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Etiopathological Aspects on some Pneumonic lesions in Slaughtered sheep in Diyala Provaince

A Thesis

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By

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Supervised by

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بسم الله الرحمن الرحيم

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا ﴿١١٤﴾

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I certify that this thesis (**Etiopathological Aspects on some Pneumonic lesions in Slaughtered sheep in Diyala Province)** has been prepared under my supervision at the College of Veterinary Medicine / University of Bagdad in Partial fulfillment of the requirements for the degree of Master of Scince in veterinary Medicine / Pathology.

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/ / 2017

In view of the available recommendations, I forward this thesis to debate by the examination committee.

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I hereby declare that this dissertation my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at university of Baghdad or other institution.

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/10/2017

Abstract

This study was designed to determine the incidence, causative agents (bacterial and parasites) and pathological changes (Macroscopic and histopathological changes) of pneumonia in local sheep in Diyala Province. For this reason, a total of 67 lungs containing pneumonic lesions were collected by examining 1000 sheep at Khanakin abattoirs and other area for the period of the first November 2016 until the end of March 2017.

The frequency rate of pneumonic lesions were 67 infectous cases (6.7%). The bacterial pneumonia constituted 64 infectious cases (95.5%) out of 67 pneumonic lung and 3 infectious cases (4.5%) were verminous pneumonia.

The total number of the bacterial isolates were 89 isolates,The bacterial species which isolated from infectious cases were *Staphylococcus aureus* 28 isolates (31.4%), *Pasteurella multocida* 20 isolates (22.4%), *Streptococcus species* 15 (16.8%), *Klebsella pneumoniae* 9 isolates (10.1%), *Proteus species* 6 isolates (6.7%), *Staphylococcus epidermidis* 5 isolates (5.6%), *Mannheimia hemolytica* 2 isolates (2.2%), *Actinomyces pyogenes* 1 (%) and *Escherichia coli* 3 isolates (3.3%).

Pneumonic lesions were classified according to the inflammatory exudate:-

Three types of bacterial pneumonia were reported: Acute to chronic suppurative pneumonia: This was found in 38 infection cases (56.7%) of pneumonic cases. This types of pneumonia characterized by suppurative exudate in the bronchius and bronchioles with inflammatory cells (neutrophils and other mononuclear cells).Most of the associated bacterial isolates were of the following species: *Staphylococcus aureus* was 20 isolates (22.4%), *Streptococcus species* was 7 isolates (7.8%),

Klebsella pneumoniae was 7 isolates (7.8%), *Pasteurella multocida* was 4 isolates (4.4%) and *Proteus species* was 6 isolates (6.7%).

The lung absesses found in 7 infectious cases (10.4%) and characterized by presence of abscess containing pus.The bacterial isolated from it as following *Staphylococcus aureus* was 2 isolates (2.2%), *Streptococcus species* was 3 isolates (3.3%), *Klebsiella pneumoniae* was 2 isolates (2.2%), *Actinomyces pyogenes* was 1 isolate (1%) and *Escherichia coli* was 1 isolate (1%).

Acute to chronic fibrinous pneumonia: This was found in 19 infectious cases (28.3%) of pneumonic cases and this type showed fibrin exudate in the bronchial and bronchioles with inflammatory cells. Most of associated bacterial isolates were of type: *Pasteurella multocida* 15 isolates (16.8%), *Staphylococcus aureus* was 3 isolates, *Streptococcus species* was 2 isolates (2.2%), *Staphylococcus epidermidis* was 2 isolates (2.2%), *Mannheimia hemolytica* was 2 isolates (2.2%) and *Escherichia coli* was 2 isolates (2.2%).

Acute to chronic interstitial pneumonia: This was found in 7 infection cases (10.4)% of pneumonic cases nd this type characterized by thekening in the alveolar wall and proliferation of type two pneumocytes. Most of associated bacterial isolates were of the types: *Staphylococcus aureu*s was 3 isolates (3.3%), *Streptococcus species* was 3 isolates (3.3%), *Staphylococcus epidermidis* was 3 isolates (3.3%) and *Pasteurella multocida* was 1 isolate (1.1%).The Chi-sequre 24.75 and the P value 0.001.

Two types of Verminous pneumonia were reported: Chronic purulent & mucopurulent Bronchopneumonia: This was found in 2 infectious cases (3%) of pneumonic cases.This type characterized by mucus exudate with supurative exudate in the bronchi and bronchioles. The most commonly identified parasites in association with

this species were *Dictyocaulus filaria* which was seen alone in one infectious case and seen associated with *Muellerius capillaris* in one other case.The Chi-sequre 0.23 and the P value 0.65.

Catarrhal Bronchopneumonia: this found in 1 infectious cases and constitutes (1.5%) of pneumonic cases. The most commonly identified parasite in association with this species were: *Muellerius capillaris*.& Dictyocaulus.filaria. These two parasites were found in one case.This type showed mucus exudate in the bronchius and bronchioles.

Dedication

To my angel in life .. To the meaning of love and financial sense of compassion and dedication ... To the smile of life and the secret of existence .. My dear father.

To the one who overwhelms me with her obedience and affection ... To the secret of my success in life .. To the most precious of love .... My beloved mother.

To those who accompanied me my life and with you walked the path step by step and still accompany me so far .. To a candle shining illuminates the darkness of my life ... My dear wife.

To my long-time companions and to those who will help me in times of distress .. Dear brothers.

Give this humble gift to you.

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CHAPTER ONE

INTRODUCTION

Livestock consider an important sources of national wealth sources in Iraq, the sheep contribute about 63.7% of the total livestock in Iraq (Ali and AL-Samee,2014). The sheep have high economic value because of their products which is benefits to the humans such as milk, meat and wool, For this reason we can see the important of the livestock and need for their development in Iraq. We should keep this kind of animals because of it is importance in the meat, milk and wool production as well as the industry of leather, where some study have pointed to preserve and set up research centers on the study of economic aspects of livestock. In Iraq the livestock constitutes an integral part of farming system. Traditionally farmers keep livestock to enhance their incomes and their basic food requirements. Sheep,goat and cattle consider most important sources of meat ,milk,wool, skin and hair (Bruere *et al.*,2002).

The pneumonia lead to decrease in the weight, high costs of the treatment and high rate of morbidity and mortality in the sheep. (Saleh *et al.,* 2014).

There are multiple agents may be infectious or non-infectious can affect the lung and cause damage to it and lead to formation of lesions in the lungs, therefor the study of the lung diseases are essential because these disease result in heavy economic losses in the flock of the sheep. Generally, the disease of the respiratory system, mainly pneumonia cause high costs of the treatment, morbidity and high mortality rate in the small ruminants specially sheep (Amaravathi *et al.*,2016).The term Pneumonia refer to lung infection caused by viruses, bacteria, fungi or parasites of the lung and is considered important respiratory disease which characterized by inflammatory reaction in the bronchioles and alveoli of the lung, this inflammation result from

exposure to the infective microorganism's lead to lung tissue consolidation and others lesion, this disease important and common in the sheep flock, it is consider complex disease because multiple causes lead to pneumonia such as host factor(non infectious agent),(immunity and physiology),infectious agent (bacteria, parasite, virus, mycoplasma and fungus) and these agent may be interact with environmental factors. When there are low host defense mechanisms and certain dose of infectious agent the pneumonia will occur (Bruere *et al.*,2002). The pneumonia may be lead to presence of pleurisy if the infection diffused and react the chest cavity lining cause adhesion between lung and pleura that result from infection.(Alley,2002). For this reasons, study of the pathogens (bacteria and parasites) and lesions of this disease in sheep in Diyala province is important, and therefore this study aimed to:

1 . Determine the frequency rate of pneumonia in sheep in Diyala province.

2 . Determine the frequency rate of both types of pneumonia (bacterial and parasitic species).

3 .Study the bacterial causes which associated with pneumonia in sheep.

4 .Study the parasitic causes which associated with pneumonia in sheep.

5.Study the pathological lesions which associated with bacterial and parasitical pneumonia in sheep.

CHAPTER TWO

2.Literatures Review

2.1.Etiology of Pneumonia in sheep:

2.1.1.The Bacterial agents:

The bacterial pneumonia caused by several species. Sawhney,(1959) isolated the *Pasteurella multocida* and *Mannheimia haemolytica* from 11cases and constituted 32% from total bacterial isolates. Ramachandran & Sharma,(1969) exanimated the sheep lungs take from slaughter house, dead animal and found the frequency rate of pneumonia were 11-19% in sheep, the isolated bacteria were *Pasteurella sp.,Staphylococcus sp & Actinomyces pyogenes* and few cases of acute pneumonic lesions reported without any bacteria.

In other study Misra *et al .,(*1970) reported that *Klebsiella sp. ,Escherichia coli ,Corynebacterium sp., Proteus, Salmonella, Staphylococcus & Pseudomonas* while the other researchers reported other bacteria isolated from nose, trachea and lung and these bacteria were *Pasteurella sp., Staphylococcus sp. and E.coli.* and the *Corynebacterium ovis* reported in 30 from 98 sheep lungs (Sarkar & Bhattacharyya,1975).

While Sharma & Dwivedi, (1976) reported the Pseudotuberculosis in lung of sheep and goat and isolated gram positive diphtheroid bacteria in the pathological lesions of it. Ojo, (1976) reported *Mycoplasm& Pasteurella multocida* in infected lung sheep. Other researchers isolated important types of bacteria and these bacteria were *P. multocida, S.pneumoniae, A. pyogenes, S. aureus, K. pneumoniae & E.coli* (Cutlip&laird,1976). Also,through examination of lung of the sheep slaughter reveal the most important bacterial isolations were *P. multocida, S. aureus* and *A. pyogenes*

which present in large numbers with few pathological lesions (Al-sultan,1976).The Maedi disease reported and the caused by Maedi visna virus (Pillae *et al,*.1979) While Banerjee and Gupta, (1979) reported Maedi disease in sheep and goat with detected the causative agent of it. In Nigeria, the tuberculosis recorded in 3 cases of the 15 sheep lungs (Alonge&Fassnmi,1979). the maedi viasina virus were isolated from sheep cases infected with pneumonia (pepin et al .,1998). But the Kimberling et al. (1998) recorded four isolates of the Parainfluenza type 3 virus and mentioned these virus associated with interstitial pneumonia together with the adenovirus isolation from the animals suffer from sever pneumonia.

Andrews *et al.*,(1985) were isolated *Haemophilus somnus* and reported the fibrinous pneumonia caused by *Pasteurella multocida*. In animals the respiratory diseases caused by many isolates, these isolates such as mainly *Pasturella multocida, Mannheimia haemolytica, Staphylococcus aureus. Klebsiella species. Mycoplasma ovipneumonia, Streptococcus spp.* and *Chlamydia* (Jubb *et al.*,1993). The important cause which are *Mycobacterium species. and Pasteurella species*. These previous isolates affecting the lung by several forms,the *Pasteurella spp.* lead to fibrinous pneumonia in most cases while the *Staphylococcus aureus* and *Streptococcus spp*. lead to lung Abscess. Also, the granulomatous reactions result from many isolates such as *Mycobacterium spp*., other histological types of pneumonia such as Interstitial pneumonia and bronchopneumonia (Haziroglu *et al.*,1994) Sheep pneumonia which occurs due to bacterial agents considered most common between the types of the pneumonia and caused by multiple species of bacteria, these bacteria were *Pasteurella multocida ,Mannheimia haemolytica , Actinomyces pyogens, E coli, Klebsiella species, Streptococcus species* and *Staphylococcus species* (Bancroft *et al.*,1996).

Other researchers isolated the *Pasteurella species* and *Mannheimia haemolytica* from the lung of the sheep and also isolated the *E coli, Streptococcus spp, Actinomyces pyogens* and *Staphylococcus spp*. from the lung infected with the pneumonia in sheep and goat (Brogden *et al.*,1998).

There are some of the bacterial species isolated from experimental and natural cases belong to sheep pneumonia and these isolated are *Proteus spp., Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus spp.* and *Pasteurella multocida* (Mukasa *et al.,* 2000).Four sheep cases with pulmonary adenomatosis and 1 sheep case with lung tumor that examined by polymerase chain reaction (PCR) and mentioned these lesion caused by jaagsiekte retrovirus (García-Got *et al .,* 2000).

In other study Tibbo *et al.*,(2001) isolated the *Streptococcus specious* from 17 case of the 74 total infected lung of sheep and recorded it as lung abscess cases. Other workers noted the relationships between *Pasteurella multocida* and *Mannheimia haemolytica* in inducing fibrinous pneumonia in the sheep and goat and mentioned in their study the *E coli* and *Actinomyces pyogenes* are common species isolated from animals and the less commonly bacterial isolates are *Proteus spp., Klebsiella spp., Shigella species* and *Staphylococcus aureus* (Douart,2002).While the other researchers isolated the *Streptococcus species* from 8 lamb dead cases in additions to the *Pasteurella multocida* (Alley,2002).

In expansions bacteriological study of the sheep pneumonic lesions the researchers found more than 20 pathogenic bacteria species, among these bacteria were *Salmonella , Staphylococcus spp. ,Pseudomonas aeruginosa, Proteus* and *Klebsiella species*. (Weiser *et al .*, 2003). Some researchers recorded the sheep pneumonia and isolated from it the *Klebsiella pneumoniae, Streptococcus species, E. coli* and

Pseudomonas aeruginosa (Ayelet *et al.*, 2004). The others reported Parainfluenza type 3 virus were isolated from infected lambs by immunohistochemistry technique (Grubor, 2004).

(Alton *et al* .,2006) numerous studies have been attempted to explain isolation of the *Haemophilus somnus* in pneumonic lung of dead sheep by immunohistochemistry study. (Daniel *et al.*,2006) Several studies investigating the *Klebsiella pneumoniae, Pseudomonas spp, Pasteurella multocida, E coli* and *Streptococcus spp*. in the lung of the sheep and observed formation of the nodule, small in the size in the lung of sheep caused by *Klebsiella pneumonia* with the formation of the abscess which occur due to *Streptococcus spp*.. Yimer and Asseged, (2007) isolated from the 192 swabs taking from different part of the respiratory tract of the normal sheep were *E.coli* more than (14%), *Staphylococcus epidermidis* less than (10%) and the *klebsiella species* (3%).

Bells,(2008) found the *Mycoplasma ovipneumonae* with the interstitial pneumonia and the *Pasteurella spp*. with the fibrinous pneumonia in addition to the *Mannheimia haemolytica* which responsible of the outbreak in the lambs. In an expansion study of bacterial pneumonia in sheep isolated several types of bacterial agents have high pathological evaluation and these isolation are *Staphylococcus epidermidis, Proteus vuligaris* and *Citrobacter spp*. which represent (9%) from the total isolation while the *Staphylococcus aureus, Pasteurella multocida* and *Streptococcus spp*. represent about (53%) of the total cases (Garedew *et al* ., 2010).Other researchers investigated the pneumonia responsible for the more economical losses in the sheep flock and in the other side the pneumonia considered the important causes of the death in the lamb (Cynthia and Scott,2010).

Other researchers show the dangerous of *Mannheimia haemolytica* because of these pathogenic agent lead to septicemia in the sheep and in similar study the researchers reported Tuberculosis in the sheep caused by *Mycobcterium avium* (Marianelli *et al* ., 2010)**.** Other study on group of the affected sheep , the results revealed isolation of the *Mycoplasma ovipneumoniae* and found it associated with suppurative , interstitial and fibrinous pneumonia ,while In the Iran there are numbers of authors isolation the *Staphylococcus aureus, Corynebacterium peudotuberculosis* and *Actinomyces pyogens* in slaughtered sheep infected with pneumonia at the Tabriz slaughter house (Azizi *et al.*,2011).

Omer *et al.,(*2012) isolated the *E. coli, Staphylococcus aureus, Corynebacterium pseudotuberculosis* and *Staphylococcus epidermidis* from the respiratory tract of the healthy lamb and also they isolated the fallowing pathogenic bacteria (*Pasteurella multocida* and *Mannheimia haemolytica*) from infected sheep and lamb with pneumonia in Ethiopia. In 2012 there are several studies recording occurrence outbreak in the sheep infected with pneumonia at western United states result from infected of the sheep with *Mycoplasma ovipneumonia* and *Mannheimia haemolytica*, Pseudotuberculosis in the lung of sheep and goat were recorded and caused by *Corynebacterium pseudotuberculosis* which isolated in the lesions of the lungs (Besser *et al* .,2012).

Emikpe *et al.,* (2013) were mentiod the influenza virus lead to pneumonia and activation of the secondary bacterial disease of the respiratory tract**.** Azizi *et al.,* (2013) were isolated others species of the bacteria from the animal without any abnormal signs in which are *Pasteurella multocida, E. coli, Staphylococcus epidermidis* and *Neisseria*. Other researchers isolated the *Staphylococcus aureus, Klebsiella pneumonae* and considered the important bacterial agents which

responsible of supurative pneumonia (Saleh, *et al.*,2014).While the Sawa,(2014) observed the Pseudomonas aeruginosa causing lung pneumonia through culture of the bacteria. Other study, the researchers recorded (9) cases of bronchopneumonia in the sheep and isolated from it *Mycoplasma ovipneumoniae*, *Pasteurella multocida* and mentioned the difference of the rate of infections ,the *Pasteurella multocida* have more than half cases and the *Mycoplasma ovipneumoniae* less than (40%), (Oruc,2015).

Asaye *et al.*,(2015) show the high costs of the treatment in addition to the more economic losses due to decrease in the production as a one sequel of bacterial pneumonia. Pawaiya,(2015) exanimated normal slaughtered lambs in the abattoir and he isolated Zygomycetes spp. from pneumonic lungs. Whereas AL-Anbagi *et al.*,(2016) reported *Pasteurella multocida* and the *Mycoplasma ovipneumonia* were more common isolates in the lung of the sheep. Rare occurrence of the Tuberculosis in the sheep and have same pathological lesions to bovine tuberculosis was caused by *Mycobacterium bovis* (Amaravath *et al.*,2016).

Other studies describing the bacterial agents when exanimated 42 sheep and the isolations of bacteria were *Proteus spp., Pseudomonase aeruginosa* from the nose of the sheep and these researchers also mentions the bacterial isolates in the lung which are the *Staphylococcus species* represent in (32%) while the *Pasteurella multocida* (15%) and recording the high morbidity and mortality rate in the sheep due to respiratory distress and pulmonary lesions (Azizi *et al.*,2016).While Sirlatha *et al.*,(2016) reported one case of pulmonary TB in the sheep. Other agents lead to pneumonia in sheep such as the viruses,also in the random study the maedi disease was diagnostic by Pall,(2016) in the one case suffered from the pneumonia .

2.1.2. Verminous pneumonia:

Gerichter, (1951) reported five types of the lung worms in the sheep and goat and these worms were *Dictyocaulus filaria, Muellerius capillaris, Cystocaulus ocreatus, Neostrongylus linearis & Protostrongylus rufescens* while the Leiper, (1957) reported the *Dictyocaulus filarial* in the Iraqi sheep and consider this parasite common in Iraq. The Nieberle & Cohrs,(1966) reported the hydatid cysts in the lung sheep. In the india the Ramachandran, (1967) reported the *P.rufesccens &D.filaria* and not reported any case with *M.capillaris* in the India.

While in USA the Wilson,(1970) mentioned the goat have ability to infected with *D.filaria* more than the sheep. Whereas in Iraq (Altaif 1970; Kadhim 1974) reported *D.filaria, P.rufescens, C.ocreatus & M.capillaris* in the sheep in Iraq and mentioned the first type of this worms was more common in period from December to March. Also other types of parasites were as aberrant parasitism and causing infections including *Schistosmoa species, Fasciola gigantica*. In addition, to the cestodes intermediate stages such as hydatid cyst (Hussein and Haroun,1976) The parasites cause high economic losses in the sheep, some of parasites inhabit the lungs and lead to parasitic or verminous pneumonia while the others worms causing damage to the lung through the life cycle (Mitchell, 1980).

In USA, slaughtered sheep in the abattoir reveal more than 52% of the lesion belong to the lung worms and about (8%) associated with hydatid cysts and in the Jordan the *E. granulosis* occupy the lung of the sheep ,goat and cattle (AL-Yaman *et al.*, 1985) Morris *et al.*,(1985) give treatment of Abendazol to group of the sheep's suffer from pulmonary cyst caused by *Echinococcus granulosus*. Alousi *et al.*,(1986) recorded the rate of the lung worm in the Iraq were more than (52%) and reported only the *Muellarius sp.* and *Dictyocaulus sp*. In morocco, examination of the 612 of the sheep

lung with verminous pneumonia show the larval stage of the *Dictyocaulus filaria , E. granulosis ,Protostrongylus rufescence* and *Muellerius capillaries* (Boljihad *et al* ,.1995). Whereas Yoshihara,(1998) recorded presence of the *Fasciola gigantica* in the lung of sheep during experimental infection and this study mentioned the most common causes of the verminous pneumonia is the lung worm. The high rate of frequency of the infection with the lung worm because of the large number of herbal areas (Ayelet *et al* .,2004) after one year and in the other area recorded the economic losses which result from respiratory distress and mentions the influence of the worm on the bronchus and bronchiole by excessive mucous production that lead to obstruction of the air ways and catarrhal bronchopneumonia is common type in these sheep (Kahn, 2005).

Gelagay *et a*l.,(2005) and mentioned the lung worm lead to pathological change and suppresser of the immunity system result in bronchitis and pneumonia. In the Turkish, there are other studies to determine presence of the lung worms in the sheep and reported *Cystocaulus ocreatus* in the (50%) of the lung which exanimated and mentioned *Cystocaulus ocreatus* responsible for warm nodules in the lung in addition to that the *Dictyocaulus filaria* which represented more than (23%) of the total lung worms (Yildiz, 2006).

More studies mentions the *Dictycoculus filaria* occupying the first stage between other parasites in the inducing infection such as the study of the Alemu, (2006) that showing *Dictycoculus filaria* has high incidence in the infected of the lung than *Muellerius capillaris* and *Protostrongylus rufescens*, while in the other next study reported only the *Protostrongylus rufescens* and *muellerius capillaris* in the sheep (Abebe,2008). Hassan, (2009) reported the infection of the sheep with *Protostrongylus rufescens* only.However, Jenkins *et al.*,(2007) described the

pulmonary lesion as a multiple nodules diffused at the surface of the lung and mentions the causes of these lesion were *Protosterngylus rufescens*. same study the researchers recorded nodular lesions (1-4) cm in the diameters in the caudal lobe of the lung sheep caused by *Neostrongylus linearis*, Inhalation pneumonia caused by inhalation of the foreign materials and mentioned The *Dictyocaulus filaria* causes nodular lesion and pneumonia (Bell,2008). Seven cases of pulmonary hydatid cyst caused by *Echinococcus granulosus* found in the lung (Barnes *et al* ., 2011).

Some studies revealed the ratio of the prevalence of the lung worm examine the 390 fecal sample for searching of the lung worm larvae , the *Dictyocaulus flaria* represent about (55.1%) and *Muellerius capillaries* about (25.75%) and (18.9%) with mixed infection of the two parasites (Moges *et a* ., 2011). Other study on the pneumonia in the India which recorded the causes of it and revealed the high level incidence of the verminous pneumonia in (72) cases from the (104) total cases of the sheep pneumonia due to *Dictyocaulus flaria, Muellerius capillaries and Protostrongylus rufescens* (Addis *et al* ., 2011).

Schock *et al*.,(2011) recorded *Sarcocystis tenella* in one dead sheep and considered it the primary causes of the pulmonary lesions and mention that this case only infected with *Sarcocystis tenella* from the 157 total pneumonic case. Some authors said that untreated area responsible for high incidence of the pneumonia in contrast treated area (Weldesenebet and Mohamed,2012). Other parasites associated with the pulmonary lesions such as Hyadatid cysts but the major causes of the verminous pneumonia caused by several types of the lung worms, these worms were *Dictyocaulus filaria* , *Protostrongylus rufescens* and *Muellerius capillaries* (Eyob and Matios, 2013). Similar findings , 54% of the mortality and morbidity of the sheep in Ethiopia due to bronchopneumonia caused by lung worms (Yagoob *et al* ., 2014).

The data from the different studies revealed unequal infection ratio between the different parasitic lung worm, this supporting by study of (Chanie *et al.,* 2014) who were recorded the rate of the infection with the verminous pneumonia, the *Dictyocaulus filaria* approximately (50%), *Muellerius capillaris* more than (2.3%) and *Protostrongylus rufescens* about (1.5%).The *Dictyocaulus flaria* caused catarrhal bronchitis in the bronchus and bronchioles at the necropsy finding of the sheep lungs (Tewodros, 2015).

The lung worm lead to inhibition of the immunity of the respiratory system and result in bronchitis or pneumonia, study the pathological effect of the pneumonia in sheep considered the Verminous pneumonia as chronic disease effected on the respiratory tract of the animal due to infected with the worm and lead to high damages in the respiratory tract of the animal (Tegegne *et al.*,2015).Other authors reported the prevalence of the verminous pneumonia more than the bacterial pneumonia according to the data analysis recorded by Adem, (2016).

2.2. Types of pneumonia, gross pathological changes:

Georgsson *et al.*, (1990) classified the sheep pneumonia in to the three types:

1.Purulent pneumonia: characterized by different degree of consolidation and the color of the effected lungs vary from bluish red to gryish white and also showed granular appearance of lesions.

2.Hemorrhagic pneumonia: the lung characterized by pale or dark red color, hardness with patchy destitution in the apical lobes.

3. Interstitial pneumonia: this type shows blue to red color .

In addition to that similar authors described the pneumonitis which characterized by congestion and edema (Georgsson *et al.*,1990).

Jubb *et al*.,(1993) reported 42 lung abscess which caused by *Staphylococcus aureus* and *Klebsiella pneumonia* and noted multiple abscessation focal and multifocal with the deferent sizes ,thickening of interlobular septa in additions to the pleural adhesions.

Whereas oruc, (2003) reported other types of pneumonia such as:

1.Bronchopneumonia: characterized by congestion, edema, and hepatizition with purulent exudate in the bronchus and bronchioles .

2.Fibrinous pneumonia: this type show consolidations, congestions, edema with presence of the fibrin on the lung surface.

Daniel *et al.*,(2006) described the gross lesions of Maedi disease and characterized by granular lesions located in the apical lobe in which white in color exudate, thickens of the bronchioles and containing pus.

The Goodwin, (2006) mentioned several types of the ovine pneumonia

1.Supurative pneumonia : this type showed hard abscess, large in size in the apical lobe.

2.Mycotic pneumonia : characterized by presence of the pseudomembranous flakes in the bronchioles and consolidations of the pleura, in additions to that there is presence of the small nodules under pleura in all the lobes of the lungs ,also edema ,congestion ,Emphysema and Atelectasi.

3.Foreign body type pneumonia: this type characterized by presence of the fluids in the bronchus and bronchioles.

4.Mycoplasma pneumonia : characterized by presence of consolidation area with dark red color, hard in consistency in addition to the presence of hemorrhagic infarctions mostly in the left diaphragmatic lobe .

5.Giant cell pneumonia: this type show solid focal dark red in color of the consolidation, collapse,emphysema and the lesions contribute in the right lobe more than left lobe while the other lobes equally distributed lesions.

6.Fibrinous pneumonia: there was presence of marble appearance on pleura , both pleura and pericardium covered by fibrin.

Griffiths *et al.*, (2010) mentioned multiple of the pneumonic lesions;

1.Granulomatous pneumonia: it represent about (1%) of the affected lung, The gross appearance characterized by presence of the nodule which variable in size in the affected portion, these nodules solid, multiple, multifocal and contain caseated center white in color surrounded by area of hemorrhage .

2.Parasitic pneumonia: The gross appearance characterized by presence of cyst in the lung inflammatory cell accompanied by infiltration ,inflammation of the bronchus and presence of bronchiectasis.

Dar *et al.*, (2012) recorded the types of pneumonia lesions in 203 cases in sheep:

1.Acute suppurative bronchopneumonia: The gross lesion show consolidation ,diffuse areas (red to gray in color) and presence of the mucopurulent to purulent exudation in the bronchi and bronchioles.

2.Acute fibrinous pneumonia: The microscopical appearance show thickening of the pleura and presence of the fibrin in the interlobular septa.

3.Sub-acute bronchopneumonia: The gross appearance showed presence of the multiple consolidation containing pus exudate.

4.Chronic bronchopneumonia:The gross appearance characterized by pale or gray color of the affected site, adhesion of the pleura, hard with shrinking surface.

5.Acute interstitial pneumonia: The gross appearance show gray color of the affected site ,firm and rubbery ,in the cut section there is foamy exudate in the bronchus and bronchioles.

6.Chronic interstitial pneumonia: Similar to the acute interstitial pneumonia and in this type there is fibrosis in the affected site.

7.Embolic pneumonia: This type characterized by presence of nodules red in color and contain necrotic area which distrubuted in the parenchyma, the necrotic site surrounded by zone of the inflammation

8.Adenomatosis : grossly the adenomatosis show focal gray nodules , in some cases show diffuse consolidation of parenchyma of the lungs, these nodules vary from 1 to 1.5 cm diameter and embedding in the parenchyma, when make cut sections there is presence of creamy exudate from the nodule.

While in anthor study the Azizi *et al.*,(2013) reported other types of pneumonia through the examination of 257 lungs of sheep in Tabreiz in Iran and observed :

1.Acute bronchopneumonia: The gross appearance characterized by consolidation and hepatization area that reddish brown or grayish in color ,yellow fluids exudate in the

bronchus and bronchioles .The diaphragmatic lobes affected mainly in the right lung. the cut section reveal oozing of the yellow color fluid from bronchus and bronchioles.

2.Fibrinous bronchopneumonia: The gross appearance show area of consolidation in the cardiac lobe, apical lobe and in the diaphragmatic lobe. The consolidation was firm on cut section. The pleura also affected.

3.Suppurative bronchopneumonia:The gross lesions are reddish to gray lungs , hardenss in consistency ,the bronchi and bronchiole contain suppurative exudate,small lung abscess in the lung parenchyma.

kumar *et al*., (2014) observed many types of the pneumonia through examination (261) sheep with pneumonia from 740 sheep cases and recorded :

1:Acute catarrhal bronchopneumonia: grossly show congestion and consolidation regions in the anterior lobe .

2 .mucopurulent bronchopneumonia: The gross appearance show Several focus with the serious degree of the consolidation regions in the anterior lobe of the lung ,presence of the purulent and mucous exudation when cutting of the lung surface.

3.Purulent necrotic bronchopneumonia: The gross appearance show more area with necrosis.

Oruc *et al*.,(2015) described the pulmonary abscess, vary from two to ten cm in the diameter and occurred as single or multiple in the pulmonary lobes ,the abscess containing pus in which white to yellow in color, odorless, in the chronic cases the abscess surrounding with the fibrous tissue.

Mahdi *et al.*,(2015) done study and recording the other types of the pneumonia through study of this disease in the lamb and mentioned the gross lesion of it :

1.Chronic non progressive pneumonia: This refer to multiple types of the pneumonic lesions , the important one are gray to red brown color at the apical lobes.

2 .Ovine progressive pneumonia: caused by lentivirus, the lungs become gray to blue in color, firm and large in the size.

Ovine adeno virus lesions also observed according to the (Griffiths *et al.*,2010) thought the study of the disease in the sheep, grossly the ovine adenocarcinoma show irregular enlargement of the lobe of the lung, this is enlargement result from tumor ,the affected part hard, dark in color than normal, the distribution differ from case to case.

In case of the verminous pneumonia, the gross pathological lesions that recorded in the sheep are sub pleural nodule and sometime surrounded by calcification (Teffera, 1993).These nodule vary from 1 to 10 cm diameter ,harden with the protrusion from the lung surface and the workers recorded that the causes of the presence of the nodules in the subpleural area and rare in the lung parenchyma belong to the good ventilations in sub pleural regions in contrast to parenchymal regions (Kassai , 1998) Other workers mentioned the causes of these nodules due to impairment of host immunity against the parasite (McGavin and Zachary, 2007).

Other workers reported the edema and inflations of the lung due to *Muellerius capillaria,* and *Dictyocaulus filaira* whereas other workers recorded presence of the lung worm in the bronchus that belong to the *Dictyocaulus filaira* (Mengestom, 2008)' (Kassahun and Mersha ,2013).

2.3. Types of pneumonia with Histopathological changes:

Hazairogglu *et al.*,(1994) observed the histopathlogical lesions of some type of the sheep pneumonia:

1.Supurative pneumonia: This type characterized by presence of the inflammatory cells specially neutrophils and inflammatory exudates in the bronchus and bronchioles and in the alveolar spaces, depositions of fibrin with the debris, in the affected area there is very sever congestion result from bronchus and bronchioles obstruction in additions to the emphysema and atelectasis.

2.Fibrinous bronchopneumonia: This type reveal congestion of capillaries with multifocal necrotic areas . In the alveolar lumen and bronchioles,there is fibrinous exudate, pleural and interlobular septal thickening result from edema and inflammatory cells .

3.Granulomatous pneumonia: This type characterized by presence of the nodules which contain area of the necrosis in the center and surrounded it the regular zone of the layers of the inflammatory cells with the fibrous tissue .

4.Parasitic pneumonia: There is mononuclear cells infiltrations in the lung in additions to bronchitis and inflammatory reactions lead to bronchiectasis.

In the other study Ours,(1997) described the other types of the pneumonia and mentioned the histopathological lesions of it :

1.Acute supurative bronchopneumonia : there is thickening of the pleura , congestion and neutrophils infiltrations lead to prominent of interlobular septa, presence of the macrophage and neutrophils in the alveoli, in severe cases the bacterial colonies seen

in alveolar lumen, neutrophils, macrophages and others inflammatory cells in the bronchus and bronchioles lumen in addition to the necrotic material.

2.Acute fibrinous pneumonia: in acute cases the interlobular septa destinations with the fibrin and thickenings of the pleura, there is fibrin and presence of the inflammatory cells (neutrophils), fibrinous exudate fill the alveoli with multifocal necrotic area.

3.Intersticial pneumonia: This type show proliferations of the fibroblasts which result in interlobular septa thickenings , presence of macrophages and edematous fluid in lumen of alveoli together with type two pneumocyte cells proliferation in the alveolar wall. in one case there is fibrosis in parenchyma of the lung . epithelial hyperplasia in bronchi and bronchioles. peribronchial and peribronchiolar lymphoid aggregations .

4.Acute interstitial pneumonia: The histopathology of this type show cuboidal cell proliferation which simulating the type 2 cell morphology, infiltrations of the plasma cells , lymphocytes result in interstitium thickening . Also the pleural necrosis was evident in one case.

5.Chronic interstitial pneumonia: There is little fibrosis, losing of the structure of the normal epithelia with the presence of the mononuclear cells.

6.Broncho-interstitial pneumonia : fibrin deposition lead to thickening of the pleura in addition to lymphocytic infiltrations. neutrophils and fibrin in the alveoli. Peribronchial lymphocytic hyperplasia. Neutrophils and necrotic material in the lumen of the air ways . bronchitis and bronchiolitis also seen .

7.Embolic pneumonia: this type showed inflammatory zone surrounded the multifocal necrotic areas. congestion and neutrophils with lymphocytic infiltrations . degenerated

cells present in the necrotic area and in the center of these necrosis there are colonies of the bacteria .

In additions, to these previous types of pneumonia, the researchers reveal the histopatholgy of the adenomatosis:

The wall of the alveoli showed single and sometime multiple layers of the cuboidal to columnar epithelia which giving appearance of the adenomatosis, if consolidation present there are adhesions of the pleura and pleuritis. structure like projection of papillae with the central core of the connective tissue protrude in to the space of the alveoli. In additions to that there is emphysema which characterized by distended of the alveoli, rupture of interalevolar septa which result in giant alveoli in addition to the hyperplasia of the bronchus and bronchioles .While the atelectasis show depressed area from the lung ,reddish or discolored than normal lung.

Kimberling *et al.*,(1998) reported in their study other histological pneumonic type and mentioned the lesions of it :

1.Acute bronchopneumonia: this type present in (49%) and the histopathological lesions characterized by the bronchiolar lumen contain serous exudate, arounded the bronchial and bronchiolar there are infiltrations of the neutrophils which much more than in the alveolar – bronchial Lumena. Alveolar septal cells proliferation result in interaleveolar septa thickening . lumen of alveoli filled with neutrophils and few mononuclear inflammatory cells .

2.Supurative bronchopneumonia: in the lung parenchyma,there is focal area of suppuration contain caseo-necrotic site and in the a round of it there is pyogenic

reaction composed of membrane including polymorpho- nuclear cells infiltration with mononuclear cells . purulent exudate present in the bronchial and bronchioles lumen.

3.Interstitial pneumonia: This type reveal bronchial and alveolar cells proliferations and desquamation . thickening of the interalveolar septae and neutrophils , lymphocytes and macrophages infilteration. Also, there is hyperplasia of peribronchiolar lymphoid tissue.

In the other study the Oruc,(2006) reported other type of the sheep pneumonia and descriptions the pathological lesions:

1.Acute catarrhal bronchopneumonia: represent (18%) from the total cases, the lesions is congestion of the alveolar capillary with few neutrophils in the lumen of bronchi and bronchioles and edema .

2.Mucopurulent bronchopneumonia: There is neutrophils present in the mucous exudate in the alveolar and bronchial , bronchioles lumina. the alveoli show alveolar macrophage proliferation and desquamation of the epithelium of the bronchioles .

3.Purulent necrotic bronchopneumonia: this type reveal more necrotic area and neutrophils . bronchi and bronchiole contain sever desquamation in the epithelia.

Mcgavin and Zachary,(2007) recorded other types of the pneumonia in lambs through study of 325 sheep and these types are :

1.Hemorrhagic pneumonia: this type characterized by collections of the red blood cells and mononuclear cells in the alveoli in additions to present of the degeneration of the RBC.

2.Pneumonitis: this type show congestion and hemorrhage in the tissue between the lobules and alveoli .

3.Verminous pneumonia: characterized by presence of the nodule containing eosinophil's and also presence of the parasite .

4.Proliferative pneumonia: this type including the lesions of pulmonary of adenomatosis and the lesions similar to the lesions of the maedi disease.

According to the Habtamu *et al.*,(2010) were reported the lesions caused by *Corynebacterium ovis* showed fibrinous pleuritis and bronchopneumonia and collapse, exudate with mononuclear cells and micro-abscess in the pulmonary paranchyma.

In the other study Dar,(2012) recorded the microscopic lesion of the fallowing types of pneumonia :

1.Suppurative bronchopneumonia: This type show supurative exudate in the bronchi and bronchioles with infiltrations of the neutrophils and plasma cell. Congestion of alveolar capillaries and present of necrosis in some area.

2.Giant cell pneumonia: Characterized by presence of the large number ofgiant cells and macrophages in the alveoli, esinophilc inclusions body in the nucleus and cytoplasm. there is thickness of the alveolar wall due to fibrin . edema and congestion.

3.Mycoplasma pneumonia: This type reveal thickening of the alveolar walls result from mononuclear cells and fibrin in additions to protein materials and macrophages in alveoli and presence of hemorrhagic infarction and venous thrombi contain fibrin and WBC.

In addition, to the previous types, similar workers noted presence of pulmonary nodules may be caused by parasite consist of scar tissue and caseous necrosis. Also mentioned the response of the body against foreign materials in the bronchioles and noted presence of the congestion ,edema , hemorrhage and atelectasis.

While the histopathological lesions which resulted from verminous pneumonia described by Emikpe,(2013) the common lesions associated with the lung worms present in the pulmonary alveoli which have nodular form and the center of these nodules contain eosinophil's surrounded the parasite which dead or calcified in the center necrotic area. out these centers, there are two layers, inner layer cellular and contain macrophages eosinophil's and lymphocytes. While the outer layer consist of capsule with the fibroblast proliferation.

Kumar,(2014) mentioned the lesions which associated with the verminous pneumonia:

1.Sub-acute catarrhal pneumonia : characterized by proliferations of the mononuclear cells and sloughing , degeneration and hyperplasia of the epithelium lined the bronchioles with much more production of the mucous together with lymphocytic proliferation a round the bronchioles. the worm present in the bronchioles while the eggs and larvae present in the alveoli seen mixed with mononuclear cells infiltration, and degeneration of alveolar septae.

2.Sub-acute bronchopneumonia: there is hyperplasia of mucous gland a rounded the bronchus .Infiltrations of the lymphocytes and plasma cells in the inter alveolar septa and sub pleural area.

3.Sub-acute catarrhal bronchopneumonia: in additions to the signs of catarrhal bronchitis the researchers noted in this type the coagulative necrosis, degeneration of epithelial cells of the bronchus and neutrophils infiltrations. There is hypertrophy in the smooth muscle of the bronchus and fibrin in the inter alveolar septa.

Other parasitic lesions showed *Dictyocaulus filaria* and *protostrongylus species* found with mucous in the lumen of the bronchioles and the eggs and larvae found in the alveoli .There is increase in the thickness of interstitial tissue which result from infiltrations of mononuclear cells, proliferation of fibrin .hyperplasia of lymphoid tissue around the bronchioles.(Mahdi ,2015) .

CHAPTER THREE

Material and methods

3.1.Materials

3.1.1.Gram stain:

We take sterile normal saline drop on the clean slide, take small colony from the culture on the slide gently , spread of the film and leave until dry by air in the atmosphere .fixing the slide which containing colony by passing over the flame quickly for three times, put slide on the staining rack and staining the slide with the crystal violate for 1 minute ,the stain wished with the tape water , after that put on the slide gram iodine for 1 minute. wash the slide and add the ethanol as decolorization for 30 seconds , wash the slide and add the carbol fucsin for one minute and washing the film and dried and then exanimated under lens of oil immersion (seif eldin and Alzarok,1996).

3.1.2.Culture media:

3.1.2.1.Blood agar:

In 1 litters of distilled water , added 37.5 grams of blood agar base ,then leave the suspension until boiling for dissolving agar completely , and then sterilized by Autoclave (121℃ under 15 lbs pressure for 15 minutes), after that cooling the solution from 45 to 50 ℃ , then adding the sterile sheep blood to the cooling solution about (7%), finally the prepared media poured in to sterile petridishes (15 ml for each one),(Murray *et a*l.,2003) .

3.1.2.2 MacConky agar:

Fifty two grams of this media added to one litter of the distilled water then boiling to dissolved completely , after that sterilizing by the Autoclaving (121℃

under 15 lbs pressure for 15 minutes), after that cooling the solution from 45 to 50 °C and poured to the sterile petridishes (Anderson and Cindy,2013).

3.1.2.3.Nutrient agar :

Nutrient agar base 42 grams added to 1 L of distal water , boiling until completely dissolved and then sterilized by Autoclaving (121°C under 15 lbs pressure for 15 minutes) , after that cooling and poured to sterile petridishes (Downes,F and Ito,K,2001).

3.1.2.4.Eosin methylene blue agar :

Suspend 37.5 grams of the medium in 1 liter of distilled water, boiling until completely dissolved the medium and then Autoclave at 121°C for 15 minutes , after that leaving until cooling and poured in to sterile petri dishes(Bachoon,D and Wendy, A,2008).

3.1.2.5.Mannitol Salt agar :

One hundred eleven grams suspended in to the one litter of the DW ,then boiling until completely dissolved , sterilized by autoclave (121°C under 15 lbs pressure for 15 minutes), after that cooling and poured in sterile petri dishes (Anderson,2013).

3.1.3.Biochemical tests :

3.1.3.1.Catalase test :

Three drops of 3% hydrogen peroxide on clean slide ,the suspected colonies placed on these drops , if gas bubble produced the result is (+) (Heit,C *et a*l.,2017).

3.1.3.2.Oxidase test :

Soaked of filter paper in 1% of oxidase test solution . then the colonies placed on the filter paper . the developed of the pink color within 5-10 minutes ,indicate positive reaction (Isenberg,2004).

3.1.3.3.Slide coagulase test:

Take drop of the normal saline and placed on the clean slide ,then added small amount of culture to the slide drop. After that added few drops of rabbit plasma to suspension . clumping of organism during 10 to 15 minutes means positive reaction(Heit,*C et al*.,2017).

3.1.3.4.Nitrate reduction test :

Tack the colonies by sterile loop and put in tube test which containing nitrate , put the test tube in the incubator at 37 ℃ for two days. after that the developed of red color means the positive test and the organisms convert the nitrate to nitrite (Williams & Wilkins,C,1967).

3.1.3.5.Indol test:

Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth, incubate at 37°C for 24-28 hours in ambient air, add 0.5 ml of Kovac's reagent to the broth culture, Positive: Pink colored rink after addition of appropriate reagent,Negative: No color change even after the addition of appropriate reagent. e.g. Klebsiella pneumoniae (Laine,L et al .,2005).

3.1.3.6.Methyle Red-Voges Proskauer (MR-VP) Broth:

Take bacteria colonies in the sterile tube containing broth , incubate for 24-48 hr ,then adding the (MR-VP) regent and reading the result (MacFaddin,1980).

3.1.3.7.Nutrient broth :

seven grams of the Nutrient broth added to 1 litters of the distilled water and for well dissolving shack the solution , after put the solutions for five ml in each tube and then sterilized by Autoclaving (121 ℃ under 15 lbs pressure for 15 minutes),(Williams & Wilkins,C,1967).

3.1.3.8.Simmons citrate :

Twenty four g of the simmons citrate broth suspended in the 1000 ml of DW and boiling until dissolving the solutions completely .Sterilization by Autoclave (121 °C under 15 lbs pressure for 15 minutes),and poured in tube until using (Brown and Smith,,2015).

3.2.Methods

3.2.1.Samples Collection:

One thousand lungs of the slaughtered sheep were examined (Khanaqin slaughterhouse, 900 sheep's and others 100 lungs from the others different sites) at Diyala Province. A total of 67 samples with pneumonic lesions were collected during November 2016 to March 2017. Sixty four lung lesions for bacteriological examinations and 86 lesions for histopathological examination.

3.2.2.The Sampling :

3.2.3.Bacteriological examination methods:

The affected samples after opening the diaphragm and exposure of the rib cage , inspected of the lungs and exanimated grossly and recorded any lesions. The pneumonic lungs send for bacteriological examination for this reason, pieces of lesions taken in sterile petri dish after cut part of affected portion using sterile scalpel blade ,scissor and Alcohol (70%) and using iodine. For bacteriological examination samples from affected portion were taken and growing in the culture media (Blood and MacConcky agar) and were incubated at 37 C for 24 – 48 hours ,the colonies were exanimated and recorded after that study the descriptions of bacterial colonies such as color, size, ,blood hemolysis and then staining the bacteria with the gram stain for each type of the colonies . The following step sub culturing of the individuals

colonies on the special media (each bacteria has specific media for growth). Finally the biochemical tests were done and recorded isolated bacteria.

3.2.4.Parasite examination methods:

For parasitic examination, the air ways, trachea, bronchus and bronchioles were opened using sterile scissors and examined the lung worms, if parasite present samples were collected and stained with lactophenol and then study the shape of the adult worm and the color in addition to the location of it. In case of presence the nodules, these nodules opened and observed the larvae and eggs of parasites by direct swab from nodule.

3.2.5.Histopathological examination:

Samples were collected and immediately sent for histopathological examination, these samples were cut to ($1mm^3$) and then preserved in (10%) neutral buffer formalin solution (Bancroft , 1996).

3.3.Identification of bacteria:

3.3.1.*Staphylococcus aureus*:

Gram positive cocci bacteria, culture on the mannitol salt agar and fermented of it. (Yellow colonies) also used nutrient agar.

The *Staphylococcus aureus* was positive for (catalase,coagulase,simmon citrate,methyl red test,nitrate reduction test and Voges Proskauer test) and negative for (motility,oxidase).

3.3.2.*Streptococcus species*:

Gram positive cocci growth on the blood agar give partial hemolysis(alpha hemolysis)or compelet hemolysis (beta hemolysis).

Streptococcus species is negative for catalase .

3.3.3.*Pasteurella meltocida*:

Gram-negative, non-motile coccobacilli or rods, Colonies of *Pasteurella multocida* on blood agar. Cultivation 24 hours, 37°C. *P. multocida* colonies on 24-h blood agar plates are 1 to 3 mm in diameter and nonhemolytic with mousy oder.

Pasteurella multocida was positive for (oxidase, catalase,indol) and negative for (simmon Citrate ,methyl red and Voges-Proskauer test).

3.3.4.*Klebsiella pneumoniae*:

Gram-negative, rod shaped bacterium, Mucous, lactose positive colonies of Klebsiella pneumoniae on MacConkey agar. Cultivation 37°C, 24 hours.

Klebisella pneumoniae is positive for (Voges-Proskauer test, simmon citrate,lactose fermentor) and negative for (indol,methyl red test).

3.3.5.*Proteus species*:

Gram negative rod non lactose fermentor on the macConky agar producing swarMming appearance.

The *Proteus species* is positive for (Methyl red, catalase,simmon citrate) and negative for (Voges-Proskauer,oxidase test and non lactose fermentor).

3.3.6.*Staphylococcus epidermidis*:

Gram positive cocci, arranged in grape-like clusters. It forms white, raised, cohesive colonies about 1–2 mm in diameter after overnight incubation, and is not hemolytic on blood agar.

Staphylococcus epidermidis is positive for catalase and ngative for coagulase.

3.3.7.*Mannheimia hemolytica:*

Gram negative bacteria,rod shape and give hemolysis on blood agar.

Mannheimia hemolytica was positive for (lactose fermentor, hemolysis,catalase and oxidase) and negative for indol test.

3.3.8.*Actinomyces pyogenes:*

Gram positive very small white, opaque and glistening colonies , with β-hemolysis (clear) on sheep blood agar and negative for catalase,oxidase.

3.3.9.*Escherichia coli:*

Gram-negative rod-shaped bacteria,growth on the Eosine methelen blue agar give green metallic shane appearance.

E.coli was positive for (catalase, MR test,indol, nitrate reduction) and negative for (oxidase,VP test,simmon citrate).

3.4.Pathological examination:

3.4.1.Gross pathology :

The gross pathological examination done immediately after animal slaughtered. Examination of the whole trachea and lung in addition to mediastinal lymph nodes and recorded gross lesion . Pieces from abnormal areas were preserved in 10% natural buffered formalin for histopathological examination (Bancroft,1996).

3.4.2.Sample fixation:

About 1 to 3 cubic cm take from the tissue lesions , after that fixed in (10%) buffer formalin for tissue preservation. The time of the fixation extended from 24 to 72 hours .

3.4.3.Tissues processing:

Pass the fixed tissue in serious concentration of solution.

3.4.4.Dehydration and Clearing:

This is important step, the tissues were passed in the ascending graduate concentration of the ethanol (50 ,70,90 100) respectively, two hours for each step and in xylol (2 steps) for clearing ,then put in paraffin wax at 56 ℃ for impregnation.

3.4.5.Embedding of the tissue:

Pieces of tissue put in paraffin wax in a melting point 54-56 ℃ in suitable size blocks and kept until hardening.

3.4.6. Microtome technique:

By the microtome the embedded tissue section were cuted at 5 Mm of thickness.

3.4.7 Manipulation of section:

On the glass slide , the tissue sections were allowed to dry for 1 hour.

3.4.8.Staining process:

The following steps were performed before staining of the sections.The wax from the tissues were removed useing xylene and then used the descending concentration of ethanol (100%,90%,70%,50%) respectively for rehydrated the tissue sections and stained the sections with Hematoxyline and eosin stains for 5-10 and 1-3minutes respectively. Finally dehydrate the sections with the ascending concentration of ethanol and clearing the slide section with xylol.

3.4.9.Mounting:

Use the Canada balsm on the stained slide section and covered with the cover slip, then allow to dry for one day and then exanimated .

3.5.Sterilization:

The glass instrument (pipettes , glass test tube) forceps , scissors , scalpel placed and sterilized at 160 °C for 1 hours at hot air oven. While the plastic instrument (media , bottle) were sterilized by autoclaving (121°C under 15 lbs pressure for 15 minutes) (Cowan and Steel .,1993).

CHAPTER FOUR

RESULTS

4.1. Bacterial isolation:

The results of this study showed The frequency rate of pneumonic lesions were 67 infectous cases (6.7%). The bacterial pneumonia constituted 64 infectious cases (95.5%) out of 67 pneumonic lung and 3 infectious cases (4.5%) were verminous pneumonia.

The total number of the bacterial isolates were 89 isolates,The bacterial species which isolated from infectious cases were *Staphylococcus aureus* was 28 isolates (31.4%), *Pasteurella multocida* was 20 isolates (22.4%), *Streptococcus species* was 15 (16.8%), *KlebsIella pneumoniae* was 9 isolates (10.1%), *Proteus species* was 6 isolates (6.7%), *Staphylococcus epidermidis* was 5 isolates (5.6%), *Mannheimia hemolytica* was 2 isolates (2.2%), *Actinomyces pyogenes* was 1 (%) and *Escherichia coli* was 3 isolates (3.3%),(Table 1).

Pneumonic lesions were classified according to the inflammatory exudate:-

Three types of bacterial pneumonia were reported: Acute to chronic suppurative pneumonia: This was found in 38 infection cases (56.7%) of pneumonic cases. This types of pneumonia characterized by suppurative exudate in the bronchius and bronchioles with inflammatory cells (neutrophils and other mononuclear cells). Most of the associated bacterial isolates were of the following species: *Staphylococcus aureus* was 20 isolates (22.4%), *Streptococcus species* was 7 isolates (7.8%), *KlebIsella pneumoniae* was 7 isolates (7.8%), *Pasteurella multocida* was 4 isolates (4.4%) and *Proteus species* was 6 isolates (6.7%).

The lung absesses found in 7 infectious cases (10.4%) and characterized by presence of abscess containing pus.The bacterial isolated from it as following *Staphylococcus aureus* was 2 isolates (2.2%), *Streptococcus species* was 3 isolates (3.3%), *Klebsella pneumoniae* was 2 isolates (2.2%), *Actinomyces pyogenes* was 1 isolate (1%) and *Escherichia coli* was 1 isolate (1%).

Acute to chronic fibrinous pneumonia: This was found in 19 infectious cases (28.3%) of pneumonic cases and this type showed fibrin exudate in the bronchial and bronchioles with inflammatory cells. Most of associated bacterial isolates were of type: *Pasteurella multocida* 15 isolates (16.8%), *Staphylococcus aureus* was 3 isolates, *Streptococcus species* was 2 isolates (2.2%), *Staphylococcus epidermidis* was 2 isolates (2.2%), *Mannheimia hemolytica* was 2 isolates (2.2%) and *Escherichia coli* was 2 isolates (2.2%).

Acute to chronic interstitial pneumonia: This was found in 7 infection cases (10.4)% of pneumonic cases nd this type characterized by thekening in the alveolar wall and proliferation of type two pneumocytes. Most of associated bacterial isolates were of the types: *Staphylococcus aureu*s was 3 isolates (3.3%), *Streptococcus species* was 3 isolates (3.3%), *Staphylococcus epidermidis* was 3 isolates (3.3%) and *Pasteurella multocida* was 1 isolate (1.1%),(Table 2).

Two types of Verminous pneumonia were reported: Chronic purulent & mucopurulent Bronchopneumonia: This was found in 2 infectious cases (3%) of pneumonic cases.This type characterized by mucus exudate with supurative exudate in the bronchi and bronchioles. The most commonly identified parasites in association with this species were *Dictyocaulus filaria* which was seen alone in one infectious case and seen associated with *Muellerius capillaris* in one other case (Table 3).

Catarrhal Bronchopneumonia: this found in 1 infectious cases and constitutes (1.5%) of pneumonic cases. The most commonly identified parasite in association with this species were: *Muellerius capillaris.*& Dictyocaulus.filaria. These two parasites were found in one case(Table 4).

There are 13 cases of pulmonary emphysema and 8 cases of pulmonary atelectasis and one case of pulmonary thrombosis.

Table (1) numbers and the percentages the species of bacteria.

Sequence	Bacterial type	Isolation number	The ratio %
1	*Staphylococcus aureus*	28	31.4 %
2	*Pasteurlla multocida*	20	22.4 %
3	*Streptococcus species*	15	16.8%
4	*Klebsiella pneumoniae*	9	10.1 %
5	*Proteus species*	6	6.7 %
6	*Staphylococcus epidermidis*	5	5.6%
7	*Mannheimia haemolytica*	2	2.2%
8	*Actinomyces pyogenes*	1	1.1%
9	*Echerishia coli*	3	3.3%
Calculation of the bacteria isolated (89)			

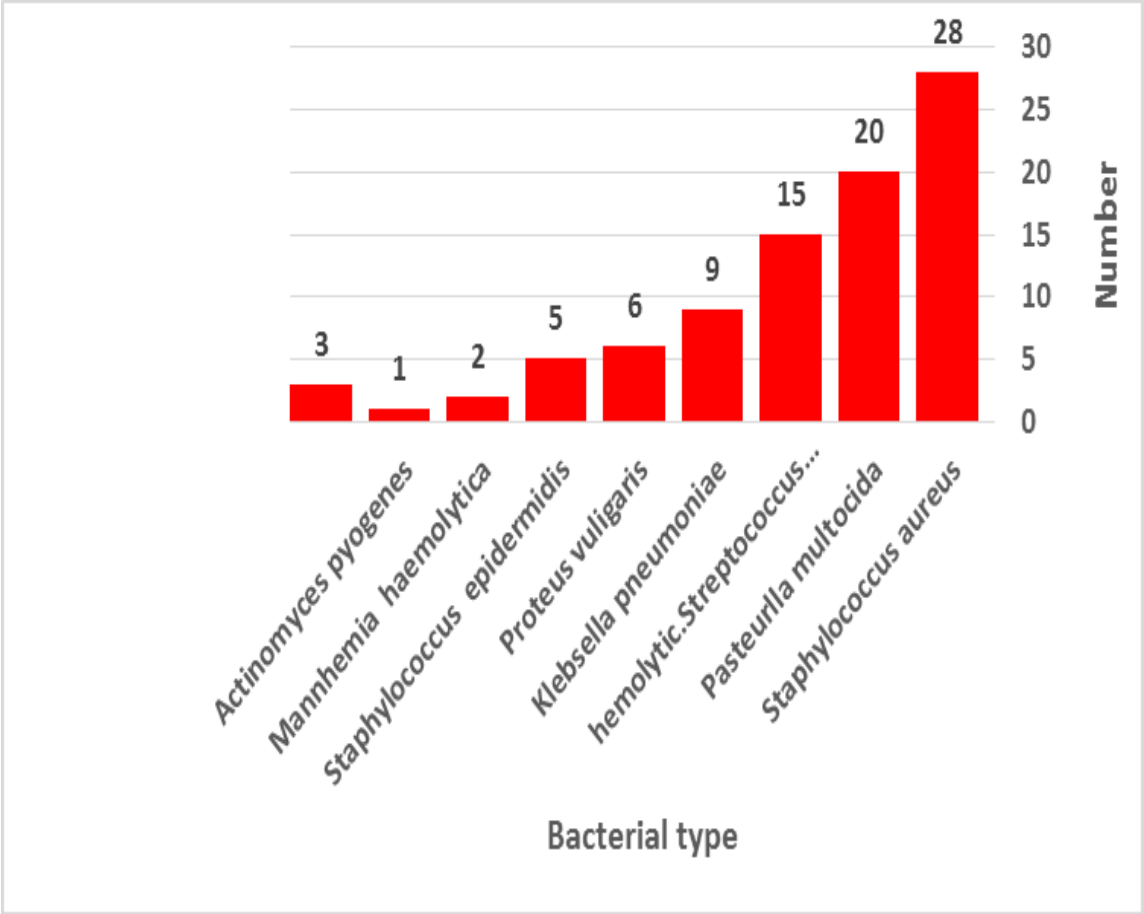

Figure (1): Biostatical analysis showing number and the percentage the species of bacteria.

Table (2) the species of Bacterial pneumonia and the isolated of it .

Pneumonic type	Number of infectious cases	Ratio %	Isolated bacteria
Suppurative bronchopneumonia	31	46.2 %	*Staphylococcus aureus (n 10) , Streptococcus(n4), Staphylococcus + Klbsella pneumonae (n 4) , Klebsiella pneumonae + Streptococcus (n 3) , Pasteurella multocida (n4) , Staphylococuus aureus + Proteus species(n 6) .*
Lung abscess	7	10.4 %	*Streptococcus(n3), Staphylococuss + Klebsiella pneumonae(n 2), E coli (n 1), Corynebacterum pyogenes (n 1).*
Fibrinous pneumonia	19	28.3 %	*Pasteurella multocida (n 8) ,P. multocida+S.aureus(n3) ,P.multocida + Streptococcus spp.(n2)* *P.multocida + Staphylococcus epidermidis (n 2) , E.coli (2),* *Mannhemia haemolytica (n 2)*
Interstitial pneumonia	7	10.4 %	*Staphylococcus aureus (n 3) ,* *Streptococcus species (n3) ,Staphylococcus epidemidis (n 3) , Pasteurella multocida (n 1)*
Calculation of infectious cases (64)			calculation of bacterial isolates (89)

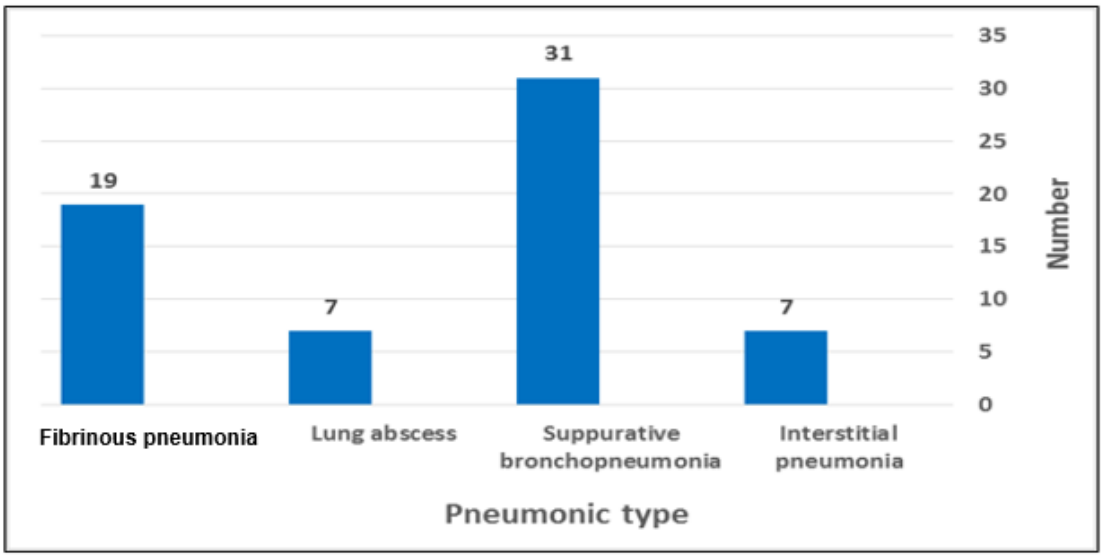

Figure (2):Biostatical analysis showing the types of bacterial pneumonia with numbers of it.

4.2.Parasitic isolations:

D.filaria were seen in purulent and mucopurulent pneumonia in one case alone and mixed with *M.capillaris* in other one case of purulent and mucopurulent pneumonia one case and Catarrhal bronchopneumonia (other case),(Table 3).

Table (3) the species of Parasites isolated from the lung sheep which infected naturally with pneumonia.

Sequence	Parasitic type	Isolation number	The ratio
1	*Dictyocaulus filarial*	3	60%
2	*Muellerius capillaris*	2	40%
Calculation of parasitic isolates (5)			

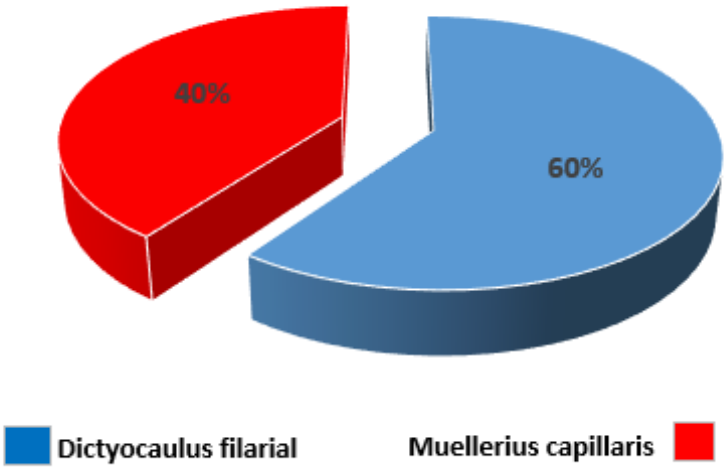

Figure (3):Biostatical analysis showing parasitic isolates with pneumonia with rates of it.

Table (4) The types of verminous pneumonia were isolated of it.

Pneumonic type	Number	Ratio %	Isolated parasite
Chronic mucopurulent Bronchopneumonia	2	3%	*Dictyocaulus filaria(n 1) , Dictyocaulus filaria& Muellerius capillaris(n 1)*
Catarrhal pneumonia	1	1.5%	*Dictyocaulus filaria& Muellerius capillaris(n 1)*
Calculation of infectious cases (3)			

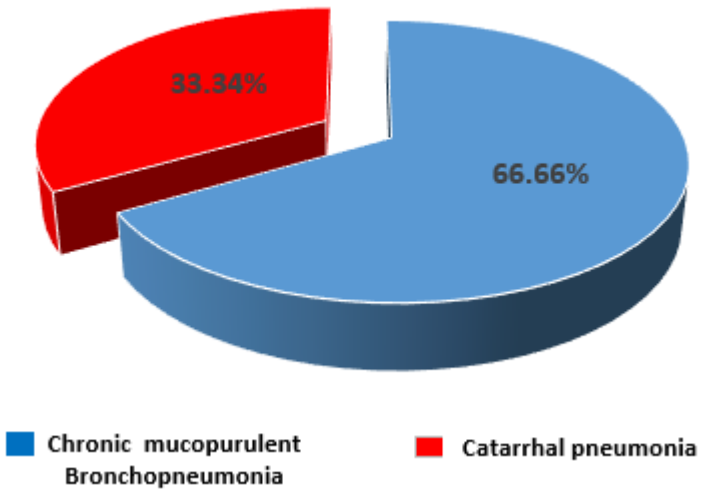

Figure (4): Biostatical analysis showing the types of parasitic pneumonia with the rates of it.

4.3.Bacterial pneumonia:

4.3.1.Gross Pathological changes and its distributions:

1 . suppurative pneumonia :

This type of pneumonia present in 38 cases (56.7%) from the total cases of the pneumonia , this type showed:

A . supurative bronchopneumonia : This present in 31 cases (46.2 %) , the lesions are characterized by solid irregular areas, red gray to white gray, also the lesion occupy the pulmonary tissue in most cases and surrounded by inflations area, Atelectasis and congestion of the lung tissue , in the cut section pus, the lesions distributed in the apical lobe in the 18 cases and other 7 cases distributed on the apical and cardiac lobes while the other lesions distributed on the different parts of the lungs (Fig:5) .

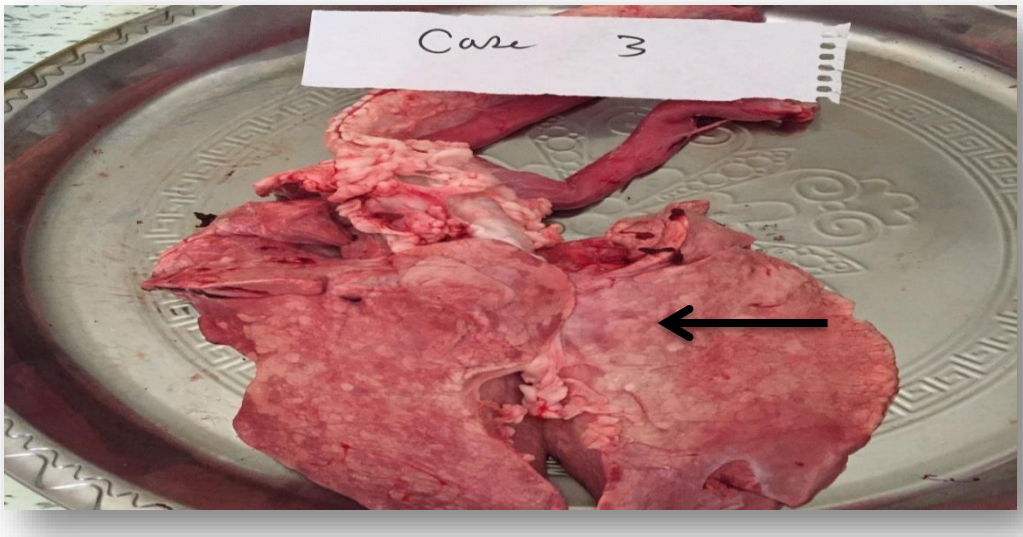

Figure (5): Gross apperance of the lung showing consolidation reddish to white grayish, lesions distributed on most area of the lung.

B. Pulmonary abscess : this lesion was seen in 7 cases (10.4%) from the total numbers of the pneumonic cases, this type present as hard swelling, pale red or yellow in color and the size of it from small (1-10 cm), also present in the two types focal abscess in 5 cases (Fig: 6) and multifocal abscess represent in 2cases (Fig:7).This swelling contain pus and sometime contain cheesy material, the multifocal abscess present in the apical lobes while the most focal abscess present on the cardiac and diaphragmatic lobes and the lesions protrude from the lung surface consist of necrotic material,pus and surrounded with fibrous tissue capsule .

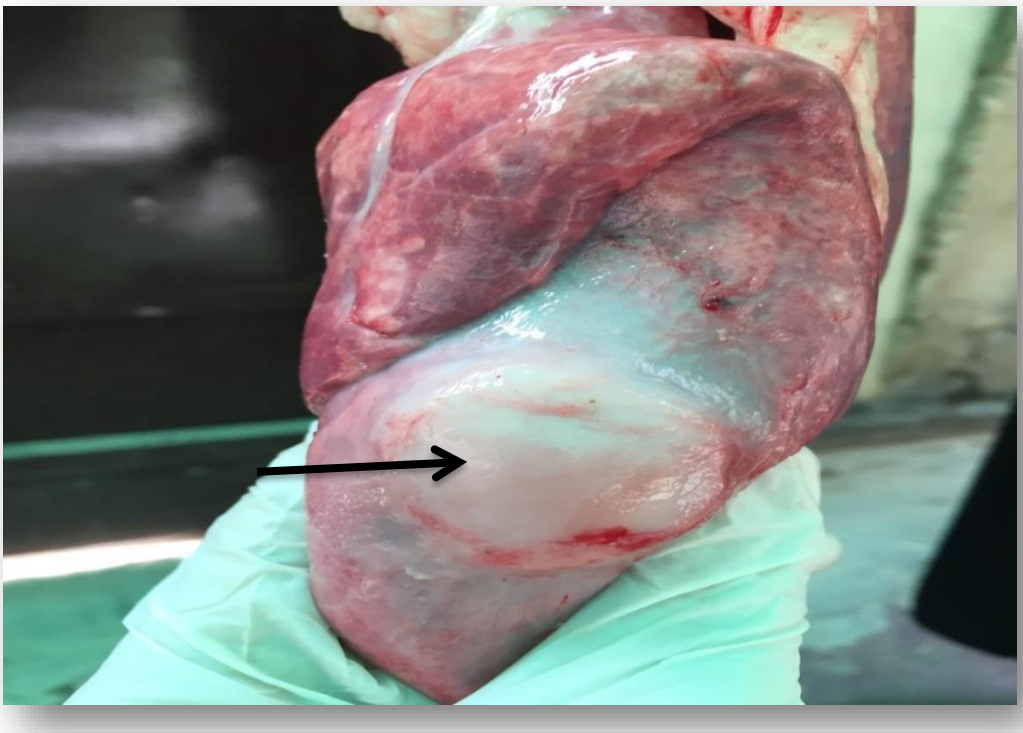

Figure (6): Gross apperance of the lung showing large area of abscess raised above the surface lung occupied the diaphragmatic lobe of the right lung lobe.

Figure (7): Gross apperance of the lung showing multifocal abscess with fibrinous exudate in the lung parenchyma.

2 .Fibrinous pneumonia and fibrino purulent pneumonia :this type of lesions present in 19 cases (28.3%) from total pneumonic cases , the lesions characterized by sold irregular area, pale or red in color , presence of atelectasis in the pulmonary tissue with the congestion in the other area of the lung , there is fibrinous material present as thin membrane on the lung surface and in one case fibrinous-pleuropneumonic lesions ,8 of the cases present on the apical lobe and cardiac lobe and in other cases distributed on the different sites of the lung surface (Fig:8) .

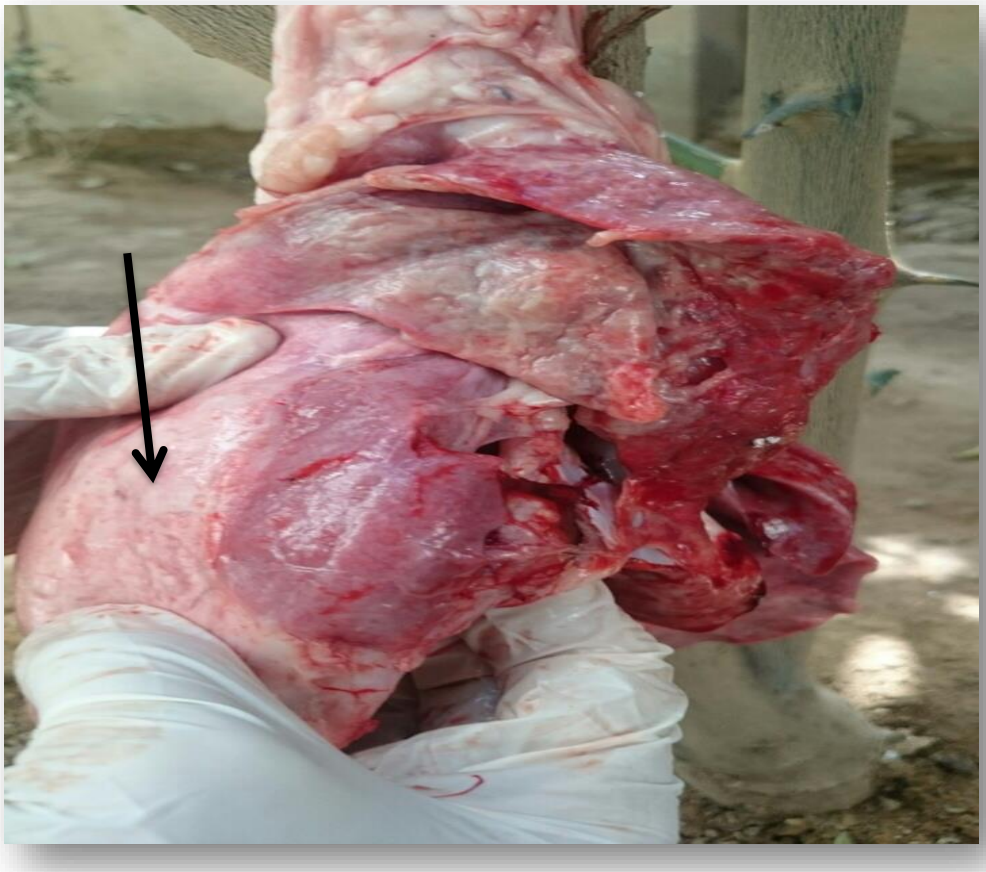

Figure (8): Gross apperance of the lung showing surface area coated with fibrin and consolidations distributed on the surface area of the left lung.

3 .Interstitial pneumonia : this type of the pneumonia present in the 7 cases (10.4%) of the total cases of the pneumonia, the gross lesions characterized by present of the consolidation, soild regular area red in color and the lesions more common in the apical lobe of the lung(Fig: 9).

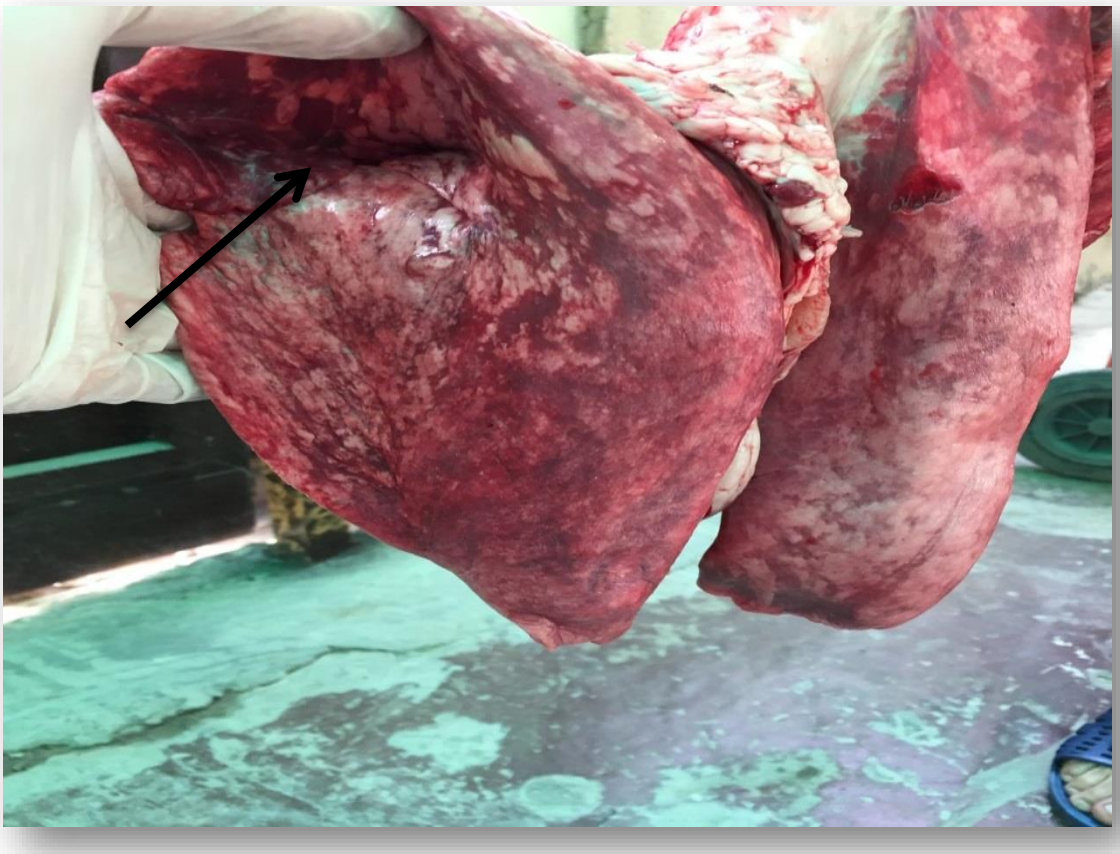

Figure (9): Gross apperance of the lung showing congestion, edema, emphysema and red hepatization, the color vary from dark red to red area.

4.Pulmonary emphysema: this type present in 13 cases, the lung large in size and inflated and compressed by fingers (Fig: 10).

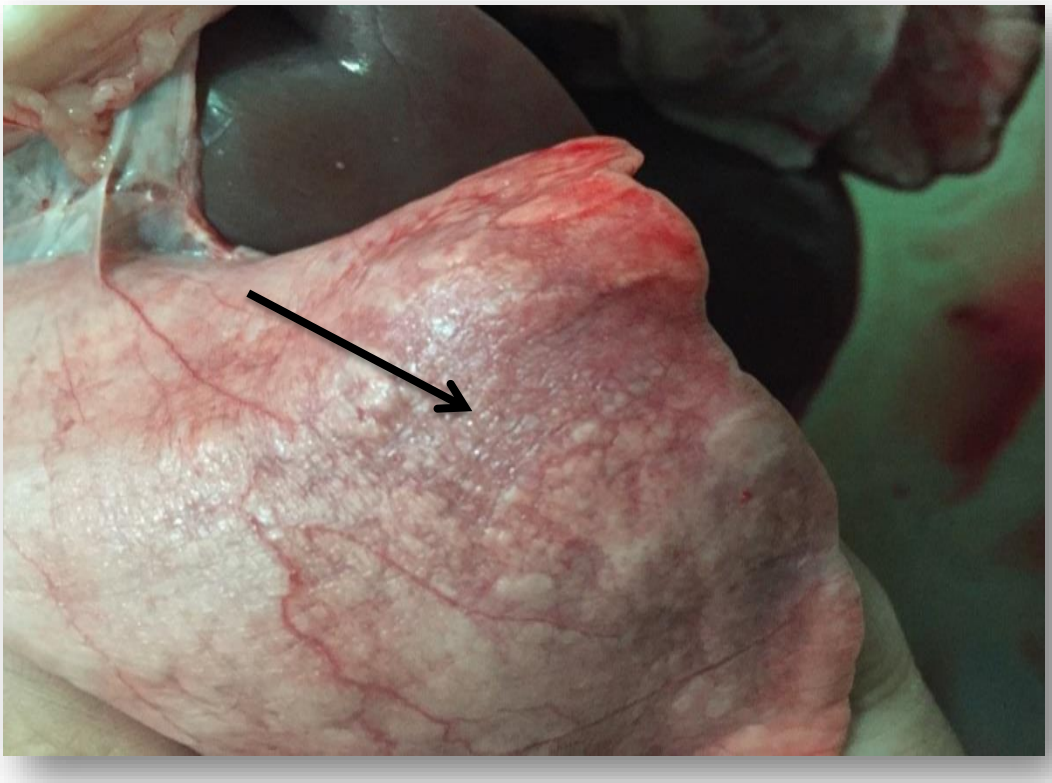

Figure (10): Gross apperance of the lung showing pale emphysematous area in the lung parenchyma easily compressed by fingers.

5.Pulmonary atelectasis: this type present in 8 cases , the lungs showed depressed areas, dark in color (Fig:11) .

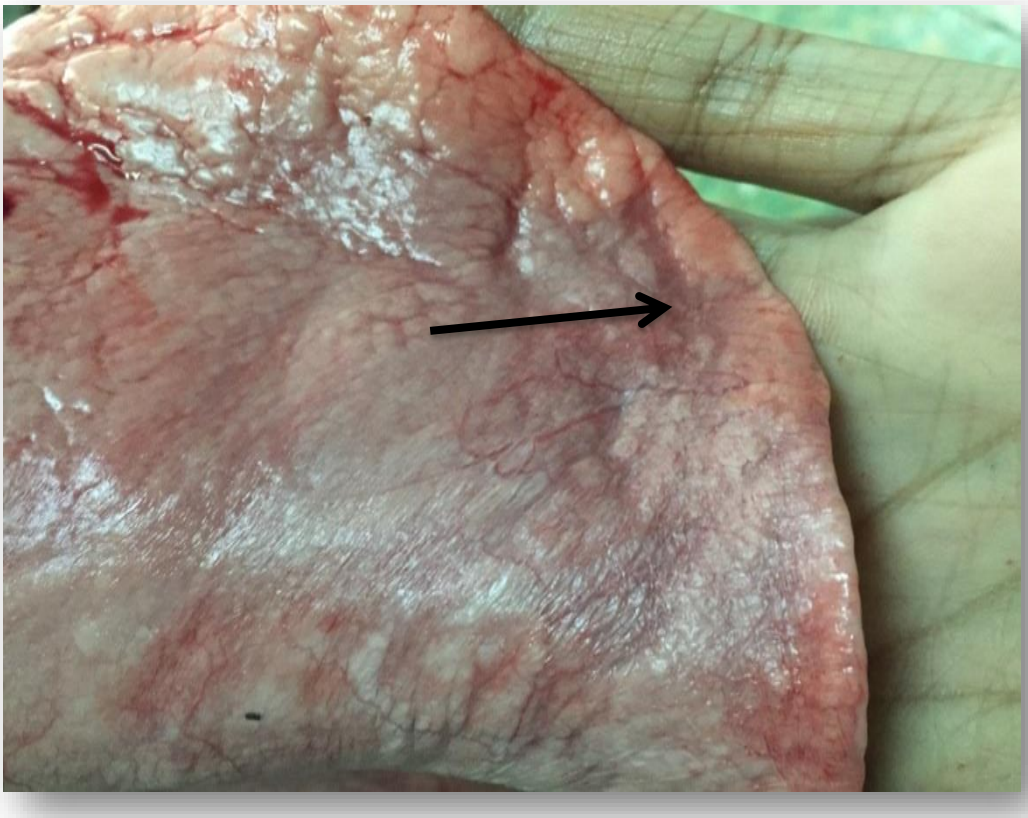

Figure (11): Gross apperance of the lung showing red pale areas and atelectasis under lung surface.

4.3.2.Histopathological changes:

1.Acute suppurative bronchopneumoni: present in the 24 cases (35.8%), there is extensive aggregation of neutrophils with few mononuclear cell aggregation (lymphocytes, macrophages and plasma cells) in the bronchial lumen,bronchiole and alveoli and also similar infiltrates were seen in the bronchial wall epithelia causing sloughing of some epithelial lining, also congestion of the blood vessels together with pulmonary emphysema and atelectasis (Fig:12) .

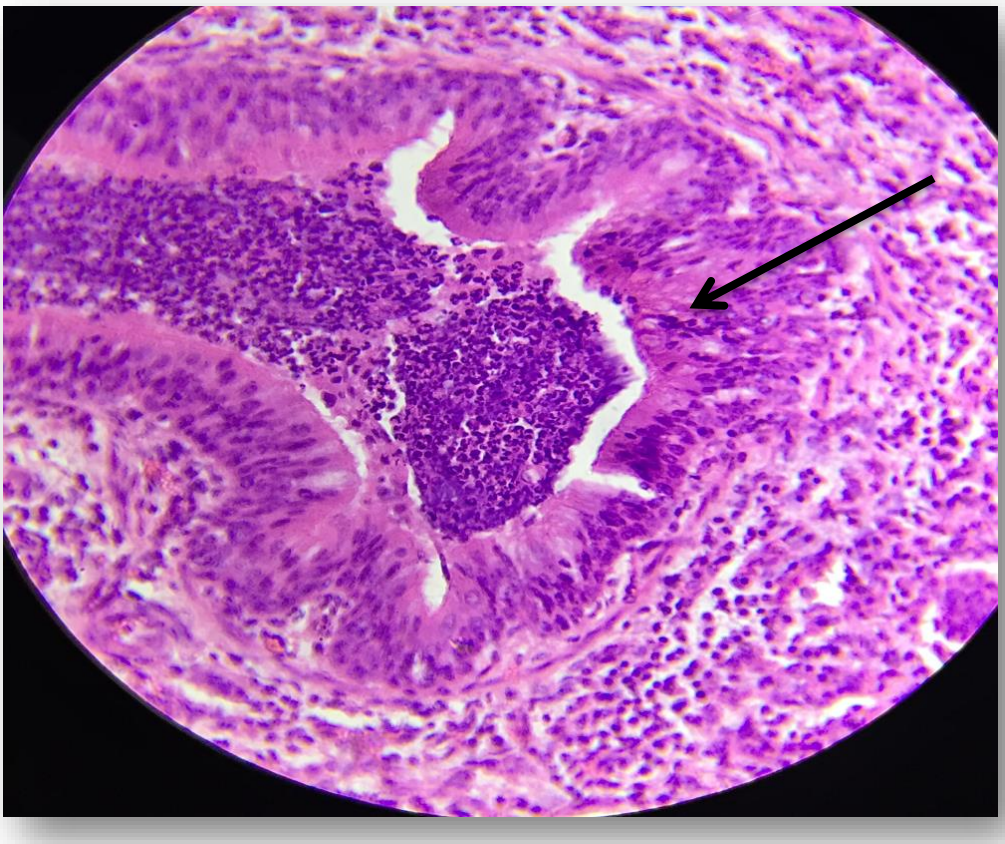

Figure (12): Histopathlogical section of the lung showing aggregation of neutrophils with few mononuclear cell (lymphocytes,macrophages and plasma cell) in the bronchial lumen,bronchiole and alveoli (H&E) X40 .

2.Chronic suppurative bronchopneumonia: present in 7 cases (10.4%), there is extensive supurative exudate of bronchi, bronchiole and alveoli with suppurative exudate composed of neutrophils with necrotic debris, the cellular infiltrate is also seen in the bronchioles and bronchiolar wall causing extensive desquamation of epithelial lining together with metaplasia of some epithelial lining to the bronchus, also there is bronchiectasia in some cases together with squamous epithelium metaplasia, atelectasis and emphysema present in most of pulmonary tissues (Fig: 13).

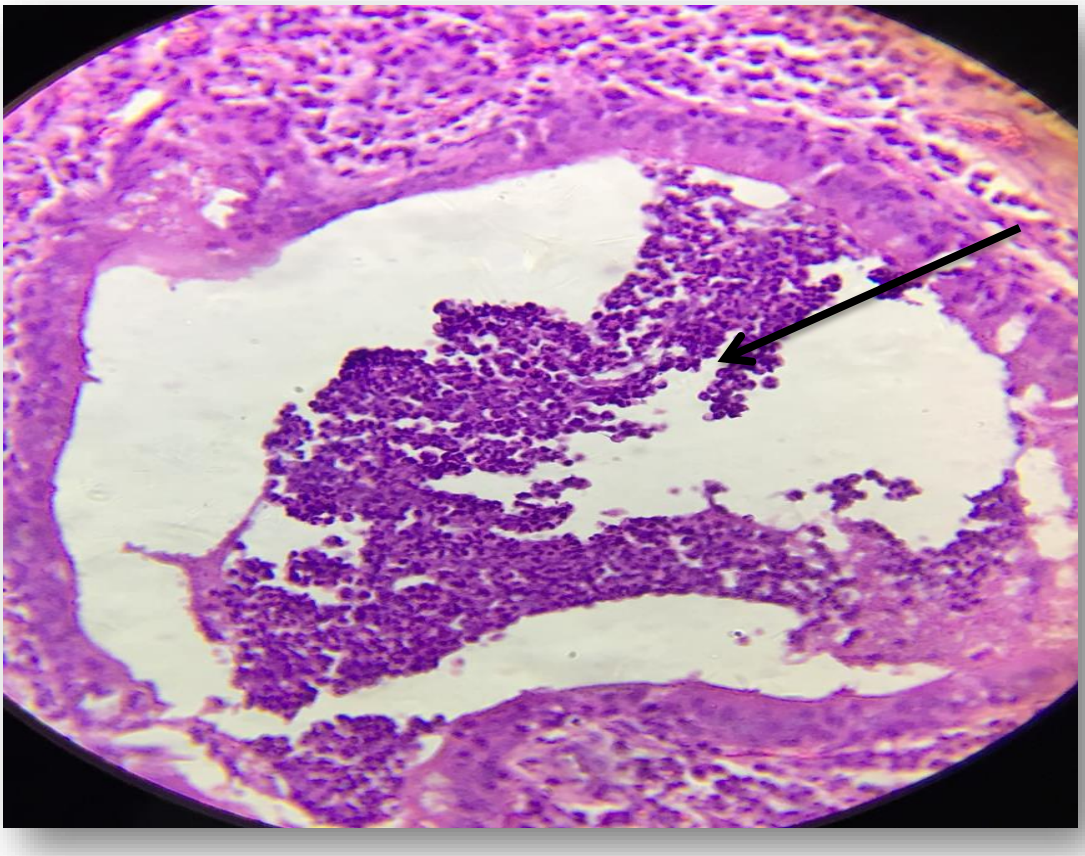

Figure (13): Histopathological section of the lung showing Squamous metaplasia of the bronchial epithelia and neutrophils in the lumen of the bronchiole and alveoli (H&E) X 40.

3.Pulmonary lung abscess :present in 7 cases (10.4%), there is multifocal area of liqufactive necrosis filled with suppurative exudate and edema, the supurative exudate consist of heavy neutrophils collections, most of neutrophils dead, other neutrophils living together with necrotic tissue debris , peribronchial lymphoid tissue hyperplasia was seen, other area adjacent to the abscess showed atrophy of pulmonary tissues , atelectasis and emphysematous were seen together with congestion of blood vessels , in other animal there is focal abscess in the pulmonary tissue with the similar pathological finding, the pulmonary tissue surrounded abscess showed extensive emphysema ,atelectasis and atrophied pulmonary tissues together with congestion and fibrosis in the chronic abscess (Fig: 14) .

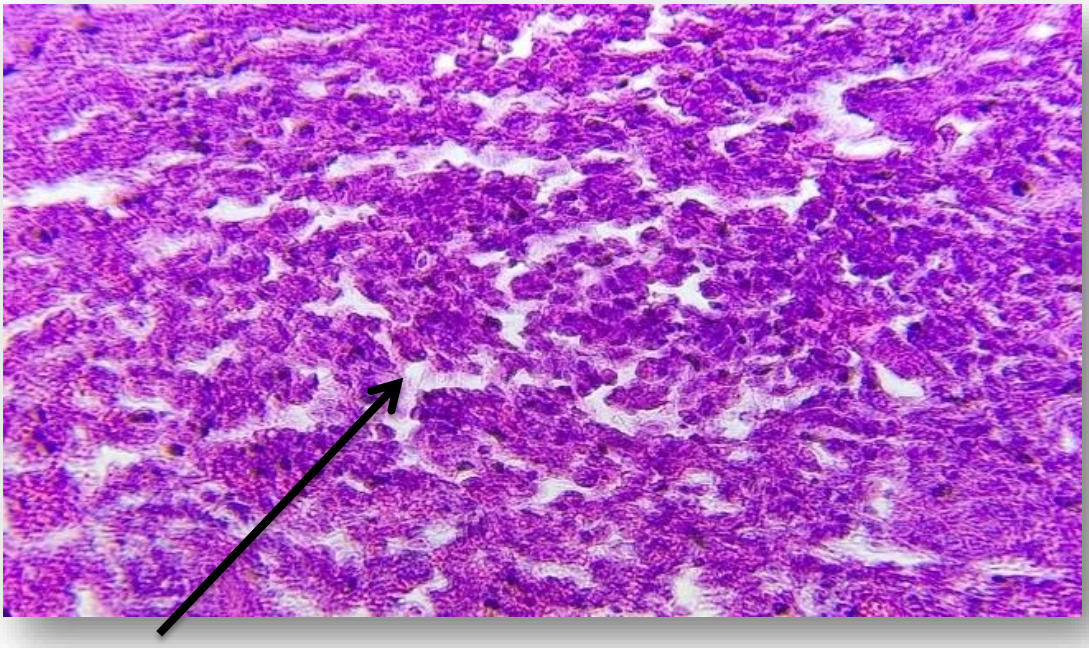

Figure (14):Histopathological section of the lung showing supurative exudate, Necrotic area and pus composed of high numbers of neutrophils surrounded by fibrous tissue proliferation (H&E) X 40.

4.Acute fibrinous pneumonia :present in 13 cases (19.4%),characterized by presence of fibrinous exudate together with neutrophils ,RBCs in the pulmonary alveoli and bronchiole replacing the whole pulmonary alveoli causeing damage to the alveolar walls. Also, there is extensive congestion of alveolar walls and pulmonary tissue,peribronchial lymphoid tissue hyperplasia seen in most of exanimated sections , In addition, there is infiltration of streaming macrophages passing through alveolar Lumina (Fig:15) .

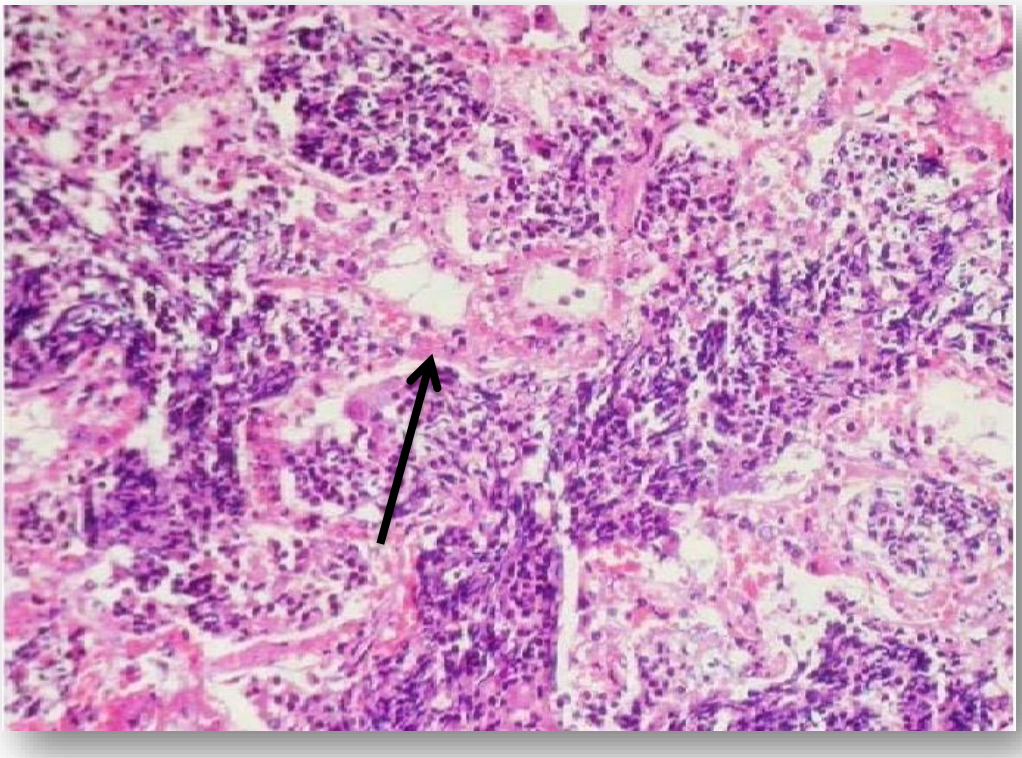

Figure (16): Histopathological section of the lung showing fibrinous exudate in the alveoli and neutrophils infilteration(H&E) X100.

5.Chronic fibrinous pneumonia: present in 6 cases (9%) there is extensive fibrin together with mononuclear cells and neutrophils in the alveolar spaces and interstitial tissue. Also, there is extensive fibrosis in the interstitial tissue of the lung together with extensive peribronchial lymphoid hyperplasia, emphysema and atelectasis , in certain cases fibrotic area replace the whole section of pulmonary tissue together with bronchiectasia, also there is fibrinous –pleuropneumonia in one case (Fig:16) .

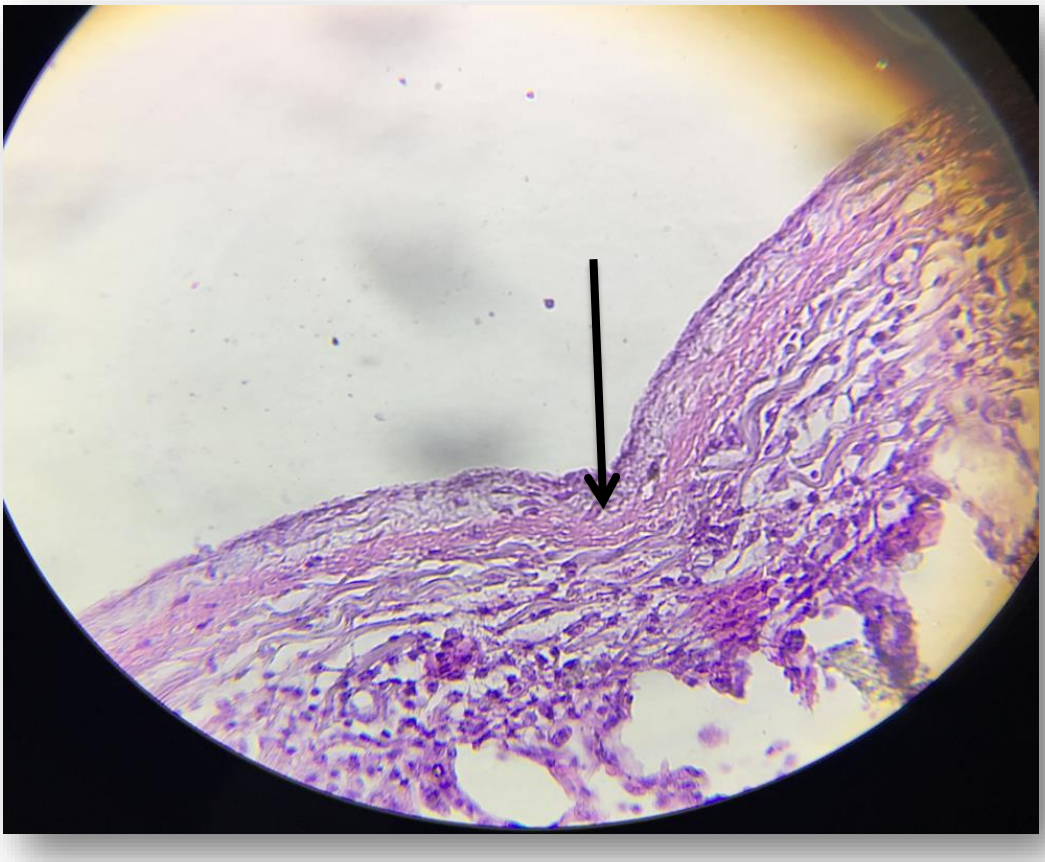

Figure (16): Histopathological section of the veseral pleura showing fibrin deposion in the pleura (H&E) X40.

6.Acute interstitial pneumonia : present in the 5 cases (7.4%) characterized by congestion of alveolar walls capillaries and infiltrations of neutrophils causing thickening of alveolar walls and narrowing of alveolar lumen.Also, there is emphysema in other pulmonary tissues together with congestion, also peribronchial lymphoid tissue hyperplasia (Fig:17).

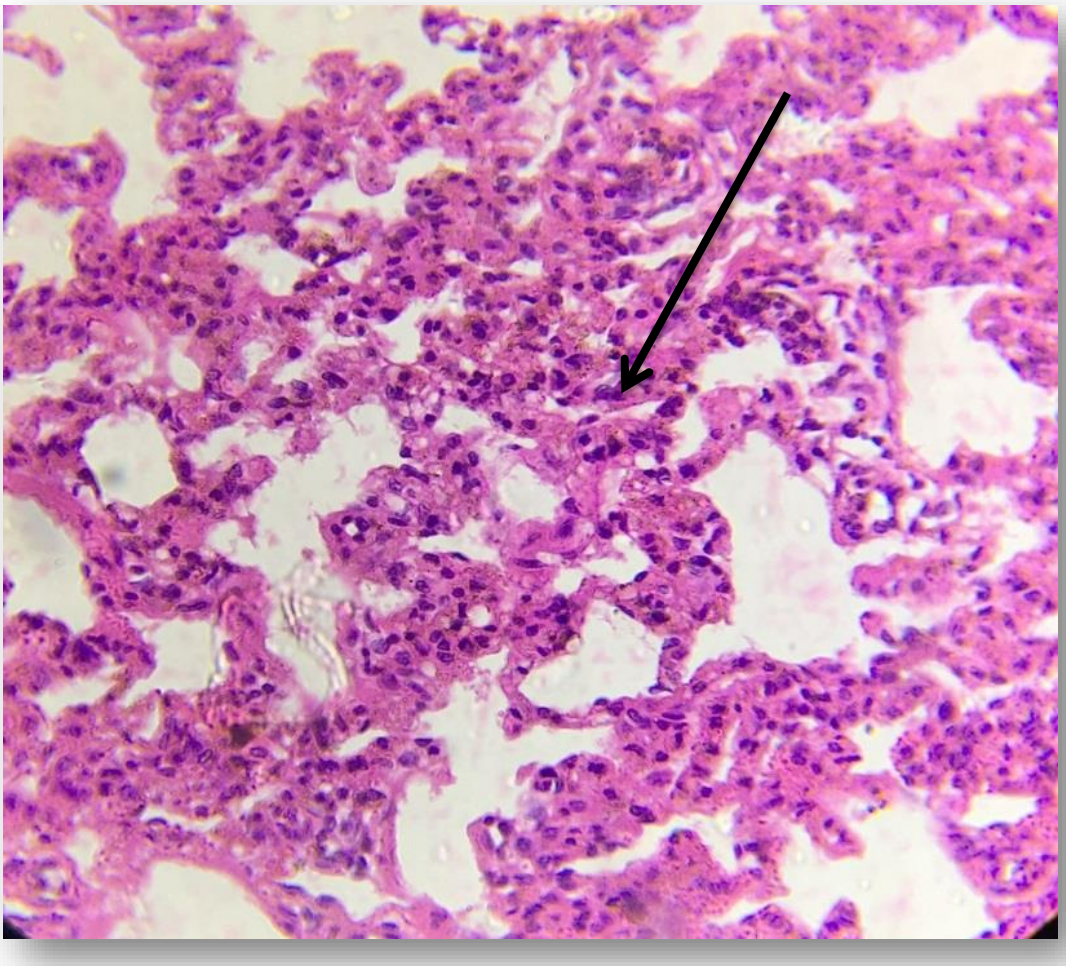

Figure (17): Histopathological section of the lung showing thickening of the alveolar septa and congestion of Blood capillaries as well as infiltration of inflammatory cells specially neutrophils in additions, to presence of emphysema.(H&E)X40.

7.Chronic interstitial pneumonia : present in the 2 cases (3%) there is extensive thickening of alveolar walls and interstitial tissue by fibroblast proliferation together with mononuclear cell infiltrations and Bv congestion in interstitial tissue alveolar walls leading to narrowing of alveolar lumen ,also there is peribroncheal lymphoid tissue hyperplasia (fig:18) .

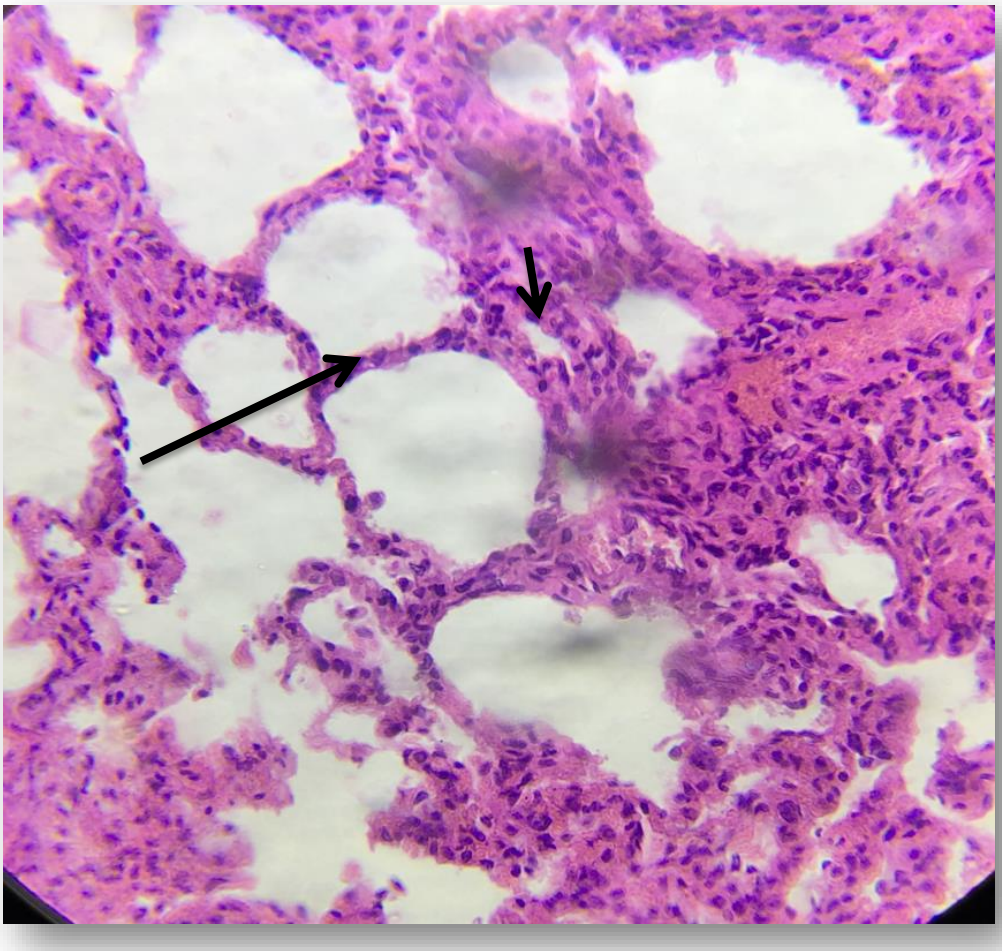

Figure (18): Histopathological section of the lung showing thichening of alveolar wall and mononuclear cells infilteration in the interstitial tissue as well as congestion of the blood vessles (H&E) X40.

8. pulmonary emphysema : this type characterized by distruction of the alveolar walls result in formation of large emphysematous spaces (fig:19).

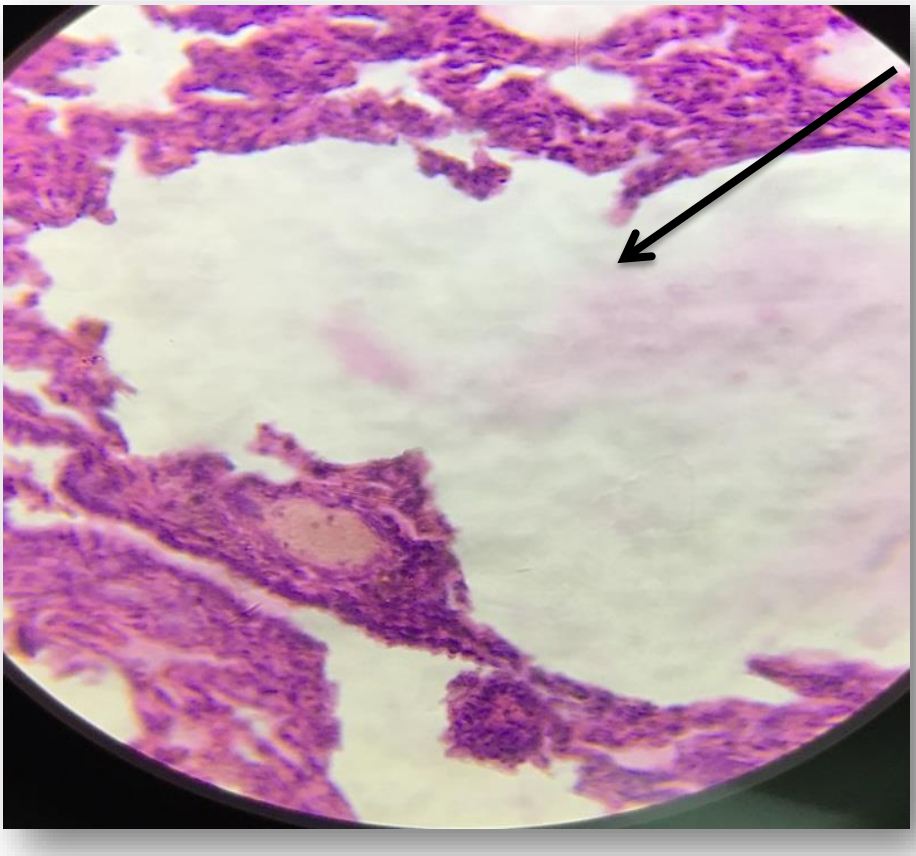

Figure (19): Histopathological section of the alveoli showing distruaction of the alveolar wall (H&E)X40.

9.Pulmonary Atelectasis: this lesions revealed collapsed (non-expanded) alveoli (fig:20).

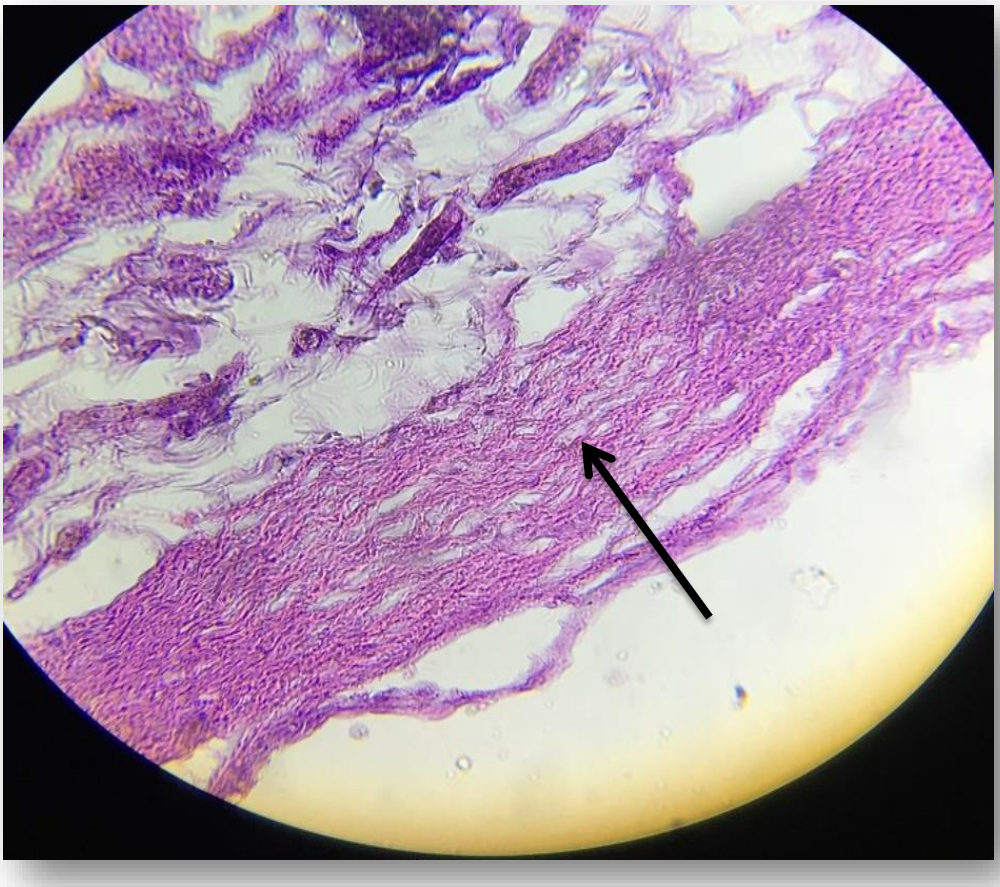

Figure (20): Histopathological section of the lung showing atelectasis of the alevuli and in the diaphragmatic lobe, most cases occur in the left lung than right lung (H&E)X40.

10.Pulmonary thrombosis showed red pink color more visible and occluded the pulmonary artery (fig:21).

Figure (21): Histopathological section of the pulmonary artery showing thrombus occluded the artery (H&E)X40.

4.4.Parasitic pneumonia:

4.4.1.Gross pathological changes:

present in 4.5 % from the total pneumonic cases and showed the following type :

1.Purulent&mucopurulentbronchopneumonia: this type present in two case and represent (3%) of total pneumonic cases. This type was in the form of soild nodules of different size and in number and ranged in size from 1 to 3 mm approx. The nodules are located under the pleura on the costal surface and the dorsal edge of the diaphragmatic lobes. The lesions were red to dark red in color and over the surface of the lung. During the cross-section lung worms present in the bronchi and bronchioles belong to *D. filaria* But in the small nodules which located deep in the lung Muellerius *capillaris* and *D. falaria* were seen (Fig:22).

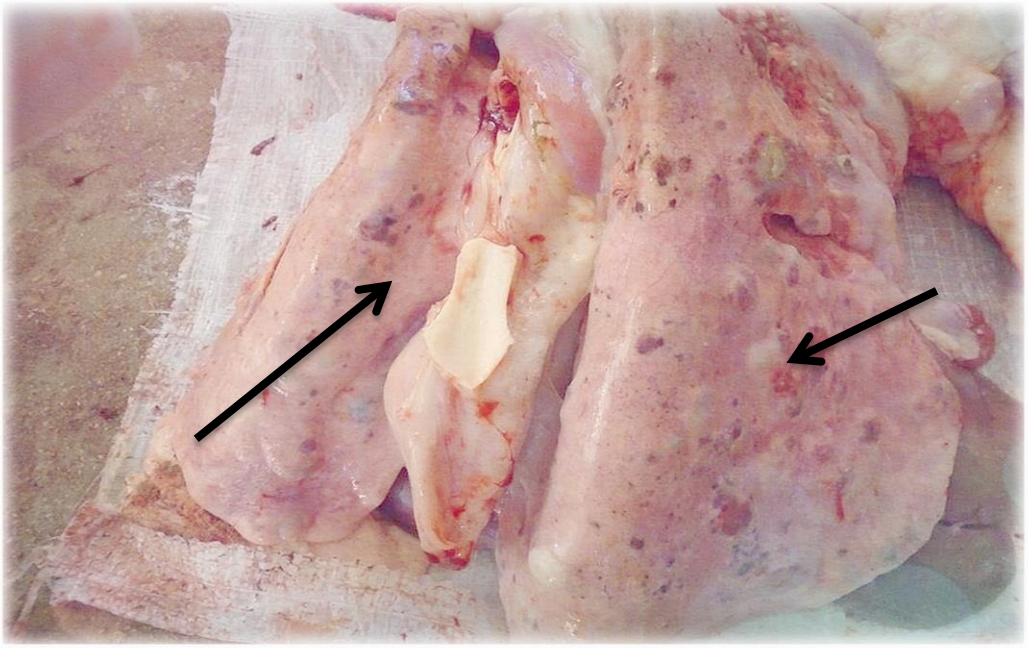

Figure number (22): Gross morphology of the lung showing pale – colored area, hemorrhage with diffuse small nodules on upper surface of the lung.

2.Catarrhal bronchopneumonia: this type represent (1.5%) of the pneumonic cases (1case). The lesion was in the form of multiple nodules solid red to white gray in color, present on the diaphragmatic lobe and close to the edge of the lobes, there are soild areas and other atelectasis in the lung tissue of both types *Muellerius capillaris* with *D. filarial* (fig:23).

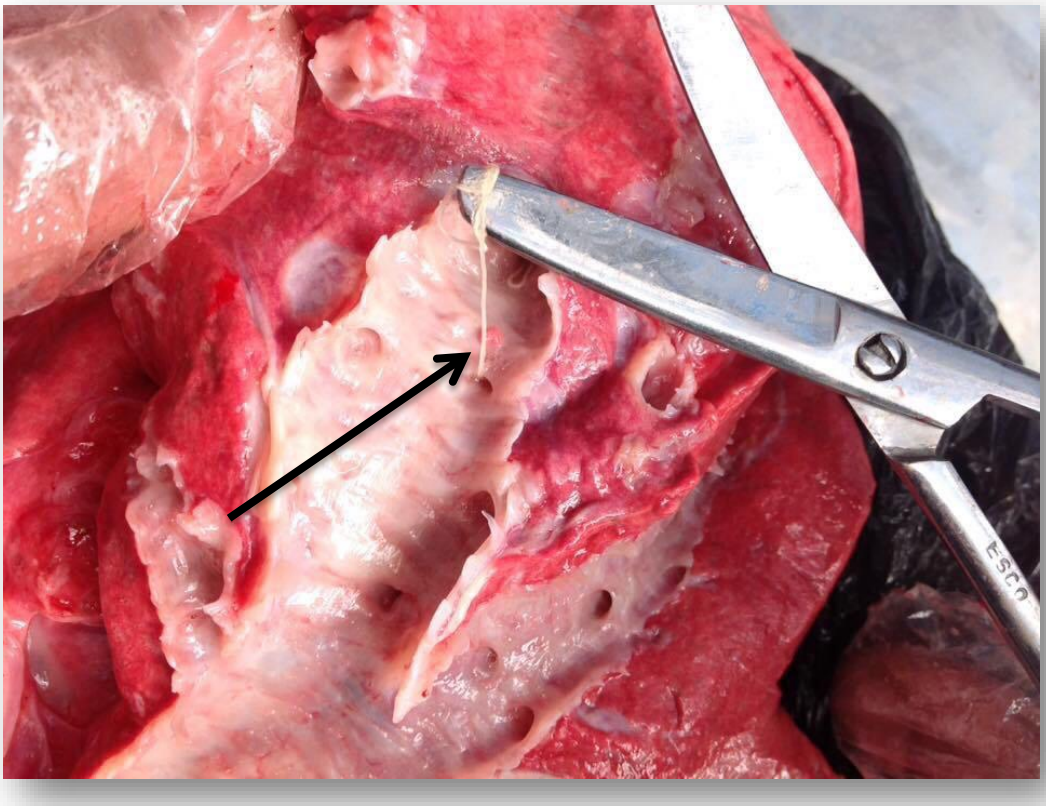

Figure number (23): Gross morphology of the lung showing *Dictycaulus filaria* worm in the bronchius during red consolidation stage, the parasite appears thread like and has a white color.

4.4.2.Histopathological changes:

1.Chronic mucopurulent bronchopneumonia: The similar pathological changes seen in the bacterial pneumonia (Purulent bronchopneumonia), but in airways mucous exudate with cross sections or longitudinal section of the adult parasites, larvae and eggs in the bronchi and bronchioles and alveoli admixed with supurative exudate also the infiltration of eosinophil's with the larvae of *Dictyocaulus filarial* and *Muellerius capillaris.* The presence of the cause damage to the wall of alveoli. (fig:24).

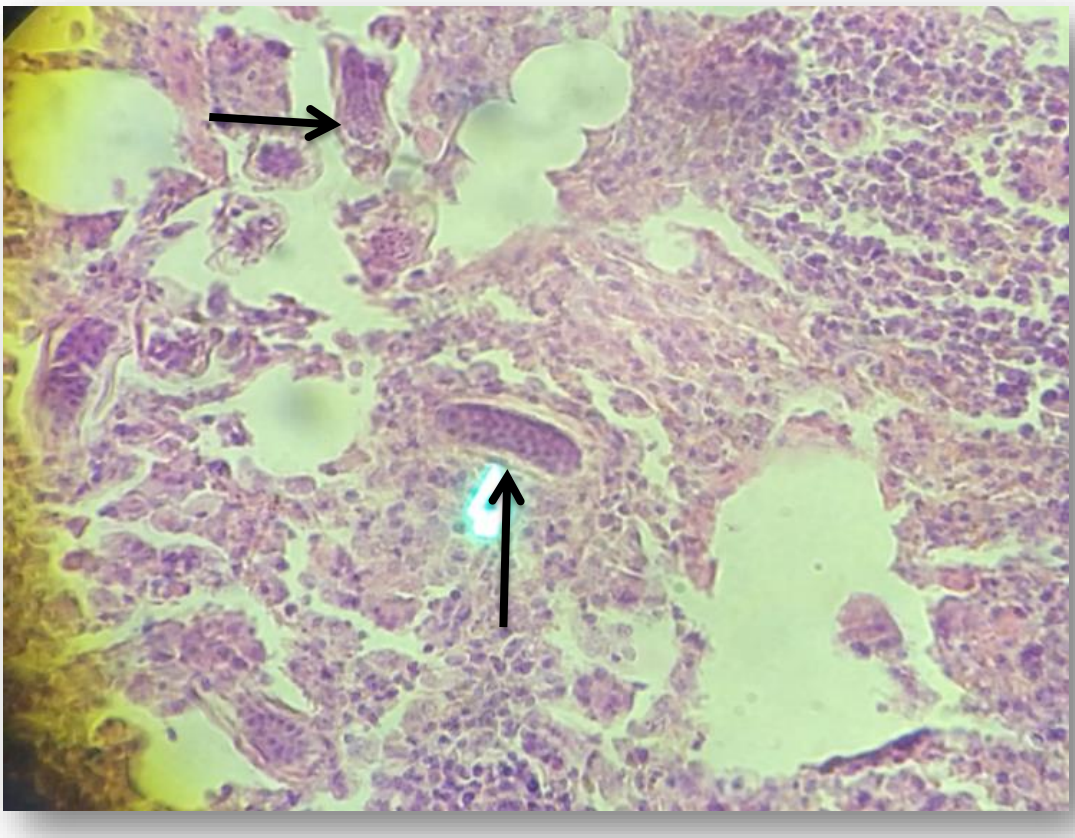

Figure number (24): Histopathological section of the lung showing suppurative exudate and different stages of *D. falaria* larvae.(H&E)X40

2.Catarrhal bronchopneumonia: similar pathological lesions reported in interstitial pneumonia. Bronchi and bronchioles contain mucus exudate admixed with mononuclear cells and eosinophils and neutrophils present in bronchi, bronchioles and alveoli. Cross section of adult parasites, ova, larvae was seen admixed with mucous exudate (Fig:25).

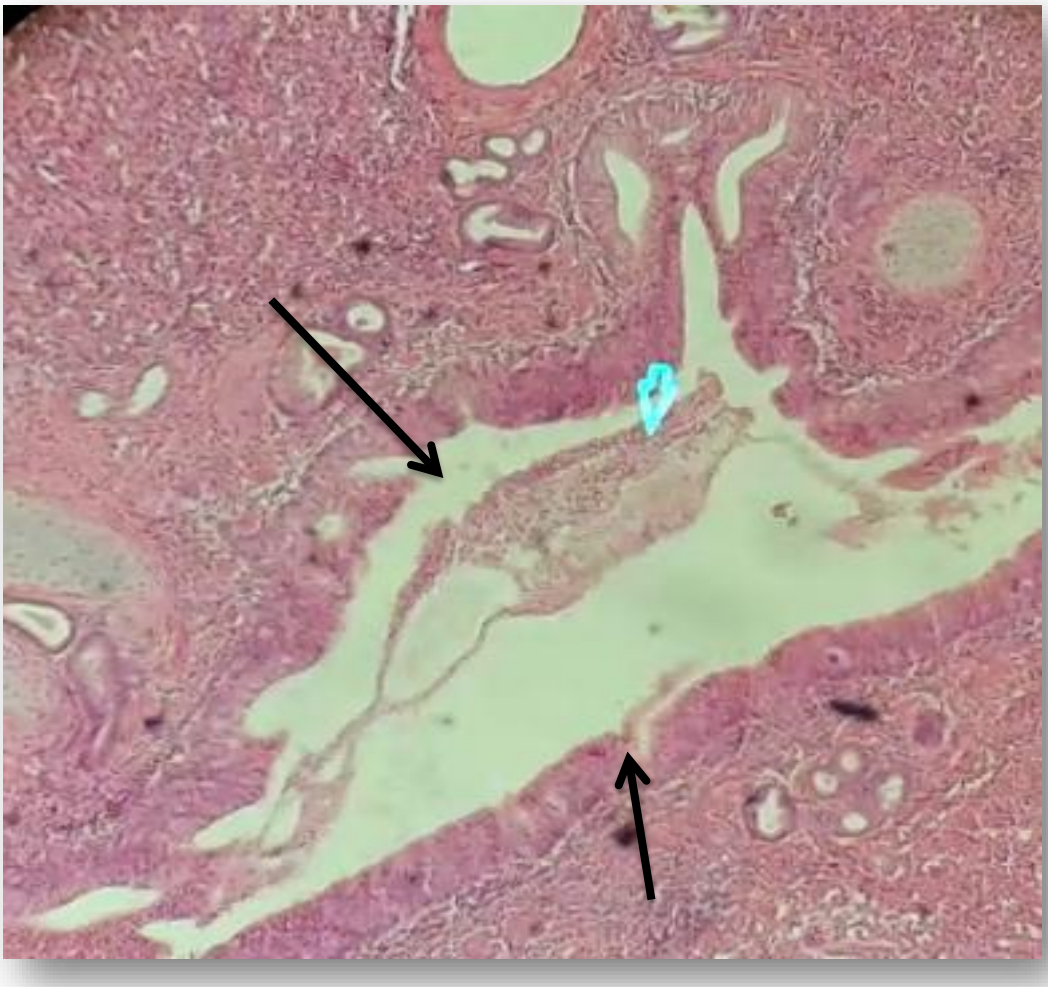

Figure number (25): (A).Histopathological section of the lung showing mucous exudate and mononuclear cells in the bronchus(B). in addition to metaplasia and desqumation (H&E)X40.

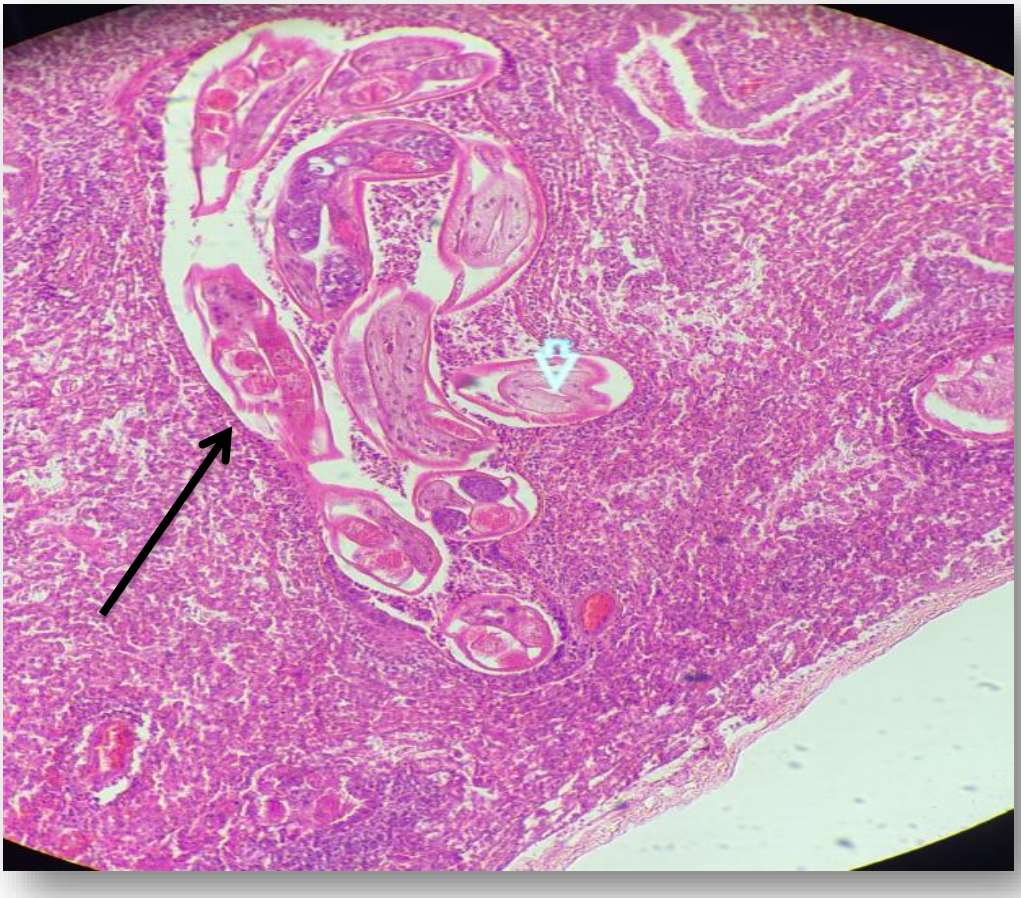

Figure number (26): Histopathological section of the lung showed adult parasite of *Dictycaulus filaria* in lung paranchyma surrounded by inflammatory cells.(H&E)X40.

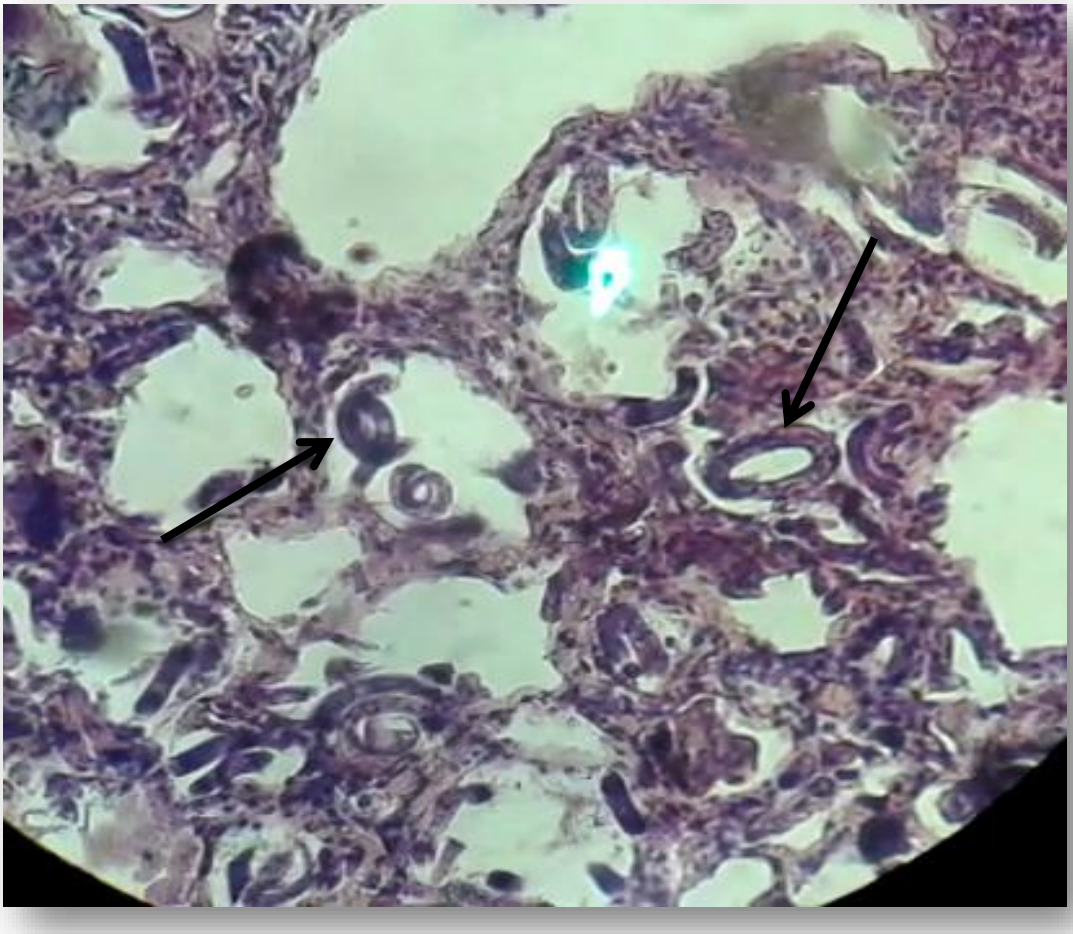

Figure (27):Histopathological section of the lung showed larvae of *Muellerius capillaris* in the subpleural region (H&E)X40.

CHAPTER FIVE

5.1.Discussion

Sheep pneumonia is considered common disease in the world and associated with high rates of morbidity,mortality and economic loses (Saleh *et al* .,2014). In this study through examinations of 1000 sloughtered sheeps, the rate of pneumonic disease in the sheep was 6.7%. The frequency rate of this disease is vary from one place to another .In Iraq and mainly in the Kirkuk slaughter the frequency rate of pneumonia show 8.4% from the total exanimated cases (kadir *et al* .,2012) while in India the frequency rate was vary from 15 to 27 % and the reference revealed the difference in the ratio of infection in same country .

In the benjab the frequency rate was 6% and in the Tara city was 2.4% (Samybal *et al* 1980) . In the Newzleand the frequency rate was 3.4% (Goodwin-Ray,2006).The deference in the frequency rate of the pneumonia may belong to the several factors including the deference in the heat and moisture, methods of animal management, stress factor , type of nutrition ,breeding of the animal and animal situation before the slaughtering (kadir *et al* .,2012).

In this study the bacterial pneumonia represent 6.4% from the total exanimated cases and the numbers of this type started to increase from mid-November to highest increase in January. This increase can be attributed to weather factors and a lack of food during this period which leads to increased susceptibility of sheep to infection with bacteria and lead to lung inflammation. In addition, to presence of infect of *D. falaria* which enhance the bacterial infection. The pulmonary worms facilitated the task of bacteria naturally present in the upper respiratory tract (Uqbazghi,1990) and these bacteria invade the lung and cause inflammation which more evident in this study.

the suppurative pneumonia is the common type and represent 56.7% from the total cases of the pneumonia. There were two types of it ,firstly the suppurative bronchopneumonia which represent 46.2% and second type the pulmonary abscess which represent 10.4%. The frequency rate of occurrence of this type of pneumonia was near to the rate of occurrence registered by other researchers, such as ,Ozyildiz et al (2013), Sheehan *et al* (2007) who registered respectively following occurrence rates:43% ,36% ,37.3%. The more isolates were *Staphylococcus aureus* and the number of the isolates were (28) isolates.

Whereas the *Streptococcus spp.*isolates were (12) isolates. Other researchers isolated both types of bacteria (*staph & streptococcus*) from naturally affected animal and reveal the ability of this bacteria to induce the suppurative pneumonia (Haziroglu *et al* ., 1994). The other bacteria were isolated from the suppurative pneumonic type are *Klebsiella pneumoniae* and the numbers of the isolates are (9), *Pasteurella multocida* and the numbers of isolates are (4), *Proteus vulgaries* and the numbers of isolates are (6) , *E. coli* and the number of isolates is (1) and *Trueperella pyogenes* and the number of isolates is (1),these bacterial agent are isolated from pneumonia (Boljihad *et al* ., 1995).

The fibrinous pneumonia represent 28.3% from the total pneumonia types. The most common types bacteria which isolated from this type were *Pasteurella multocida*, This type of bacteria is responsible for induction of this type of pneumonia due to their endotoxin causing localized necrosis and is endotoxin cause damage to endothelial cells of the capillary vessels which result in fibrin exudation. This type of pulmonary response (fibrinous pneumonia) is recordered conveniently and caused by this bacteria in natural and experimental pneumonia in sheep and lambs (Azizi *et al* ., 2015). The infiltration of streaming macrophages and oat cell that present in this study

described as important characterization of the pneumonic manhemiosis (AL-Daragji *et al* ., 1982).There are other bacterial isolation from this type of pneumonia : *Staphylococcus epidermidis* (2) isolates ,*Escherichia coli* (2) isolates and *Mannhemia haemolytica* (2) isolates, *Staphylococcus aureus* (3) isolates and the hemolytic *Streptococcus alph & Beta* (2) isolates . the last two bacteria found associated with *P. multocida* are responsible to induced the pus in the fibrinous pneumonia (Daniel *et al* ., 2006).

The interstitial pneumonia represent 10.4% of the total cases of the pneumonia .It is less types of bacterial pneumonia in this study, and this is consistent with the reports of Abubaker *et al.*(1980). It is known that the bacteria usually cause lesions (exudate within the alveoli) in the lung but in case of interstitial type lesion the bacterial agent may induce mild lesion (interstitial type lesion) without exudation in alveolar lumina similar finding observed in this study. More isolates were *Staphylococcus aureus* and hemolytic *Streptococcus alpha & beta* with the evidence of the purulent histological response in the lung.

While Sharma,(1977) and Abubaker, (1980) reported interstitial pulmonary response in the infected sheep. Also, in this study there are other bacteria recorded in the interstitial type of pneumonia and these bacteria were *Staphylococcus epidermidis* (3 isolates) and *Pasteurella multocida* (1isolate) similarly other researchers isolated these bacteria from the interstitial pneumonia(Symbal *et al.*, 1980) and reported that the bacterial may induce mild lesion in interstitial tissue without exudation of alveolar lumina Al-jeboori,(1984).

As for the pathological changes as well as macroscopic and their distribution in the lungs in these types of pneumonia recorded in this study it was similar to referred to (Bhagwan & Singh 1972; Abubaker *et al* 1980).

The frequency rate of verminous pneumonia was 4.5% of pneumonic cases studied, and their induced differed from records of the disease in sheep in Iraq, where it was 38% (AL-sultan,1976) and 45% according to the ministry of Iraqi agriculture recorded (2010). That most cases of verminous pneumonia recorded from February to end of March. with the same period recorded in Iraq, most cases of pneumonia parasites in sheep(Altaif,1970) similar recorded by AL-jeboori, (1984).

In this study, the reasons of the low incidence rate of infection with the verminous pneumonia in the Diyala provinces is due to the use of anti-lung worm therapy widely, the lack of green agricultural areas, increasing temperature compared with the previous years, also most of the areas studied exposed to chemical poisons in the air and deposited on the ground by the military operations that took place in the Diyala province and that eliminate the vectors of larvae from an animal to another.

The type of worm in this study were *Dictyocaulus filaria* which found in two cases with *Muellerius capillaris* in one case found alone. The infection with *Dictyocaulus filaria* consider most commonly between other type of the verminous pneumonia in sheep which shows that this animal is sensitive to this type of worms. Similar findings reported byDhar&sharma.(1978), Sissay, (1996). AL-jeboori,(1984) reported similar finding in the goats. The helping factors to spread the infection with these worms is the shorting of the life cycle, it has been found that after 7 days of the descent of the first stage larvae become infective larvae without having to intermediate host that it needed for other types of lung worms (Nuraddis and Yared,2012;AL-

jeboori,1984).Whereas the decrease of the presence of *M. capillaris* in lung affection relate to the need of intermediate host (snail) in order to complete its life cycle, while *P. rufescens* needs three types of snails of the genus Hellicela to complete their life cycle to be infectious to sheep but not available the appropriate factors to complete their life cycle of these worms, making the frequency rate very low with these parasites (AL- jeboori,1984).

The purulent & mucupurulent bronchopneumonia which was constitutes (3%) associated with of the verminous pneumonia.The frequency rate of this type of pneumonia can be explained on the basis that the pulmonary response to worms were mucous accompanied by suppuration which main feature characterize this type of pneumonia (purulent exudate or mucus),(Tewodros et al .,2012;Villarroel, 2013), and explains why this response due to the irritation caused by adult worms to the mucous membranes of the bronchi and bronchioles leading to mucous production with infiltration neutrophils, but bacterial secondary infection and this actually found in this study through the bacterial isolate was *Staphylococcus aureus.*

The Catarrhal pneumonia represent also (1.5%) of the total pneumonic cases which associated with *M. capillaries* In one cases and the lesions of this type of pneumonia. The intensity of lesion can be attributed to the absence of an immune response by the host against the parasite whereas the localized lesion due to resistance of body against parasites due to previous infection exposition (Nimo,1979). In this study, there is infiltration of eosinophils in large numbers, the presence of these cells due to sensitizing the animals to parasitic components or as result of previous exposure (Ghanem, 2009).

CHAPTER SIX

6.1.CONCLUSIONS:

1 .This study showed the pneumonia is a significant lesion in Diyala province.

2 .The frequency rate of bacterial pneumonia more than parasitic pneumonia.

3 .The Bacterial isolation were mostly *Staphylococcus aureu , Pasteurella multocida ,Streptococcus species, Klebsiella pneumonia, Proteus species, Staphylococcus epidrmidis, Mannhemia haemolytica, Actinomyces pyogenes and E. coli.*

4 .The types of pneumonic lesions which associated with bacteria were acute and chronic supurative bronchopneumonia, Lung abscess , acute and chronic fibrinous pneumonia,acute and chronic interstitial pneumonia, pulmonary emphysema, atelectasis and pulmonary thrombosis.

5 .The Parasitic isolation were *Dictyocaulus filarial* and *Muellerius capillaris.*

6.The lesions relation with parasites were Purulent & mucopurulent bronchopneumonia and Catarrhal pneumonia.

6.2.RECOMMENDATIONS:

1 . Study of genetic mutations of bacteria causing pneumonia.

2 .Characterize the virulent gene associated with most common bacterial causes.

3. Done the prevention of health and control of diseases entering the province, especially from surrounding areas

3 . Study the pneumonic lesion under scanning and transmission electron microscopy.

4. Building a healthy slaughter houses in Diala province because the khnankin slaughter house is bad.

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الخلاصة

في دراسة صممت لمعرفة نسبة الحدوث, المسببات المرضية (البكتيرية والطفيلية) والتغيرات المرضية (العيانية والنسيجية) لمرض ذات الرئة في الاغنام المحلية في محافظة ديالى , جمعت 67 رئة مصابة تحوي على افات التهابية من خلال فحص 1000 من الاغنام ذبحت في مجزرة خانقين ومناطق مختلفة من المحافظة للفترة من اول تشرين الثاني 2016 ولغاية نهاية اذار 2017.

كان عدد الرئات المصابة 67 رئة مصابة (6.7%) حيث شكلت حالات الالتهاب الرئوي ذو السبب البكتيري 64 حالة مصابة (95.5%) من مجموع 67 حالة مصابة بينما شكلت حالات الالتهاب الرئوي ذو السبب الطفيلي 3 حالات مصابة (4.5%) من مجموع 67 حالة مصابة.

صنفت الحالات المرضية لذات الرئة حسب نوع النضحة الالتهابية, حيث كانت انواع ذات الرئة البكتيري المشخصة هي ثلاث انواع وهي:

اولا: ذات الرئة القيحي الحاد والمزمن: كان عدد الحالات المصابة بهذا النوع 38 حالة مصابة (56.7%) وقسم الى قسمين حيث كان الاول هو التهاب الرئة القصبي القيحي ومثل 31 حالة مصابة (46.2%) وكان بنوعين الحاد 24 حالة مصابة (35.8%) وكان يتميز بارتشاح اعداد كبيرة من العدلات اضافة الى عدد قليل من الخلايا الالتهابية وحيدة النواة وظهور القيح في القصبات والقصيبات الرئوية والثاني وهو النوع المزمن حيث مثل 7 حالات مصابة (10.4%) وكان يتميز بظهور الخلايا الالتهابية وحيدة النواه التي تظهر في النوع المزمن. وكانت العزلات البكتيرية المشخصة في هذا النوع هي *Staphylococcus aureus* وكان عددها 20 عزلة, *Streptococcus species* وكان عددها 7 عزلات, *Klebsiella pneumonia* وكان عددها 7 عزلات, *Pasteurella multocida* وكان عدد عزلاتها 4 عزلات و *Proteus species* وكان عدده 6 عزلات.

اما النوع الاخر من الالتهاب القيحي فهوا الخراج الرئوي حيث مثل 7 حالات مصابة (10.4%) حيث كانت 5 حالات ذو خراج واحد وحالتين متعددة الخراج ويتميز هذا النوع من الالتهاب بظهور قيح داخل جيب ليفي يكون مرتفع من على سطح الرئة وذو احجام مختلفة , وكانت اهم العزلات البكتيرية المشخصة في هذا النوع هي Streptococcus species وكان عددها 3 عزلات, *Staphylococcus aureus* وكان عددها عزلتين, *Klebsella pneumonia* وكان عددها 2 اما *Actinomyces pyogenes* و *Escherichia coli* حيث كانت هناك عزلة لكل واحدة منها.

ثانيا: ذات الرئة اليفيني الحاد والمزمن: كان عدد الحلات المصابة بهذا النوع 19 حالة مصابة (28.3%) وقسم الى قسمين الاول هو التهاب الرئة الليفيني الحاد حيث مثل 13 حالة مصابة (19.4%) وكان يتميز بظهور الفيبرين في القصبات والقصيبات مع ارتشاح كبير للعدلات والنوع المزمن حيث مثل 6 حالات مصابة (9%) وكان يتميز بارتشاح الفيبرين مع الخلايا الالتهابية وحيدة النواه مع قليل من العدلات وكانت العزلات البكتيرية المشخصة مع هذا النوع هي *Pasteurella multocida* وكان عددها 15 عزلة,

Staphylococcus aureus وكان عددها 3 عزلات, *Streptococcus speicies* , *Mannheimia hemolytica* , *E coli* و *Staphylococcus epidermidis* كان عددها عزلتين لكل نوع.

ثالثا: التهاب الرئة الخلالي الحاد الى المزمن: كان عدد الحالات المصابة بهذا النوع هي 7 حالات (10.4%) وكان بنوعين التهاب الرئة الخلالي الحاد حيث مثل 5 حالات مصابة (7.4%) ويتميز هذا النوع بتثخن الجدار الخلالي مع تكاثر النوع الثاني من الخلايا السنخية وارتشاح كبير للعدلات مع قليل من الخلايا الالتهابية وحيدة النواه. اما النوع الثاني فكان يتميز بتثخن كبير للجدار السنخي نتيجة الخلايا الالتهابية وحيدة النواة والخلايا اللمفية مع قليل من العدلات.

ايضا كان هناك 13 حالة مصابة بالنفاخ الرئوي, و8 حالات مصابة بالانغماس الرئوي وحالة واحدة هي تجلط في الشريان الرئوي.

كان العدد الكلي للبكتيريا المشخصة والمعزولة هو 89 عزلة بكتيرية وهي:

Streptococcus , عزلة 20 *Pasteurella multocida*, عزلة 28 *Staphylococcus aureus* *speicies* 15 عزلة, *Klebsiella pneumonia* 9 عزلات,*Proteus species* 6 عزلات,*Staphylococcus epidermidis* 5 عزلات, *E coli* 3 عزلات, *Mannheimia hemolytica* عزلتين و*Actinomyces pyogenes* عزلة واحدة.

اما بالنسبة الى ذات الرئة الطفيلي فقد شكل 3 حالات (4.5%) من المجموع الكلي للحالات المصابة وكان بنوعين الاول ذات الرئة القصبي القيحي المزمن والمخاطي وقد تمثل بحالتين ويتميز بوجود نضحة قيحية في المجاري التنفسي مع ارتشاح للعدلات وايضا هناك مخاط نتيجة تخرش الانسجة بسبب الطفيليات وقد عزل من اول حالة طفيلي *Dictyocaulus filaria* وفي الحالة الثانية فقد تم عزل *Dictyocaulus filaria* و *Muellerius capillaris* .

اما النوع الثاني من ذات الرئة الطفيلي والذي تم تشخيصه هو ذات الرئة النزلي القصبي والذي تمثل بحالة واحدة ويتميز بوجود نسبة عالية من المخاط نتيجة وجود الطفيلي في القصبات الهوائية وقد عزل من هذه الحالة طفيليان هما *Dictyocaulus filaria* و *Muellerius capillaris* .

لقد كان عدد العزلات الطفيلية 5 عزلات وهي *Dictyocaulus filarial* ب 3عزلات و *Muellerius capillaris* عزلتين.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بغداد

كلية الطب البيطري

الجوانب السببية المرضية لبعض افات ذات الرئة في الاغنام المذبوحة في محافظة ديالى

رسالة مقدمة الى

مجلس كلية الطب البيطري – جامعة بغداد وهي جزء من متطلبات نيل درجة

الماجستير في علوم الطب البيطري / الأمراض

من قبل

محمد عبد محمود ابراهيم

بأشراف

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