Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024
https://doi.org/10.71375/djvs.2024.02303

The Toxic Effects on Lung Tissues in Albino Male Rabbits infected by Klebsiella pneumonia

Hiba Yasseen Abbas1 , Al-Khafaji Nazar2 , Anas A. Humadi3

1,2,3 Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Diyala

Corresponding author: anas.a@uodiyala.edu.iq

Abstract:

Klebsiella pneumoniae is an important member of the Klebsiella genus in the Enterobacteriaceae family. It is a type of bacteria that can survive with or without oxygen, not motile, has a rod-like shape, and is classified as gram-negative.

Thirty male rabbits used in study and divided into three groups: 1st group (GI): given (1CC /animal) an oral dose of phosphate buffer saline (PBS) by a stomach tube as a control group for 60 days, 2nd group (GII): One dose weekly were given 1CC viable *K. pneumonia* (1 X 10^6 CFU/ml) orally by stomach tube for 60 days, 3rd group (GIII): twice dose weekly were given 1CC viable *K. pneumonia* (1 X 10^6 CFU/ml) orally by stomach tube for 60 days. After 60 day of experiment the tissue sample from lung were taken for pathological examination & stained by hematoxylin & eosin stain.

The results in 2nd group showed edema, interstitial pneumonia, hemorrhage with ballooning emphysema, severe infiltration of mononuclear cells, congested of blood vessels with interstitial pneumonia, While in 3rd group showed emphysema, artery arteriosclerosis with granuloma, fibrosis of bronchus, hyperplasia of epithelium and alveoli atelectasis.

The aim and importance of this to explain the effects of *K. pneumonia* on lung tissue after exposure orally.

Keywords: Klebsiella pneumonia, liver and kidney, pathological changes ,Albino male rabbits, Vitek-2 technique

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Introduction:

Klebsiella pneumoniae is a Gram-negative rod, facultative anaerobic, non-motile, possessing a prominent polysaccharide capsule, ubiquitous endemic pathogen. It is regarded as one of the most significant members of the Enterobacteriaceae family's Klebsiella genus, which is found in the gastrointestinal tracts of both people and animals as normal flora and is responsible for recurring infections in both animals and immunocompromised humans. The majority of Klebsiella species infections are brought on by eating tainted food, such as rotten seafood or tainted water (1&2). Additionally, *Klebsiella pneumoniae* typically live in soil, water, and plant environments (3).

In neonatal facilities, Klebsiella has become a prominent nosocomial infection. Additionally, extremely problematic are nosocomial Klebsiella infections, especially in premature newborns and critical care units (ICUs). Klebsiella species can readily invade pediatric patients. The principal sources of nosocomial outbreaks are colonization of the intestines and the oropharynx. In fact, it has been noted that K. pneumoniae is a common cause of infections in people who have indwelling urinary catheters. (4&5).

K. pneumoniae caused severe pathological changes in different organs of the host, particularly lung tissue, kidneys, and GIT. These lesions may be related to its pathogenicity and virulent factors. A pathogen's pathogenicity is defined as its capacity to cause disease, and its degree of pathogenicity is determined by its virulent components. The terms are synonymously applied (6).

Vegetables could potentially harbor K. pneumoniae bacteria. Raw veggies are frequently included in salads and other dishes. *Klebsiella pneumoniae* is often present in the oral cavity, skin,

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

and intestines and is also prevalent in healthcare environments and medical equipment (7).

Materials and methods:

After the two-week adaption period, this investigation was carried out in the animal house of the department of internal medicine and preventive medicine at the University of Diyala's Faculty of Veterinary Medicine. A total of 30 male rabbits were divided into three groups: 1st group (GI): given (1CC /animal) an oral dose of phosphate buffer saline (PBS) by a stomach tube as a control group for 60 days, 2nd group (GII): One dose weekly were given 1CC viable *K. pneumonia* (1 X 10^6 CFU/ml) orally by stomach tube for 60 days, 3rd group (GIII): twice dose weekly were given 1CC viable *K. pneumonia* (1 X 10^6 CFU/ml) orally by stomach tube for 60 days.

Isolation of *Klebsiella pneumoniae* from human samples:

Isolation from Urine, blood and stool specimens.

1- Urine: 5-10ml, urine samples which were collected in sterile tube, transport to the laboratory, Department of Medicine; College of Veterinary Medicine; University of Diyala. The sample centrifuged at (3000 rpm for three minutes), floating was neglected, and apart of sediment taken by loop and cultured on media (MacConkey, nutrient & EMB) for 24 hours at 3 °C in incubator.

2- Blood samples from 125 patients were placed in a specific container with BHI broth for accelerated growth. The samples ranged in volume from 2-4 ml for patients under ten years old to 5-10 ml for patients over ten years old, then put in a bacterial or alert apparatus to detect the presence of bacterial infection. After that, take a droop by syringe and put on a culture media plate, then incubate in an incubator overnight at 37 °C (Analysis done in the microbiology department in Al-Batol hospital). The VITEK-2 technology was utilized to automatically identify the isolated microorganisms.(Biomeriux, Germany) (8).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

3- Stool specimens: 2 grams formed stool or 2 mL liquid stool (preferred specimens); were collected in a sterile clean, dry, plastic jar, using an applicator swab, collected a small amount of feces. from areas with visible blood or mucous, if present. which were cultured over surface of nutrient, MacConkey, Sabroied dexroied agar by streeking method, Incubate all media at 36°C in incubator for 24 hours (overnight) (9).

Vitek-2 is a smart colorimetric method used for the identification of clinical isolates by a computerized microbiology program system (10).

Preparation the experimental dose:

The infective dose of *Klebsiella pneumoniae* required to induce infection in experimental animals was determined using the McFarland method, After the mixture was well-shacked, it was stored at 4ºC in the dark in a test tube with a screw top. Before used, the solution is well mixed to produce a turbidity equal to 1 x 106 CFU/ml, which is used to compare it to bacterial turbidity. (11&12).

Histopathological changes examination:

After 60 days of experiment, samples of one centimeter in length were obtained from lung and fixed in a 10% formaldehyde solution for a duration of 72 hours. Immediately after the lung were extracted, the specimens were rinsed with tap water prior to further processing.

This included increasing the alcohol concentration from 70% to a pure 100% for a duration of two hours at each concentration. The tissues were then treated with xylol to remove any impurities. Finally, the tissues were saturated with semi-liquid paraffin wax at a temperature of 58 °C, which was done in two separate phases. The specimens are divided into sections using a rotating microtome at a thickness of 5 mm for all kinds of tissue. The hematoxylin and eosin (H & E) stain was applied to all tissues for staining purposes (13).

Results:

Control group: Not significant any pathological changes in lung.

2nd group: Showed edema, interstitial pneumonia, hemorrhage with ballooning

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

emphysema (fig. 1), in other section showed mononuclear cells infiltration with thickening artery and edema (fig. 2), also showed increase in epithelial column cells of bronchus (fig. 3), severe infiltration of mononuclear cells with interstitial pneumonia (fig. 4), congested of blood vessels with interstitial pneumonia (fig. 5).

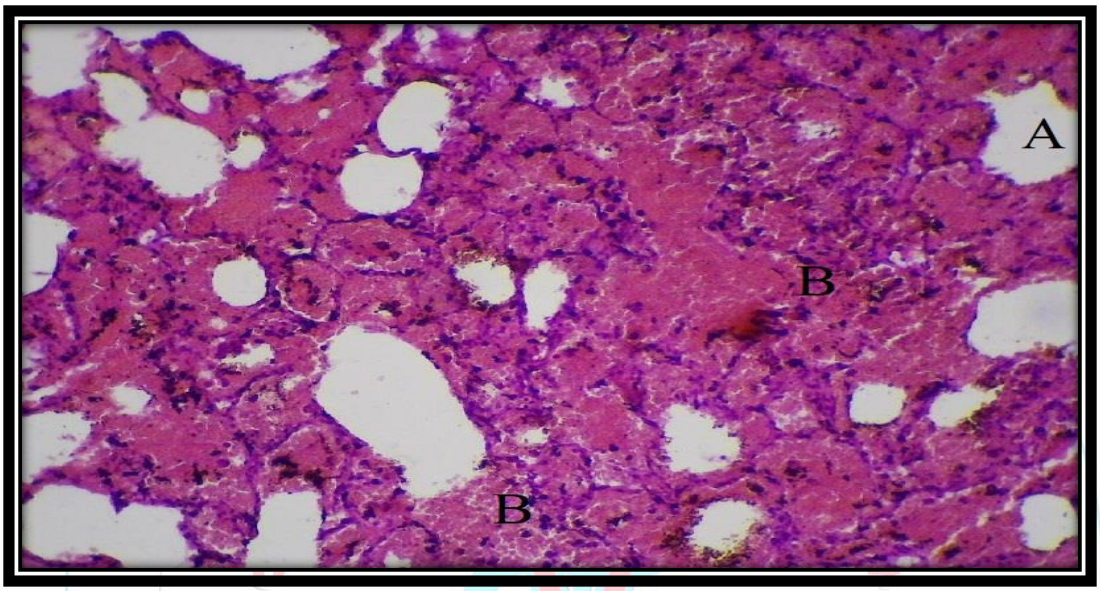
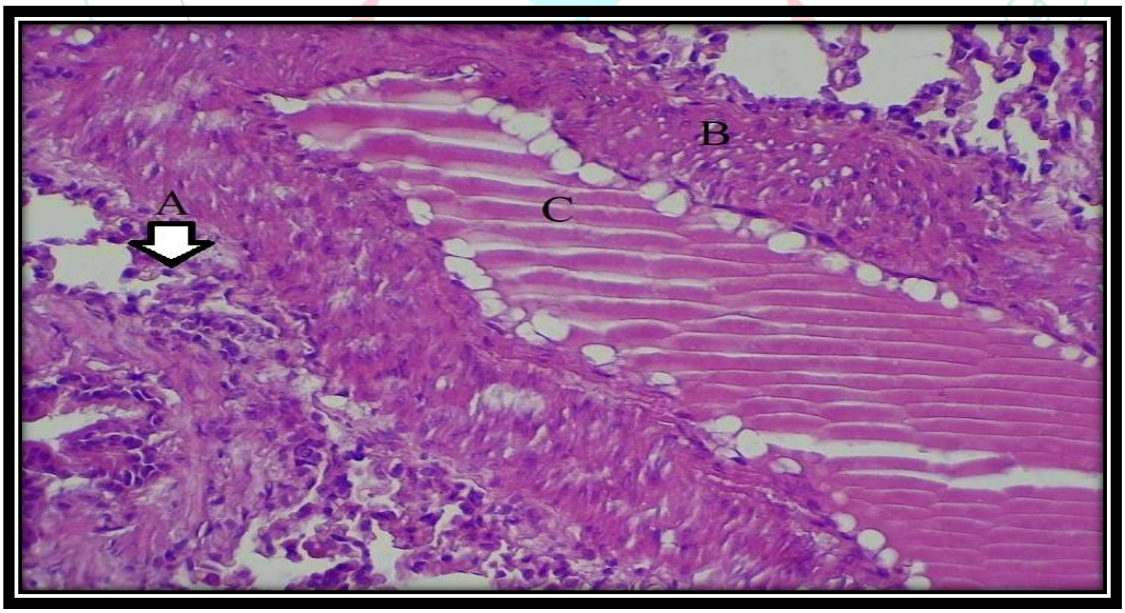


Fig. (1): Histopathological section of lung in 2nd group showed A- Ballooning emphysema, B-Hemorrhage (H & E stain; X20).



Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Fig. (2):Histopathological section of lung in 2nd group showed A- mononuclear cells infiltration B- thickening artery C- edema (H and E stain; X20).

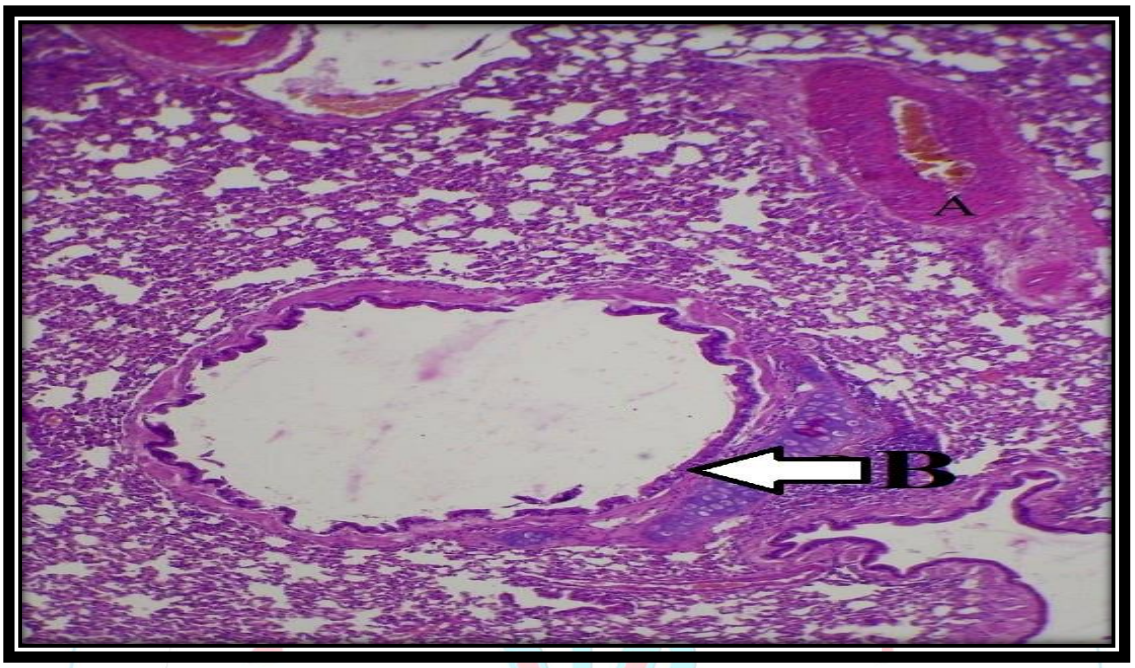
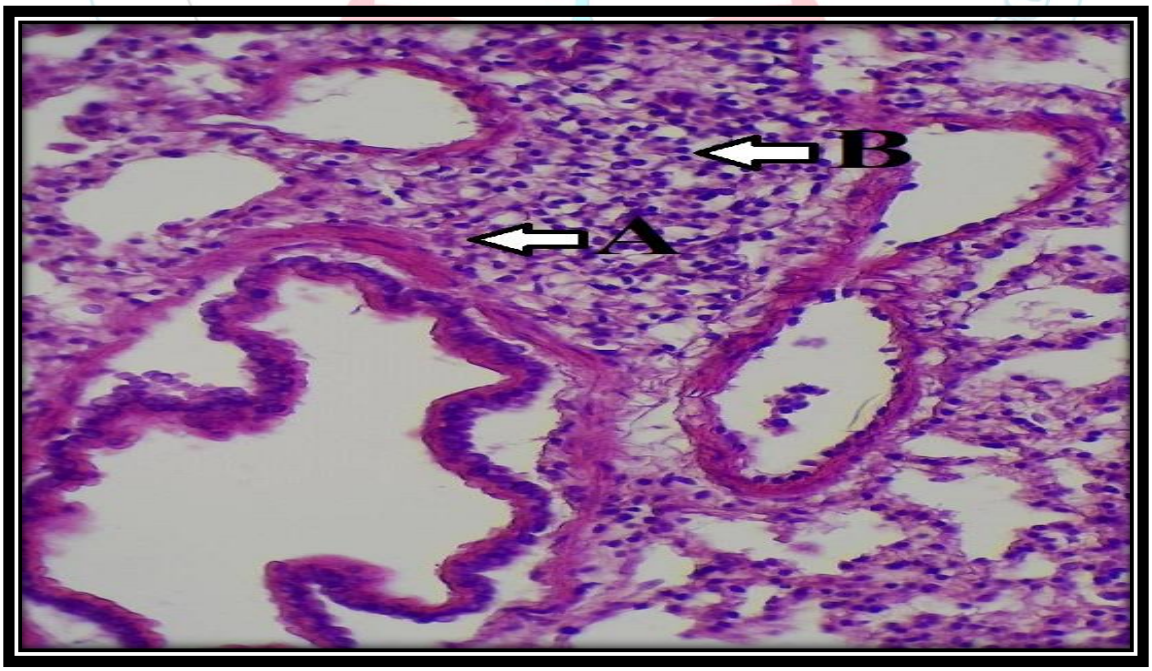


Fig. (3):Histopathological section of lung in 2nd group showed A- Blood vessel (artery) congestion &thickening B- Increase in epithelial column cells of bronchus (H and E stain; X40).



Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Fig. (4):Histopathological section of lung in 2nd group showed A- interstitial pneumonia B- Mononuclear cells infiltration (H and E stain; X40).

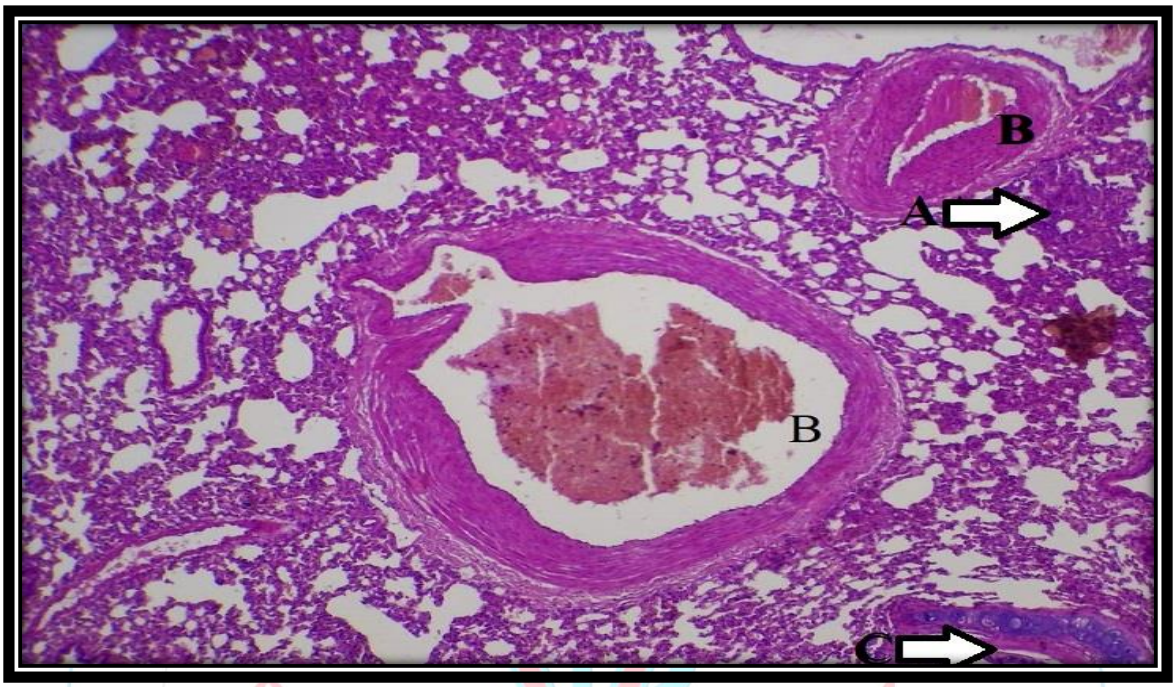


Fig. (5):Histopathological section of lung in 2nd group showed: A- Interstitial pneumonia B- Congested Blood vessels C- bronchiectasis (H& E stain; X20).

3rd group: Showed emphysema, artery arteriosclerosis with polymorphic cells (fig. 6), also showed emphysema, interstitial pneumonia, arteriosclerosis and emphysema (fig. 7), in other section also showed fibrosis of bronchus, hyperplasia of epithelium with emphysema (fig. 8), also showed arteriosclerosis, alveoli atelectasis, hemorrhage and emphysema (fig. 9).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

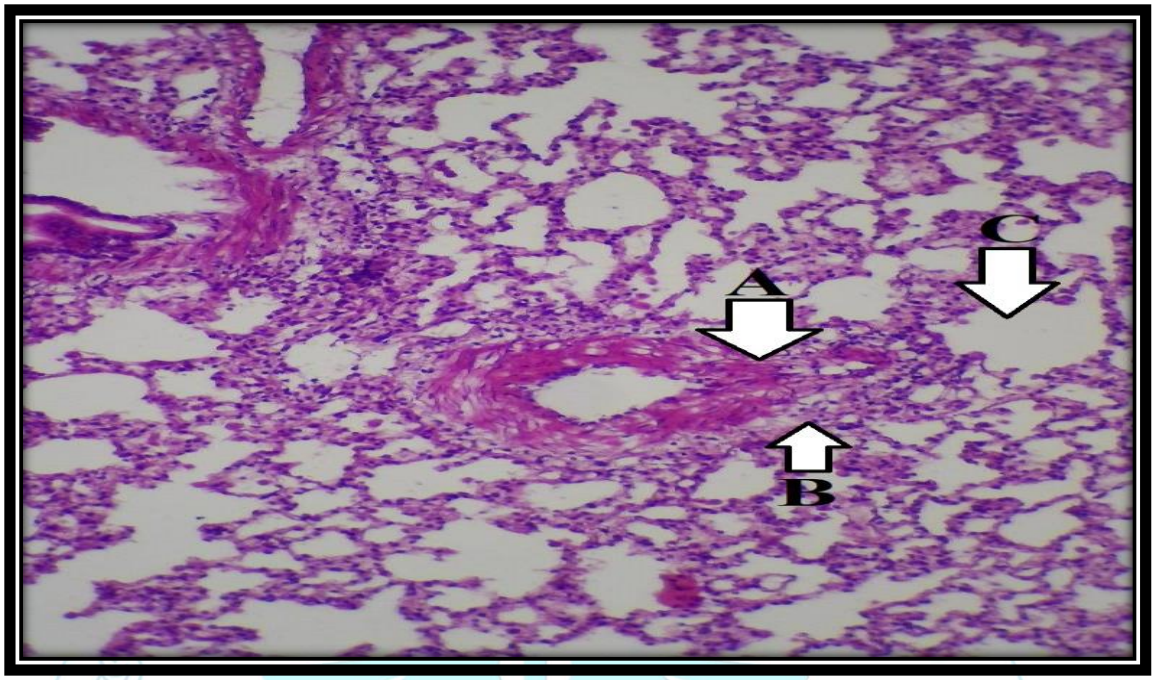


Figure (6): Histopathological section of lung in 3nd group showed: A- arteriosclerosis B- Polymorphic cells infiltration C- Emphysema (H&E stain; X20).

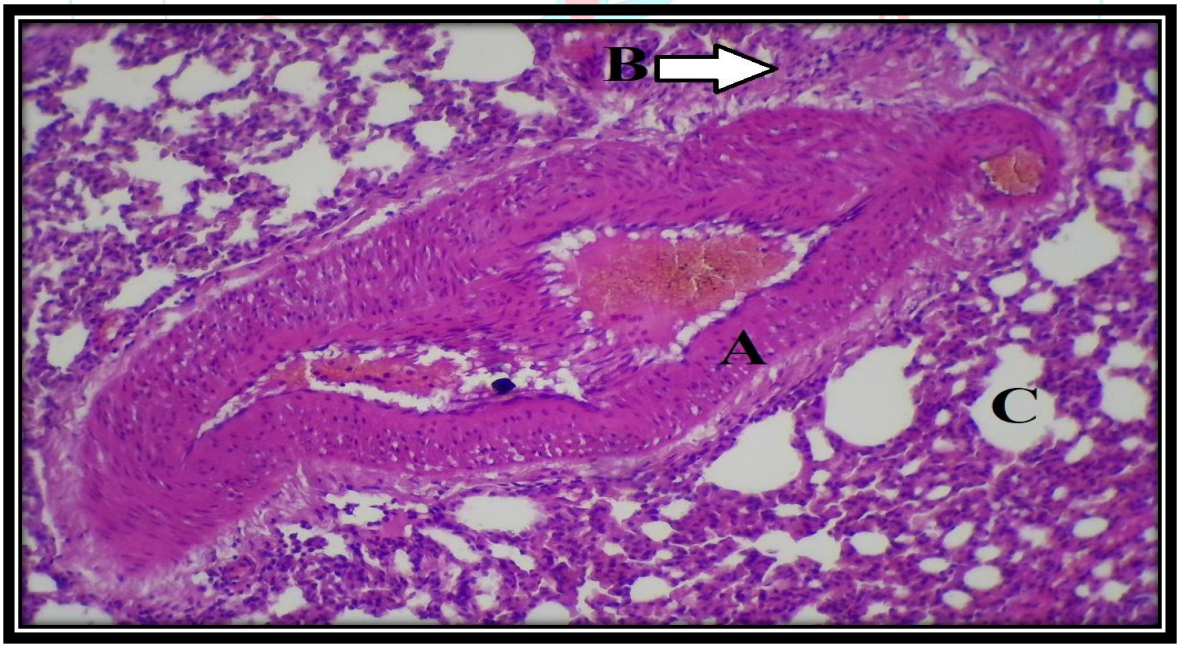


Figure (7):Histopathological section of lung in 3nd group showed A- Arteriosclerosis B- Interstitial Pneumonia C- Emphysema (H&E stain; X40).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

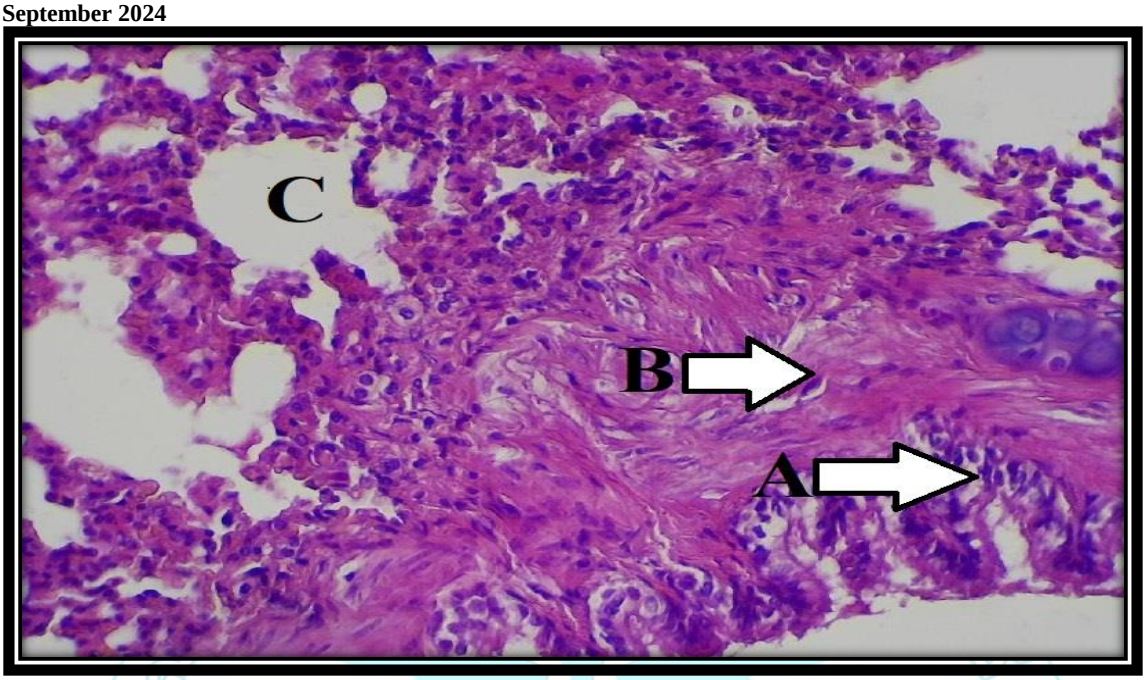


Figure (8):Histopathological section of lung in 3nd group showed A- Hyperplasia epithelium B- Fibrosis of bronchus C- Emphysema (H&E stain; X20).

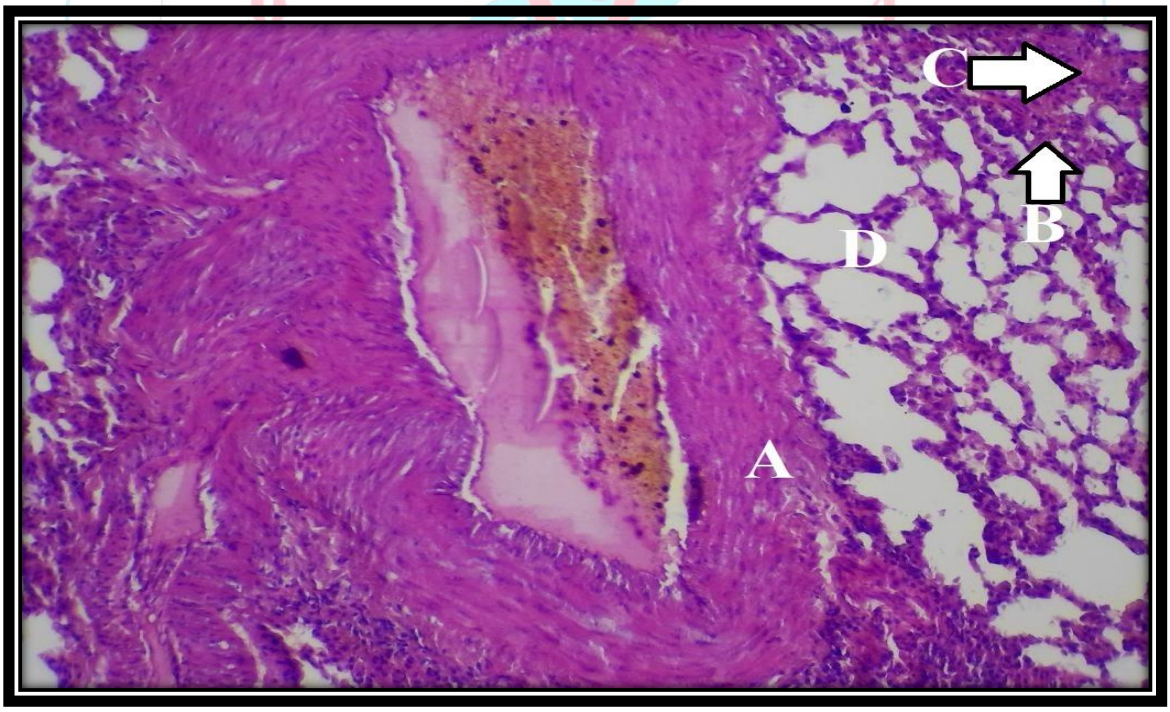


Figure (9):Histopathological section of lung in 3nd group showed A- Arteriosclerosis B- Alveoli atelectasis C- Hemorrhagic D- emphysema (H&E stain; X20).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Discussion:

The histopathological changes of lung by *K. pneumonia* infection showed edema, interstitial pneumonia, hemorrhage with ballooning emphysema, dilated blood vessels, mononuclear cells infiltration with thickening artery, increase in epithelial column cells of bronchus, hyperemia of artery, interstitial pneumonia with emphysema, thickening in interlobular septa, also showed congested of blood vessels, artery arteriosclerosis, granuloma, fibrosis of bronchus, hyperplasia of epithelium and finally alveoli atelectasis and bronchiectasis, these results agree with (14&15) who showed most of the above results.

Also these results agree with (16) who showed that the infected mice with *K. pneumonia* showed lung inflammation in response to gut injury, Increased granulopoiesis in the spleen expands circulating neutrophils, which infiltrate the lung and the colon. This may indicate a spillover effect where neutrophils produced to respond to the gut insult also infiltrate the lungs and subsequently promote inflammation and lung damage. Neutrophil infiltration is the primary marker of inflammation in the lung.

The severe pneumonia with infiltration of mononuclear cells (MNCs) after infection by *K. pneumonia* , this results agree with (17) who recorded that the respiratory response against *K. pneumonia* infection lead to release caspase-11, also agree with (18) who, a few hours after being infected with K. pneumonia, displayed severe acute inflammatory changes in mice. These changes were characterized by fibrin, edematous fluid present in interstitial tissues, blood vessel congestion, and PMN infiltration, primarily neutrophils.

In response to chronic inflammation brought on by K. pneumonia, there is a rise in macrophage activation and recruitment to the infection site. This leads to an increase in proinflammatory cytokines, particularly TNF, which is the primary signal essential for the formation of granulomas. The theory put forth by (19) who showed how a K. pneumonia infection can lessen the host's innate immune response by generating proinflammatory cytokines, is also supported by (20).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Furthermore, the study's findings are consistent with those of (21) who noted significant lung damage 48–72 hours after infection, showing blood vessel congestion and polymorphonuclear cell infiltration in mice infected with K. pneumonia. Additionally, I concur with (22) who demonstrated that female sick mice exposed to ozone exhibited a greater inflammatory response in their lungs than did male mice. Furthermore, (21) demonstrated increased lung intra-alveolar septal thickness as a result of inflammatory cell infiltration and clogged capillary blood vessels.

Finally the histopathological changes of lung in this study agree with (23) who demonstrated the *K. pneumonia* causes cellular damage particularly in apoptosis due to mitochondrial damage which represent an important role in many kinds lead to alterations in function of mitochondrial ,calcium dysregulation and mitochondrial dysfunction were stimulated, which resulted in cell damage.

Conclusion:

The lung infection caused by K. pneumoniae resulted in changes to the histology of the lung tissue and an increase in proinflammatory cytokines, particularly TNF, which was the predominant indicator of the development of granulomas.

References:

1- Lau HY, Clegg S, Moore TA. Identification of Klebsiella pneumoniae genes uniquely expressed in a strain virulent using a murine model of bacterial pneumonia. Microbial pathogenesis. 2007 Apr 1;42(4):148-55.
2- Priyanka A, Akshatha K, Deekshit VK, Prarthana J, Akhila DS. Klebsiella pneumoniae infections and antimicrobial drug resistance. Model organisms for microbial pathogenesis, biofilm formation and antimicrobial drug discovery. 2020:195-225.
3- Abbas R, Chakkour M, Zein El Dine H, Obaseki EF, Obeid ST, Jezzini A, Ghssein G, Ezzeddine Z. General Overview of Klebsiella pneumonia: Epidemiology and the Role of Siderophores in Its Pathogenicity. Biology. 2024 Jan 27;13(2):78.
4- Haryani Y, Noorzaleha AS, Fatimah AB, Noorjahan BA,

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Patrick GB, Shamsinar AT, Laila RA, Son R. Incidence of Klebsiella pneumonia in street foods sold in Malaysia and their characterization by antibiotic resistance, plasmid profiling, and RAPD–PCR analysis. Food control. 2007 Jul 1;18(7):847-53.

5- Gorrie CL, Mirčeta M, Wick RR, Edwards DJ, Thomson NR, Strugnell RA, Pratt NF, Garlick JS, Watson KM, Pilcher DV, McGloughlin SA. Gastrointestinal carriage is a major reservoir of Klebsiella pneumoniae infection in intensive care patients. Clinical infectious diseases. 2017 Jul 15;65(2):208-15.

6- Aljanaby AA, Alhasani AH. Prevalence of blaTEM and blaSHV genes in multidrug resistant Klebsiella pneumoniae isolated from hospital patients with burns infections in Al-Najaf governorate-Iraq. World J Pharmaceut Res. 2015 Apr 27;4(7):145-54.

7- Abu-Zaid AA, Sehrawy MH, Mahmoud H, Nemari AH. Detection of Klebsiella pneumonia in raw food and their antibiotic resistance. Advances in Environmental Biology. 2016 Apr 1;10(4):80-92.

8- Radhakrishnan V. Stool culture and blood culture protocol. BMJ. 2022;421:12.

9- World Health Organization. A WHO network building capacity to detect, control and prevent food borne and other enteric infections from farm to table. Laboratory Protocol: WHO. 2010.

10- Aziz ZS, Al-Muhanna AS, Salman AJ, Alzuhairi MA. Klebsiella and Raoultella biotyping and probability of identification by Vitek-2 system. IJIRSET. 2014;3(4):11289-94.

11- McFadden JF. Biochemical Tests for Identification of Medical Bacteria. 3rded. Lippincott Williams and Wilkins, USA. 2000.

12- Andrews JM. Determination of minimum inhibitory concentrations. Journal of antimicrobial Chemotherapy. 2001 Jul 1;48(suppl_1):5-16.

13- Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. Elsevier health sciences; 2018.

14- Munther, A.G. Isolation and identification of Klebsiella pneumonia of human and animal origins in Baqubah city, in addition to study of its pathogenicity and sensitivity to plant extracts. Degree of master of science in Veterinary Medicine/Microbiology/College of Veterinary Medicine/University of Diyala, Iraq, 2018.

15- Tawfeeq Jassim S, Mohammed Jwad B. Molecular detection and

pathological modifications of Klebsiella pneumoniae in trachea and lung of rabbits after infected by intranasal instillation route, Revis Bionatura 2023; 8 (3) 76.

16- Raftery AL, O'Brien CA, Harris NL, Tsantikos E, Hibbs ML. Development of severe colitis is associated with lung inflammation and pathology. Frontiers in Immunology. 2023.

17- Perlee D, De Beer R, Florquin S, van der Poll T, van't Veer C, De Vos AF. Caspase-11 contributes to pulmonary host defense against Klebsiella pneumoniae and local activation of coagulation. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2020 Jul 1;319(1):L105-14.

18- Ibrahim ZI, Jawad ZJ. Histopathologic Study in lung & kidney of mice post Infection with Klebsiellae pneumonia.The Ninth International Scientific Academic Conference. Istanbul. Turkey.. 2018 Aug 25.

19- Mohammed, A. M. Immunopathological study of Klebsiella pneumoniae as major ESKAPE pathogens in AL Qadisiyah province [Ph.D. dissertation]. Baghdad, Iraq: University of Baghdad; 2019.

20- Chang RK, Miller M, Shahin K, Batac F, Field CL, Duignan P, Struve C, Byrne BA, Murray MJ, Greenwald K, Smith WA. Genetics and pathology associated with Klebsiella pneumoniae and Klebsiella spp. isolates from North American Pacific coastal marine mammals. Veterinary Microbiology. 2022 Feb 1;265:109307.

21- Abadullah SM, Zghair ZR. Isolation of Klebsiella pneumoniae from urine of human and cattle in Baghdad city with histopathological study experimentally in mice. Int J Adv Res Biol Sci. 2016;3:38-45.

22- Mikerov AN, Cooper TK, Wang G, Hu S, Umstead TM, Phelps DS, Floros J. Histopathologic evaluation of lung and extrapulmonary tissues show sex differences in Klebsiella pneumoniae-infected mice under different exposure conditions. International journal of physiology, pathophysiology and pharmacology. 2011;3(3):176.

23- Cheng J, Zhang J, Yang J, Yi B, Liu G, Zhou M, Kastelic JP, Han B, Gao J. Klebsiella pneumoniae infection causes mitochondrial damage and dysfunction in bovine mammary epithelial cells. Veterinary Research. 2021 Dec;52:1-2.