Original Article

Role of Tumor Necrosis Factor-α in Experimental *Streptococcus pneumoniae* Infection in Lambs

Abbas A Hamza[1*], **Zainab I Ibrahim**[1*]

1. Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Use your device to scan and read the article online

How to Cite This Article Hamza, A. A., & Ibrahim, Z. I. (2025). Role of Tumor Necrosis Factor-α in Experimental *Streptococcus pneumoniae* Infection in Lambs. *Iranian Journal of Veterinary Medicine, 19*(1), 11-22. http://dx.doi.org/10.32598/ijvm.19.1.1005527

http://dx.doi.org/10.32598/ijvm.19.1.1005527

ABSTRACT

Background: *Streptococcus pneumoniae* is a common bacterial pathogen causing various diseases in humans and animals.

Objectives: We aimed to evaluate the role of tumor necrosis factor (TNF)-α against infection with *Streptococcus pneumoniae* by intratracheal route in lambs as an experimental animal model.

Methods: Six male un-weaned lambs, aged 1 to 2 months and weighing 5 to 7 kg, were exposed to *S. pneumoniae* strain ATCC 6303 serotype 3 at 2×10^6 CFU/mL via intratracheal route to induce pneumonia. Pneumonic clinical signs were monitored daily, and blood samples were collected before exposure (day 0) and on days 3, 6, and 14 post-exposure for total and differential white blood cells (WBC) counts and TNF-α assessments. Additionally, on days 6 and 14 post-exposure, trachea and lung tissue samples were collected for macroscopic and microscopic pathological evaluation.

Results: The findings revealed a significant increase ($P<0.001$) in total WBC counts from day 3 post-exposure, maintaining elevated levels on days 6 and 14 compared to day 0. Differential WBC counts indicated an early, significant rise in neutrophils, with sustained elevation in lymphocytes and monocytes. TNF-α levels peaked on day 3 and gradually declined by day 14 post-exposure, reflecting an acute inflammatory response to the infection. Gross pathology at 6-14 days post-exposure showed pulmonary congestion and edema of affected lungs, emphysema, swelling, and congestion of the trachea. Histopathologically marked epithelial degeneration and necrosis with inflammatory processes in tracheitis and focal interstitial pneumoniae.

Conclusion: The present study concluded the pivotal role of TNF-α in the immune response against *S. pneumoniae* infection in lambs.

Keywords: Lambs, *Streptococcus pneumoniae*, TNF-α

Article info:
Received: 18 Jun 2024
Accepted: 21 Sep 2024
Publish: 01 Jan 2025

*** Corresponding Author:**
Zainab I Ibrahim, Professor.
Address: *Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.*
Phone: *+964 (770) 3912918*
E-mail: *zainabaalrubaei@covm.uobaghdad.edu.iq*

Introduction

Sheep and goats play an important socio-economic role within conventional animal husbandry systems, primarily providing high-quality animal protein and income (Al-Joboury et al., 1889; Ukwueze & Kalu, 2015; Rabana et al., 2022).

The small ruminant respiratory complex is one of the significant causes of morbidity and mortality in sheep flocks. The contributing factors comprise exposure to adverse weather conditions, animal movement, overcrowding, and stress, which increase the susceptibility of animals to viral and bacterial infections (Scott, 2011; Baghezza et al., 2024).

Respiratory diseases, particularly lamb pneumonia, stem from a multifaceted interplay of host factors (immunological and physiological), etiological agents (viruses, bacteria, mycoplasma), and environmental factors (Amit et al., 2012; Brogden et al., 1998; Kumar et al., 2011). Among various causative agents (Ali et al., 2005), *Streptococcus pneumoniae*, a commensal bacterium in the nasopharynx of animals, stands implicated in a majority of lamb pneumonia cases, precipitating reduced growth, increased mortality, and substantial economic burdens in terms of treatment costs and abattoir condemnations (Goodwin et al., 2004; Jones et al., 1982).

S. pneumoniae, a prominent gram-positive bacterium, is renowned for its involvement in respiratory tract infections, including pneumonia, in both human and animal populations (Proctor & Manning, 1990; Zivich et al., 2018). In veterinary medicine, *S. pneumoniae* poses a significant concern, particularly among livestock like lambs, where it causes notable morbidity and mortality (Kumar et al., 2013). While similarities exist between *S. pneumoniae* infections in lambs and humans (Alnajjar et al., 2021), the intratracheal route serves as a means of pathogen challenge, directly administering the pathogen into the animal's respiratory tract (Borsa et al., 2019). Key aspects of *S. pneumoniae* pathogenesis warrant investigation, including bacterial colonization of the respiratory tract, adhesion to respiratory epithelial cells, evasion of host immune defenses, and induction of inflammatory responses leading to tissue damage (Sharma et al., 2020).

Upon bacterial invasion, the host mounts an immune response characterized by activating white blood cells (WBCs), notably neutrophils and lymphocytes (Korkmaz & Traber, 2023). Neutrophils, or polymorphonuclear leukocytes, play crucial roles in early infection stages through pathogen phagocytosis and cytokine-mediated immune responses such as tumor necrosis factor (TNF)-α release (Lee et al., 2022). Lymphocytes, encompassing B and T cells, are essential for specific immune responses against *S. pneumoniae* (He et al., 2021). Monocytes differentiate into macrophages, which engage in pathogen engulfment and digestion (Anandachar et al., 2023). TNF-α, a proinflammatory cytokine, facilitates immune cell recruitment and instigates inflammatory cascades (Silva et al., 2019).

Utilizing the lamb model of intratracheal route-induced pneumonia, this research endeavors to advance the understanding of *S. pneumoniae* infections in veterinary medicine and contribute to developing innovative strategies for preventing and treating *S. pneumoniae* in livestock populations.

Materials and Methods

Animals and housing

Six male lambs of the local Iraqi breed, aged 1-2 months and weighing between 5 and 7 kg, were procured from a local source in Al-Najaf Province, Iraq. The lambs were transported to the animal farm facility at the College of Veterinary Medicine, University of Baghdad, where they underwent a one-week acclimatization period to adapt to their new environment and diet.

Bacterial inoculum preparation

The *S. pneumoniae* strain ATCC 6303, serotype 3, was obtained from a local supplier. The bacteria were cultured on 5% sheep blood agar plates and incubated at 37 ˚C with 5% CO_2 for 18 hours (Ashrafi et al., 2022). Subsequently, the bacterial suspension was prepared in sterile phosphate-buffered saline and incubated in brain heart infusion broth for 6 hours. The bacterial concentration was adjusted to 2×10^6 colony-forming units (CFU)/mL for experimental use (Alwash et al., 2017).

Experimental design

A single exposure to the prepared *S. pneumoniae* suspension was administered via injections precisely directed at the midsection of the trachea.

Bacterial suspension (3 mL) containing 2×10^6 CFU/mL was injected between two tracheal rings in the middle portion of the trachea. The procedure was conducted under controlled conditions to ensure consistent animal exposure.

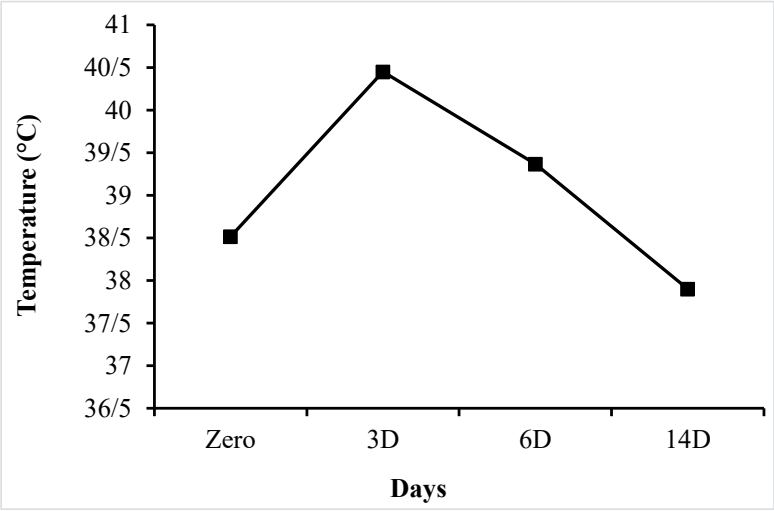

Figure 1. Body temperature during experimental infection

Clinical evaluation

Following inoculation, lambs were monitored daily for clinical signs of pneumonia, including coughing, dyspnea, tachypnea, fever, and sputum production. Observations were recorded to track the progression and severity of the infection (Al-Khafagi et al., 2016).

Blood sample collection

Blood samples were collected from the jugular vein at predetermined time points for hematological and biochemical analyses. Blood samples were evaluated in EDTA-anticoagulant tubes for total and differential WBC count using an automated hematology analyzer (Getein BHA-5000, China) (Karaşahin et al., 2023). For TNF-α analysis, blood was collected in gel tubes, centrifuged at 3500 rpm for 5 minutes, and the serum was stored at -20 ˚C. Serum TNF-α levels were measured using a Sheep TNF-α ELISA Kit (Beijing Solarbio Science & Technology CO., China) following the manufacturer's protocol (Shihab et al., 2022).

Bacterial isolation from lung tissues

To evaluate the persistence of *S. pneumoniae* post-intratracheal exposure, lung tissue samples were collected from the lambs at days 6 and 14 post-exposure. The tissues were processed under sterile conditions and cultured on blood agar plates. The presence of *S. pneumoniae* colonies was assessed qualitatively (AL Kutbi et al., 2001; Mohammed et al., 2020).

Histopathological examination

Tissue samples from the trachea and lungs were collected from euthanized lambs and subjected to histopathological analysis (Hashim, 2021). The samples were fixed, processed, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined under a light microscope (Luna, 1968; Spitalnik & Witkin, 2017).

Statistical analysis

Data were analyzed using the statistical analysis system (SAS) software, version 2018. The least significant difference (LSD) test was employed for pairwise comparisons of means in the analysis of variance (ANOVA) (Omar & Ibrahim, 2023).

Results

Clinical signs

Following the onset of *S. pneumoniae* infection, clinical manifestations became evident, marked by symptoms such as coughing and nasal discharge accompanied by a mild elevation in body temperature (Figure 1).

Examination of *S. pneumoniae* post-infection

All lung tissue samples from lambs, collected 6 and 14 days after exposure, yielded positive results for bacterial colonies consistent with *S. pneumoniae*. Characteristic colony morphology observed on sheep blood agar included round, grayish-white, translucent colonies with alpha-hemolysis (Figure 2A). Microscopic examination

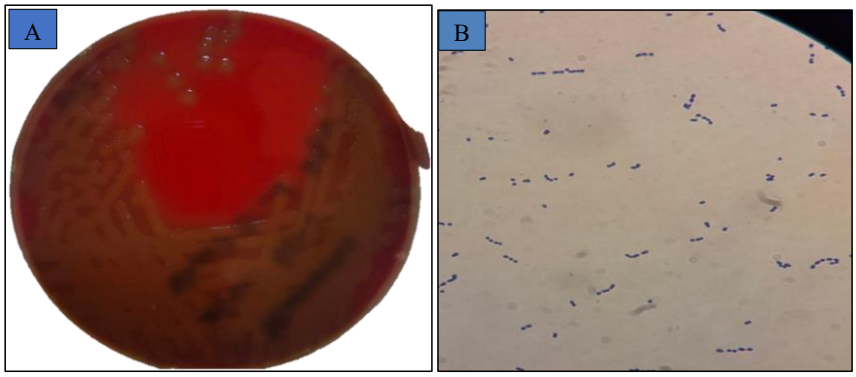

Figure 2. Microbial culture

A) Growth culture of *S. pneumoniae* with round or circular in shape slightly raised or convex colonies on agar surfaces and grayish-white with α-hemolysis, B) Microscopic shapes of Pneumococci in duplicate and short chain coccid (X100)

further -confirmed the presence of gram-positive cocci arranged in pairs and chains (Figure 2B).

Subsequent analysis utilizing the VITEK 2 system provided a 98% probability of the isolates being *S. pneumoniae*, validating the initial observations and supporting the identification of the pathogen responsible for the infection in the lambs.

At day 6 post-exposure, *S. pneumoniae* was isolated from all three lambs (lamb 1: +, lamb 2: +, lamb 3: +). Similarly, at day 14 post-exposure, all three lambs exhibited the presence of *S. pneumoniae* in their lung tissues (lamb 4: +, lamb 5: +, lamb 6: +). This process was crucial in confirming the establishment and persistence of the *S. pneumoniae* infection in the lamb model, providing valuable insights into bacterial colonization dynamics following inhalation exposure.

The consistent presence of the bacteria in lung tissues at both time points served as compelling evidence of successful infection, shedding light on the progression and duration of the infection post-exposure.

Hematological parameters during the induction of pneumonia in lambs

The white blood cell (WBC) counts in lambs exposed to *S. pneumoniae* through the intratracheal route were measured pre-infection and post-infection. A significant increase in WBC counts was observed on day 3 post-infection ($22.6\pm0.788\times10^9$/L), followed by slightly lower counts on day 6 ($21.2\pm0.738\times10^9$/L) and day 14 ($20.75\pm0.046\times10^9$/L), compared to day 0 ($8.97\pm0.316\times10^9$/L), indicating an early and vigorous immune response. Differential WBC counts were also assessed, including neutrophils, lymphocytes, and monocytes. There was a significant increase in neutrophils on day 3 post-exposure ($8.14\pm0.758\times10^9$/L) compared to day 0 ($2.44\pm0.393\times10^9$/L), indicating an early neutrophilic response. Neutrophil counts gradually decreased by days 6 ($6.83\pm0.701\times10^9$/L) and 14 ($4.67\pm2.009\times10^9$/L) but remained significantly higher compared to day 0.

Intratracheal exposure to *S. pneumoniae* significantly increased the lymphocyte count on days 6 ($10.5\pm1.706\times10^9$/L) and 14 ($15.9\pm0.717\times10^9$/L) post-exposure compared to day 0 ($6.43\pm0.951\times10^9$/L).

Lambs exposed to *S. pneumoniae* through the intratracheal route exhibited a significant increase in monocytes on day 3 post-exposure compared to day 0, indicating an early and robust monocyte response. Monocyte counts on days 6 and 14 post-exposure gradually decreased but were significantly higher than day 0 (Table 1).

The total WBC count and the breakdown of different types of white blood cells in blood samples from male lambs were examined following experimental intratracheal exposure to *S. pneumoniae*.

TNF-α level by intratracheal exposure

This study aimed to find changes in TNF-α levels after pneumococcal administration by intratracheal injection. The final results are shown below. On day 3 post-exposure, TNF-α levels increased significantly ($P<0.001$) compared to day 0, indicating an acute inflammatory response. TNF-α levels increased at intervals of 6 to 14 days but decreased steadily with subsequent testing. Although still greater than day 0, the decrease indicates continued resolution of inflammation and a return to homeostasis. Intratracheal exposure to *S. pneumoniae*

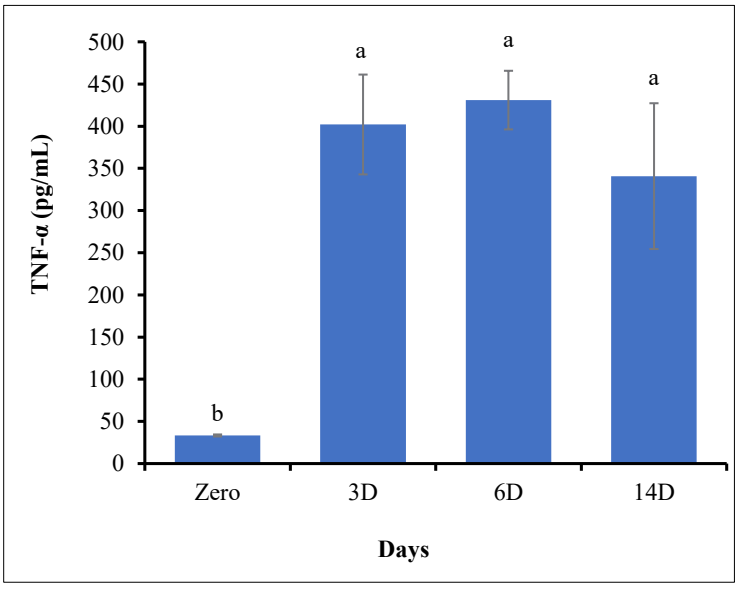

Figure 3. Levels of tumor necrosis factor-α (pg/mL) before and after infection

Notes: Values are presented as Mean±SEM, n=6. Means with different superscripts within a column differ significantly (P≤0.05).

in lambs significantly increases TNF-α levels, indicating an intense inflammatory response. TNF-α levels decrease over time, indicating that inflammation gradually resolves Figure 3.

Pathology gross and microscopic

Gross changes

The experimental intratracheal infection with *S. pneumoniae* in lambs revealed affected lungs at day 6 post-infection, represented by moderate to mild pulmonary congestion, hemorrhagic spots, and linear congestion of the mucosal internal layer along the trachea (Figures 4A, and 4B). At day 14, post-infection shows mild enlargement, congestion of trachea with dorsal hemorrhagic spots, apical lobular congestion and edematous fluid, ecchymotic hemorrhage in ventral lobes and hemorrhage, emphysema in lower lobes (Figures 4C and 4D).

Table 1. WBCs, neutrophils, lymphocytes, and monocytes post-exposure compared to those at day 0

Day	Mean±SEM			
	WBC (×10⁹/L)	Neutrophile (×10⁹/L)	Lymphocyte (×10⁹/L)	Monocyte (×10⁹/L)
0	8.97±0.316^{b}	2.44±0.393^{c}	6.43±0.951^{c}	0.01±0.003^{c}
3	22.6±0.788^{a}	8.14±0.758^{a}	8.51±0.909^{bc}	0.63±0.074^{a}
6	21.2±0.738^{a}	6.83±0.701^{ab}	10.5±1.706^{b}	0.27±0.044^{b}
14*	20.75±0.046^{a}	4.67±2.009^{bc}	15.9±0.717^{a}	0.03±0.006^{c}
P	<0.001	0.0004	0.0018	<0.0001
LSD	2.575	3.259	3.525	0.1401

LSD: Least significant difference.

*n=6 for day 14=3.

Notes: Means with different superscripts within a column differ significantly (P≤0.05).

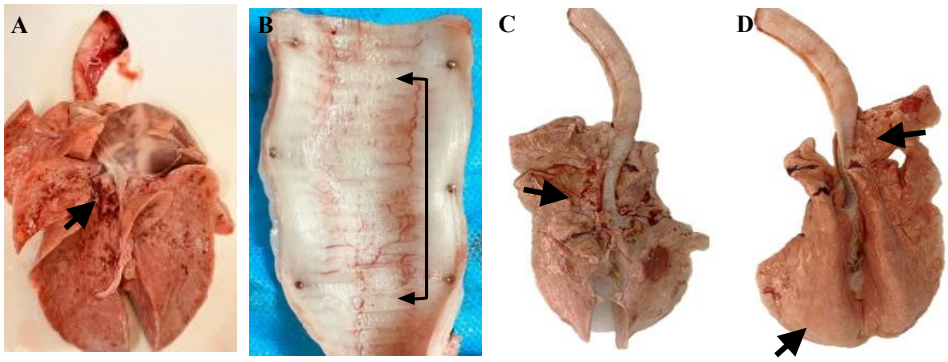

Figure 4. Gross appearance of A) Affected lung at day 6 post infection with *S. pneumoniae* by intratracheal route showing lung congestion and ecchymotic hemorrhage (arrow), B) Linear congestion of internal mucosa of trachea (arrow), C) Congestion of trachea in the ventral site of lungs showing hemorrhage and edema (arrow), D) At day 14 post infection, swelling and congestion of trachea, apical lobular congestion (arrow) and emphysema (arrow)

Microscopic examination

The microscopic examination of lambs' post-infection intratracheally with *S. pneumoniae* revealed marked disruption of tracheal and bronchial mucosal epithelia represented by vacuolar degeneration of mucosal epithelia (Figure 5a), intraepithelial and subepithelial infiltration of mononuclear cells (MNCs) mainly lymphocytes and polymorph neutrophils (PMNs), also in submucosa to serosa (Figure 5b) at day 6. The microscopic examination At day 14 post-infection (Figure 5c) shows massive infiltration of inflammatory exudate replaced the normal mucosa and submucosa of pulmonary passages (trachea and bronchi), focal proliferation of lymphoid cells in submucosa was the prominent lesion (Figure 5d). On day 6, the lung tissues (Figures 5e, and 5f) revealed mild to moderate thickening of interalveolar septa due to hyperemia of alveolar capillaries and infiltration of inflammatory cells, mainly lymphocytes, macrophages, and few neutrophils in interalveolar septa with bronchitis and bronchiolitis as necrotic damage of mucosal epithelia and infiltration of inflammatory cells. On day 14, post-infection, focal MNCs around the bronchioles were found (Figure 5g). Figure 5h shows lobular interstitial thickening of the lungs.

Discussion

The pathogenesis of pneumococcal infection is complex and involves the host, infectious agent, and environmental factors (Narayan et al., 2023). Serotype 3 is the most common serotype isolated from severe infections in animals and humans (Wong et al., 2023). Different isolates of *S. pneumoniae* can cause various diseases, from fatal systemic diseases to asymptomatic ones. This difference in disease severity is not related to genome size, serotype, sequence type, or virulence-associated phenotypes in vitro (Norman, 2015; Chaguza et al., 2022; Jacques et al., 2023; Wong et al., 2023). The importance of understanding the pathogenesis and immune response induced by pneumococci is underscored by the appearance of clinical symptoms after infection (Morimura et al., 2021), as observed in this study by signs of respiratory disease such as rhinorrhea, mild cough, and mild temperature depending on the time of infection. After 6 and 14 days, Arends et al. (2022) have reported typical clinical signs, such as cough, nasal discharge, and a slight increase in body temperature, which are often associated with pneumococcal respiratory infections and indicate the body's effort to confront invading pathogens (Arends et al., 2022). These clinical observations were consistent with the study of (Small et al., 1986), who observed that a moderate increase in body temperature corresponded with activation of the host immune response against pneumococci.

Fever, which is often mediated by the release of pyrogenic cytokines, such as interleukin 1 beta and TNF-α, is vital to prevent bacterial growth and enhance immune cell function, which explains the significant rise in serum TNF-α levels and increased number of immune cells. On days 6 and 14 after infection with *S. pneumoniae*, neutrophils emerge to reduce the number of pathogens and eliminate their harmful effects in target tissues by stimulating immune responses through the action of proinflammatory cytokines of interleukins and tumor necrosis factors. They cause an increase in temperature in infected lambs due to infectious tissue injury and pyrogen production combined with the innate immune response, increased reconstitution of polymorphonuclear neutrophils in the pulmonary tissue of the trachea and lungs, and finally, increased levels of lymphocytes and macrophages (MNCs), which represent the adaptive immune response. This finding is consistent with Jimistan's

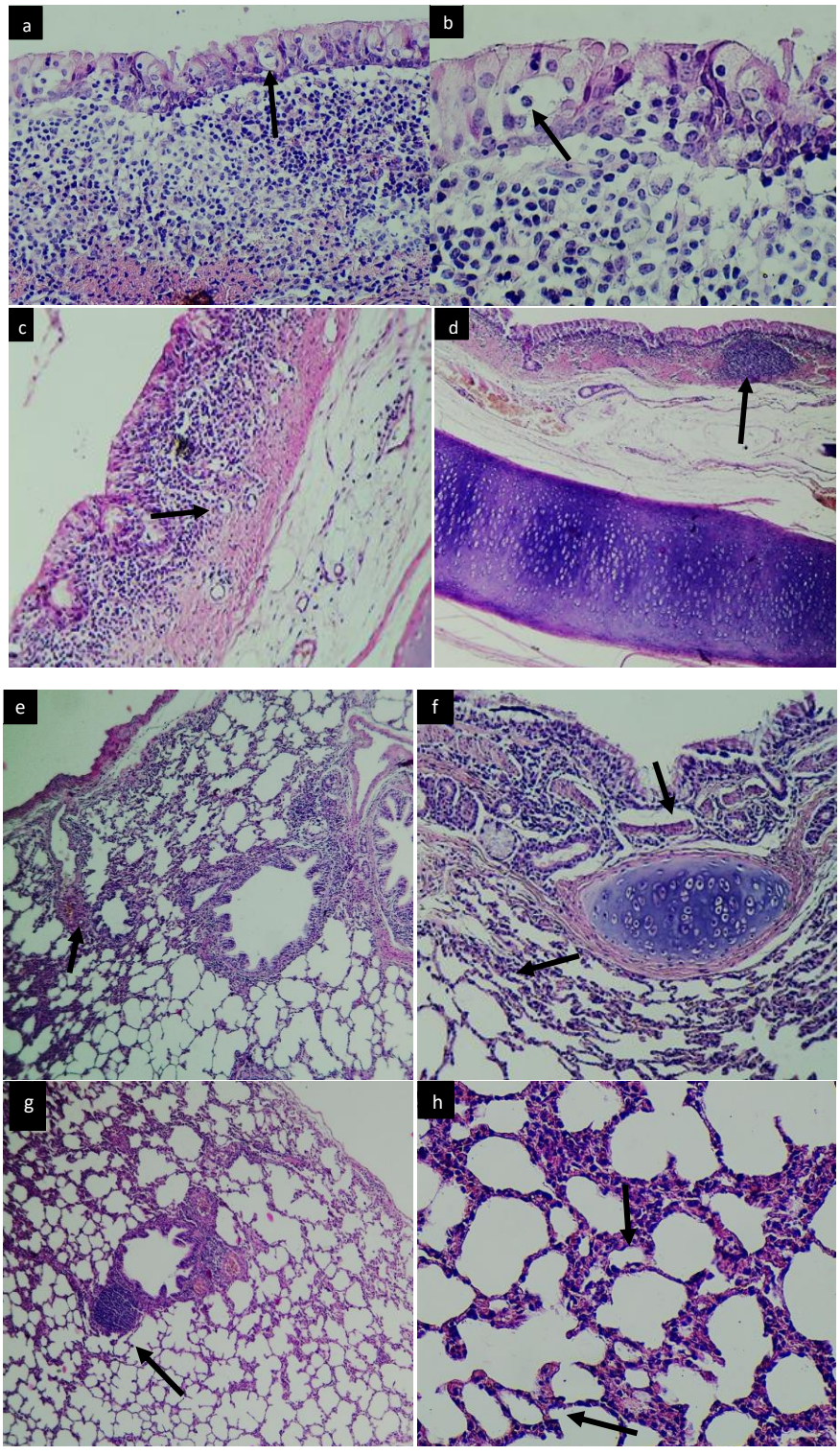

Figure 5. Histopathological section of trachea from lambs infected intratracheally With *S. pneumoniae* 6 and 14 days post infection

Notes: a) Shows complete loss of cilia and vacuolar degeneration of mucosal epithelial cells, b) (Arrow) intraepithelial and subepithelial infiltration of MNCs and few PMNs in mucosa, submucosa, c) Shows necrosis of stratified columnar epithelium and severe infiltration of inflammatory cells and damage of mucosal gland in submucosa (arrow), d) Presence of focal MNCs aggregation in submucosa (arrow), e) Thickening of intra-alveolar septa (arrow), f) Necrotic bronchitis and atelectasis (arrow), g) Peri bronchial aggregation of lymphoid cells (arrow). h) Interstitial thickening with emphysema (arrow) (H&E stain, X400, X40, X100).

results, who recorded a peak body temperature on the third day after infection. The initial stages of the immune response, characterized by the recruitment of neutrophils and macrophages to the site of infection, are followed by the initiation of adaptive immune responses (Periselneris et al., 2014).

The subsequent decrease in body temperature on days 6 and 14 marks the end of the acute phase of infection, indicating successful containment and elimination of the pathogen. This observation is consistent with the idea that pneumococci usually elicit local protective immune responses, as evidenced by mild clinical signs in the present investigation. These local responses may include activating mucosal immunity and producing specific antibodies targeting pneumococcal antigens, contributing to the resolution of infection without major systemic complications. This finding is consistent with Wong et al. (2023) results, who reported a significant increase in total WBC counts, neutrophils, and monocytes, agreeing with the present findings of higher total WBCs and differential neutrophil counts on day 3 in both groups and increased numbers of lymphocytes on days 6, 14 along with high percentages of monocytes reached on day 14 after infection. This rise in leukocyte count can be attributed to bacterial invasion, where colonization causes infection of epithelial tissue and endothelial cells, leading to the release of growth factors, cytokines, and other inflammatory mediators, thus stimulating the proliferation and maturation of white blood cells.

Respiratory illness-induced stress triggers the release of endogenous corticosteroids, affecting the regulation of circulating leukocyte concentrations in moderate to severe cases of pneumonia (Sayad et al., 2002; El-Naser and Khamis, 2009; Saleh & Allam, 2014; Donia et al., 2018). These virulent pathogens have polysaccharide capsules, M protein, and group A streptococci. Hyaluronidase is one of the enzymes of pathogenic bacteria *Streptococcus* spp., *Staphylococcus* spp., and *Clostridium* spp., which hydrolyzes hyaluronic acid from connective tissue and facilitates the invasion of bacteria into the depths of layers of affected tissue (Borriello, 1998; Paton et al., 1993; Wong et al., 2023).

S. pneumoniae infection triggers an inflammatory response in exclusively infected respiratory organs. *S. pneumoniae* is characterized by a severe inflammatory reaction, as occurred in the present study, due to direct stimulation by cell wall components of pneumococci and pneumolysin, which has been shown to stimulate TNF-α production. by human monocytes in vitro (Heumann et al., 1994; Houldsworth et al., 1994). Increased levels of TNF-α have been observed in serum and cerebrospinal fluid in patients with acute lower respiratory tract infection and meningitis, respectively, caused by *S. pneumoniae* (Glimåker et al., 1993; Nohynek et al., 1991). In addition, TNF-α is an important mediator of inflammation and tissue damage in pneumococcal meningitis (Xu et al., 2017). However, the role of TNF-α in the pathogenesis of pneumococcal pneumonia is unclear. Takashima et al. created a model of pneumonia in mice with pneumocystis pneumonia that closely mimics the situation in humans (Takashima et al., 1996). Using this model, endogenous TNF-α production and its role in pneumonia were studied by administration of anti-TNF-α antibody, demonstrating the protective role of the endogenous product (TNF-α) in pneumococcal pneumonia, which may explain the persistent respiratory infection during the trial period.

Post-mortem examination of affected lambs revealed gross signs of inflammation, such as redness, swelling, congestion of the affected lungs, and bleeding (petechiae and ecchymosis). Gross changes in the trachea showed linear congestion of the internal mucosa. The histopathological changes caused by intratracheal pneumococcal infection on days 6 and 14 after infection revealed focal interstitial bronchitis represented by focal infiltration of mainly MNCs. A few lymphocytes and macrophages were also seen around the bronchi, bronchioles, and perivascular cuffs, reflecting the host immune response against the invading microorganisms from the action of cytokines and chemokines to the site of pulmonary infection with bacterial toxins, the same results suggested by Lucas et al., (2020). The cytokines of interest in the present study, TNF-α, were elevated in their levels a few days after pneumonia as proinflammatory cytokines at the time of pathogen invasion and petechial and ecchymotic hemorrhages in the affected lungs.

Thickening of the barrier between the alveoli and the pleura was observed 14 days after the injury due to congestion of alveolar capillaries and the accumulation of edema in the interstitium, as well as infiltration of lymphocytes, and the same pathological changes occurred in an experiment by Alnajjar et al. (2021), who demonstrated pneumonia as a secondary bacterial infection after viral pneumonia or viral-bacterial coinfection (Madhi & Klugman, 2004; Smith et al., 2013). They used the lamb rather than the mouse model to understand that viral-bacterial coinfections are crucial for studying treatments. Sheep are also permissive to *S. pneumoniae* infection and have served as a model for sepsis because they show clinical signs similar to human infection (Legesse Garedew et al., 2010; Zaghawa et al., 2010). In conclusion, pneumonia causes pathological lesions of

epithelial cell degeneration and necrosis that represent acute lung injury in the trachea and bronchi that may facilitate coinfection with other pathogens and increase morbidity and even mortality.

Conclusion

S. pneumoniae, as a pathogenic agent, has a significant role in inducing inflammatory reactions in air passages when introduced directly into the trachea.

Ethical Considerations

Compliance with ethical guidelines

Ethical approval was granted by the Local Committee of Animal Care and Use at the College of Veterinary Medicine within the University of Baghdad, Baghdad, Iraq (Code: 2152/PG).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the mnuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors appreciate and thank everyone who helped with laboratory and theoretical devices.

References

AL Kutbi, S. H., & Alwan, M. J. (2001). Pathological and histochemical changes in the lungs of rabbits experimentally infected with P. seudomonas aeruginosa. *The Iraqi Journal of Veterinary Medicine, 25*(1), 24-39. [DOI:10.30539/ijvm.v25i1.1144]

Ali, J. S., & Badri Abboud, H. (2005). Bacterial causes of upper & lower respiratory tract infection in Sheep. *The Iraqi Journal of Veterinary Medicine, 29*(1), 1- 10. [DOI:10.30539/iraqijvm.v29i1.858]

Al-Joboury, K. H., & Al-Darraji, A. M. (1989). A study on caprine pneumonia in IRAQI local goats etiological findings. *The Iraqi Journal of Veterinary Medicine, 13*(1), 170-179. [DOI:10.30539/ijvm.v13i1.1668]

Al-Khafagi, A. M., & Zghair, Z. R. (2016). Histopathological and diagnostic study of Toxoplasmosis in human and sheep by using ELISA in Kut city. *The Iraqi Journal of Veterinary Medicine, 40*(2), 94-9. [DOI:10.30539/iraqijvm.v40i2.119]

Alnajjar, S., Sitthicharoenchai, P., Gallup, J., Ackermann, M., & Verhoeven, D. (2021). Streptococcus pneumoniae serotype 22F infection in respiratory syncytial virus infected neonatal lambs enhances morbidity. *Plos One, 16*(3), e0235026. [DOI:10.1371/journal.pone.0235026] [PMID]

Alwash, B., Saed, N., & ALqusay, S. (2017). The effect of alcoholic extract eriobotrya japonica leaves on some bacterial genes and some chemical and microbiological properties of beef. *Iraqi Journal Of Agricultural Science, 48*(5), 1231-1238. [DOI:10.36103/ijas.v48i5.332]

Amit, K., Verma, A., Gangwar, N., & Anu, R. (2012). Isolation, characterization and antibiogram of Mycoplasma bovis in sheep pneumonia. *Asian Journal of Animal and Veterinary Advances, 7*(2), 149-157. [DOI:10.3923/ajava.2012.149.157]

Anandachar, M. S., Roy, S., Sinha, S., Boadi, A., Katkar, G. D., & Ghosh, P. (2023). Diverse gut pathogens exploit the host engulfment pathway via a conserved mechanism. *Journal of Biological Chemistry, 299*(12). [Link]

Arends, D. W., Alkema, W., Hapsari Putri, I., van der Gaast-de Jongh, C. E., Eleveld, M., & Langereis, J. D., et al. (2022). Differential pneumococcal growth features in severe invasive disease manifestations. *Microbiology Spectrum, 10*(3), e0005022. [DOI:10.1128/spectrum.00050-22] [PMID]

Ashrafi, F., Azari, A. A., & Fozouni, L. (2022). Prevalence and antibiotic resistance pattern of mannheima haemolytica and pasteurella multocida isolated from cattle lung samples from an industrial abattoir: A study from Northeastern Iran. *Iranian Journal of Veterinary Medicine, 16*(4), 414-422. [Link]

Baghezza, S., Azizi, A., Derrar, F., Adnane Smadi, M., Djeghim, H., & Ghougal, K., et al. (2024). Epidemiological study of bovine parainfluenza 3 virus in sheep: Seroprevalence, risk factors, and distribution in two regions of algeria. *Iranian Journal of Veterinary Medicine, 18*(2), 159-168. [DOI:10.32598/IJVM.18.2.1005387]

Borriello, S. (1998). Pathogenesis of clostridium difficile infection. The Journal of Antimicrobial *Chemotherapy, 41*(suppl_C), 13-19. [DOI:10.1093/jac/41.suppl_3.13] [PMID]

Borsa, N., Pasquale, M. D., & Restrepo, M. I. (2019). Animal models of *Pneumococcal* pneumonia. *International Journal of Molecular Sciences, 20*(17), 4220. [DOI:10.3390/ijms20174220] [PMID]

Brogden, K. A., Lehmkuhl, H. D., & Cutlip, R. C. (1998). Pasteurella haemolytica complicated respiratory infections in sheep and goats. *Veterinary Research, 29*(3-4), 233-254. [Link]

Chaguza, C., Ebruke, C., Senghore, M., Lo, S. W., Tientcheu, P. E., & Gladstone, R. A., et al. (2022). Comparative genomics of disease and carriage serotype 1 pneumococci. *Genome Biology and Evolution, 14*(4), evac052. [DOI:10.1093/gbe/evac052] [PMID]

Donia, G. R., El Ebissy, I. A., & Wassif, I. M. (2018). Biochemical and immunological studies on the respiratory diseases in sheep in North Western coast. *European Journal of Biomedical, 5*(12), 34-41. [Link]

El-Naser, E., & Khamis, G. (2009). Some hematological and blood serum biochemical indices associated with respiratory affections by camels. *Assiut Veterinary Medical Journal, 55*(123), 1-10. [DOI:10.21608/avmj.2009.174979]

Glimåker, M., Kragsbjerg, P., Forsgren, M., & Olcén, P. (1993). Tumor necrosis factor-α (TNFα) in cerebrospinal fluid from patients with meningitis of different etiologies: High levels of TNFα indicate bacterial meningitis. *The Journal of Infectious Diseases, 167*(4), 882–889. [DOI:10.1093/infdis/167.4.882] [PMID]

Goodwin, K. A., Jackson, R., Brown, C., Davies, P. R., Morris, R. S., & Perkins, N. R. (2004). Pneumonic lesions in lambs in New Zealand: Patterns of prevalence and effects on production. *New Zealand Veterinary Journal, 52*(4), 175-179. [DOI:10.1080/00480169.2004.36425] [PMID]

Hashim, M. (2021). Histopathomicrobial examination of echinococcosis in lung and liver infected with klebsiela pneumonia in sheep of IRAQ. I*RAQI Journal of Agricultural Sciences, 52*(1), 129-135. [DOI:10.36103/ijas.v52i1.1244]

He, S. W. J., van de Garde, M. D. B., Pieren, D. K. J., Poelen, M. C. M., Voß, F., & Abdullah, M. R., et al. (2021). Diminished pneumococcal-specific CD4+ t-cell response is associated with increased regulatory t cells at older age. *Frontiers in Aging, 2,* 746295. [DOI:10.3389/fragi.2021.746295] [PMID]

Heumann, D., Barras, C., Severin, A., Glauser, M. P., & Tomasz, A. (1994). Gram-positive cell walls stimulate synthesis of tumor necrosis factor alpha and interleukin-6 by human monocytes. *Infection and Immunity, 62*(7), 2715-2721. [DOI:10.1128/iai.62.7.2715-2721.1994] [PMID]

Houldsworth, S., Andrew, P. W., & Mitchell, T. J. (1994). Pneumolysin stimulates production of tumor necrosis factor alpha and interleukin-1 beta by human mononuclear phagocytes. *Infection and Immunity, 62*(4), 1501-1503. [DOI:10.1128/iai.62.4.1501-1503.1994] [PMID]

Jacques, L. C., Green, A. E., Barton, T. E., Baltazar, M., Aleksandrowicz, J., & Xu, R., et al. (2023). Influence of Streptococcus pneumoniae within-strain population diversity on virulence and pathogenesis. *Microbiology Spectrum, 11*(1), e0310322. [DOI:10.1128/spectrum.03103-22] [PMID]

Jones, G. E., Field, A. C., Gilmour, J. S., Rae, A. G., Nettleton, P. F., & McLauchlan, M. (1982). Effects of experimental chronic pneumonia on bodyweight, feed intake and carcase composition of lambs. *The Veterinary Record, 110*(8), 168-173. [DOI:10.1136/vr.110.8.168] [PMID]

Karaşahïn, T., Dursun, S., Aksoy, N. H., İpek, H., & Şentürk, G. (2023). Hematological parameters in hair goats during and out of breeding season hair goats seasonal hematological parameters. *Iranian Journal of Veterinary Medicine, 17*(2), 113-118. [DOI:10.32598/IJVM.17.2.1005334]

Korkmaz, F. T., & Traber, K. E. (2023). Innate immune responses in pneumonia. *Pneumonia, 15*(1), 4. [DOI:10.1186/s41479-023-00106-8] [PMID]

Kumar, A., Verma, A. K., Sharma, A. K., & Rahal, A. (2013). Isolation and antibiotic sensitivity of Streptococcus pneumoniae infections with involvement of multiple organs in lambs. *Pakistan Journal of Biological Sciences: PJBS, 16*(24), 2021-2025. [DOI:10.3923/pjbs.2013.2021.2025] [PMID]

Kumar, A., Verma, A., & Rahal, A. (2011). Mycoplasma bovis, A multi disease producing pathogen: An overview. *Asian Journal of Animal and Veterinary Advances, 6*(6), 537-546. [DOI:10.3923/ajava.2011.537.546]

Lee, M., Lee, S. Y., & Bae, Y. S. (2022). Emerging roles of neutrophils in immune homeostasis. *BMB Reports, 55*(10), 473-480. [DOI:10.5483/BMBRep.2022.55.10.115] [PMID]

Legesse Garedew, G. A., Yilma, R., Zeleke, A., & Gelaye, E. (2010). Isolation of diverse bacterial species associated with maedi-visna infection of sheep in Ethiopia. *African Journal of Microbiology Research, 4*(1), 014-021. [Link]

Lucas, R., Hadizamani, Y., Gonzales, J., Gorshkov, B., Bodmer, T., & Berthiaume, Y., et al. (2020). Impact of bacterial toxins in the lungs. *Toxins, 12*(4), 223. [DOI:10.3390/toxins12040223] [PMID]

Luna, L. G. 1968. Manual of histologic staining methods of the armed forces institute of pathology. In: L.G Luna (Eds), *Manual of histologic staining methods of the armed forces institute of pathology*. New York: McGraw-Hill. [Link]

Madhi, S. A., Klugman, K. P., & Vaccine Trialist Group (2004). A role for Streptococcus pneumoniae in virus-associated pneumonia. *Nature Medicine, 10*(8), 811-813. [DOI:10.1038/nm1077] [PMID]

Mohammed, Y., Mustafa, J., & Abdullah, A. (2020). Isolation and molecular study of some bacterial urinary tract infections of sheep in Basrah province. *IRAQI Journal of Agricultural Sciences, 51*(3), 885-893. [DOI:10.36103/ijas.v51i3.1043]

Morimura, A., Hamaguchi, S., Akeda, Y., & Tomono, K. (2021). Mechanisms underlying pneumococcal transmission and factors influencing host-pneumococcus interaction: A review. *Frontiers in Cellular and Infection Microbiology, 11,* 639450. [DOI:10.3389/fcimb.2021.639450] [PMID]

Narayan, K. G., Sinha, D. K., & Singh, D. K. (2023). Agent, host, and environmental factors. In K. G. Narayan, D. K. Sinha& D. K. Singh (Eds.), *Veterinary public health & epidemiology* (pp. 55-61). Singapore: Springer. [DOI:10.1007/978-981-19-7800-5_6]

Nohynek, H., Teppo, A. M., Laine, E., Leinonen, M., & Eskola, J. (1991). Serum tumor necrosis factor-α concentrations in children hospitalized for acute lower respiratory tract infection. *The Journal of Infectious Diseases, 163*(5), 1029–1032. [DOI:10.1093/infdis/163.5.1029] [PMID]

Norman, E. M. (2015). Characterization of virulence in clinicalisolates of streptococcus pneumoniae [MSc thesis]. Solna: Karolinska Institutet. [Link]

Omar, F., & Ibrahim, A. (2023). The prevalence of integron class I and II among multi-drug resistance producing klebsiella pneumonia. *Iraqi Journal of Agricultural Sciences, 54*(3), 619-629. [DOI:10.36103/ijas.v54i3.1775]

Paton, J. C., Andrew, P. W., Boulnois, G. J., & Mitchell, T. J. (1993). Molecular analysis of the pathogenicity of Streptococcus pneumoniae: The role of pneumococcal proteins. *Annual Review of Microbiology, 47*, 89-115. [DOI:10.1146/annurev.mi.47.100193.000513] [PMID]

Periselneris, J., José, R. J., & Brown, J. S. (2014). Pulmonary immune response to Streptococcus pneumoniae. *Shortness of Breath, 3*(4), 147-158. [DOI:10.11138/sob/2014.3.4.147]

Proctor, M., & Manning, P. J. (1990). Production of immunoglobulin A protease by Streptococcus pneumoniae from animals. *Infection and Immunity, 58*(9), 2733-2737. [DOI:10.1128/iai.58.9.2733-2737.1990] [PMID]

Rabana, L. J., Bukola, Y. Z., Mustapha, M., & Adamu, L. (2022). Indigestible Foreign Materials Impaction of Small Ruminants in Gombe State, Nigeria. *Iranian Journal of Veterinary Medicine, 16*(1), 1-14. [Link]

Saleh, N. S., & Allam, T. S. (2014). Pneumonia in sheep: Bacteriological and clinicopathological studies. *American Journal of Research Communication, 2*(11), 73-88. [Link]

Sayad, A., Ali, A., Mottelib, A., & Abd EI-Rahman, A. (2002). Bronchopneumonia in buffalo calves in Assiut governorate studies on bacterial causes, clinical haematological and biochemical changes associated with the disease. *Assiut Veterinary Medical Journal, 46*(92), 138-155. [DOI:10.21608/avmj.2002.180165]

Scott, P. R. (2011). Treatment and control of respiratory disease in sheep. *The Veterinary Clinics of North America. Food Animal Practice, 27*(1), 175–186. [DOI:10.1016/j.cvfa.2010.10.016] [PMID]

Sharma, L., Feng, J., Britto, C. J., & Dela Cruz, C. S. (2020). Mechanisms of epithelial immunity evasion by respiratory bacterial pathogens. *Frontiers in Immunology, 11,* 91. [DOI:10.3389/fimmu.2020.00091] [PMID]

Shihab, O. H., Abdullah, D. S., & Abdulrahman, E. G. (2022). Estimation of Some Genetic Parameters of Some Productive Traits of local and Turkish Awassi Sheep. *Tikrit Journal for Agricultural Sciences, 22*(3), 60-69. [DOI: 10.25130/tjas.22.3.7]

Silva, L. B., Neto, A., Maia, S. M., dos Santos Guimarães, C., Quidute, I. L., & Carvalho, A. d. A., et al. (2019). The role of TNF-α as a proinflammatory cytokine in pathological processes. *The Open Dentistry Journal, 13,* 332-338. [Link]

Small, P. M., Täuber, M. G., Hackbarth, C. J., & Sande, M. A. (1986). Influence of body temperature on bacterial growth rates in experimental pneumococcal meningitis in rabbits. *Infection and Immunity, 52*(2), 484-487. [DOI:10.1128/iai.52.2.484-487.1986] [PMID]

Smith, A. M., Adler, F. R., Ribeiro, R. M., Gutenkunst, R. N., McAuley, J. L., & McCullers, J. A., et al. (2013). Kinetics of coinfection with influenza A virus and Streptococcus pneumoniae. *Plos Pathogens, 9*(3), e1003238. [DOI:10.1371/journal.ppat.1003238] [PMID]

Spitalnik, P. F. (2016). *Histology laboratory manual (2016-2017)*. New York: Columbia College of Physicians and Surgeons Scholarly. [Link]

Takashima, K., Tateda, K., Matsumoto, T., Ito, T., Iizawa, Y., & Nakao, M., et al. (1996). Establishment of a model of penicillin-resistant Streptococcus pneumoniae pneumonia in healthy CBA/J mice. *Journal of Medical Microbiology, 45*(5), 319-322. [DOI:10.1099/00222615-45-5-319] [PMID]

Ukwueze, C. S., & Kalu, E. (2015). Prevalence of haemoparasites in red Sokoto goats slaughtered at Ahiaeke market, Umuahia, Abia State, Nigeria. *Scientific Journal of Microbiology, 4*(1), 1-5. [Link]

Wong, H. E., Tourlomousis, P., Paterson, G. K., Webster, S., & Bryant, C. E. (2023). Naturally-occurring serotype 3 Streptococcus pneumoniae strains that lack functional pneumolysin and autolysin have attenuated virulence but induce localized protective immune responses. *Plos One, 18*(3), e0282843. [DOI:10.1371/journal.pone.0282843] [PMID]

Xu, D., Lian, D., Wu, J., Liu, Y., Zhu, M., & Sun, J., et al. (2017). Brain-derived neurotrophic factor reduces inflammation and hippocampal apoptosis in experimental Streptococcus pneumoniae meningitis. *Journal of Neuroinflammation, 14*(1), 156. [DOI:10.1186/s12974-017-0930-6] [PMID]

Zaghawa, A., Hassan, H., & El-Sify, A. (2010). Clinical and etiological study on respiratory affections of sheep. *Minufiya Veterinary Journal, 7*(1), 93-103. [Link]

Zivich, P. N., Grabenstein, J. D., Becker-Dreps, S. I., & Weber, D. J. (2018). Streptococcus pneumoniae outbreaks and implications for transmission and control: A systematic review. *Pneumonia (Nathan Qld.), 10,* 11. [DOI:10.1186/s41479-018-0055-4] [PMID]

This Page Intentionally Left Blank