



## Immunopathological Changes of *Streptococcus pneumoniae* Causing Respiratory Infection in Lambs

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### A B S T R A C T

*Streptococcus pneumoniae* are common bacterial pathogens that can cause a range of diseases in humans and animals. This study attempts to clarify the course and consequences of pneumonia caused by this bacterial infection. A total of 6 male un-weaned lambs aged between 1 to 2 months and weighing 5 to 7 kg were subjected to *S. pneumoniae* strain ATCC 6303 serotype 3 at  $2 \times 10^6$  CFU/mL by inhalation to induce pneumonia. Pneumonic clinical signs were monitored daily throughout the study period. The blood samples were collected from all animal groups at day zero before pneumoniae induction and 3, 6, and 14 days post infection for total and differential white blood cell count (WBCs) and tumor necrosis factor-alpha (TNF- $\alpha$ ) assessments. Additionally, on days 6 and 14 post-exposure, trachea and lung tissue samples were harvested for macroscopic and microscopic pathological changes evaluation. The results showed that there was a significant increase ( $P < 0.001$ ) in total WBC counts from day 3 post-exposure and maintaining elevated levels on days 6 and 14 compared to day zero. Differential WBC counts showed an early, significant rise in neutrophils, with sustained elevation in lymphocytes and monocytes. TNF- $\alpha$  levels significantly elevated on day 3 and gradually declined by day 14 post-exposure ( $P < 0.001$ ). On day 6 post-exposure, the gross pathological changes in the lung showed pulmonary edema, and emphysema, with mild to moderate lung congestion and emphysematous changes observed on day 14 post-exposure. Histopathologically, severe erosion and necrosis in the trachea and bronchus epithelium, along with inflammation in adjacent mucosa and submucosa, accompanied by focal inflammatory infiltration and emphysema in the lung by day 6 post-exposure, were observed. By day 14, these changes progressed to marked epithelial vacuolation and necrosis in the trachea, with lung sections revealing perivascular cuffing, peri bronchiolitis, mild bronchiectasis, atelectasis, and alveolar collapse. This study is attempt for a better understanding of *S. pneumoniae* infection in lamb and contributing to the development of preventive and management strategies in Iraq.

**Keywords:** *Streptococcus pneumoniae*, respiratory infection, immunopathology, lamb

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### INTRODUCTION

Respiratory problems, such as lamb pneumonia, result from the combined influence of different variables, including the host's immunological and physiological characteristics, the causative agent (such as a virus, bacteria, or mycoplasma), and environmental issues factors (1-3). Among several causative agents, *Streptococcus pneumoniae* (*S. pneumoniae*), a bacterium

that normally resides in the nasopharynx of animals, has been linked to an important amount of cases of illness and death in young lambs caused by pneumonia (4). *S. pneumoniae* infection results in reduced development and death in lambs and have a substantial economic impact due to the expenses incurred for treatment and the condemnation of animals in abattoirs (5, 6).

*S. pneumoniae*, a prominent Gram-positive bacterium, is recognized for its role in causing respiratory tract

infections, including pneumonia, in both humans (7) and animals (8). In veterinary medicine, *S. pneumoniae* causes respiratory infection in livestock such as lambs, where it can lead to significant morbidity and mortality (4). Also, *S. pneumoniae* caused human infections (9). Inhalation is a route of infection for *S. pneumoniae* in animals, including lambs, reflecting natural exposure scenarios on farms (10). Key aspects of *S. pneumoniae* pathogenesis to be investigated include bacterial colonization of the respiratory tract, adhesion to respiratory epithelial cells, evasion of host immune defenses, and induction of inflammation leading to tissue damage (11). One of the histopathological changes, interstitial pneumonia represents in the lung of sheep (12).

When bacteria invade, the host's immune system is activated specifically by neutrophils and lymphocytes (13), neutrophils, or polymorphonuclear leukocytes, are essential in early infection stages by phagocytosing and destroying pathogens. They also release cytokines like TNF- $\alpha$ , which regulate the immune response (14). Lymphocytes, including B and T cells, are essential for specific immune responses against *S. pneumoniae* (15). Monocytes differentiate into macrophages, which engulf and digest pathogens (16). TNF- $\alpha$ , a pro-inflammatory cytokine, promotes the recruitment of immune cells and inflammatory cascade (17).

Studying the respiratory infection disease caused by *S. pneumoniae* and investigate the pathological changes and immune response by using the lamb model of inhalation-induced pneumoniae infections seeks to enhance our understanding in veterinary medicine and contribute for avoidance and treatment of *S. pneumoniae* in livestock populations.

## MATERIALS AND METHODS

### Ethical Approval

The study was carried out with the authorization of the local Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad (Approval Number: 2152/P.G. dated October 10, 2023).

### Animals and Management

In this investigation, a total of six male lambs of the native Iraqi breed, aged between one and two months, unweaned, and weighing between 5 and 7 kg. The animals were acquired from a nearby location in the Al-Najaf region, Iraq, and then transferred to the animal facility at the College of Veterinary Medicine, University of Baghdad. Upon arrival at the animal farm facility, the lambs were given a week-long acclimatization period to adapt to their new environment and diet. During this period, they were housed in normal field conditions of temperature, ventilation, and lighting. They were provided with milk for artificial feeding and dry hay, and clink tests were conducted to ensure that the lambs were safe from respiratory infections.

### Inoculum Preparation of *S. pneumoniae*

*S. pneumoniae* strain ATCC 6303, serotype 3, was sourced from a local supplier (Media Diagnostic Center, Erbil, Iraq). For the initial culture, the bacteria were grown on 5% sheep blood agar plates (Liofilchem, Italy) in an incubator set at 37 °C with 5% CO<sub>2</sub> for 18 hours (18, 19, 20). Following the incubation period, the bacteria were harvested, rinsed, and resuspended in sterile phosphate-buffered saline (PBS). The bacterial suspension was then transferred to brain heart infusion broth (BHI, Liofilchem, Italy) and incubated without agitation for an additional 6 hours under the same conditions to promote further bacterial growth (21).

Post-incubation, the bacterial cultures were subjected to centrifugation (2000 rpm for 15 min) then washed and rinsed frequently with sterile PBS to ensure purity and removal of any non-bacterial components. To ascertain the infectious dose, colony-forming units (CFU) were counted by plating serial dilutions of the *S. pneumoniae* ATCC 6303 suspensions on fresh 5% sheep blood agar plates. These plates were then cultured at 37°C with 5% CO<sub>2</sub> for 24 h to allow for accurate CFU determination. The final working inoculum was adjusted to a target concentration of  $2 \times 10^6$  CFU/mL (9). This concentration was verified through densitometric measurements using the Densi CHEK system (BioMérieux, France) and confirmed by spectrophotometric analysis at a 600 nm wavelength (Shimadzu, Japan). The precision and accuracy of the bacterial concentration for the experimental inoculum were further validated using the standard plate counting method, ensuring consistency and reliability in the dosage used for the animal experiment.

### Experimental Design

The experimental design involved a single exposure to the prepared *S. pneumoniae* suspension. The inhalation method was chosen as the route of administration to mimic natural infection pathways. For this, a nebulizer system (Mesh Nebulizer, China) was utilized. Pre-inoculation, each lamb's face was sterilized with 70% alcohol to ensure aseptic conditions. The lambs then inhaled 3 mL of the bacterial suspension ( $2 \times 10^6$  CFU/mL) through the nebulizer. The entire procedure was conducted under controlled conditions to ensure consistent animal exposure (22).

### Clinical Signs

Post-inhalation exposure, lambs were observed daily for any clinical signs indicative of pneumonia, such as coughing, dyspnea, tachypnea, fever, and sputum production. These observations were recorded to track the progression and severity of the infection over time.

### Blood Sample Analysis

On day 0 (pre-inhalation exposure) and days 3, 6, and 14 (post-inhalation exposure), blood samples (about 5 mL) were drawn from the jugular vein into EDTA-anticoagulant tubes for total and differential white blood cells (WBC) count using an automated hematology analyzer (Getein

BHA-5000, China). For tumor necrosis factor-alpha (TNF- $\alpha$ ) analysis, blood was collected in gel tubes, centrifuged at 3500 rpm for 5 minutes, and the serum was stored at -20 °C. Serum TNF- $\alpha$  levels were measured using a Sheep TNF- $\alpha$  ELISA Kit (Beijing Solarbio Science & Technology CO., China) following the manufacturer's protocol.

### Bacterial Isolation from Lung Tissues

To evaluate the persistence of *S. pneumoniae* post-inhalation exposure, bacterial isolation from lung tissues was performed on days 6 and 14 post-inhalation exposure (n=3/timepoint). Lung tissue specimens were taken from the lambs during necropsy and subjected to bacterial culture techniques to detect the presence of *S. pneumoniae*. The collected tissues underwent processing under sterile conditions, the lung surface was sterilized with a flame, incised with a sterile blade, and wiped with a sterile swab. Next, they were transferred to Brain Heart Infusion Broth (BHI, Liofilchem, Italy), where they were incubated for 24 h and monitored for the presence of bacterial growth. After incubation, bacterial growth was cultured on plates containing 5% sheep blood agar. The plates were cultured at a temperature of 37°C with a concentration of 5% carbon dioxide for 24 h to facilitate the proliferation of bacteria. Post-incubation, the presence of *S. pneumoniae* colonies was assessed qualitatively, recording the results as either present (+) or not present (-). The microscopic morphology of *S. pneumoniae* for diagnostic under light microscope was also performed.

### Histopathological Examination

For histopathological analysis, three lambs were ethically euthanized according to Islamic slaughtering manner, on day 6 and the remaining three on day 14. Tissue specimens from the air passageway (trachea) and lungs were obtained and preserved in a solution of 10% buffered formalin for 48 h. The samples subsequently completed a series of dehydration procedures in an automated tissue processor (Histo-Line ATP700, Italy). After dehydration, tissues were fixed in paraffin (HESTION TEC2800-C, China) and sectioned at 4-5  $\mu$ m using a semi-automatic microtome (Histo-Line MRS3500, Italy). Segments were stained with Hematoxylin and Eosin (H&E, Dakocytomation, Denmark) and examined under a microscope (Olympus, Japan) at various magnifications to evaluate the pathological changes (23,24).

### Statistical Analysis

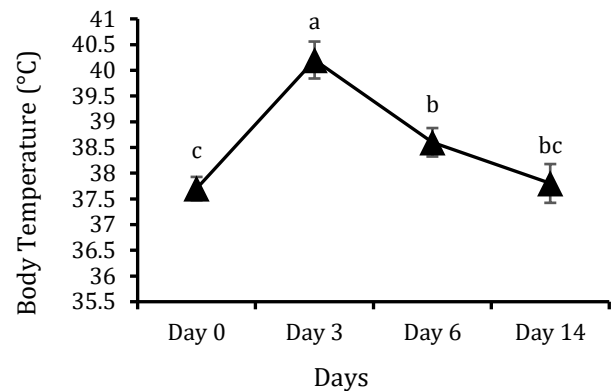
Data were analyzed using the Statistical Analysis System (SAS) version 2018. Data were subjected to one-way analysis of variance (ANOVA) at each time point and followed by the Least Significant Difference (LSD) post hoc test to detect the significant means at  $P \leq 0.05$ .

## RESULTS

### Clinical Signs

Following the onset of *S. pneumoniae* infection, clinical signs such as coughing, nasal discharge, and mild elevation

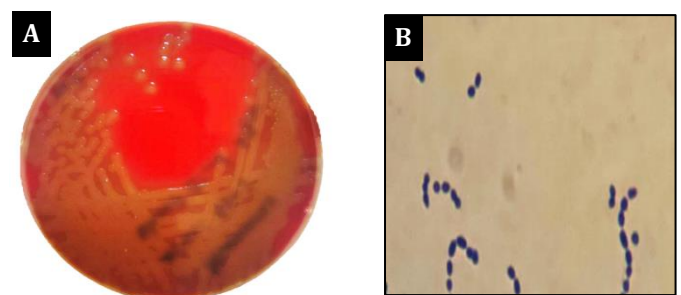
in the body temperature were observed at 3rd day. A gradual decrease in the body temperature at day 6 and 14 compared to day 0 (Figure 1).



**Figure 1.** Body temperature (°C) pre and post infection with *Streptococcus pneumoniae* in male lambs. Each point denotes the average mean  $\pm$  SE (error bars) where, n =6, n= 3 at day 14. Statistically significant differences were denoted by differing

### Examining *S. pneumoniae* Post-infection

Post-infection, all lamb lung tissue samples at day 6 and 14 were tested positive for the presence of bacterial colonies consistent with *S. pneumoniae*. The colony morphology observed on sheep blood agar was characteristic of *S. pneumoniae*, displaying round, grayish-white, and translucent colonies with alpha-hemolysis (characterized by a greenish discoloration around the colonies due to partial hemoglobin breakdown from red blood cells in the agar) (Figure 2A). Microscopic examination revealed Gram-positive cocci in pairs and chains (Figure 2B).



**Figure 2. (A)** Growth culture of *S. pneumoniae* with round or circular in shape slightly raised or convex colonies on agar surfaces and grayish white in color with  $\alpha$ -hemolysis. **(B)** Microscopic shapes of pneumococci in duplicate and short chain coccid (100 $\times$  oil emersion)

On day 6 post-exposure, *S. pneumoniae* was successfully isolated from all three lambs. On day 14 post-exposure, all three lambs also showed the presence of *S. pneumoniae* in their lung tissues. This procedure was integral to confirming the establishment and persistence of the *S. pneumoniae* infection in the lamb model. The presence of

the bacteria in lung tissues at both time points provided evidence of successful infection and gave insights into the bacterial colonization dynamics post-inhalation exposure.

### Total and Differential WBC count

The WBC counts in lambs exposed to *S. pneumoniae* through the inhalation route were measured at day 0 pre-infection and post-infection as summarized in (Table 1). A significant increase in WBC counts at day three post-infection ( $22.41 \pm 0.628 \times 10^9/L$ ) then at day 6 and 14 ( $21.24 \pm 0.405$ ,  $18.75 \pm 0.531 \times 10^9/L$ , respectively) compared to day 0 ( $8.732 \pm 0.274 \times 10^9/L$ ). These results indicated an early and vigorous immune response.

The results of differential WBCs, neutrophils, lymphocytes, and monocytes in blood samples of lambs infected with *S. pneumoniae* through the inhalation route are shown in Table 1. The results showed that there was a significant increase in neutrophils count on day three post-exposure ( $12.4 \pm 0.980 \times 10^9/L$ ) compared to day 0 ( $2.14 \pm 0.275 \times 10^9/L$ ). This finding indicates an early neutrophilic response. The neutrophil counts elevation gradually decreased by days 6 ( $10.8 \pm 0.875 \times 10^9/L$ ) and day 14 ( $9.67 \pm 1.406 \times 10^9/L$ ) but remained significantly higher compared to the day 0. Inhalation exposure to *S. pneumoniae* significantly increased ( $P < 0.002$ ) the lymphocyte count on days 6 ( $9.67 \pm 0.84 \times 10^9/L$ ) and 14 ( $15.3 \pm 2.31 \times 10^9/L$ ) post-exposure when compared to the day 0 ( $6.43 \pm 0.95 \times 10^9/L$ ). Lambs exposed to *S. pneumoniae* through inhalation exhibited a significant increase in monocytes on the third day post-exposure compared to the day 0, indicating an early and robust monocyte response. Monocyte counts at the 6-day and 14-day time points gradually decreased but were significantly higher compared to the day 0.

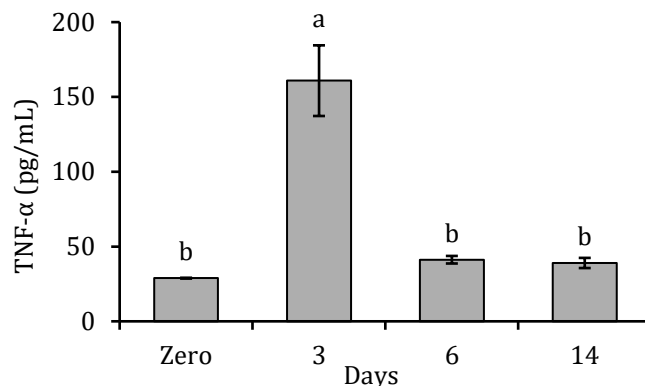
**Table 1.** Total white blood cell and differential (neutrophils, lymphocytes, and monocytes) count of male lambs at pre- and post-inhalation exposure to *S. pneumoniae*

Days	WBC ( $\times 10^9/L$ )	Neutrophils ( $\times 10^9/L$ )	Lymphocyte ( $\times 10^9/L$ )	Monocytes ( $\times 10^9/L$ )
Zero	$8.732 \pm 0.274$ c	$2.14 \pm 0.275$ b	$6.43 \pm 0.95$ c	$0.02 \pm 0.005$ d
3	$22.41 \pm 0.628$ a	$12.4 \pm 0.980$ a	$7.97 \pm 1.16$ bc	$2.62 \pm 0.157$ a
6	$21.24 \pm 0.405$ a	$10.8 \pm 0.875$ a	$9.67 \pm 0.84$ b	$1.54 \pm 0.079$ b
14*	$18.75 \pm 0.531$ b	$9.67 \pm 1.406$ a	$15.3 \pm 2.31$ a	$0.63 \pm 0.155$ c
P-value	<0.001	<0.001	0.002	<0.001

Values mean  $\pm$  SEM, n = 6. \*n = 3 at day 14. Means with different small letters within a column differ significantly ( $P \leq 0.05$ )

### Serum TNF- $\alpha$

On the third day post-infection, there was a significant increase ( $P < 0.001$ ) in TNF- $\alpha$  levels ( $160.6 \pm 18.14$  pg/mL) compared to the levels on day 0 ( $30.74 \pm 0.13$  pg/mL) (Figure 3). By day 6 post-exposure, the serum TNF- $\alpha$  levels had significantly decreased to  $41.2 \pm 2.53$  pg/mL. By the 14th day, the TNF- $\alpha$  levels had further decreased to  $38.9 \pm 3.40$  pg/mL, still higher than the levels on day 0 ( $30.74 \pm 0.13$  pg/mL).



**Figure 3.** Serum levels of TNF- $\alpha$  (pg/mL) pre and post infection with *Streptococcus pneumoniae* in male lambs. Each bar denotes the average mean  $\pm$  SEM (error bars), n = 6, n for day 14 = 3. Statistically significant differences are denoted by differing superscript letters ( $P \leq 0.05$ )

### Gross Pathological Findings

On day 6 post-inhalation exposure to *S. pneumoniae* the gross examination of the lung showed pulmonary edema and emphysema, the lungs exhibiting a friable consistency and flabby appearance along their edges (Figure 4A, B).

By day 14 post-inhalation exposure, there was pulmonary congestion, particularly evident in the apical lobe. Additionally, areas of emphysema, where the lung tissue exhibits a pale and bloated appearance (Figure 4D).

### Histological Findings

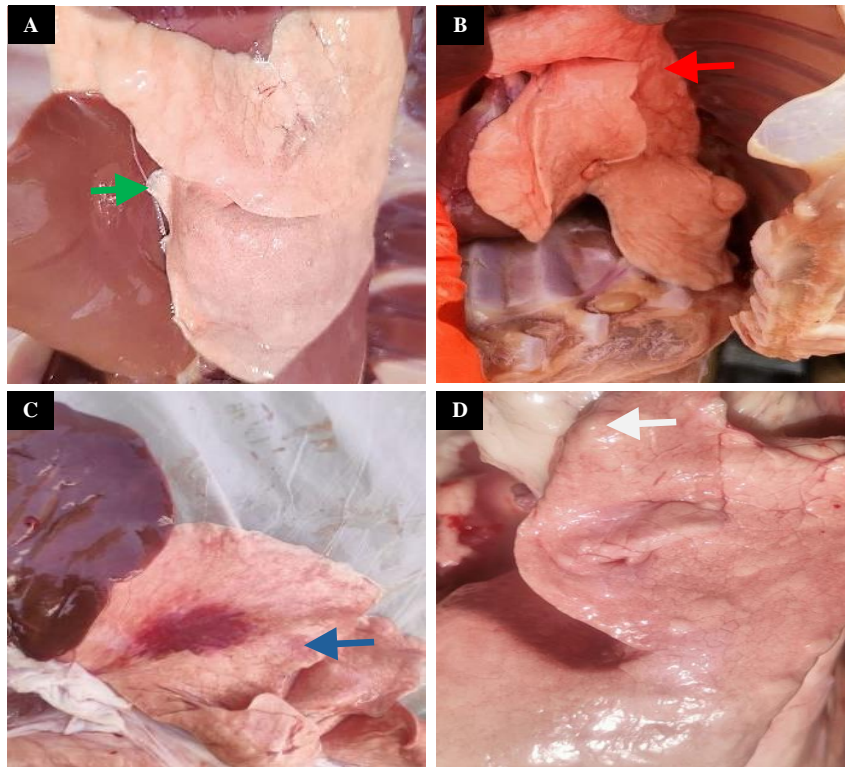
The histopathological results at day 6 post-infection, revealed massive destruction of pseudostratified columnar epithelium, complete loss of cilia, and infiltration of inflammatory cells extended beyond to submucosa to the serosa and desquamation of mucosal epithelium of trachea (Figure 5A, 5C). The bronchus showed peri-inflammatory cells (Figure 5B).

At day 14, (Figure 5D) showed necrotic atrophy of mucosa marked erosive tracheitis was evident, characterized by the total absence of cilia and erosion of tissue and necrosis of the pseudostratified columnar epithelium.

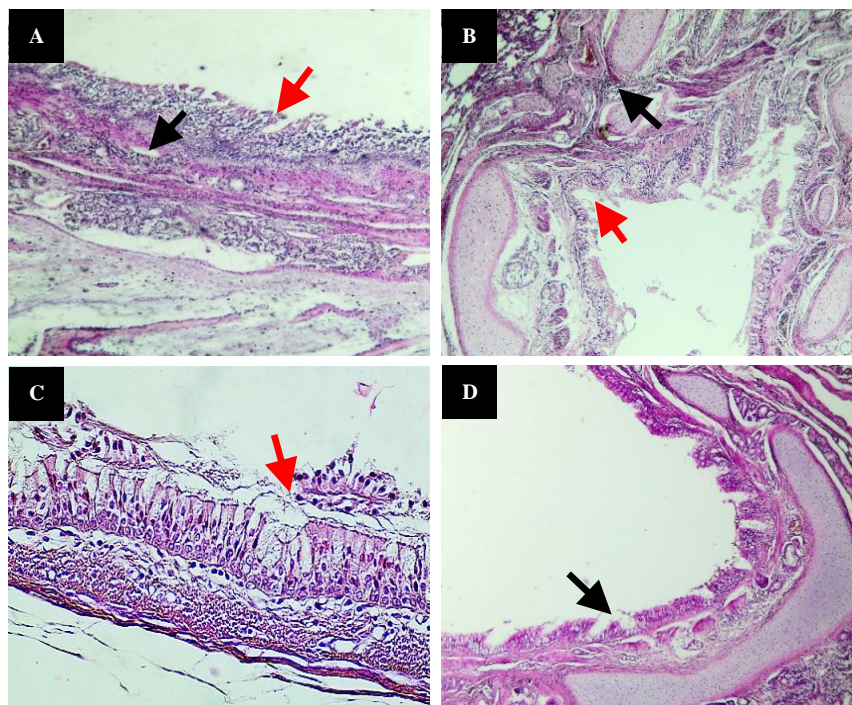
The primary histological lesions observed in the lungs of infected lambs 6- and 14 days post-infection were focal interstitial bronchopneumonia, bronchiolitis, focal infiltration of inflammatory cells around bronchioles (Figure 6A), and thickening of interalveolar septa (Figure 6B), which caused over-inflation (emphysema) of alveoli, in (Figure 6C).

Focal aggregations of mononuclear cells, predominantly lymphocytes and macrophages, with a few polymorphonuclear neutrophils present perivascular and around bronchioles (Figure 6D).

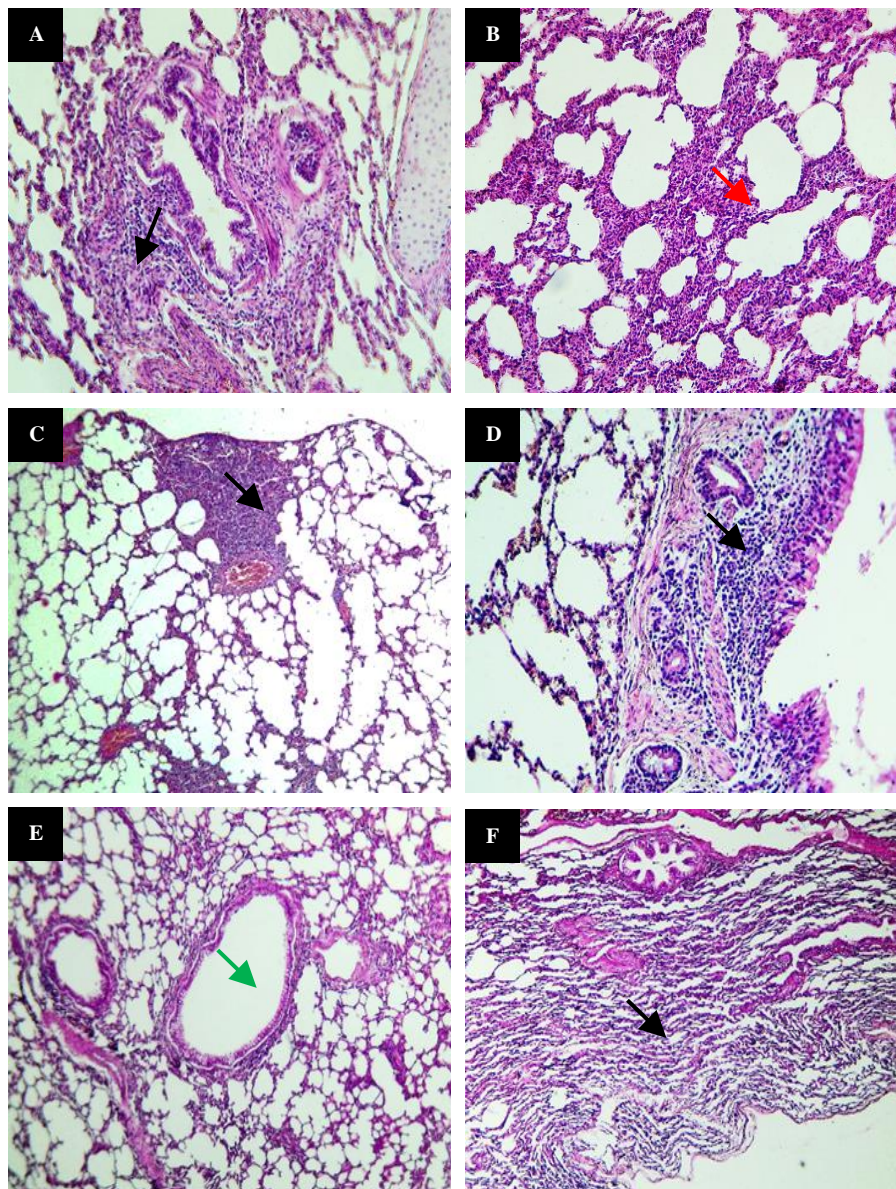
The sequel of interstitial bronchopneumonia was distorted of alveolar function from emphysema (Figure-6E) and atelectasis (Figure 6F).



**Figure 4.** Gross appearance of lung from male lambs experimentally infected with *S. pneumoniae* by inhalation. On day 6: **(A)** there were emphysema with friable consistency of lungs as flabby appearance of their edges (green arrow), **(B)** there were pulmonary edema (red arrow). On day 14: **(C)** There were congestion of lung with edematous fluid (like mosaic appearance), haemorrhagic aspirated spot (blue arrow), **(D)** There were mild pulmonary congestion at the apical lobe and emphysema (white arrow)



**Figure 5.** The histopathological section in trachea **(A)** and bronchus **(B)** on day 6 post-infection showed severe erosion and necrosis of ciliated pseudostratified columnar epithelium (red arrow), inflammatory cells infiltration in the mucosa and submucosa in trachea and perinflammatory cells in the bronchus **(B)** (black arrow) (H&E stain, 100 $\times$ , 200 $\times$ ). The histopathological section in the trachea on day 14 showed **(C)** marked desquamation of mucosal epithelium (red arrow) **(D)** necrosis in mucosa and pseudostratified columnar epithelium (black arrow) (H&E stain, 400 $\times$ , 40 $\times$ )



**Figure 6.** Histopathological sections of lung tissues from male lambs experimentally infected with *S. pneumoniae* by inhalation. On day 6 post-inhalation exposure shows: **(A)** focal infiltration of inflammatory cells around bronchioles (black arrow), **(B)** thickening of interalveolar septa and emphysema (red arrow) (H&E stain, 100 $\times$ ). On day 14 post-inhalation exposure shows: **(C)** perivascular cuffing (black arrow), **(D)** peribronchiolitis; infiltration of inflammatory cells in mucosa and submucosa (red arrow), **(E)** mild bronchioectasis (green arrow), **(F)** atelectasis and collapsed of alveoli (black arrow). (H&E stain, 40 $\times$ , 200 $\times$ , 200 $\times$ ).

## DISCUSSION

One of the significant health challenges affecting humans and animals is inflammation of the respiratory system, particularly in the upper and lower parts (25). Due to the risk factors associated with secondary or mixed infections involving viruses and bacteria in pneumonia cases, especially in young animals and children, this condition has garnered increasing attention in infectious disease research (22, 26).

The lungs are constantly exposed to inhaled stimuli, including microorganisms, chemicals, and dust (27). Establishing a robust immune response to opportunistic microorganisms is crucial for preventing infections.

However, when this response is dysregulated, it may lead to allergic airway inflammation and immunopathological disorders (28).

The present study investigated *S. pneumoniae* infection in lambs as an experimental model. Morbidity was observed in all lambs in the infected inhalation group on days 3, 6, and 14, compared with day 0. The clinical signs of respiratory infection included mild elevations in body temperature during these time points.

The total leukocyte count increased throughout the infection, with a notable rise in neutrophil numbers on days 3 and 6, followed by a decline by day 14. Lymphocyte counts increased significantly by day 6 and peaked on day 14, indicating an adaptive immune response against

pneumococci. Monocytes were also prominent on day 14 post-infection, reflecting a sustained inflammatory response.

One of the virulence factors of *S. pneumoniae* is its ability to adhere to the ciliated pseudostratified columnar epithelium, enabling invasion and colonization of epithelial cells (29). This mechanism leads to pathological changes in pulmonary tissues, as observed in the current study, including degeneration and necrosis of epithelial cells and infiltration of inflammatory cells in the trachea and lungs on days 6 and 14. Adhesion is facilitated by fimbriae and haemagglutinins, while the bacterial capsule protects against phagocytosis (30). Previous studies have demonstrated that *Streptococcus suis* infiltrates the tonsils, migrates to lymph nodes via lymphatic channels, and disseminates infected monocytes throughout the body (27).

*S. pneumoniae* triggers an intense inflammatory response in affected respiratory organs, characterized by elevated levels of TNF- $\alpha$ . In this study, TNF- $\alpha$  levels were detected in the blood samples of infected lambs on days 3, 6, and 14 post-infection. TNF- $\alpha$  plays a critical role in promoting inflammation and causing tissue damage in pneumococcal infections, such as meningitis and lower respiratory tract infections (31-33). Takashima et al. (34) highlighted the protective role of endogenous TNF- $\alpha$  in pneumococcal pneumonia in mouse models, demonstrating its involvement in the inflammatory response.

In the present study, inhalation exposure to *S. pneumoniae* in lambs resulted in a significant increase in TNF- $\alpha$  levels, indicative of an intense inflammatory response. By day 14, TNF- $\alpha$  levels decreased, suggesting the gradual resolution of inflammation and a shift towards healing and homeostasis.

Pathological changes observed in the trachea and lungs of experimental lambs infected with *S. pneumoniae* included tracheitis and pneumonia. Focal interstitial bronchopneumonia was the most prominent pulmonary lesion, characterized by infiltration and aggregation of MNCs, including lymphocytes, macrophages, and a few neutrophils, around the bronchi and bronchioles (perivascular cuffing). The phagocytic activity of neutrophils and macrophages, along with the production of TNF- $\alpha$  by T-lymphocytes and injured epithelial cells, was consistent with previous findings (34).

Co-infections involving *S. pneumoniae* and other pathogens, such as mycoplasmas, may exacerbate the invasive properties of pathogens in the lower respiratory tract, leading to heightened damage to epithelial cells and enhanced production of pro-inflammatory cytokines (35, 36). This could explain the immunopathological changes observed in the experimental lambs. Additionally, thickening of the interalveolar septa and bronchiectasis were recorded in the present study, consistent with findings from earlier research on co-infections involving *S. pneumoniae* (22, 37).

Bronchiectasis, observed on day 14 post-infection, likely resulted from increased invasion and colonization of pathogens in the injured bronchial epithelium, leading to compensatory emphysematous alveoli adjacent to

collapsed alveoli. These changes align with the progression of interstitial pneumonia and alveolar insufficiency caused by *S. pneumoniae*.

In conclusion, *S. pneumoniae* induced pneumonia in experimental lambs via inhalation, leading to significant immune-pathological changes in the lower respiratory organs. Understanding the pathogenesis of *S. pneumoniae* is crucial for developing effective prevention strategies in both human and veterinary medicine.

## ACKNOWLEDGEMENTS

N/A.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## التغيرات المناعية المرضية في عدوى العقديّة الرئوية التجريبية في الحملان

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### الخلاصة

العقدية الرئوية هي مسببات الأمراض البكتيرية الشائعة التي يمكن أن تسبب مجموعة من الأمراض في البشر والحيوانات. تعرض ما مجموعه 6 ذكور من الحملان غير المفطومة الذين تتراوح أعمارهم بين 1 إلى 2 شهر ووزن 5 إلى 7 كجم لسلسلة الالتهاب الرئوي S. الرئوي ATCC 6303 النمط المصلي 3 عند  $1.0 \times 10^6$  CFU/مل عن طريق الاستنشاق للحث على الالتهاب الرئوي. تم رصد العلامات السريرية للالتهاب الرئوي يومياً طوال فترة الدراسة. قبل تحريض الالتهاب الرئوي (اليوم 0) وفي الأيام 3 و 6 و 14 يوماً بعد التعرض، تم جمع عينات دم من جميع الحيوانات لتقييم إجمالي عدد خلايا الدم البيضاء (WBCs) وعامل نخر الورم ألفا (TNF -  $\alpha$ ). فضلاً عن ذلك، في اليومين 6 و 14 بعد التعرض، تم حصاد عينات القصبية الهوائية وأنسجة الرئة لتقييم التغيرات المرضية العيانية والمجهريّة. أظهرت النتائج أن هناك زيادة كبيرة ( $P < 0.001$ ) في إجمالي تعداد خلايا الدم البيضاء من اليوم الثالث بعد التعرض والحفاظ على مستويات مرتفعة في اليومين السادس والرابع عشر مقارنة باليوم صفر. أظهرت أعداد خلايا الدم البيضاء التفاضلية ارتفاعاً ميكروبياً وكبيراً في العدلات، مع ارتفاع مستمر في الخلايا الليمفاوية والخلايا الوحيدة. ارتفعت مستويات TNF -  $\alpha$  بشكل كبير في اليوم 3 وانخفضت تدريجياً بحلول اليوم 14 بعد التعرض ( $P < 0.001$ ). في اليوم بعد التعرض، أظهرت التغيرات المرضية الإجمالية في الرئة ونمة رئوية، واحتقان الدم، وتغيرات انتفاخية لوحظت في اليوم السادس واستمرت حتى يوم الرابع عشر بعد التعرض، مع احتقان خفيف إلى متوسط في الرئة. من الناحية النسيجية المرضية، لوحظ تآكل شديد ونخر في القصبية الهوائية وظهارة القصبية الهوائية، إلى جانب التهاب في الغشاء المخاطي المجاور وتحت المخاطية، مصحوباً بارتشاح التهابي بؤري وانتفاخ رئوي في الرئة بحلول اليوم السادس بعد التعرض. بحلول اليوم الرابع عشر، تطورت هذه التغيرات إلى فجوة ظهارية ملحوظة ونخر في القصبية الهوائية، حيث كشفت أقسام الرئة عن الأصفاد المحيطة بالأوعية الدموية، والتهاب القصبية المحيطة، وتوسع القصبية الخفيف، والانخماص، والانهيار السنخي. هذه الدراسة هي خطوة أولى نحو فهم أفضل لهذا النوع من العدوى في الحيوانات في سياق غير شائع، مما يساهم في تطوير استراتيجيات الوقاية والإدارة في العراق.

الكلمات المفاحية: العقديّة الرئوية، العدوى، الباثولوجيا المناعية، الباثولوجيا النسيجية