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Original Research

Evaluation of Recent Techniques in Diagnosis of Induced Caprine Pneumonia

Shimaa M. Abdullah[1*], Abdel Khalek R. El-Sheikh[1], Abdel Raouf M. Mahmoud[1], Noura E. Attia[1], Nora El-Seddawy[2]

[1]Department of Animal Medicine (Internal Medicine), Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.
[2]Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.

***Correspondence**
Shimaa M. Abdullah
Department of Animal Medicine (Internal Medicine), Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.
E-mail address: shimaamohamed282016@gmail.com

Abstract

The goal of the current study was to assess the new techniques for diagnosing of ammonia-induced pneumonia in goats, primarily using radiography and Computed tomography (CT) examinations coupled with some selective biochemical parameters. The experimental induction of pneumonia using ammonia vapor inhalation (33%), was performed on five female goats weighing 20 to 30 kg and ranging in age from 10 months to one year. A thorough clinical examination was performed on all goats. Goats' jugular veins were used to collect blood samples in order to monitor changes in the pulmonary function test and some selective biochemical parameters. A clinical examination of goats showed that exposure resulted in a significant ($P< 0.05$) rise in mean rectal temperature at day 14, as well as heart and respiration rates at day 7. On the 7th day, dullness, coughing, dyspnea, bilateral mucopurulent or mucoid/purulent nasal discharge, and abnormal lung auscultation were recorded. On the 14th day, the severity increased. When compared to zero-day results (auto-control group), serum biochemical examination showed significant ($P< 0.05$) increases in haptoglobin, serum amyloid A at 7 days, and malondialdehyde levels at 14 days, but there was a significant ($P< 0.05$) decrease in total antioxidants capacity at 14 days after inhalation. When ammonia was inhaled, pCO_2, tCO_2, pO_2, and HCO_3 changed significantly ($P< 0.05$) at day 7 and pH decreased significantly at day 14. Upon ultrasonography, pneumonic consolidation displays an echotexture resembling the liver with the presence of hypoechoic patches and loss of reverberation artefacts. The chest x-ray showed increased opacity (more gray or white) with a cotton-wool-like look. On the 7th day, a lung CT revealed focused areas of ground-glass opacity that later became diffuse. An edematous, hyperemic lung with consolidation was observed during the postmortem examination. Lung histopathology revealed serofibrinous pneumonia. Therefore, in order to diagnose ammonia-induced pneumonia in goats, ultrasonographic, radiographic, and CT examinations, coupled with selective biochemical parameters and pulmonary function tests, are useful.

KEYWORDS
Caprine pneumonia, Computed tomography, Acute Phase proteins, Blood gases, CT

INTRODUCTION

For farmers in rural regions, goats are regarded as the most valuable small ruminants, which can be best utilized to escape the poverty trap since they bring real benefits such as financial income from sales, meat, waste, skins, and fiber (Abd-Allah *et al*., 2016). Goats are susceptible to a wide range of diseases, although respiratory diseases are the most common since they are spread mostly through the air and the blood (Tesfaye and Mekonnen, 2016).

The primary disease restricting the progress of animal production in the tropics is still pneumonia, a respiratory disorder caused by an inflammatory reaction of the lung parenchyma (Attoh-Kotoku *et al*., 2018). Caprine pneumonia is a syndrome resulted from interactions between micro-organisms, environmental stressors, and the host immunity (Mohamed and Abdelsalam, 2008).

The most frequent environmental and managemental factors that affect goats' respiratory systems include exposure to cold weather during winter, heavy rains, housing in poorly ventilated areas, inhalation of dust and irritating vapors like ammonia, which is abundant in the atmosphere of animal buildings, dirty barns, inadequate drainage systems, high humidity, and transportation stress (Islam *et al*., 2006).

One of the hazardous air pollutants, ammonia has a terrible smell in high concentrations and is harmful for both human and animal health (Tao *et al*., 2019). Ammonia from the atmosphere is inhaled by both humans and animals and enters their respiratory tracts as NH_4^+, which damages or removes their respiratory cilia. Damaged cilia on the mucosal surface cannot stop bacteria from entering the respiratory system, increasing the risk to disease (Ghaly and MacDonald, 2013).

Clinical diagnosis of pneumonia usually includes physical examination, imaging, serology, and identification of the causative agent from nasal swabs, bronchial lavages, and even faeces (for verminous pneumonia) (McRae *et al*., 2016). For an accurate diagnosis, these attempts should be supplemented with postmortem investigation of the lungs either at necropsy or slaughter (Di

Provvido *et al*., 2018).

The preferred approach for determining whether a patient has lung disease in humans is computed tomography (CT) of the thorax, which is now increasingly applied in veterinary patients (Rizzi *et al*., 2011). Due to the lack of superimposition of anatomical structures, thoracic CT has shown to be useful for determining the extent of lung disease and for providing better anatomic details (Prather *et al*., 2005). Therefore, the purpose of this study was to assess modern techniques, particularly radiography and CT examinations together with some selective biochemical parameters, for the diagnosis of ammonia-induced pneumonia in goats.

MATERIALS AND METHODS

The study protocol was approved by the animal care committee of the Faculty of Veterinary Medicine, Zagazig University, Egypt No. ZU-IACUC/2/F/99/2022.

Animals

The experimental induction of pneumonia by ammonia vapor inhalation was performed on five female goats weighing 20 to 30 kg and ranging in age from 10 months to one year. Throughout the adaptation period, the goats were monitored daily.

According to Constable *et al*. (2017), a thorough clinical history and examination were conducted on each animal to verify their health status. This included a general clinical examination for temperature, pulse, respiration, mucous membranes, superficial palpable lymph nodes, rumen motility, heart rate, and a physical examination of the respiratory system by percussion and auscultation. On days 0, 7, and 14 after induction, all goats underwent physical examinations, thoracic auscultation, hematobiochemical analysis, thoracic ultrasound, radiography, and computed tomography. After mortality or emergency slaughter, postmortem and histopathological examination were applied. The goats were kept in the farm of the Faculty of Veterinary Medicine at Zagazig University under medical supervision, with regular examinations, and 15 days of acclimatization to the research room before the experiment. The housing was appropriate, with a known diameter chamber measuring about 9 m2. Water was available ad libitum, and crushed corn (0.5 kilogram per head per day), bran (0.100 kg per head per day), wheat straw (0.250 kg per head per day), and berseem (1.250 kg per head per day) were fed to goats supplemented with a mineral and vitamin mixture. The goats were subjected to concentrated ammonia solution 33% daily until the experiment was completed (14 days).

Sampling

Two blood samples were collected from each animal before exposure and served as auto control samples, at the 7^{th}, 14^{th} days from pneumonia induction.

Blood serum samples

About 10 ml of blood from the jugular vein is allowed to flow freely into a clean, dry, and labelled centrifuge tube without using an anticoagulant. The tubes are then placed in a slope position at room temperature for 2 hours to coagulate until the clot retracts, after which the sera are removed with a Pasteur pipette and clarified by centrifugation at 3000 rpm for 15 minutes. Finally, clear, non-hemolyzed serum transferred by using sterilized pipette into dry, sterile, and labeled vial (Kaneko *et al*., 1997). Then preserved in a deep freezer at -20 °C for detection of acute phase proteins (Serum amyloid A, SAA and haptoglobin, Hp) and oxidative stress biomarkers (malondialdehyde, MDA and total antioxidants capacity, TAC).

Heparinized blood samples

A blood gas analyzer was used to collect anaerobically venous blood from the jugular vein, which was then rapidly analyzed for blood pH, partial oxygen pressure (pO_2), carbon dioxide pressure (pCO_2), and bicarbonate (HCO_3). The samples were taken at day 0 (control), day 7, and day 14 following exposure to ammonia vapor.

Ultrasonography

An ultrasound machine with a 3.5 MHz linear probe was utilized to examine each goat's lungs for ultrasonographic findings of lung abnormalities (edema, congestion, or consolidation) (Njoroge *et al*., 2000) at day 0, day 7, and day 14 following exposure to ammonia vapor.

Radiography

The radiography was taken to confirm the pneumonia and help in evaluating the various pulmonary function scores in pneumonic goats (Smith and Sherman, 2009) at day 0, day 7, and day 14 following exposure to ammonia vapor.

Computed tomography (CT)

The CT scan was performed at a private CT center, at EL-Sharkia Governorate, Egypt. A General Electric (GE) light speed ultra-system (a third generation multi-slice helical CT scanner) was used with eight body slices per rotation and soft tissue window width was 500 HU (Hounsfield unit). Its features were 53.2 KW generator, 6.3 MHU tube size, a fast rotation time of 0.5 seconds.

Histopathological examinations

For histopathology, small lung specimens with a thickness of 0.5 cm were taken from recently dead or emergency slaughtered goats with respiratory problems. As previously done by Ghanem *et al*. (2015), lung tissues were inflated with formalin to maintain the architecture of the pulmonary alveoli.

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA), using Statistical Package for Social Sciences software, version 16.0 (SPSS Inc., Chicago, IL). The results are expressed as means ± standard error (SE). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Clinical signs

Goats exhibited a normal appetite, normal general systemic status, light rosy red and slightly bluish conjunctival mucous membranes without any secretion or edema, a normal resonant sound on lung percussion, and a normal vesicular sound on auscultation at zero day. The mean values of temperature, pulse and respiration rates were 39.06±0.08 °C, 74.40±1.36/ min and

23.80±0.80/ min respectively.

The goats were slightly affected on the 7th day, exhibiting decreased appetite, accelerated shallow breathing, pale mucous membranes, dry cough, scanty serous to mucoid nasal discharge, incomplete dull sound on lung percussion, and hyper-vesicular sound on auscultation. Compared to zero-day findings, there was a significant elevation in pulse and respiration rates (83.40±1.07/ min and 31.20±1.06/ min respectively), while temperature revealed non-significant change (39.24±0.23°C).

On the 14th day, the goats were severely affected, exhibiting dyspnea, anorexia, depression, and recumbency, congested mucous membranes, moist painful cough, mucopurulent to purulent nasal discharge, incomplete to complete dull sounds on lung percussion, dry, moist and crepitant rales, crepitation, and wheezes on auscultation with audible heart sound was heard. In comparison to zero-day findings, the mean values of temperature, pulse, and respiration rates significantly increased (40.06±0.13°C, 100.80±2.81/ min and 42.20±1.56/ min respectively).

Acute phase proteins (SAA, Hp)

At the 7th day, goats showed significant increase in the mean values of SAA and Hp. While at the 14th day, the mean values of them revealed highly significant increase when compared with zero-day results (Table 1).

Table 1. Serum amyloid A (SAA), haptoglobin (Hp), and total antioxidants capacity (TAC), malondialdehyde (MDA) at zero, the 7th and the 14th days from pneumonia induction.

Parameter	Zero day	7th day	14th day
SAA (mg/l)	88.48 ± 0.77[c]	95.99 ± 1.58[b]	115.68 ± 1.67[a]
Hp (g/l)	2.91 ± 0.10[c]	3.80 ± 0.16[b]	7.36 ± 0.39[a]
TAC (ng/ml)	15.03 ± 0.11[a]	14.53 ± 0.18[a]	12.15 ± 0.18[b]
MDA (nmol/ml)	6.91 ± 0.18[b]	7.44 ± 0.25[b]	10.63 ± 0.13[a]

Values are represented as mean ± SE. Means within the same row carrying different superscripts (a, b, and c) are significant at $p < 0.05$.

Oxidative stress biomarkers (TAC, MDA)

At the 7th day, goats showed nonsignificant alteration in the mean values of TAC and MDA. While at the 14th day, the mean values of them revealed significant decrease in TAC and significant increase in MDA when compared with zero-day data (Table 1).

Blood gas analysis

At the 7th day, goats showed nonsignificant alteration in the mean value of pH, significant decrease in the mean value of pO_2 and significant increase in the mean value of pCO_2, tCO_2 and HCO_3. While at the 14th day, there were significant decrease in the mean values of pH, and highly significant decrease in the mean value of pO_2 with highly significant increase in the mean value of pCO_2, tCO_2 and HCO_3 when compared with zero-day results (Table 2).

Ultrasonographic imaging

At the 7th day, lung ultrasonography revealed loss of reverberation artifacts with presence of hypoechoic areas. While at the 14th day, lung tissue appeared more echogenic resembled hepatic architecture with hyperechoic nodules (Figure 1).

Table 2. Blood gas analysis at zero, the 7th and the 14th days from pneumonia induction.

Parameter	Zero day	7th day	14th day
pH	7.38 ± 0.02[a]	7.35 ± 0.01[a]	7.27 ± 0.01[b]
pO_2 (mmHg)	40.10 ± 0.38[a]	36.54 ± 0.29[b]	31.44 ± 0.32[c]
pCO_2 (mmHg)	39.95 ± 0.14[c]	43.19 ± 0.14[b]	47.88 ± 0.63[a]
tCO_2 (mmol/l)	19.70 ± 0.41[c]	22.14 ± 0.31[b]	24.38 ± 0.33[a]
HCO_3 (mmol/l)	19.67 ± 0.23[c]	21.31 ± 0.18[b]	24.28 ± 0.38[a]

Values are represented as mean ± SE. Means within the same row carrying different superscripts (a, b, and c) are significant at $p < 0.05$.

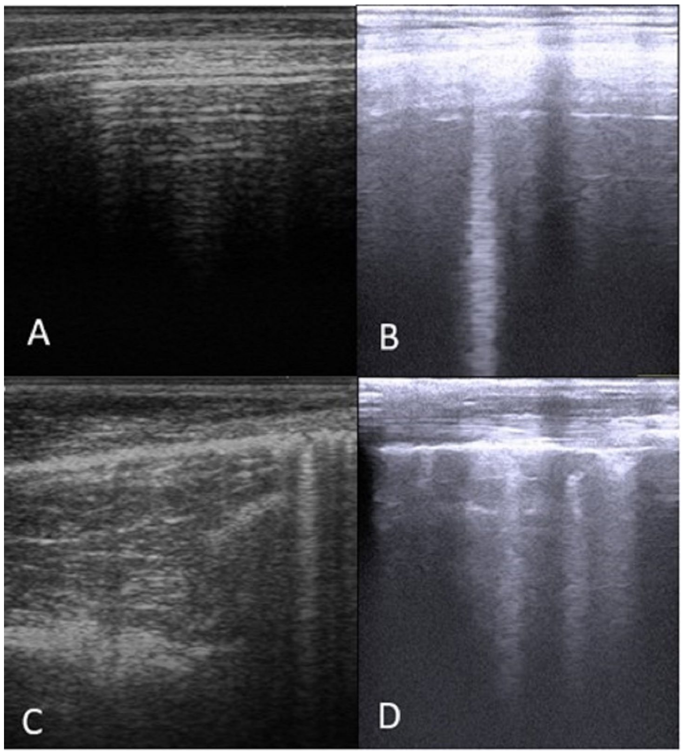

Figure 1. Ultrasonography of lung. A: Normal reverberation artifacts at zero day. B: Comet tail artifact at the 7th day. C: Consolidation with echoic part and comet tail artifact and D: Comet tail with thickened pleura at the 14th day.

Radiographic changes

Lung radiography showed gradual increase in the radio opacity (grey or white) at the 7th and 14th days respectively when compared with the normal pulmonary pattern (quite black) (Figure 2).

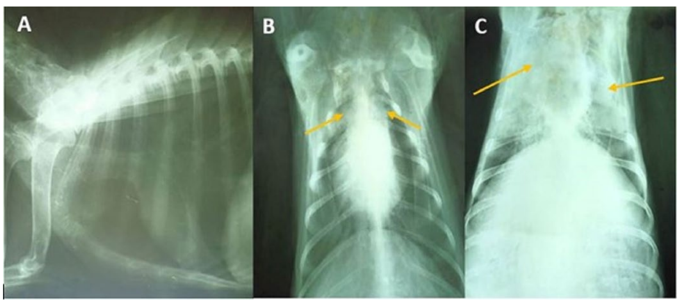

Figure 2. Radiography of lung. A: Normal radiograph at zero day. B, C : Alteration of pulmonary patterns in pneumonic goats by increase the radio opacity of the lung (arrows) that may be more gray or white at the 7th ,14th days respectively.

Computed Tomography (CT)

At the 7th day, lung CT showed focal areas of ground-glass opacity (increase in lung opacity) especially in the cranio-ventral area. While at the 14th day, the ground-glass opacity was diffused (Figure 3).

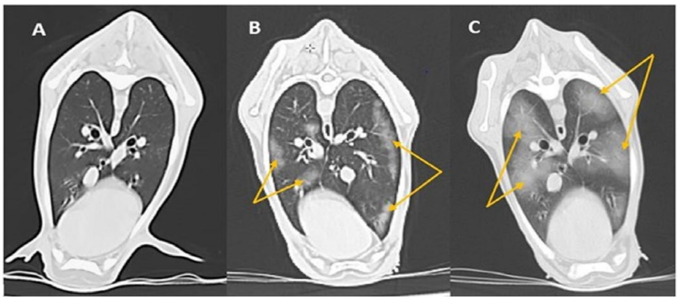

Figure 3. CT of lung. A: Normal CT at zero day. B: localized areas of Ground Glass Opacity (GGO) at day 7. C: Diffused GGO at day 14.

Postmortem findings

The pneumonic lung displayed areas of pulmonary congestion and edema, represented by several congested foci on lung tissue, white froth in the trachea and major bronchi, and oozing frothy fluid on cut sections, areas of red hepatization (early stage of consolidation), represented by dark red coloration and firm in consistency, and other areas of grey hepatization (grey coloration, firm in consistency like liver tissue) (Figure 4).

Histopathological findings

The lungs showed consolidation with exudates and serofibrinous pneumonia which characterized by some alveoli filled with serous exudates and other filled with fibrin threads with mononuclear cells infiltrations in the interalveolar septa. Few alveoli were filled with detached alveolar macrophages. Focal aggregation of mononuclear cells replaced some alveolar tissues. The pleura and interstitial septa showed thickening with fibrin threads and leukocytic infiltration (Figure 5).

DISCUSSION

Infected goats exhibit severe mental and physical depression, poor appetite, and dullness at the onset of respiratory disease. They quickly develop a high temperature, anorexia, rapid shallow breathing, and copious mucopurulent secretions from the nose and eyes. Most infected animals later develop a productive cough that is worsened by movement or physical exertion. In extremely severe stages of the disease, severe dyspnea with an expiratory grunt may be noticed (Lopez, 2001). These symptoms may be caused by hypercapnia, which increases respiratory rate and causes polypnea. Additionally, excessive ammonia formation and accumulation, especially in poorly ventilated houses, can cause irritation and inflammation of nasal mucosa, nasal discharge and abnormal rales by exuding inflammatory fluids into the respiratory tract and alveolar lumen (Metwally *et al*., 2017). The nasal mucus membranes inflammatory alterations may also be responsible for the nasal discharges. To counteract the hypoxia in goats

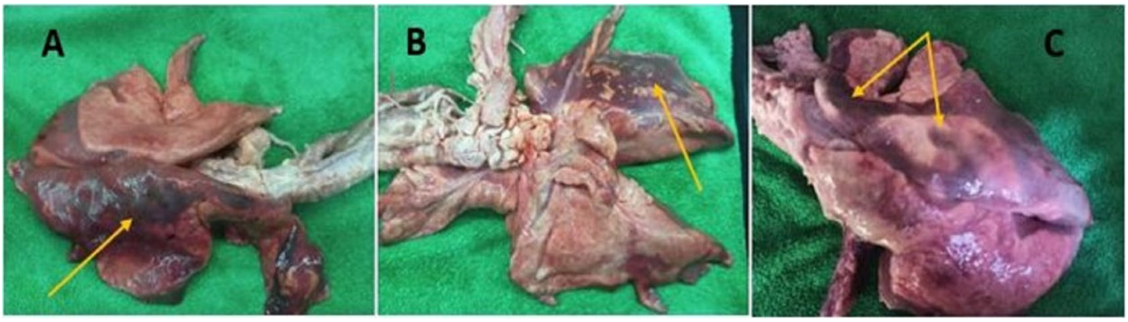

Figure 4. Gross lesion. A, B: Consolidation with areas of congestion and oozing blood. C: edematous and hyperemic lungs with consolidation.

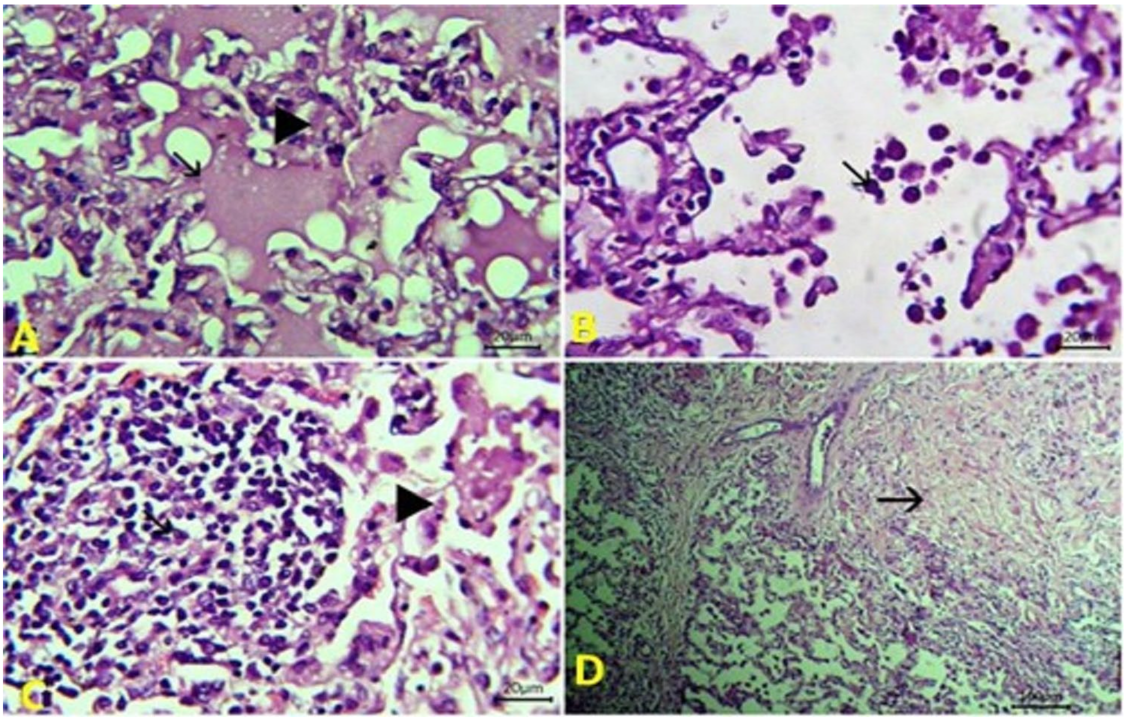

Figure 5. Photomicrograph of lung tissue stained with HE. A: Alveoli filled with serous exudates (arrow) and mononuclear cells infiltrations in the interalveolar septa (arrow head), bare 20. B: Alveolar macrophages (arrow), bare 20. C: Focal aggregation of mononuclear cells(arrow) and alveoli filled with fibrin thread (arrow head), bare 20. D: The interstitial septa showed thickening with fibrin thread and leukocytic infiltration (arrow), bare 100.

with pneumonia, heart rate was increased. Moreover, a strong cough, depression, and decreased appetite (Kumar *et al*., 2018).

Hepatocytes and peripheral tissues both produce acute phase proteins in response to inflammation (Ceciliani *et al*., 2012). It has been suggested that acute phase proteins are early and sensitive indicators of inflammatory processes in ruminants (González *et al*., 2011). This explains the significant rise in Hp and SAA levels on the 7^{th} day, which may have been caused by the inflammation in pneumonic goats' lungs, which emphasizes their function in host immunity (Orro *et al*., 2011). These results suggest that the best indicators for ruminant respiratory disease detection were Hp and SAA (Abdelbaset *et al*., 2013). A change in protein profile reflects alterations in the acute phase response (El-Seidy *et al*., 2003).

According to Metwally *et al*. (2017), lipid peroxide MDA showed a significant elevation while the mean value of total antioxidant capacity (TAC) showed a significant reduction. The current investigation reveals that, as compared with zero-day data at the 14^{th} day after ammonia inhalation, TAC significantly decreased, and MDA significantly increased. This could be attributed to pulmonary endothelium's destruction (Lykkesfeldt and Svendsen, 2007). Therefore, this damage will result in poor perfusion in the pulmonary tissue, which could lead to the initiation of free radical processes and free radical peroxidation (Chilvers, *et al*., 1989).

According to prior studies, pneumonic goats displayed a significantly higher level of pCO_2, tCO_2, and HCO_3 and a significantly lower level of pH and pO_2 when compared to the control group (Fararh *et al*., 2017). The current study's findings showed that pH significantly decreased at day 14 while pO_2, pCO_2, tCO_2, and HCO_3 significantly changed on the 7^{th} day of exposure. High respiratory frequency and aerobic metabolism to lower CO_2 levels may be responsible for the rise in PCO_2, HCO_3, and tCO_2 and decline in PO_2 levels (Smith, 2014). The use of oxygen during metabolism as a result of the increase in WBCs could be the cause of the decrease in PO_2 in addition to the disturbance in gas exchanges (Hussein and Aamer, 2013). Respiratory acidosis, which results in hypoxic conditions and pulmonary hypoventilation associated with pneumonia, may be the source of these findings. These findings in agreement with those of Ghanem *et al*. (2015). The renal retention of bicarbonate in exchange for chloride to maintain electrical neutrality in response to the respiratory acidosis may also be responsible for the rise in HCO_3 levels (Kumar *et al*., 2018).

The lesions were easily distinguished by ultrasonography from the bright linear reverberation artefacts of the normal lungs in control animals (Smith, 2017). Due to the constant inhalation of ammonia, consolidation was first noticed predominantly cranio-ventrally at day 7 and then spread to include both lungs by day 14. Pneumonic lung imaging with ultrasonography showed consolidation and loss of reverberation artefact. According to Bayoumi *et al*., (2022) an inflamed portion of the pleura appeared as a localized echogenic patch, followed by a comet tail artefact and lung lesion was more ventrally located. For the case prognosis, the percentage of the visible consolidated lung lobes in the entire lung capacity is essential (Asare *et al*., 2016). In a study by Emikpe *et al*. (2013) the right lung's cranial region was mainly affected.

Normal lung radio density changed in accordance with lung inflation during radiography, with expiration processes having a higher radiodensity than inspiration processes. Goats with pneumonic disease had considerable opacity on the radiograph. At the 7^{th} and 14^{th} days, lung radiography revealed a gradually increasing radio opacity (grey or white), which may be related to the pneumonia's increasing severity. High gray- or white-density areas, such as opacity, had the look of cotton wool was mainly located cranioventrally (Bayoumi *et al*., 2022). According to the radiographic examination, the severity of pneumonia in goats was confirmed and rated as 1, 2, and 3 by comparing the affected areas of the lung to healthy goats and detecting infiltration density and contrast (Kattimani *et al*., 2020). Radiographic examination of the lungs that reveals severe lung alterations may be used to determine the condition's severity and prognosis (Smith and Sherman, 2009).

The severity of the disease on radiography and CT did not significantly correlate with the clinical respiratory scores. The hypothesis that clinical disease and the appearance of imaging abnormalities occur later than expected is consistent with the mismatch between clinical severity and imaging severity (Fowler *et al*., 2017). The current study's lung CT revealed localized areas of ground-glass opacity (increase in lung opacity) particularly in the cranio-ventral region at the 7^{th} day. The ground-glass opacity was diffused on day fourteen. Ground-glass opacity is described as a hazy elevation in lung opacity without obstruction of the underlying vasculature or bronchi and is caused by the partial replacement of alveolar gas by fluid or cells or by an increase in interstitial thickness (fluid or cells) (Hansell *et al*., 2008).

The affected lungs were edematous and showed areas of congestion, red hepatization (dark red coloration and resemble liver in consistency) and grey hepatization (grey coloration, firm in consistency like liver tissue). The result in this study was in agreement with those recorded by Constable *et al*. (2017).

Histopathological findings of pneumonic goats' lungs showed consolidation with exudates and serofibrinous pneumonia which characterized by some alveoli filled with serous exudates and other filled with fibrin thread with mononuclear cells infiltrations in the interalveolar septa. Few alveoli were filled with detached alveolar macrophages. Focal aggregation of mononuclear cells replaced some alveolar tissues. The pleura and interstitial septa showed thickening with fibrin threads and leukocytic infiltration. This agreed with Sastry and Rama Roa (2002).

CONCLUSION

The current study suggested that various recent diagnostic techniques, mainly ultrasonographic radiographic and CT examination along with some selective biochemical parameters including Acute phase proteins (SAA and Hp), Oxidative stress biomarkers (TAC and MDA) and blood gas analysis which were supported by a thorough clinical examination, postmortem and histopathological examination, may be helpful in confirmatory diagnosis of ammonia induced pneumonia in goats.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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