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Hematological, Biochemical, and Ultrasonographic Changes in Sheep Diagnosed Clinically with Bacterial Pneumonia

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Abstract

PNEUMONIA continues as a serious issue of great impacts on the sheep industry. Therefore, this study aims to evaluate the clinical respiratory score (CRS), hematobiochemical and ultrasonographic alterations in sheep diagnosed with bacterial pneumonia. Totally, 130 Baladi sheep, 120 suffered pneumonia, and 10 healthy, were selected and used. Based on clinical and physical examination, pneumonic sheep were shown the signs of anorexia, coughing, nasal discharge, dyspnea, abnormal lung sounds, and a significant increase in values of body temperature, pulse rate, and respiratory rate. The incidence of pneumonia was high in adult females and sheep with poor body condition scores. Bacteriological examination revealed that the most common bacteria were *Pasteurella haemolytica*, *Staphylococcus aureus (staph* aureus), *Escherichia coli (E coli)*, and *Proteus*. Further investigation of 15 pneumonic sheep by ultrasonography revealed the presence of abnormal lung patterns (pulmonary consolidation, abscess, and pleural effusion). Hematological analysis of pneumonic sheep detected a significant decrease in values of total erythrocytes, hemoglobin, packed cell volume, and lymphocytes, with a significant increase in neutrophils and the total white blood cells count. Biochemical analysis indicated a significant decreasing in vitamin D, iron, albumin, and albumin / globulin ratio. Substantial significant increases in serum amyloid A, total protein, and globulin in pneumonic sheep were seen when compared to values of healthy controls. In conclusion, bacterial pneumonia in sheep can significantly impact various hematological and biochemical parameters; in addition to ultrasonography, combined with clinical and inflammatory mediators, could be used as diagnostic tools in ovine pneumonia.

Keywords: Hematology, Ovine respiratory infections, Bacteria, Vitamin D, Iron

Introduction

Pneumonia is a major respiratory issue of various animals including sheep, which occurs due to environmental and management factors resulting in severe economic losses and serious animal welfare concerns [1]. It is known as inflammation of the pulmonary parenchyma, which may be associated with pleurisy or comprises an inflammation of bronchioles. The hallmarks of pneumonia include respiratory embarrassment and toxemia [2]. A combination of different types of bacterial and viral diseases, physiological stress, and unfavourable climatic conditions can result in respiratory disorders in sheep and goats. Ovine pneumonia could be acute or chronic. The main cause of acute respiratory diseases is usually bacterial, while the chronic type is usually viral [3]. The respiratory diseases that affect small ruminants can be divided into different bacterial and viral pneumonia are usually caused by (ovine parainfluenza 3, ovine pulmonary adenocarcinoma, Peste des petits ruminants (PPR), and ovine respiratory syncytial virus). Parasitic pneumonia is usually caused by *Dictyocaulus filaria*, *Protostrongylus rufescens*, *Muellerius capillaris*, and nasal myiasis. Fungal pneumonia is usually caused by *Aspergillus fumigatus* [4,5]. Diagnosing of respiratory infections in sheep usually depends on history, physical examination, clinical findings, laboratory tools, and imaging assays as ultrasonography [26]. Ruminants suspected of having pneumonia may express different symptoms such as coughing, depression, inappetence, respiratory distress, nasal or ocular discharge, and others. Each sign appears on animals, take the score on the chart, and then sum the scores with each other to obtain the total respiratory score. If the total respiratory score of

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animal having five or more, it classified as an infected animal with respiratory disease [7]. Chest auscultation is necessary for diagnosing and distinguishing between the normal and abnormal (crackles, wheezes, and pleural rub) respiratory sounds. Auscultation also evaluates airflows *via* the trachea-bronchial tree [8]. Ultrasonography is a non-invasive diagnostic device that has many applications in veterinary medicine, including respiratory infections. Furthermore, lung ultrasonography enabled rapid grouping of affected animals according to the degree of pneumonic lesion. Ultrasonography is more sensitive in describing and evaluating respiratory illnesses in cows than other diagnostic techniques like radiography [9]. Hematological and biochemical analyses is a simple, effective and rapid laboratory diagnostics for assessing infectious inflammatory responses [10].

Recent research has revealed the role of vitamin D in several cellular functions, such as differentiation, wound healing, repair, cellular proliferation, and regulatory systems like inflammation, immunology, and host defense. There are correlations between elevated incidence of inflammatory disorders, impaired pulmonary functions, and low level of serum vitamin D. It has been shown that vitamin D can reduce pulmonary inflammation [11]. Following harmful events, such as respiratory illness, iron homeostasis shifts, and nutritional immunity is strengthened. Nutritional immunity, a defense mechanism, restricts Pathogenic bacteria that acquire iron and other essential elements. Pathogenic bacterial growth and virulence may be inhibited by decreased iron availability at the infection site [12].

An increase in certain acute-phase proteins (APPs), such as serum amyloid A, is a hallmark of the acute-phase reaction in sheep. Since APPs are thought to be more sensitive, specific, and effective than hematological testing, they are useful markers for identifying inflammation in sheep. Serum amyloid A (SAA) is the main positive APP released by small ruminants [13]. Therefore, this survey study aimed to record the incidence of pneumonia in sheep at Qalioubia Governorate. Moreover, evaluate the role of clinical respiratory score, hematobiochemical, and ultrasonographic examination as diagnostic and prognostic tools of bacterial pneumonia in sheep.

Material and methods

Animals

Totally, 130 Baladi sheep, 120 clinically with pneumonia and 10 sheep apparently healthy, used as controls, were selected for the current study based on clinical and physical examinations during September 2023 to October 2024. The study sheep comprised different sexes (50 male and 80 female), body weights, and ages (up to 4 years). The current study was conducted on sheep cases admitted to the Veterinary Teaching Hospital in the Faculty of Veterinary Medicine (Benha University), as well as sheep admitted to private veterinary animal clinics and private flocks in Qalioubia Governorate (Egypt). These field cases showed clinical signs of pneumonia, including fever, coughing, depression, anorexia, respiratory distress, nasal or ocular discharge, and others.

Based on the bacteriological and virological examination, we included 15 pneumonic sheep with bacteriological infection only and negative virological infection for further hematological, biochemical analysis, and ultrasonographic examinations.

Ethical approval

All studies were carried out after approval by the ethical and animal care and use committees (ACUC) at the Faculty of Veterinary Medicine at Benha University with the ethical number BUFVTM 29-09-23. All samples were collected after the owner consented.

Clinical examination of animals

The clinical examination was recorded and conducted to detect clinical signs, depending on the data [1]. Ruminants suspected of having pneumonia undergo visual inspections to look for symptoms such as coughing, depression, inappetence, respiratory distress, nasal or ocular discharge, and others. Each sign appears on animals, take the score on the chart, and then sum the scores with each other to obtain the total respiratory score. If the total respiratory score of the sheep with a score of five or more, it was classified as a diseased animal, as shown in Table 1. [7,14,15].

Samples for bacteriological and virological examinations

Nasal swabs were collected aseptically from pneumonic sheep to isolate and identify the causative agents [6,16].

Identification of the causative agent of sheep pneumonia

The most popular method for identifying the causative agents in pneumonic sheep is swab-based nasal screening, which involves analyzing various swabs for the main causative agent [16]. For 24 hours, the nasal swab samples were ino culated independently in the nutritional broth to activate the microbe at 37 °C. On blood agar, paired barker media, and MacConkey's agar, a sterilized loop full of the broth containing the active microbe was immediately subcultured. After 24 and 48 hours, plate readings were taken. Identification of bacteria [17]. The collected isolates were subcultured, and colony morphology, Biochemical tests, and the Gram stain were used for their identification [18]. Observing colony morphology under a microscope and using certain biochemical tests, such as hemolysis, motility, indole production, glucose, lactose, methyl red, Voges-Proskauer, and citrate. The biochemical characteristics of the bacterial isolates were determined using MacFaddin's methodology [19].

Molecular identification of the viruses was done according to the procedures of previous studies [20-24]. Oligonucleotide Primers used for the molecular identification of viruses by Polymerase chain reaction (PCR) are listed in Table 2.

Samples for hematological and biochemical examinations

Two blood samples were collected from pneumonic and control sheep from the jugular vein. All samples were quickly chilled on crushed ice and sent to the laboratory for further hematological and biochemical analysis. The first blood sample was collected in a labelled test tube containing an anticoagulant (potassium salt of EDTA). The Ethylenediaminetetraacetic acid (EDTA) blood samples were used for hematological parameters. The second blood sample was collected in a plain tube (without anticoagulant). The plain tubes were centrifuged at 3000 r/min for 15 minutes. Only clear sera were collected, aliquoted, and stored at -20°C for biochemical analysis.

Ultrasonographic examination

The ultrasonographic examination of the chest is performed using the real-time B-mode Sonoscape A5 ultrasound machine linked to a 5.0 MHz sector transducer. A first field configuration of 6-7 cm permits in-depth analysis of the pleurae and superficial lung parenchyma, which may then be expanded to 12–16 cm to assess the scope of any lesions fully [25,26].

Hematological analysis

A hematological analyzer (Mindray BC_3000 plus Edan H30) was used to determine hematological parameters.

Biochemical analysis

Spectrophotometry was used to determine serum total protein and albumin concentrations using commercial kits (Bio-Diagnostics Ltd., Egypt). The variations between total protein and albumin were used to calculate serum globulin [29]. The albumin value was divided by the globulin value to determine the A/G ratio [29]. Serum amyloid A concentration was measured using ELISA kits (Shanghai Coon Koon Biotech, Ltd.; China).

Serum concentrations of potassium (K), sodium (Na), and chloride (Cl) were assessed by the kits of (Bio-diagnostic Company- Egypt), according to the methods specified. The analyses were carried out according to the manufacturer’s prescriptions. The providers' standard procedures used commercial kits to quantify iron (UK: Abcam Co). Commercial ELISA kits were used to determine the amounts of vitamin D in the serum (Beijing Savant Biotechnology Co., China).

Statistical analysis

The statistical analysis was conducted using the *t*-test in the SPSS Software (IBM Corp. Released 2013). Data were treated as a complete randomization design, differences between values were considered significant at $p<0.05$ [27].

Results

Clinical Findings and Epidemiology

The most prevalent and easily detectable clinical symptoms included fever, shallow rapid respiration, varied degrees of depression, off-food, nasal discharge, cough, weight loss, Dyspnea with mouth breathing, extension of head and neck, congested conjunctival mucous membrane, and ocular discharge. The frequency of clinical signs was demonstrated in (Table 3)

Chest auscultation of pneumonic sheep showed numerous abnormal lung sounds, such as loud wheezing, moist rales, and crackling. Exaggerated vesicular sounds and frictional sounds were also detected. The pneumonic sheep with nasal discharge was classified into 4 scores according to color, consistency, and amount of nasal discharge as shown in (Fig. 1). Sheep were evaluated for clinical indicators of respiratory disease to assess the clinical respiratory score (Table 4).

The pneumonic sheep showed a significant increase ($P<0.05$) in body temperature, pulse rate, and respiratory rate compared to the control group (Table 5). Pneumonia was more common in sheep younger than one year of age. Analysis by sex showed that respiratory tract infections were more common in females than in males. Animals with poor body conditions develop lung infections more frequently and with greater severity (Table 6).

Isolation and identification of the causative agent of bacterial pneumonia

The bacteriological examination of cultured swabs collected from the pneumonic sheep revealed that the isolated pathogen, *Pasteurella haemolytica*, was the most predominant bacteria, *Staph. aureus*, *E. coli*, and *Proteus*.

Identification of causative agents of viral pneumonia in pneumonic sheep

PCR results for ovine parainfluenza 3, ovine pulmonary adenocarcinoma, PPR, ovine respiratory syncytial virus, and ovine adenovirus genes showed negative amplification of genes in the tested samples.

Ultrasonographic findings

Normal lung tissue could not be seen on the ultrasound of the sheep's chest in the control group's 7th intercostal region because of its air content, but comet-tail reverberation artefacts in the shape of echogenic bands flowing were visible parallel to the lung surface. The thoracic wall's muscles and the lungs' surface formed a smooth hyperechoic line representing the pulmonary and costal pleura, a distinction that was not always possible. It was evident that the lungs were moving along with respiration (Fig. 2; A).

Chest ultrasonography of pneumonic sheep revealed (8/15) (53.34%) animals showed pulmonary consolidation, and (3/15) (20%) showed lung abscesses. In addition, 26.67% (4/15) of sheep exhibited pleural effusion and lung collapse.

Ultrasonography of pneumonic sheep with lung consolidation revealed consolidated lung tissue as a heterogeneous hypoechoic region, accompanied by anechoic artery cross-sections and ramified hyperechoic air bronchograms. Lacking the distinct pleural surface line, the hypoechoic fluid indicates a pleural effusion (Fig. 2; B).

Pneumonic sheep chest ultrasonography revealed numerous lung abscesses, with the pyogenic center represented by central echogenic septic pulmonary clusters and a less echogenic peripheral surrounding it. Areas of consolidation showed up as diverse areas of hypoechoic (Fig. 3; C).

Pneumonic sheep with pleural effusion and lung collapse showed the hypoechoic fluid which indicates pleural fluid displaced the lung surface up to 1.5 cm from the thicker pleural surface, resulting in a thick hyperechoic band. Pleural effusion showed up as granular or echogenic clusters (Fig. 3; D).

Hematological findings

Hematological analysis of pneumonic sheep showed a significant ($P<0.05$) decrease in RBC count, Hb content, PCV, and lymphocyte count and a significant ($P<0.05$) increase in neutrophils and WBCs (Table 7).

Biochemical findings

Biochemical analysis showed a significant ($P<0.001$) decrease in serum vitamin D and serum iron. While a significant ($P<0.01$) rise in serum Amyloid A (SAA) was observed. On the other hand, total protein and serum globulin showed a significant ($P<0.001$) increase. While values of serum albumin as well as albumin / globulin ratio were reduced significantly ($P<0.05$). Analysis of serum minerals revealed that serum Na and Cl showed a significant ($P<0.001$) decrease. Serum K showed a significant ($P<0.001$) increase in pneumonic sheep compared to healthy controls (Table 8).

Discussion

Pneumonia in sheep continues to be a serious issue that impacts flocks of sheep. The disease's numerous origins frequently include predisposed environmental factors, management factors, and infectious origins. Sheep pneumonia leads to decreased live weight and mortality, which costs lamb farmers a lot of money. Examples of economic losses are unthrifitness, treatment costs, and non-fatal case prevention. The disorder raises significant concerns for animal welfare [18].

The observed clinical signs of pneumonia were shallow rapid breathing, which indicates dyspnea, and hypoxia, which may be caused by severe inflammation in the bronchioles and alveoli that impairs gas exchange and respiration [5]. Moreover, some bacteria's cell walls include lipopolysaccharide, an example of an exogenous pyrogen that releases prostaglandin E2 (PGE2) and results in fever. The elevated body temperature causes an increase in heart rate, pulse, and respiratory rate to compensate for hypoxia. Inflammation in the nasal mucus may cause the observed nasal discharge, which varies according to the virulence factors of bacterial infection [28].

Lung auscultation revealed a variety of abnormal lung sounds, such as moist rales, loud wheezing, and crackling caused by pneumonia-related exudates from inflammatory and goblet cells. Exaggerated vesicular and frictional sounds were also heard. Similar results were previously reported by [29].

The prevalence was higher in sheep less than 1 year of age compared to adult sheep, which may be attributed to the progressive waning of passive immunity, increasing their susceptibility to microbial infection compared to adult sheep [35]. Sex-wise analysis revealed a higher occurrence of respiratory tract infections in females, followed by males, due to the stress of the transition period in females, which lowers immunity; this result agreed with [32]. The lung infections themselves might be reducing the health status of the animals. This is because the lungs constitute a central organ, and any lung infection has a wider impact on general health. Pneumonia or any other infections of the lungs render the animal stressed, resulting in a disorder in the normal body metabolism, leading to the loss of appetite and rapid catabolism of the body reserves, resulting in weight loss [33]. Thus, lung infections were more frequent and severe in animals with poor body condition compared to animals with good body condition. These results coincided with [34].

The accurate diagnosis of lung and pleural cavity disorders is the main reason for ultrasonographic examination of the respiratory system [9]. Pneumonic sheep's chest ultrasonography revealed collapsed lung tissue and pleural effusion. Moreover, Lung consolidation and numerous lung abscesses have been observed. These ultrasonography findings support. Similar results were previously recorded by [36, 37]. The typical lung contains air, which stops the ultrasonic waves from deeply reaching the lung parenchyma. As a result, the parenchyma and normal lung tissue appear as a consistently hyperechoic line on ultrasonography [9]. Finding hypoechoic pulmonary parenchyma and any bronchograms or vessels inside it served as the basis for the ultrasonographic diagnosis of pulmonary parenchymal consolidation [28]. One early indication of consolidation may be the irregularity of the lung's visceral pleural surface brought on by an imbalance in the air content of the lung periphery. Comet-tail artefacts emerge from these non-aerated regions, produced by tiny collections of blood, mucus, oedema fluid, exudate, or tumor cells [37]. They also have reported that echogenic regions with comet-tail artefacts indicate consolidation.

Pulmonary abscesses appeared more echogenic**.** The lung tissue appears hypoechoic if alveolar air is replaced with fluid [38].

The hypoechoic fluid, which represents pleural effusion, displaced the thicker pleural surface up to 1.5 cm, causing the damaged lung surface to appear as a thick hyperechoic band. This could be explained by the initial bacterial infection that resulted in a local inflammatory response. Causing fluid to escape quickly and capillary microvascular permeability to rise. Pleural effusion is caused by inflammatory cells being present in the pleural space [39].

When the pulmonary air content is decreased and the lung appears liver-like, a useful ultrasonographic assessment of the lung tissue can be made. The visceral pleural surface's irregularity can be the initial indication of consolidation [40]. An anechoic gap between the lung, thoracic wall, diaphragm, and heart is known as a pleural effusion. With profound lesion acoustic amplification and frequently within which the septa float. The visceral and parietal pleurae are distinct from each other. Transudate is represented by anechoic fluid in the pleural cavity, and greater echogenicity indicates a higher cell total protein concentration or count [38].

The hematological analysis of the pneumonic sheep showed anemia, which was expressed as a significant decrease in RBC count, PCV (%), and Hb (gm/dl), which agreed with [30]. This alteration in parameters was attributed to respiratory infections, especially pneumonia. Anemia caused by erythropoiesis suppression may result from decreased protein and calorie intake or from iron being sequestered in bone marrow macrophages and hepatocytes during infection, preventing it from being utilised in hemoglobin synthesis [41]. When inflammation occurs, phagocytic cells rise and trap a significant amount of iron needed for the bone marrow to synthesize red blood cells. This lowers Hb production and leads to anemia [42]. Furthermore, the breakdown of red blood cells caused by metabolites like superoxide and peroxides that are created and released by organisms may be a causes of anemia [41].

According to our findings, the pneumonic sheep's WBC and neutrophil counts significantly increased, but there was a significant decrease in lymphocytes compared to the control group. These results agreed with [30,31]. and comparable to the results recorded in pneumonic sheep [30]. The significant decrease in lymphocytes, on the other hand, may be due to a high endotoxin concentration, which can cause lymphocyte lysis and severe lymphopenia in sheep with respiratory infections [31].

Furthermore, this reduction in lymphocytes might be the result of the stress reaction and endogenous production of corticosteroids, or it could be related to the stimulation of the adrenal gland with the tissue invaded by bacterial toxins. Corticosteroids perform a supporting function in the recirculating lymphocytes' redistribution, which results in their sequestration in the lymphoid tissues instead of penetrating the circulation and efferent lymph to contribute to the emergence of inflammation. High cortisol levels may cause lymphocytes to sequester in lymphoid tissue rather than release into the bloodstream to contribute to the inflammatory response, which may lead to lymphopenia [41].

The serum biochemical analysis of pneumonic sheep revealed a significant decrease in albumin and the A/G ratio and a significant increase in total protein and globulin. These results agreed with [29,30]. Increased immunoglobulin synthesis after antigenic stimulation and decreased albumin production are the primary causes of the significant decrease in the A/G ratio and increase in total protein [2]. According to reports, 30– 40% of the hepatic protein anabolic capacity is utilized during the acute phase response to produce positive acute phase proteins; as a result, other protein production must be reduced, leading to hypoalbuminemia [43,44].

APPs are modifications in plasma proteins, some of which have a reduction in concentration (negative APPs), such as albumin, as well as other substances that increase in concentration (positive APPs), like SAA. According to our studies, the acute phase response significantly increases the demand for amino acids for the production of the positive acute phase proteins, requiring a reordering of the hepatic production of proteins [44]. The main negative acute-phase protein is serum albumin [31]. Rapidly following tissue damage or inflammation, the host's nonspecific inflammatory response is known as the acute phase response.

Serum biochemical analysis of the pneumonic sheep showed a significant increase in SAA. These results agreed with [30,45]. The massive increase in

SAA is due to the role of SAA in host immunity. It attaches itself to Gram-negative bacteria, enabling phagocytic cells to destroy them [47]. The main positive APPs, like SAA, have low physiological levels at first but rise quickly in the hours following the inflammatory stimulation and return to normal as soon as it stops. It has been suggested that APPs are quick and sensitive markers of ruminant inflammatory processes [48]. Because APPs are radially quantifiable in serum and can even distinguish between acute and chronic inflammation, their presence in the blood may be an excellent biomarker of inflammation [49]. APPs have the role of preventing further harm to the body, getting rid of infectious agents, getting rid of harmful substances and residues, and starting the healing process that is necessary for the organism to resume its regular functions and recover [46].

The serum minerals and electrolytes levels revealed a significant decrease in Fe, Na, and Cl, and a significant rise in K. Similar findings were previously recorded by [29,30]. Maintaining low iron levels in the lungs is essential for maintaining the pulmonary defense against inhaled infections and preventing oxidative stress**.** It has recently been suggested that pro-inflammatory cytokines, such as IL-6, cause an increase in the expression and secretion of hepcidin in the liver of animals suffering from respiratory diseases. The systemic iron deficiency and anemia in these animals are likely caused by hepcidin, which degrades ferroportin (FPN) and lowers cellular iron release into the bloodstream [50]. Reduced energy and protein intake or iron sequestration in bone marrow macrophages and hepatocytes during infection, which prevents iron from being used for erythropoiesis, could cause the decreased Fe [2].

The decreased Na and Cl levels could be related to respiratory illnesses that negatively impact electrolytes, acid-base balance, and body fluids. Hyponatremia could be caused by false hyperglycemia due to respiratory illnesses' stressful effects, which can result in Gluconeogenesis [51]. Respiratory acidosis, which results from CO_2 retention in the blood (hypercapnia), causes a drop in sodium and chloride levels and an increase in potassium levels. This showed up as biochemical hyponatremia, hypochloremia, and hyperkalemia in response to respiratory acidosis [5,52].

Vitamin D deficiency is an important predisposing factor for pneumonia. Low vitamin D levels were associated with more symptoms and prolonged symptom duration than pneumonic cases with normal levels. The results demonstrate a significant decrease in vitamin D that impacts the incidence of pneumonia as well as the severity of the illness, These results agree with[28]. Vitamin D has long been known to have bactericidal, bacteriostatic, and bacteriolytic properties both in vitro and in vivo. More recently, research has linked low serum 25-hydroxyvitamin D levels to the incidence of respiratory tract infections. The antimicrobial peptides cathelicidin and beta-defensin-2, which are crucial components of the innate immune response to infection, are produced in reaction to vitamin D [53]. The expression of genes that produce proteins in the immune system is regulated by vitamin D3. These proteins are involved in the removal of both endogenous and external infections. The microbes decrease immunological responsiveness and ultimately increase their chances of survival by dysregulating the vitamin D receptors [54].

Deficiencies in vitamin D are associated with an increased incidence of lower respiratory tract infections in sheep. 25(OH)D3 plays a crucial role in endothelial function, cell proliferation, and immunity by strengthening the immune system and lowering inflammation. Vitamin D supplements may decrease the incidence of pneumonia and improve its treatment [55]**.** Because vitamin D may be activated in the lungs, vitamin D supplementation may help prevent interstitial pneumonia by inhibiting pulmonary fibrosis [56]**.**

Conclusion

In conclusion, adult female and low body weight sheep are more susceptible to bacterial pneumonia. Clinical respiratory scores and hematobiochemical analysis play an impact role as diagnostic tools for pneumonia. Moreover, chest ultrasonography is a non-invasive diagnostic tool that aids in diagnosing pneumonia. APPs are more sensitive, specific, and useful markers for identifying inflammation in sheep. Iron and Vitamin D deficiency influence the incidence of pneumonia as well as the severity of the illness. Further investigations related to the addition of Vitamin D and iron to the therapeutic protocol for pneumonia are recommended.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Clinical respiratory score

Parameter	Score 0	Score 1	Score 2	Score 3
Temperature	38.6:39.7°C	39.8:40°C	40.1:40.5°C	≥40.5°C
Nasal discharge	No	Serous to mucoid	Mucoid to purulent	Purulent
Ocular discharge	No discharge	Slight	Moderate	Heavy ocular
Cough	None	Cough	Cough	Cough
Head carriage	poll of head above the level of withers	poll of head with the level of withers	poll of head under the level of withers	Sustained low level
Conjunctival mm	Rosy red	Congested	Severe congested	Cyanosed
Appetite	Normal	Slightly normal	Inappetence	Anorexia
Rumen fill	Normal	slight depression in the rumen fill	Moderate depression in rumen fill	severe depression in rumen fill
General Appearance	Normal	Slightly depressed	Depressed	Highly depressed
Respiratory signs	Normal	Hyperpnoea	Dyspnea	Distressed
Panting	No	Slight	Moderate	Fast

TABLE 2. Primer sequences, target genes for SYBR green RT-PCR.

Target gene	Primers sequences	Reference
Ovine parainfluenza 3	AGGTAGGCAATCCACCAAAGC CCCGATTGGTAAAGAACCTGAT	[20]
Ovine pulmonary adenocarcinoma (retrovirus)	TGGGAGCTCTTTGGCAAAAGCC CACCGGATTTTTACACAATCACCGG	[21]
PPR	TCTCGAAATCGCCTCACAGACTG CCTCCTCCTGGTCCTCCAGAATCT	[22]
Ovine respiratory syncytial	GTGCAGTTAGTAGAGGTTATCTTAGT TAGTTCTTTAGATCAAGTACTTTGCT	[23]
Ovine adenovirus	ATTCARGTWCCWCARAARTTTTTTGC CCWGAATAHRIAAARTTKGGATC	[24]

TABLE 3. Frequency of clinical findings in pneumonic sheep (Total number = 120)

Clinical signs	Number of cases	Percentage
Dyspnea (respiratory distress)	109	90.83%
Anorexia	83	69.16%
Nasal discharge	96	80%
Ocular discharge	48	40%
Cough	92	76.67%
Congested conjunctival mucous membrane	87	72.5%
Wholly abdominal Breathing pattern	22	18.33%

TABLE 4. Score of respiratory diseases in pneumonic sheep according to modified CRSC (Total number = 120)

Score	Number of animals	Percentage
5	22	18.33%
6	38	31.66%
7	45	37.5%
8	11	9.16%
9	4	3.33%

TABLE 5. Temperature, respiratory, and heart rate of control and pneumonic sheep (mean±SE) (Total number = 120).

Parameter	Control group(N=10)	Pneumonic group(N=15)
Temperature (C°)	39.34±0.27	40.24±0.47*
Pulse (beat/min)	76.86±3.13	84.57±3.95*
Respiration (breath/min)	26.43±2.99	34.86±2.67*

Significant * ($P<0.05$)

TABLE 6. Epidemiological data of animals

P.O.C	Sex		Age		Body weight	
	Male	Female	≤ 1 year	1:4 year	10:40kg	40:60kg
Total number	50	80	77	53	84	46
Percentage	38.46%	61.53%	59.23%	40.76%	64.61%	35.38%

TABLE 7. Hematological parameters of control and pneumonic sheep (mean±SE).

Parameter	Control group(N=10)	Pneumonic group(N=15)
Hb (g/dL)	9.42±0.12	9.09±0.09*
RBCs (106/µL)	11.10±0.81	9.27±0.90*
PCV (%)	29.56±1.21	27.12±1.13*
WBCs (10^3/µL)	8.06±1.09	12.71±0.52*
Neutrophil (%)	4.54±0.50	6.91±0.5*
Lymphocyte (%)	4.86±0.52	2.97±0.43*
Monocyte (%)	0.52±0.07	0.45±0.07
Eosinophils (%)	0.18±0.10	0.16±0.03

Significant * (P<0.05)

TABLE 8. Biochemical and Mineral analysis in control and pneumonic sheep (mean±SE).

Parameter	Control group(N=10)	Pneumonic group(N=15)
Vitamin D (µg/dL)	42.24±2.41	18.34±1.50*
Amyloid A protein (mg/L)	2.62±0.12	3.35±0.15*
Total protein (g/dL)	7.54±0.18	8.11±0.08*
Albumin (g/dL)	3.00±0.12	2.52±0.08*
Globulin (g/dL)	4.55±0.22	5.59±0.12*
A/G ratio	0.67±0.06	0.45±0.02*
Na (mmol/L)	146.87±1.63	135.14±3.58*
K (mmol/L)	4.98±0.11	6.04±0.13*
Iron (µmol/L)	161.23±6.81	117.60±2.85*
Chloride (Cl) (mmol/L)	106.12±0.85	103.57±0.61*

Significant * (P<0.05)

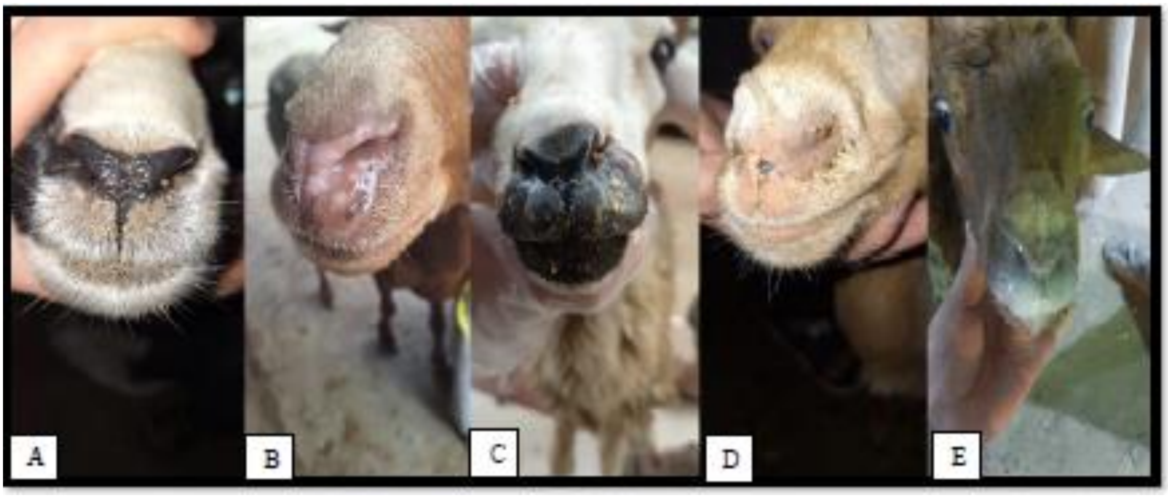

Fig. 1. Nasal discharge score. No discharge (A) score 0, serous discharge (B) score 1, sero-mucoid discharge (C) score 2, thick mucus discharge (D), and mucopurulent thick discharge (E) score 3.

Fig. 2. Ultrasonography of a sheep chest (A): normal lung: comet-tail reverberation artefacts (R), pulmonary pleura (P), the thoracic wall (TW). (B): lung consolidation: consolidated lung tissue (C) represented by a heterogeneous hypoechoic area; with ramified hyperechoic air bronchiograms and anechoic vessel cross-sections (arrow); with surrounded hypoechoic fluid representing pleural effusion (F) & without the clear line of the pleural surface.

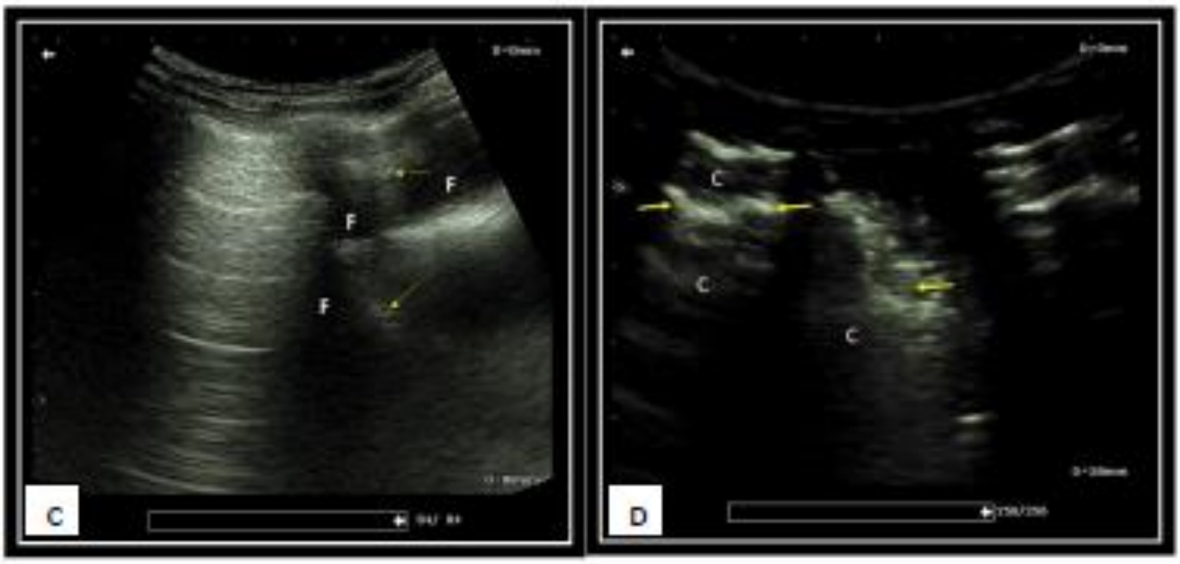

Fig. 3. (C) Ultrasonography of sheep chest with pleural effusion and collapsed lung tissue: the lung surface (L) appeared thick hyperechoic band and displaced up to 1.5 cm from the thickened pleural surface by the hypoechoic fluid representing pleural effusion (F); Cellular content of pleural effusion of the fluid appeared echogenic clusters or granular appearance (arrow). (D) Ultrasonography of the sheep chest with multiple lung abscesses: Abscess appeared as central echogenic septic pulmonary clusters (arrow) representing the pyogenic center and surrounded by the less echogenic periphery. Areas of consolidation appeared as heterogeneous hypoechoic areas (C).

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التغيرات التنفسية والدموية والكيميائية الحيوية والموجات فوق الصوتية في الأغنام المشخصة سريريا بالالتهاب الرئوي البكتيري

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الملخص

يمثل الالتهاب الرئوي في الأغنام مشكلة خطيرة تؤثر على قطعان الأغنام. لذلك، هدفت دراسة المسح هذه إلى تقييم النتيجة السريرية للجهاز التنفسي (CRS) بالإضافة إلى التغيرات الكيميائية الحيوية والدموية والموجات فوق الصوتية في الالتهاب الرئوي البكتيري في الأغنام. ولتحقيق هذا الهدف، تضمنت الدراسة اجماليا 130 من الأغنام البلدي، 120 مصابة بالالتهاب الرئوي و10 اصحاء ظاهريا. كشف الفحص السريري والجسدي للأغنام المصابة بالالتهاب الرئوي عن فقدان الشهية والسعال وإفرازات الأنف وضيق التنفس وأصوات الرئة غير الطبيعية وزيادة كبيرة في درجة حرارة الجسم ومعدل النبض ومعدل التنفس. كان معدل الإصابة بالالتهاب الرئوي مرتفعًا لدى الإناث البالغات والحيوانات ذات درجات الحالة الجسدية السيئة. كشف الفحص البكتريولوجي أن البكتيريا الأكثر شيوعًا هي الباستريلا هيموليتكا والاستاف اوريس والاي كولاي والبروتيس وأسباب بكتيرية مختلطة أخرى. بناءاً على الفحوصات البكتريولوجية، تم اختيار 15 خروفًا مصابًا بالتهاب رئوي لأجراء مزيد من الفحوصات. كشفت فحوصات الموجات فوق الصوتية عن أنماط رئوية غير طبيعية مثل تصلد الرئة والخراج ووجود سوائل بين غشائي البلورا. أظهر التحليل الدموى للاغنام المصابة بالالتهاب الرئوي انخفاضا كبيرا في عدد خلايا الدم الحمراء والهيموجلوبين والباكد سيل فوليم والخلايا الليمفاوية مع زيادة في خلايا الدم البيضاء وخلايا النيتروفيل. أظهر التحليل الكيميائي الحيوي انخفاضًا كبيرًا في فيتامين د والحديد والألبومين ونسبة الألبومين إلى الجلوبيولين وزيادة كبيرة في أميلويد أ والبروتين الكلي والجلوبيولين في الأغنام المصابة بالتهاب رئوي مقارنةً بالمجموعة الضابطة. كشفت الدراسة أن الالتهاب الرئوي البكتيري في الأغنام يؤثر بشكل كبير على مختلف المعايير الدموية والكيميائية الحيوية؛ بالإضافة الى الموجات فوق الصوتية والتغيرات التنفسية يمكن استخدامها كأدوات تشخيصية في الالتهاب الرئوي في الأغنام.

الكلمات الدالة: الدم، اصابات الجهاز التنفسي في الأغنام، البكتيريا، فيتامين د، الحديد.