء. كشف التشريح المرضي النسيجي عن نتائج مميزة في مراحل مختلفة من BRD. تشير الدراسة إلى أن ارتفاع بروتينات المرحلة الحادة، والغلوبولينات المناعية، والعدلات، ومجموعات خلايا الدم البيضاء المحددة، إلى جانب انخفاض عدد خلايا الدم الحمراء، يمكن أن يساعد في تشخيص BRD في العجول. يوصى بإجراء المزيد من الأبحاث لمقارنة هذه المؤشرات الحيوية باضطرابات الجهاز التنفسي الأخرى واستكشاف BRD في مراحل مختلفة، بما في ذلك التشريح المرضي النسيجي.

الكلمات الدالة: الإمكانات التشخيصية؛ أمراض الجهاز التنفسي البقري؛ بروتين المرحلة الحادة؛ السيتوكينات، الغلوبولين المناعي، المؤشرات الحيوية المؤكسدة.