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`Role of TNF-α in Experimental *Streptococcus Pneumoniae* infection in Lambs

TNF-α in S. Pneumoniae Infection: Lambs

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

OBJECTIVE The aim of study was to evaluate the role of TNF-α against infection with *Streptococcus pneumonia* by intratracheal route in lambs as experimental lamb model.

MATERIAL AND 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-exposures for total and differential WBC counts and tumor necrosis factor-alpha (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 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 zero. 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 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 *Streptococcus pneumoniae* infection in lambs.

KEYWORDS: lambs, *Streptococcus pneumoniae*, TNF-α

Introduction

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

small ruminant respiratory complex is one of the major 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 influences (Amit *et al*., 2012; Brogden *et al*., 1998; Kumar *et al*., 2011). Among various causative agents (Ali et al.,2005), *Streptococcus pneumoniae* (*S. 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 and Manning, 1990; Zivich *et al*., 2018). In veterinary medicine, *S. pneumoniae* poses a

significant concern, particularly among livestock like lambs, where it engenders 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 the activation of white blood cells (WBCs), notably neutrophils and lymphocytes (Korkmaz and Traber, 2023). Neutrophils, or polymorphonuclear leukocytes, play crucial roles in early infection stages through pathogen phagocytosis and cytokine-mediated immune responses such as TNF-α release (Lee *et al*., 2022). Lymphocytes, encompassing B and T cells, are essential for specific immune responses against *S. pneumonia* (He *et al*., 2021). Monocytes differentiate into macrophages, which engage in pathogen engulfment and digestion (Anandachar *et al*., 2023). TNF-α, a pro-inflammatory cytokine, facilitates immune cell recruitment and instigates inflammatory cascades (Silva *et al*., 2019).

By utilizing the lamb model of intratracheal route-induced pneumonia, this research endeavor aims to advance the understanding of *S. pneumoniae* infections in veterinary medicine and contribute to the development of innovative strategies for the prevention and treatment of *S. pneumoniae* in livestock populations.

Materials and Methods

Ethical Approval

This study received ethical approval from 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 Housing

Six male lambs of local Iraqi breed, aged 1-2 months and weighing between 5 to 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% CO2 for 18 hours (Ashrafi *et al*., 2022). Subsequently, the bacterial suspension was prepared in sterile phosphate-buffered saline (PBS) and incubated in brain heart infusion broth (BHI) for an additional 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 the injections were 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 exposure among all animals.

Clinical Evaluation

Following inoculation, lambs were monitored daily for clinical signs suggestive 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 into EDTA-anticoagulant tubes for total and differential white blood cells (WBC) count using an automated hematology analyzer (Getein BHA-5000, China) (Karaşahin *et al*., 2023). For tumor necrosis

factor-alpha (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 (Al-Tai, 2018).

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 to evaluate pathological changes (Luna, 1968; Spitalnik and Witkin, 2017).

Statistical analysis

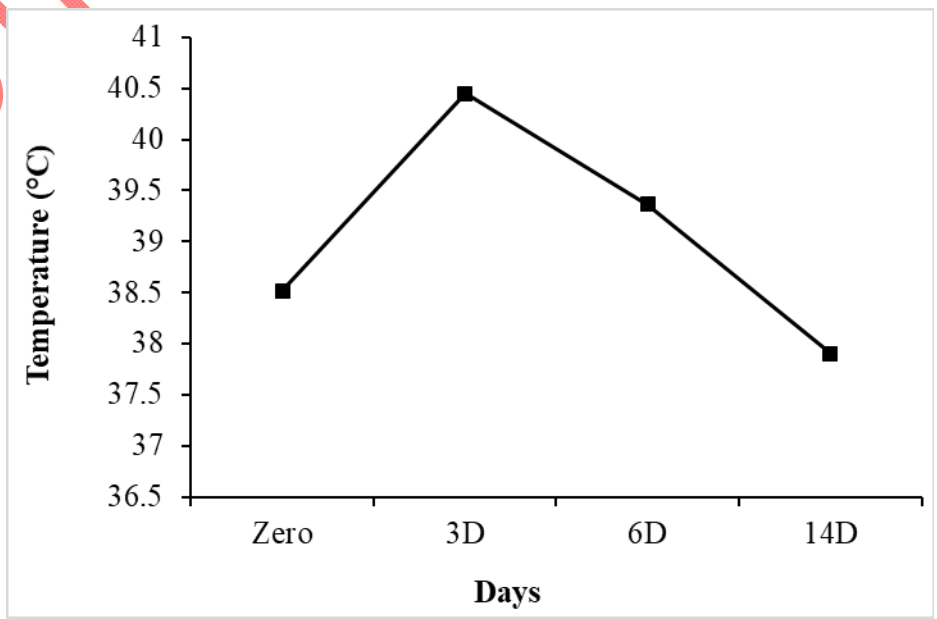
Data were analyzed using the Statistical Analysis System (SAS) software. The Least Significant Difference (LSD) test was employed for pairwise comparisons of means in the Analysis of Variance (ANOVA)(Omar and Ibrahim, 2023).

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, as depicted in the provided Figure1.

Figure.1 Body temperature during experimental infection



In the examination of *Streptococcus pneumoniae* post-infection.

All lung tissue samples from lambs, collected at both 6- and 14-days post-exposure, yielded positive results for the presence of 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 further confirmed the presence of Gram-positive cocci arranged in pairs and chains figure (2B).

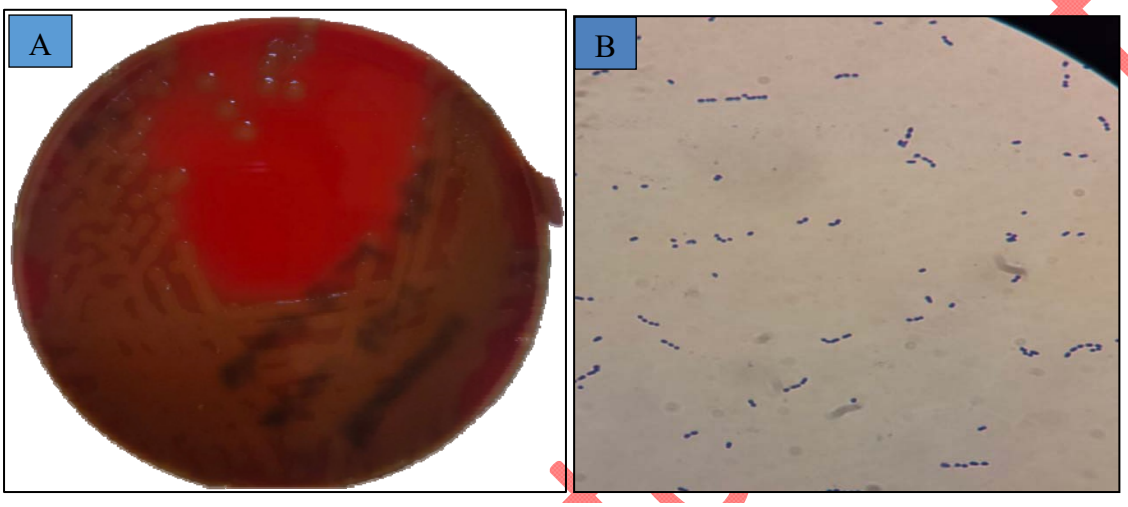


Figure 2. A. growth culture of S. pneumoniae with round or circular in shape slightly raised or convex colonies on agar surfaces and grayish-white in color with α-hemolysis. B. microscopic shapes of pneumococci in duplicate and short chain coccid (100X

Subsequent analysis utilizing the VITEK 2 System provided a 98% probability of the isolates being *S. pneumoniae*, validating the initial observations and strengthening 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 were monitored 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 at day zero pre-infection and post-infection. A significant increase in WBC counts was observed at day three post-infection (22.6±0.788 × 10^9/L), followed by slightly lower counts at day 6 (21.2±0.738 × 10^9/L) and day 14 (20.75±0.046 × 10^9/L), compared to day zero (8.97±0.316 × 10^9/L), indicating an early and vigorous immune response. Differential WBC counts, including neutrophils, lymphocytes, and monocytes, were also assessed. There was a significant increase in neutrophils on day three post-exposure (8.14±0.758 × 10^9/L) compared to day zero (2.44±0.393 × 10^9/L), indicating an early neutrophilic response. Neutrophil counts gradually decreased by days 6 (6.83±0.701 × 10^9/L) and 14 (4.67±2.009 × 10^9/L) but remained significantly higher compared to day zero.

Intratracheal exposure to *S. pneumonia* significantly increased the lymphocyte count on days 6 (10.5±1.706 × 10^9/L) and 14 (15.9±0.717 × 10^9/L) post-exposure compared to day zero (6.43±0.951 × 10^9/L).

Lambs exposed to *S. pneumonia* through the intratracheal route exhibited a significant increase in monocytes on the third day post-exposure compared to day zero, 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 day zero as summarized in **(Table 1)**

Table 1. The total white blood cell 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*. The values of WBC, neutrophils, lymphocytes, and monocytes post-exposure were compared to those at day zero.

Days	WBC ($\times 10^9$/L)	Neutrophile ($\times 10^9$/L)	Lymphocyte ($\times 10^9$/L)	Monocyte ($\times 10^9$/L)
Zero	8.97±0.316 b	2.44±0.393 c	6.43±0.951 c	0.01±0.003 c
3D	22.6±0.788 a	8.14±0.758 a	8.51±0.909 bc	0.63±0.074 a
6D	21.2±0.738 a	6.83±0.701 ab	10.5±1.706 b	0.27±0.044 b
14D	20.75±0.046 a	4.67±2.009 bc	15.9±0.717 a	0.03±0.006 c
P-value	<0.001	0.0004	0.0018	<0.0001
LSD	2.575	3.259	3.525	0.1401

Values are mean ± SEM, n =6. *n for day 14=3. Means with different superscripts within a column differ significantly (P≤0.05).

TNF-α level by intratracheal exposure

The 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 were increased at intervals of 6 to 14 days, but decreased steadily with subsequent testing. Although still greater than day zero, the decrease indicates continued resolution of inflammation and a return to homeostasis. Intratracheal exposure to *S. pneumoniae* in lamb's results in a significant increase in TNF-α levels, indicating an intense inflammatory response. TNF-α levels decrease over time, indicating that inflammation gradually resolves figure 3.

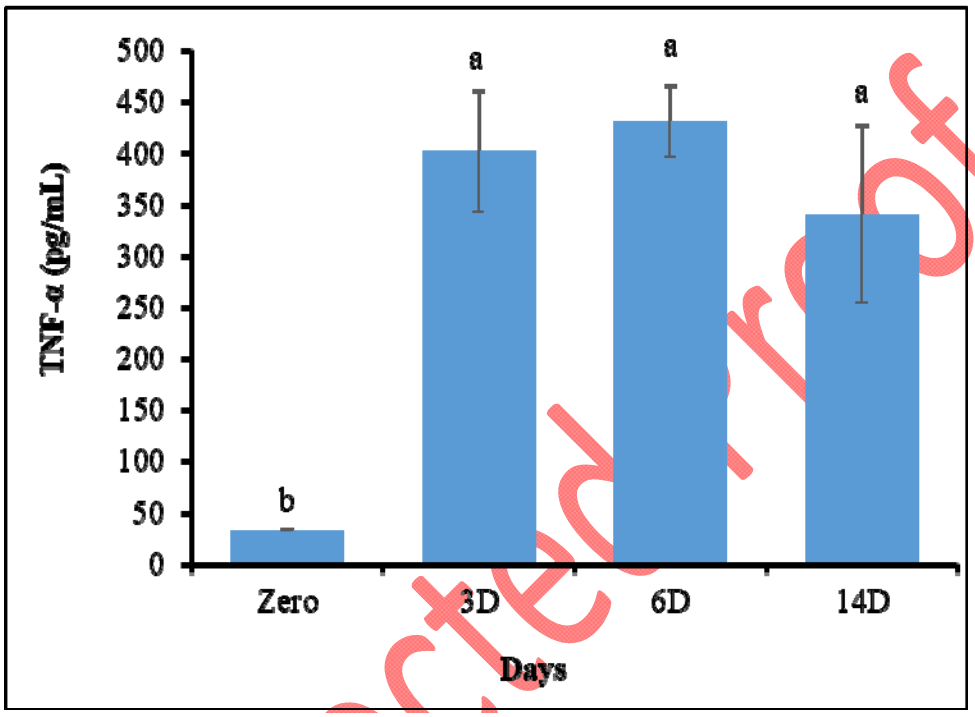


Figure 3. Levels of TNF-α (pg./ml) pre and post infection Values are mean ± SEM, n =6. Means with different superscripts within a column differ significantly (P≤0.05).

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 also hemorrhagic spots seen and linear congestion of mucosal internal layer along the trachea (Figure-4. A, B). 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 (Figure-4.C,D).

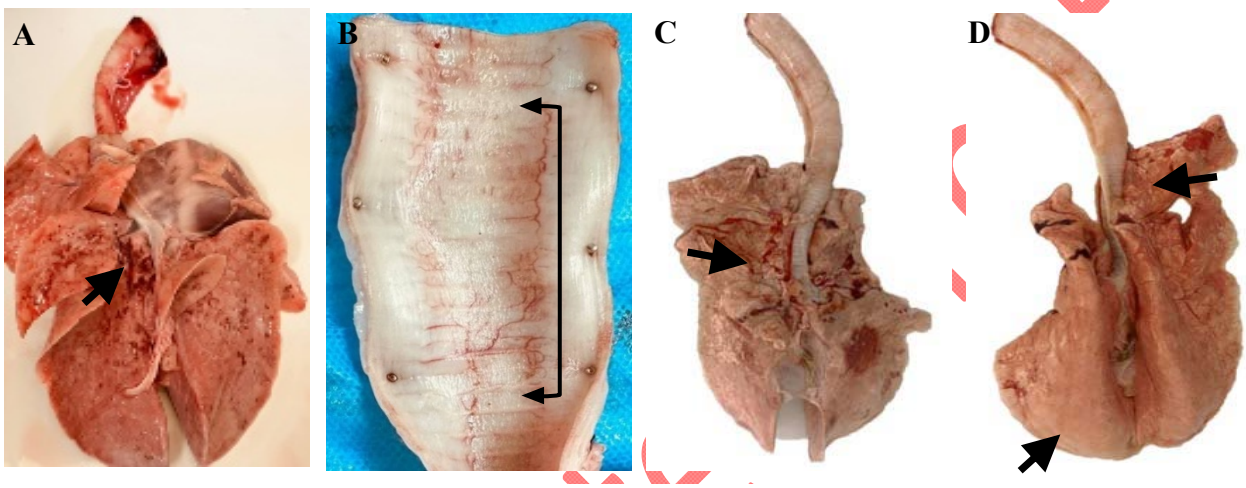


Figure 4. Gross appearance of A: affected lung at day 6 post infection with *S. pneumoniae* by intratracheal route shows lung congestion and ecchymotic hemorrhage (arrow). B: linear congestion of internal mucosa of trachea (arrow). C: congestion of trachea in the ventral site of lungs shows hemorrhage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

The microscopic examination of lambs' post infection intratracheally with *S. pneumoniae* revealed at day six marked disruption of tracheal and bronchial mucosal epithelia represented by vacuolar degeneration of mucosal epithelia **(Figure-5 a)**, intraepithelial and subepithelial infiltration of mononuclear cells (MNCs) mainly lymphocytes and polymorphoneutrophils (PMNs), also in submucosa to serosa **(Figure-5 ,b).** At day 14 post infection in **(figures-5 c**) 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-5 d).** the lung tissues at day 6 (**Figure-5 e, f**) revealed mild to moderate thickening of inter alveolar 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. At day 14 post infection focally mononuclear cells around bronchioles (**Figure-5 g). (Figure-5 h**) Lobular interstitial thickening of lungs

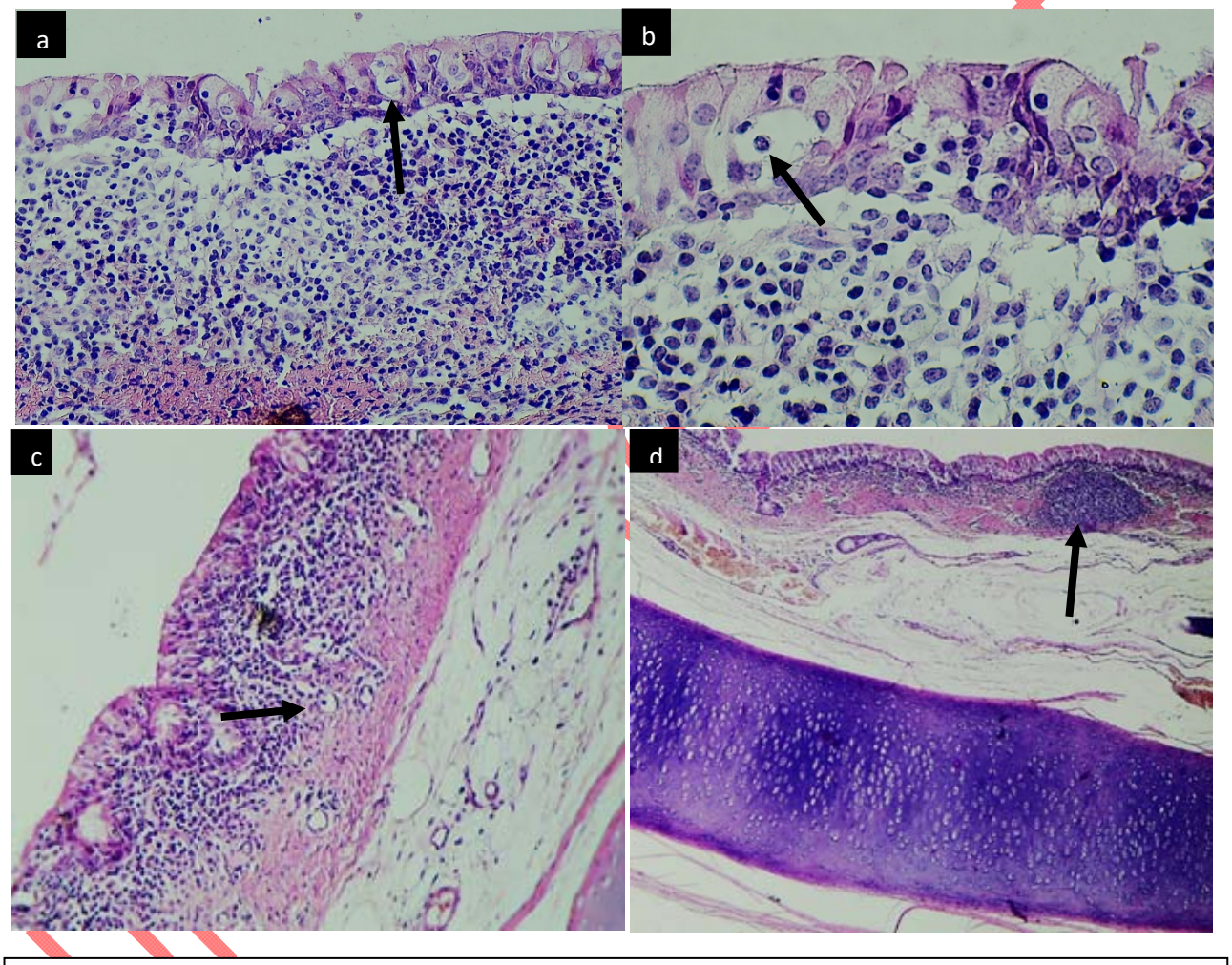


Figure 5. Histopathological section of trachea from lambs infected intratracheally with *S. pneumoniae* 6 days and 14 days post infection; a: shows complete loss of cilia and vacuolar degeneration of mucosal epithelial cells. b: (arrow) intraepithelial and subepithelial infiltration of MNCs and few PMNs in mucosa, submucosa. c: shows necrosis of stratified columinar epithelium and severe infiltration of inflammatory cells and damage of mucosal gland in submucosa (arrow). D: presence of focal mononuclear cells aggregation in submucosa (arrow). e: thickening of intraalveolar septa (arrow), f: necrotic bronchitis and atelectasis (arrow). g: peri bronchial aggregation of lymphoid cells (arrow). h: interstial thickening with emphysema(arrow). (H&E stain, 400X,40X, 100X).

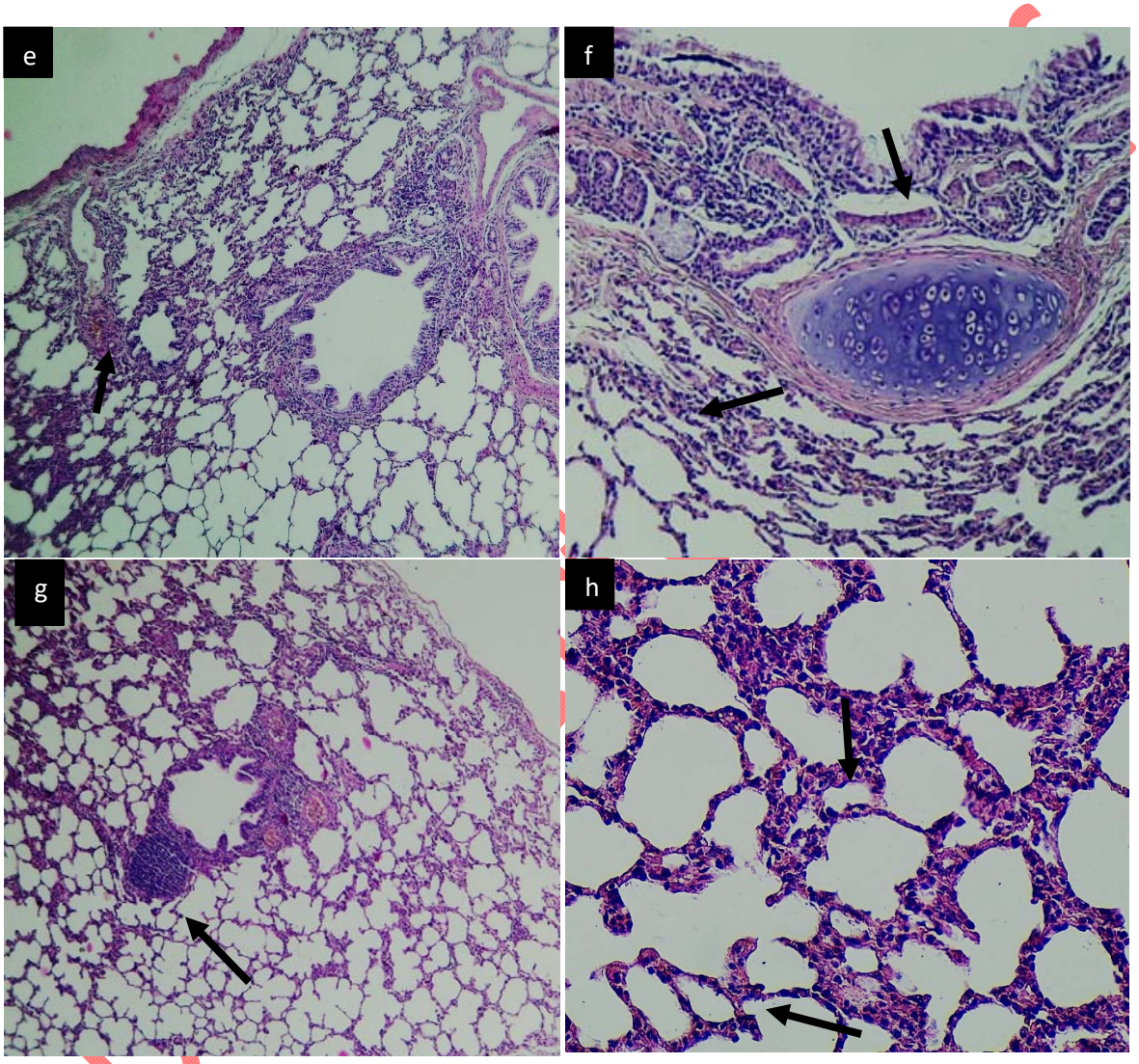


Figure 5. Histopathological section of trachea from lambs infected intratracheally with *S. pneumoniae* 6 days and 14 days post infection; a: shows complete loss of cilia and vacuolar degeneration of mucosal epithelial cells. b: (arrow) intraepithelial and subepithelial infiltration of MNCs and few PMNs in mucosa, submucosa. c: shows necrosis of stratified columinar epithelium and severe infiltration of inflammatory cells and damage of mucosal gland in submucosa (arrow). D: presence of focal mononuclear cells aggregation in submucosa (arrow). e: thickening of intraalveolar septa (arrow), f: necrotic bronchitis and atelectasis (arrow). g: peri bronchial aggregation of lymphoid cells (arrow). h: interstial thickening with emphysema(arrow). (H&E stain, 400X,40X, 100X).

Discussion

The pathogenesis of pneumococcal infection is complex and involves host, infectious agent, and environmental factors (Narayan *et al*., 2023). Serotype 3 is the most common serotype isolated from severe infections in both animals and humans(Wong *et al*., 2023). Different isolates of *S. pneumoniae* can cause a range of diseases, ranging from fatal systemic disease to an asymptomatic course. 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 subsequent to 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. Daan and collagen 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, cytokines such as interleukin 1 beta and tumor necrosis factor alpha, 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. Neutrophils on days 6 and 14 after infection with *S. pneumoniae* to reduce the numbers of

pathogens and eliminate them to reduce their harmful effects in target tissues by stimulating immune responses through the action of proinflammatory cytokines of interleukins and tumor necrosis factors and causing 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 after infection on days 6 and 14, and finally increased levels of lymphocytes and macrophages (mononuclear cells), which represent the adaptive immune response. This is consistent with Jimistan, who recorded a peak body temperature on the third day after infection, which is consistent with body temperature. The initial stages of the immune response, which are characterized by the recruitment of neutrophils and macrophages to the site of infection, 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 normally elicit local protective immune responses, as evidenced by mild clinical signs as seen in the present investigation. These local responses may include activation of mucosal immunity and production of specific antibodies targeting pneumococcal antigens, contributing to resolution of infection without major systemic complications. This is consistent with (Wong *et al*., 2023) where they reported a significant increase in total white blood cell counts (WBCs), 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 and 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. and 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, 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 has been shown to be 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 not clear. Takashima and colleagues 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, and congestion of the affected lungs, as well as bleeding (petechiae and ecchymosis). Gross changes in the trachea showed linear congestion of the internal mucosa. histopathological changes caused by intratracheal pneumococcal infection on days 6 and 14 after infection revealed focal interstitial bronchitis represented by focal infiltration of mainly mononuclear cells; 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 observed to be 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 (Alnajjar *et al*., 2021).who demonstrated pneumonia as a secondary bacterial infection after viral pneumonia or viral-bacterial co-infection (Madhi and Klugman, 2004; Smith *et al*., 2013) and used the lamb model rather than the mouse model for the purpose of understanding viral-bacterial co-infections is crucial for studying treatments. That struggles with both. 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 co-infection with other pathogens and increase morbidity and even mortality.

Conclusion

The significant role of *Streptococcus pneumoniae* as pathogenic agent to induce inflammatory reaction in air passages when introduced directly in trachea.

Author Contributions

Both authors Abbas A. Hamza and Zainab I. Ibrahim designed, performed the experiment collect the data and analyzed also prepared the tissue slices and examined to be photographed using an optical microscope camera, wrote the manuscript and agreed to the published of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

Ethics statement

Ethical approval was granted through the local committee of the animal care and use at the College of Veterinary Medicine within the University of Baghdad (Number 2152/PG) before starting this study.

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. https://doi.org/10.30539/ijvm.v25i1.1144

Ali, J. S. (2005). Bacterial causes of upper & lower respiratory tract infection in Sheep: Jamal Salman Ali, Hayder Badri Abboud. The Iraqi Journal of Veterinary Medicine, 29(1), 1- 10. https://doi.org/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.

Al-Khafagi, A. M., & Zainab, R. Z. (2016). Histopathological and diagnostic study of Toxoplasmosis in human and sheep by using ELISA in Kut city. Iraqi J. Vet. Med, 40(2), 94-9.

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. https://doi.org/10.1371/journal.pone.0235026

Al-Tai, B. F. (2018). Evaluated genetic parameters of some growth, productive and reproductive traits in Turkish Awassi sheep in Iraq. Master's Thesis-College of Agriculture–University of Tikrit-Iraq. https://doi.org/10.36103/ijas.v51i3.1043

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. The Iraqi Journal of Agricultural Science, 48(5), 1231-1238. https://doi.org/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. https://doi.org/10.3923/ajava.2012.149.157

Anandachar, M. S., Agyekum, B., Sinha, S., Roy, S., Ibeawuchi, S.-R., Gementera, H., Amamoto, A., Katkar, G. D., & Ghosh, P. (2023). Diverse Gut Pathogens Exploit the Host Engulfment Pathway via a Conserved Mechanism. bioRxiv, 2023.2004. 2009.536168. https://doi.org/10.1101/2023.04.09.536168

Arends, D. W., Alkema, W., Hapsari Putri, I., van der Gaast–de Jongh, C. E., Eleveld, M., Langereis, J. D., de Mast, Q., Meis, J. F., de Jonge, M. I., & Cremers, A. J. (2022). Differential Pneumococcal Growth Features in Severe Invasive Disease Manifestations. Microbiology Spectrum, 10(3), e00050-00022. https://doi.org/10.1128/spectrum.00050-22

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). http://dx.doi.org/ 10.22059/IJVM.2021.315021.1005144

Baghezza, S., Azizi, A., Derrar, F., Smadi, M. A., Djeghim, H., & Ghougal, Kh., 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. http://dx.doi.org/10.32598/ijvm.18.2.1005387

Borriello, S. (1998). Pathogenesis of Clostridium difficile infection. The Journal of antimicrobial chemotherapy, 41(suppl_3), 13-19. https://doi.org/10.1093/jac/41.suppl_3.13

Borsa, N., Di Pasquale, M., & Restrepo, M. I. (2019). Animal models of Pneumococcal pneumonia. International journal of molecular sciences, 20(17), 4220. https://doi.org/10.3390/ijms20174220

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. https://doi.org/10.3923/ajava.2011.537.546

Chaguza, C., Ebruke, C., Senghore, M., Lo, S. W., Tientcheu, P.-E., Gladstone, R. A., Tonkin-Hill, G., Cornick, J. E., Yang, M., & Worwui, A. (2022). Comparative genomics of disease and carriage serotype 1 pneumococci. Genome biology and evolution, 14(4), evac052. https://doi.org/10.1093/gbe/evac052

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.

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), 154-162. https://doi.org/10.21608/avmj.2009.174979

Glimåker, M., Kragsbjerg, P., Forsgren, M., & Olcen, P. (1993). Tumor necrosis factor-α (TNFα) in cerebrospinal fluid from patients with meningitis of different etiologies: high levels of TNFα indicate bacterial meningitis. Journal of Infectious Diseases, 167(4), 882-889. https://doi.org/10.1093/infdis/167.4.882

Goodwin, K., Jackson, R., Brown, C., Davies, P., Morris, R., & Perkins, N. (2004). Pneumonic lesions in lambs in New Zealand: patterns of prevalence and effects on production. New Zealand Veterinary Journal, 52(4), 175-179. https://doi.org/10.1080/00480169.2004.36425

Hashim, M. (2021). HISTOPATHOMICROBIAL EXAMINATION OF ECHINOCOCCOSIS IN LUNG AND LIVER INFECTED WITH KLEBSIELA PNEUMONIA IN SHEEP OF IRAQ. Iraqi Journal of Agricultural Sciences, 52(1), 129-135.

He, S. W., van de Garde, M. D., Pieren, D. K., Poelen, M. C., Voß, F., Abdullah, M. R., Hammerschmidt, S., & van Els, C. A. (2021). Diminished pneumococcal-specific CD4+ t-cell response is associated with increased regulatory t cells at older age. Frontiers in Aging, 2, 746295.

Heumann, D., Barras, C., Severin, A., Glauser, M., & 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. https://doi.org/10.1128/iai.62.7.2715-2721.1994

Houldsworth, S., Andrew, P., & Mitchell, T. (1994). Pneumolysin stimulates production of tumor necrosis factor alpha and interleukin-1 beta by human mononuclear phagocytes. Infection and immunity, 62(4), 1501-1503. https://doi.org/10.1128/iai.62.4.1501-1503.1994

Jacques, L. C., Green, A. E., Barton, T. E., Baltazar, M., Aleksandrowicz, J., Xu, R., Trochu, E., Kadioglu, A., & Neill, D. R. (2023). Influence of Streptococcus pneumoniae within-

strain population diversity on virulence and pathogenesis. Microbiology Spectrum, 11(1), e03103-03122. https://doi.org/10.1128/spectrum.03103-22

Johnston Jr, R. B. (1991). Pathogenesis of pneumococcal pneumonia. Reviews of infectious diseases, 13(Supplement_6), S509-S517. https://doi.org/10.1093/clinids/13.Supplement_6.S509

Jones, G., Field, A., Gilmour, J., Rae, A., Nettleton, P., & McLauchlan, M. (1982). Effects of experimental chronic pneumonia on bodyweight, feed intake and carcase composition of lambs. The Veterinary Record, 110(8), 168-173. https://doi.org/10.1136/vr.110.8.168

Karaşahin, 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. http://dx.doi.org/10.32598/ijvm.17.2.1005334

Korkmaz, F. T., & Traber, K. E. (2023). Innate immune responses in pneumonia. Pneumonia, 15(1), 1-26. https://doi.org/10.1186/s41479-023-00106-8

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. https://doi.org/10.3923/pjbs.2013.2021.2025

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. https://doi.org/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. https://doi.org/10.5483/BMBRep.2022.55.10.115

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.

Lucas, R., Hadizamani, Y., Gonzales, J., Gorshkov, B., Bodmer, T., Berthiaume, Y., Moehrlen, U., Lode, H., Huwer, H., & Hudel, M. (2020). Impact of bacterial toxins in the lungs. Toxins, 12(4), 223. https://doi.org/10.3390/toxins12040223

Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology. In Manual of histologic staining methods of the Armed Forces Institute of Pathology (pp. xii, 258-xii, 258). doi.org/10.29079/vol14iss2art34922

Madhi, S. A., & Klugman, K. P. (2004). A role for Streptococcus pneumoniae in virus-associated pneumonia. Nature medicine, 10(8), 811-813. https://doi.org/10.1038/nm1077

Mohammed, Y., Mustafa, J., & Abdullah, A. (2020). Isolation and molecular study of some bacterial urinary tract infections of sheep in Basrah province. The Iraqi Journal of Agricultural Science, 51(3), 885-893. https://doi.org/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. https://doi.org/10.3389/fcimb.2021.639450

Narayan, K. G., Sinha, D. K., & Singh, D. K. (2023). Agent, Host, and Environmental Factors. In Veterinary Public Health & Epidemiology: Veterinary Public Health-Epidemiology-Zoonosis-One Health (pp. 55-61). Springer. https://doi.org/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. Journal of Infectious Diseases, 163(5), 1029-1032. https://doi.org/10.1093/infdis/163.5.1029

Norman, E. M. (2015). Characterization of virulence in clinical isolates of Streptococcus pneumoniae. Inst för mikrobiologi, tumör-och cellbiologi/Dept of Microbiology, Tumor and ….

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. https://doi.org/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(1), 89-115. https://doi.org/10.1146/annurev.mi.47.100193.000513

Periselneris, J., José, R. J., & Brown, J. S. (2014). Pulmonary immune response to Streptococcus pneumoniae. Shortness of Breath, 3(4), 147-158. https://doi.org/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. https://doi.org/10.1128/iai.58.9.2733-2737.1990

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). http://dx.doi.org/10.22059/IJVM.2021.297539.1005166

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

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. https://doi.org/10.21608/avmj.2002.180165

Scott, P. R. (2011). Treatment and control of respiratory disease in sheep. Veterinary Clinics: Food Animal Practice, 27(1), 175-186. https://doi.org/10.1016/j.cvfa.2010.10.016

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. https://doi.org/10.3389/fimmu.2020.00091

Silva, L. B., dos Santos Neto, A. P., Maia, S. M., dos Santos Guimarães, C., Quidute, I. L., Carvalho, A. d. A., Júnior, S. A., & Leão, J. C. (2019). The role of TNF-α as a proinflammatory cytokine in pathological processes. The Open Dentistry Journal, 13(1). https://doi.org/10.2174/1874210601913010332

Small, P., Täuber, M. G., Hackbarth, C., & Sande, M. (1986). Influence of body temperature on bacterial growth rates in experimental pneumococcal meningitis in rabbits. Infection and immunity, 52(2), 484-487. https://doi.org/10.1128/iai.52.2.484-487.1986

Smith, A. M., Adler, F. R., Ribeiro, R. M., Gutenkunst, R. N., McAuley, J. L., McCullers, J. A., & Perelson, A. S. (2013). Kinetics of coinfection with influenza A virus and Streptococcus pneumoniae. PLoS pathogens, 9(3), e1003238. https://doi.org/10.1371/journal.ppat.1003238

Spitalnik, P. F., & Witkin, J. (2017). Histology laboratory manual. College of Physicians and Surgeons Columbia University Patrice. pfs2101@ Columbia. edu .

Takashima, K., Tateda, K., Matsumoto, T., Ito, T., Iizawa, Y., Nakao, M., & Yamaguchi, K. (1996). Establishment of a model of penicillin-resistant Streptococcus pneumoniae

pneumonia in healthy CBA/J mice. Journal of medical microbiology, 45(5), 319-322. https://doi.org/10.1099/00222615-45-5-319

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. http://dx.doi.org/10.5455/jva.20150207123646

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.

Xu, D., Lian, D., Wu, J., Liu, Y., Zhu, M., Sun, J., ... & Li, L. (2017). Brain-derived neurotrophic factor reduces inflammation and hippocampal apoptosis in experimental Streptococcus pneumoniae meningitis. Journal of neuroinflammation, 14(1), 1-13. https://doi.org/10.1186/s12974-017-0930-6 PMid:28778220

Zaghawa, A., Hassan, H., & El-Sify, A. (2010). Clinical and Etiological study on respiratory affections of sheep. Minufiya veterinary journal, 7(1), 93-103. https://doi.org/10.5455%2Fjavar.2023.j705

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, 10, 1-15. https://doi.org/10.1186/s41479-018-0055-4