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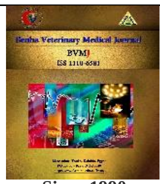

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Original Paper

Hematological changes and serum minerals concentrations in pneumonic sheep

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ABSTRACT

The present study was performed to investigate the crosstalk interactions of hematological profile, minerals and electrolytes concentrations and determine the histopathological variations associated with pneumonia in sheep. Fifty adult sheep were examined and divided into healthy (n = 20) and pneumonic sheep (n = 30). Pneumonia was confirmed by clinical and histopathological examinations in sheep. Liver, kidney function tests and mineral profile were calorimetrically determined via spectrophotometer by the method specified. Serum calcium, phosphorus, sodium and chloride concentrations were decreased in pneumonic sheep compared to healthy ones (P < 0.05). Additionally, the mean values of red blood cells (RBCS), hemoglobin (Hb), and packed cell volume (PCV) were significantly reduced while, the mean value of white blood cells (WBCS) and neutrophils were significantly increased in pneumonic sheep compared to healthy sheep (P < 0.05). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine and potassium concentrations were significantly increased in pneumonic sheep than healthy ones (P < 0.05). Lung histopathology revealed interalveolar septal thickening with vascular congestion, hydropic degeneration of bronchiolar mucosa, and lymphocytic infiltration. It was concluded that, pneumonic sheep were associated with altered hematological, minerals and electrolytes parameters. Decreased phosphorus concentrations could also be involved in the pathogenesis of erythrogram count. Hyperkalemia is associated with hyponatremia in pneumonic sheep.

1. INTRODUCTION

At present, the estimated global sheep populations are 1.2 billion heads, respectively. The African sheep population is estimated to be above 400 million heads (FAO 2021). Sheep are essential economically since they are primarily raised for lamb production, followed by milk and wool production for a huge section of the world's population, mostly in rural and arid areas. As a result, millions of individuals in numerous nations throughout the world, including Egypt, can benefit (Ali et al. 2009).

Pneumonia is considered a major source of loss in the sheep industry (Daniel et al. 2006). Pneumonia in sheep is caused by a variety of agents, including bacterial agents, which have attracted attention due to the wide range of clinical presentations, severity of disease, and re-emergence of strains resistant to a variety of chemotherapeutic treatments (Woldemeskel et al. 2002).

Clinical evaluation and hemogram profile are commonly used to diagnose a variety of disorders and determine an animal's nutritional state. The results of the blood tests would back up the physical examination, and when combined with the patient's medical history, they would provide a solid foundation for medical judgement. It would also aid in determining the level of tissue and organ damage, as well as the patient's defensive system response and diagnosis (Carlos et al. 2015). Electrolyte imbalances are typically caused by electrolyte loss, electrolyte shifts, or relative changes in concentrations due to water loss (Radostits et al. 2007; Kaneko et al. 2008), changes in these parameters were always important criteria to diagnosis, treatment, and prognosis of diseases affecting the respiratory system because the respiratory system plays such an important role in regulating acid-base status and thus serum concentrations of some minerals and electrolytes (Tanritanir et al. 2010).

The goals of this study were to investigate pneumonic sheep's hematology, minerals and electrolytes concentrations with also special reference to histopathological examination to diagnose acute pneumonia in sheep.

2. MATERIAL AND METHODS

2.1. Animals:

Fifty adult male and female sheep were examined in various farms and places throughout the Menofia Governorate in Egypt from December 20, 2020, to May 30, 2021, and were separated into two groups based on clinical examinations including healthy sheep (n = 20) with no clinical signs of illness and free from external and internal parasites and pneumonic sheep (n = 30) with bilateral nasal discharge, congested mucus membrane, inappetence, dyspnea, cough, and a harsh sound of lungs.

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2.2. Sampling:

Blood was collected from both healthy and pneumonic sheep into tubes with EDTA for hematological examination and also on plain serum clot tubes that centrifuged at 4000 × g for 10 minutes. Serum was aliquoted into smaller volumes and stored at -80°C until biochemical analysis. Moreover, 40 grams of lung tissues were collected and sectioned from healthy and animals displaying symptoms of respiratory manifestation at the abattoir for histopathological examination and kept on formalin 40% till examination.

2.3. Clinical and hematological examination:

All animals' case histories were compiled. All animals were subjected to a complete clinical examination according to (Radostits et al. 2007)**.** This study assessed hematological parameters RBCS, Hb, PCV, total and differential leukocytic counts. RBCs, Hb, and PCV values were used to calculate mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). These parameters were carried out according to the routine hematological procedures approved by (Meyer and Harvey 2004).

2.4. Biochemical and mineral profile:

Serum AST, ALT, BUN, creatinine, serum calcium, phosphorous, potassium, sodium and chloride were assessed by the kits of (Bio-diagnostic Company- Egypt), according to the methods specified.

2.5. Histopathological examination:

Lung tissues were taken from sheep who had pneumonia. For 72 hours, samples were fixed in 10% neutral buffered formalin. Trimmed, rinsed, dehydrated, embedded in paraffin wax, and sectioned into 3-5 m thick sections, tissue samples were used. Tissues were then stained with hematoxylin and eosin and examined under a Leica DMLB microscope (Leica, Wetzlar, Germany).

2.6. Statistical analysis:

The data were normally distributed and reported as means with standard deviations (Mean ± SD). Comparison between two groups was conducted by t-test using IBM SPSS Statistics 16 (IBM Corporation, Armonk, NY, USA). Pearson correlation (r) was determined between variables. Significance was determined at P < 0.05.

3. RESULTS

Clinical findings

The pneumonic sheep had a history of bilateral nasal discharge, elevated body temperature, increased heart and respiratory rates, cyanosed mucus membrane, inappetence, dyspnea, cough, and a harsh sound of lungs by stethoscope.

Hematological profile of apparently healthy and pneumonic sheep

In pneumonic sheep, complete blood counts revealed a significant decrease in the mean value of red blood cells, hemoglobin, and packed cell volume, as well as a significant increase in the mean value of white blood cells and neutrophils, when compared to apparently healthy sheep (Table 1; P < 0.05), while the mean levels of MCV, MCH, MCHC, lymphocytes, eosinophils and basophils did not differ significantly between healthy and pneumonic sheep (Table 1; P > 0.05).

Biochemical and mineral profile of apparently healthy and pneumonic sheep

The mean values of liver enzymes including (AST and ALT) and kidney function indicators (BUN and creatinine) were significantly higher in pneumonic sheep compared to control ones (Table 2; P < 0.05). The mean values of calcium, phosphorus, sodium and chloride were statically decreased, while the mean value of potassium was significantly increased within pneumonic sheep compared to control ones (Table 2; P < 0.05).

Table 1 Hematological profile of apparently healthy and pneumonic sheep.

Variables	Healthy sheep (n = 20)	Pneumonic sheep (n = 30)
RBCS (10^6/µl)	9.72±0.67	6.23±0.8*
Hb (g/dl)	13.02±0.5	9.17±1.6*
PCV (%)	31.67±0.86	19.8±3.37*
MCV (fi)	32.72±2.4	31.7±1.89
MCH (pg)	13.45±0.96	14.76±1.8
MCHC (%)	41.13±1.67	46.81±7.1
WBCS (10^3/µl)	10.26±1.56	15.76±7.8*
Neutrophils (10^3/µl)	2.86±0.98	4.27±3.5*
Lymphocytes (10^3/µl)	9.02±3.24	9.37±2.86
Monocytes (10^3/µl)	1.51±0.35	2.07±1.58
Eosinophils (10^3/µl)	0.03±0.03	0.034±0.04
Basophils (10^3/µl)	0.021±0.02	0.004±0.006

RBCS; red blood cells, WBCS; white blood cells, MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration. *P < 0.05.

Table 2 Biochemical and mineral profile of apparently healthy and pneumonic sheep.

Variables	Healthy sheep (n = 20)	Pneumonic sheep (n = 30)
AST (U/L)	27.75±2.5	36.69±1.9*
ALT (U/L)	23.12±2.03	28.4±1.6*
BUN (mmol/l)	1.8±0.4	2.9±0.6*
Creatinine (µmol/L)	47.8±3.17	55.09±3.3*
Calcium (mmol/l)	2.29±0.3	1.32±0.2*
Phosphorus (mmol/l)	1.5±0.3	0.97±0.13*
Potassium (mmol/l)	3.5±0.33	3.9±0.35*
Sodium (mmol/l)	135.2±3.19	124.6±2.6*
Chloride (mmol/l)	104.9±3. 5	94.8±1.4*

AST; Serum aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen. *P < 0.05.

Correlation between measured variables in pneumonic sheep

Serum phosphorus concentrations were positively associated with RBCS count and Hb concentrations in pneumonic sheep (r = 0.62; P < 0.05; r = 0.50; P < 0.05), respectively. Serum potassium was inversely correlated with sodium in pneumonic sheep (r = - 0.52; P < 0.05). However, no association was found between other variables.

Histopathological findings

A macroscopic examination of cases obtained from slaughtered and dead sheep revealed that some affected lungs were swollen, wet, and when sectioned, yellowish fluid exuded freely. Some lungs had varying degrees of congestion, as indicated by dark red patches. Histopathological examination of lungs revealed thickening of interalveolar septa with vascular congestion and hydropic degeneration of bronchiolar mucosa (Fig. 2A), other lung sample showed sever thickening of interalveolar septa with vascular congestion (Fig. 2B), lung tissue showed intensive inflammatory cell infiltration and hemosiderophages (Fig. 2C), acute pneumonia: showing thickening of interalveolar septa with lymphocytic infiltration (Fig. 2D).

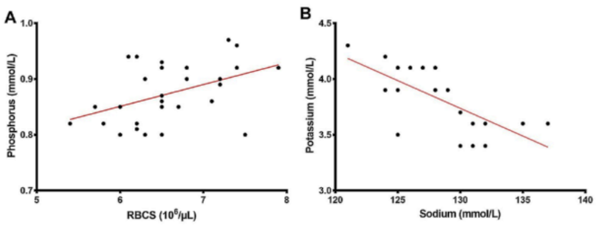

Figure 1 Pearson positive correlation between serum phosphorus and RBCS count. (B) Pearson negative correlation between potassium and sodium concentrations in pneumonic sheep. * P < 0.05.

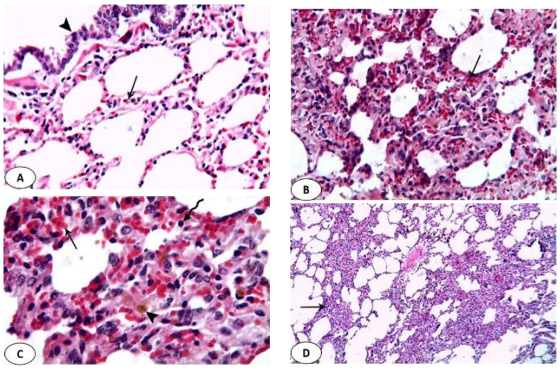

Figure 2A Lung, Sheep. Acute pneumonia: showed thickening of interalveolar septa with vascular congestion (arrow) and hydropic degeneration of bronchiolar mucosa (arrowhead). Fig. 2B Lung, Sheep. Acute pneumonia: showed sever thickening of interalveolar septa with vascular congestion (arrow). Fig. 2C Lung, Sheep. Acute pneumonia: showed sever thickening of interalveolar septa with vascular congestion (arrow), intensive inflammatory cell infiltration (bended arrow) and hemosiderophages (arrowhead). Fig. 2D Lung, Sheep. acute pneumonia: showed thickening of interalveolar septa with lymphocytic infiltration (arrow).

4. DISCUSSION

Adult sheep with abrupt severe sickness, inappetence, cough, pyrexia, cyanosed mucus membrane, significant toxemia, and tachypnea consistent with endotoxemia are presumptively diagnosed with acute respiratory disease (Fthenakis, 2011).

Regarding the hematological profile, the mean values of the red blood cells count, hemoglobin and packed cell volume were significantly decreased as well as a statistically higher mean value of white blood cells and neutrophils in pneumonic sheep compared to control ones ($P <0.05$), almost comparable findings have been reported by (Saleh and Allam, 2014).

Anemia in pneumonic sheep could be due to microbial secretions which lead to destroying red blood cells (Mondal et al. 2004) or sequestration of iron in bone marrow macrophages and hepatocytes, making it unavailable for use in hemoglobin synthesis, resulting in erythropoiesis suppression (Mosa, 2000) or oxidative damage to hemoglobin cause structural and functional changes, resulting in denaturation and precipitation of hemoglobin (Aytekin et al. 2011). Other inflammatory cytokines, such as IL1, may contribute to anemia by suppressing erythroid precursor proliferation in the bone marrow or inhibiting erythropoietin production in the kidney; in addition, TNF-mediated damage to erythrocyte membranes (Weiss and Goodnough 2005). Of interest, decreased serum phosphorus concentrations were positively associated with reduced erythrogram count, making it a likely factor involved in the pathogenesis of this condition.

In pneumonic sheep, leukocytosis and lower PCV levels were observed, which might be related to the presence of bacterial infection and the direct influence of their toxins on the sheep's hematopoietic system (Esmaeilnejad et al. 2012). The higher concentrations of total WBC count and neutrophils could be attributed to bacterial infection and tissue injury, which stimulate the release of growth factors, cytokines, and other mediators of the inflammatory response, also in moderate and severe pneumonia, the stress of respiratory disease causes the release of endogenous corticosteroids, which play a key role in regulating the circulating WBC concentration (El-Naser and Khamis, 2009). Increase in leucocytic count was found to be more prevalent in acute inflammatory disorders, especially those caused by bacterial infections. This could be because infectious agents and tissue injury products stimulate a variety of cells to release growth factors, cytokines, and other mediators of inflammation that act as rapid stimuli and are all interrelated in causing an elevation in total white blood cell count and more production, proliferation, maturation, and the release of mature and immature neutrophils from the bone marrow (Sayed et al. 2002).

In pneumonic sheep, the mean ALT and AST concentrations were substantially greater than in healthy sheep. The same conclusions were reached by (Saleh and Allam, 2014). This rise could be attributed to organ failure, including hepatic degenerative and necrotic alterations brought on by bacterial infection and toxins (Aytekin et al. 2011). Pneumonic sheep had significantly higher mean values of creatinine and BUN concentrations than healthy sheep. This could be related to kidney damage caused by free radicals created during the inflammatory process and the antioxidants' failure to counteract them. Increased catabolism of body protein could explain the rise in urea concentration, which could lead to an infection reaction, while an increase in serum creatinine could be a result of renal dysfunction following a respiratory tract infection, according to (Radostits et al. 2007).

Pneumonic sheep may have calcium and phosphorus deficiencies due to low protein levels, poor food intake, intestinal disturbances, and renal failure (Kaneko et al. 2008). In terms of changes in serum electrolytes, the findings indicated that serum sodium and chloride concentrations were dramatically reduced, while serum potassium levels were significantly elevated, the same results were reported by (Donia et al. 2018). Hyponatremia and hypochloremia could be attributed to hyperpyrexia in the acute stage of pneumonia, as well as liver and kidney infection that leads to hepatic and renal failure (Radostits et al. 2007). High potassium levels in the blood have been linked to respiratory disorders especially if there is acidosis as H^+ ions deposited in the extracellular fluid, causing hyperkalemia by exchanging with potassium in the intracellular fluid (Saleh and Allam 2014). Interestingly, increased potassium concentrations were negatively associated with hyponatremia in pneumonic sheep suggesting hypoaldosteronism. However, serum aldosterone concentrations were not measured in this study.

Histopathologically, the most prevalent histological changes produced by respiratory infections were bronchitis, bronchiolitis, alveolar emphysema, inflammatory and non-inflammatory edema, serous bronchopneumonia, and interstitial pneumonia. In the bronchioles and neighboring alveoli, neutrophils, macrophages, fibrin, cell debris, and mucus are found in various concentrations. Various researchers have reported non-inflammatory and inflammatory pathological lesions affecting the trachea and lungs in small and large ruminants (Akbor et al. 2007; Radostits et al. 2007; Jubb et al. 2012).

Acute pneumonia was linked with widespread cellular exudates, congestion, and capillary bleeding (Mohamed and Abdelsalam, 2008), although neutrophil aggregations may be present, oat cells (intense basophilic elongated and spindle-shaped nucleus) appear to be the most common cell populating the alveoli. In the alveoli, serofibrinous exudates and disseminated intravascular coagulation (DIC) were discovered (Mugale and Balachandran, 2019).

5. CONCLUSION

Throughout the course of the disease, pneumonia induces changes in hematological, biochemical, and histological markers, indicating increased organ functions, particularly in the liver and kidney. Hyperkalemia is linked to hyponatremia; phosphorus deficiency could possibly play a role in the pathogenesis of erythrogram count in pneumonic sheep.

6. REFERENCES

1. Akbor M, Haider MG, Ahmedullah F, et al 2007 . Pathology of trachea and lungs of buffaloes. Bangladesh J Vet Med 5:87–91.
2. Ali BA, El-Hanafy AA, Salem HH 2009 . Genetic biodiversity studies on IGFBP-3 gene in Egyptian sheep breeds. Biotechnol Anim Husb 25:101–109.
3. Aytekin I, Mamak N, Ulucan A, Kalinbacak A 2011 . Clinical, haematological, biochemical and pathological findings in lambs with peste des petits ruminants. Kafkas Univ Vet Fak Derg 17:349–355.
4. Carlos MML, Leite J, Chaves DF, et al 2015 . Blood parameters in the Morada Nova sheep: influence of age, sex and body condition score. J Anim Plant Sci 25:950–955.
5. Daniel JA, Held JE, Brake DG, et al 2006 . Evaluation of the prevalence and onset of lung lesions and their impact on growth of lambs. Am J Vet Res 67:890–894**.**
6. Donia GR, El Ebissy IA, Wassif IM 2018 . Biochemical and immunological studies on the respiratory diseases in sheep in northwestern coast. Eur J Biomed 5:34–41.
7. El-Naser EMA, Khamis GFA 2009 . Some hematological and blood serum biochemical indices associated with respiratory affections by camels. Assiut Vet Med J 55:154–162.
8. Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B 2012 . Evaluation of antioxidant status and oxidative stress in sheep naturally infected with Babesia ovis. Vet Parasitol 185:124–130. https://doi.org/10.1016/j.vetpar.2011.10.001
9. FAO 2021 . “Livestock Primary”. www.fao.org**.**
10. Fthenakis GC 2011 . Christos Brozos, DVM, PhDa, Vasia S. Mavrogianni, DVM, PhDb. Ther Control Sheep Goat Dis an Issue Vet Clin Food Anim Pract 27:105.
11. Jubb KVF, Kennedy PC, Palmer N 2012 . Pathology of domestic animals. Academic press
12. Kaneko JJ, Harvey JW, Bruss ML 2008 . Clinical biochemistry of domestic animals. Academic press
13. Meyer DJ, Harvey JW 2004 . Veterinary laboratory medicine. 3rd. Edn, London, W B Saunders Co, PP 17–24.
14. Mohamed RA, Abdelsalam EB 2008 . A review on pneumonic pasteurellosis respiratory mannheimiosis with emphasis on pathogenesis, virulence mechanisms and predisposing factors. Bulg J Vet Med 11:139–160.
15. Mondal D, Pramanik AK, Basak DK 2004 . Clinico-haematology and pathology of caprine mycoplasmal pneumonia in rain fed tropics of West Bengal. Small Rumin Res 51:285–295.
16. Mosa YB 2000 . Investigation of respiratory problems in calves. MV Sc
17. Mugale M, Balachandran C 2019 . An abattoir based histopathological survey of pulmonary pathology in small ruminants. Int J Livest Res 9:335–341.
18. Radostits OM, Gay CC, Hinchcliff KW, Constable PD 2007 . Veterinary Medicine, 10. Ed Saunders Elsevier, Philadelphia, USA 673–748.
19. Saleh NS, Allam TS 2014 Pneumonia in Sheep: Bacteriological and Clinicopathological Studies. Am J Res Commun 2:73–88.
20. Sayed AS, Ali AA, Mottelib AA, Abd El-Rahman AA 2002 . Bronchopneumonia in buffalo-calves in assiut governorate 1-studies on bacterial causes, clinical, haematological and biochemical changes associated with the disease. Assiut Vet Med J 46:138–155.
21. Tanritanir P, Ragbeth C, Değer Y, Ceylan E 2010 . The Effect of Draxxin Treatment on Blood Gases Levels of Montofon Calves with Pneumonia. Asian J Anim Vet Adv 5:72–76.
22. Weiss G, Goodnough LT 2005 . Anemia of chronic disease. N Engl J Med 352:1011–1023.
23. Woldemeskel M, Tibbo M, Potgieter LND 2002 . Ovine progressive pneumonia Maedi-Visna : An emerging rBancroft, J., Gamble, M., 2002. Theory and practice of histological techniques. 5th. Edinburgh. Churchill Livingstone Pub 172, 593-620.