



## Pathological study of the main pulmonary infections isolated from sheep in Holy Kerbala province

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### Abstract

In this paper we updating some data previously publishing at 2019, present work was designed to implement two goals ,the first one aimed to isolate the most important causes (parasites and bacteria) of pulmonary infection in sheep. The second goal endeavored to experimental study the effect the main bacterial isolation in mice .The first goal achieved by examining 100 lungs at different area of Karbalaa for the period from November 2017 to March 2018. A total of 52 lungs of sheep were examined macroscopically and histopathological and the main condemned diseases in this study were reported hydatidosis. The total number of bacterial isolates which identified by culturing and biochemical tests were 51 samples. They were examined grossly for pulmonary lesion, 2 pieces of lesion were collected in 10% natural buffered formalin for fixation and sent to the laboratory for histopathological examination .The samples for bacteriological studies were taken directly from lungs aseptically after slaughter the animals and from each sample bacterial isolation were done on macConky agar ,blood agar and mannitol salt agar and biochemical tests .These bacterial samples were include: *Klebsiella pneumonia* 10 (19.6%), *Staphylococcus aureus* 9 (17.6%), *Escherichia coli* 8 (15.7%), *Pasturella multocida* 7 (13.7%), *Streptococcus pneumonia* 6 (11.8%), *Escherichia coli* O157 4 (7.8%), *Corynebacterium psudotuberculosis* 3 (5.9%), *Proteus spp* 2 (3.9%) and *Enterobacter spp* 2 (3.9%). Pneumonic lesions were classified microscopically into four types according to the inflammatory exudates: 1-Chronic interstitial Pneumonia was found in 19 pneumonic infected (44.2%) and characterized by thickening in alveolar wall and interstitial tissue due to infiltration of inflammatory cells .Most of the associated bacterial isolates were: 6 isolate of *Klebsiella pneumonia*, 5 isolate of *Pasurella multocida* and 4 isolate of *Streptococcus pneumonia* and 4 isolate of *Staphylococcus aureus*. 2-Suppurative bronchopneumonia was found in 15 pneumonic infected (34.9%) and characterized by suppurative exudates in bronchioles and bronchi with infiltration of inflammatory cells composed mainly of neutrophils .Most of the associated bacterial isolates were :5 isolate of *Staphylococcus auerus*, 4 isolate *Klebsiella pneumonia*, 2 isolate of *Streptococcus pneumonia* , 2 isolate of *Proteus spp* and 2 isolate of *Escherichia coli* O157. 3.fibrinous pleuropneumonia was found in 6 of pneumonic infected (13.9%) and characterized by fibrinous exudates in pulmonary tissue with cellular infiltration .Most of associated bacterial isolates were :2 isolate of *Escherichiacoli* O157 ,2 isolate of *Enterobacter spp* and 2 isolate of *Pasturella multocida*. 4.Granulomatous pneumonia was found in 3 ( 7 %) of pneumonic infected and characterized by granulomatous inflammatory reaction which consisted of central caseous necrosis surrounded by inflammatory cell and surrounded by fibrous connective tissue. Most of associated bacterial isolates were :3 isolate of *Corynebacterium psudotuberculosis*. Conclusion shows that examined lungs samples from different cities in Kerbala provelinc were infected with parasites and bacterial causes which associated with histopathological lesion mainly granulomatous and fibrinouse and suppurative pnumonia that considers the main cause for depletion and economic loses of animals.

**Keywords:** Sheep, Pulmonary infections, Pathology, Kerbala province.

### Introduction

more virulence pathogenic organisms can infect respiratory system of sheep, and most sheep appear normal because of immunity function so effective clearance occurs against these organisms. But if there are factors make disruption of this function

will result in impaired or damaged to respiratory tissues which allow entrance of dangerous pathogens to upper and lower respiratory tract component's . So that will initiation an inflammation or severe disease. Different microbes as viruses, bacteria, parasite, fungi, as well as host defense and environmental factors cane produced abnormal

tissues structure's and function's (Quinn et al.,2004). Inflammation of pulmonary tissues named Pneumonia mainly concerned with lower respiratory structure's as bronchioles and alveoli, many infective agents resulting in the consolidation of lung tissue. Pulmonary infection caused by a complex interaction, involving interaction between host (physiological and immunological), various agent (viral, bacterial, parasitic and mycoplasma), environmental factors and poor management. Pathological changes observations showed several histological forms are mild to severe, acute to chronic and exudative to proliferative interstitial (Bruereet al.,2002). Pneumonia lead to decline in the weight, high cost of treatment and high rate of morbidity and mortality in the sheep (Salihet al.,2014). Iraqi researcher's data reported that livestock as sheep goat and cattle have important traditional and popular benefit for all live aspect (milk, meat and others economic uses) (Bruereet al.,2002). Most harmful effects reported in sheep are Pneumonia of different types, pulmonary abscess, pleurisy (adhesion), emphysema, Hydrothorax, pulmonary tuberculosis, melanosis and parasitic infestations (Jones et al.,1997). The highest prevalence rates of gross pathological lesions of the lung were pneumonia of various types and lightest was observed in T. B. like lesions was 3. 34% and 0. 04% out of total slaughter sheep, respectively. While histopathological lesion were recorded interstitial pneumonia, edema, emphysema, bronchopneumonia, atelectasis, verminous pneumonia, granuloma, necrosis and hemorrhage in variable percentage (Hadeel, 2017). There are multiple agents may be infectious or noninfectious can affect the lung and cause damage to it and lead to formation of lesions in the lungs, therefore the study of the lung disease are essential because they result in heavy economic losses in the flock of the sheep. Generally, the disease of respiratory system, mainly pneumonia cause high cost of the treatment, morbidity and high mortality rate in the small ruminant especially sheep (Amaravathi et al.,2016). . For this reasons, study of the pathogens (bacteria and parasites) and their lesions in sheep in Karbala province is important, and therefore this study aimed to: 1. Isolation the most infectious agents (either parasite or bacteria) from infected lung. 2- Infestatioin about histopathological associated with isolated and infected tissue's.

### Material and Methods

**Samples Collection:** One hundred suspected infected lungs of the slaughtered sheep were examined from the different sites of Karblaa such as Al-Hussainia, center of Karblaa and Al-Badaa) in Karblaa province. These samples were collected from November 2017 to March 2018. Each sample was placed in a separate sterile container labeled with animal, tissue and date identification and the sterile instruments (scissor, scalpel and thumb forceps) used for lung specimen collection. Gross lesion were recorded by making cut surface and incision to observe the inner lesion and recorded any abnormalities. Incision was pieces of lesion were taken for bacteriological studies and histopathological examination. The samples were collected in plastic bag immediately after slaughtered and transported in refrigerated box contained ice bag to the microbiology lab. of University of Karblaa. Part of lung was conserved in 10% neutral buffered formalin solution for histopathological study.

**Parasite Examination Methods (Direct smear).** Infected lung with hydatid cyst fluids were examined in special test tubes (5-8 ml), separated by centrifugation at 4000 rpm for 30 minutes (Jones et al., 1997). Then one drop was taken from supernatant to see protoscolex under light microscope to detect viability of PSC.

### Isolation of bacteria from infected lung sample by two methods :

**A- The surface** of infected lung was sterilized by hot scalpel blade, then incised after cooling and enter cotton swab or platinum wire –loop then cultured on blood, MacConky and mannitol salt agars.

**B- Part of lesion** has been taken from infected lung after sterilization, put in sterilized test tubes which contain 5 ml of peptone water broth, then incubated for 24-48hrs. at 37°C and cultured on blood, mannitol salt and MacConky agar(Quinn et al., 2004).

**Identification of bacterial Isolates:** Identification of bacterial isolates made by staining reaction, cell morphology, colony characteristics on different media, biochemical characteristics and hemolysis on blood agar.

### Results and Discussion

**1. Parasitic and bacterial isolation:** A total number of samples 100 sheep that had pulmonary diseases caused by parasite (Hydatid cysts) or bacterial infection were collected. In our study isolated 52(52%)samples infected with hydatidcysts, bacterial infections 43 (43%), showed in Tables (1)( 2)( 3).

**Table (1): the number and percentage of parasitic and bacterial isolated from infected sheep's lung.**

Parasitic samples	Isolation of samples		Total No.
	Positive	Negative	
<i>Hydatid cyst</i> (No=100)	52(52%)	48(48%)	100 (100%)
Bacterial infection (No=100)	43(43%)	57(57%)	100(100%)
Unknown (No= 100)	5(5%)	95(95%)	100(100%)

Chi square =3. 56 df=1, P= 0. 0001

**Bacterial isolation:** This Table showed bacterial isolate types and number out of 51/100 samples were positive and the following organisms were isolated from infectious cases were *Klebsiella pneumonia* 10 (19. 6 %), *Staphylococcus aureus* 9 (17. 6 %), *Escherichia coli* 8 (15. 7%), *Pasturellamultocida* 7 (13. 7%), *Streptococcus pneumonia* 6 (11. 8%), *Escherichia coli* O1574 (7. 8%), *Corynebacterium psudotuberculosis* 3(5. 9 %),

*Enterobactorspp* 2 (3. 9%)*Proteusspp*2 (3. 9%). This study showed that association between bacteria and pulmonary diseases can classified into different groups depended on microscopically finding of cases were : Chronic interstitial pneumonia 19 (44. 2%). B- Suppurative bronchopneumonia 15 (34. 9%). C- Fibrinous pneumonia 6(13. 9%). D- Granulomatous pneumonia 3 (7%).

**Table(2): shows number and percentage of bacterial isolated from sheep lungs**

Types of isolated bacteria	Samples No.	Percentage %
<i>Klebsilla pneumonia</i>	10	19. 6%
<i>Staphylococcus aureus</i>	9	17. 6 %
<i>Escherichia coli</i>	8	15. 7%
<i>Pasturellamultocida</i>	7	13. 7 %
<i>Streptococcus pneumonia</i>	6	11. 8 %
<i>Escherichia coli</i> O157	4	7. 8%
<i>Corynbacterium pseudotuberculosis</i>	3	5. 9 %
<i>Enterobactorspp</i>	2	3. 9%
<i>Proteus spp.</i>	2	3. 9%
Total	51	100%

**Table (3): Shows number and percentage of bacterial isolate according to different pneumonia**

Pulmonary lesion	Samples No.	Percentage
Interstitial pneumonia	19	44. 2 %
Suppurative bronchopneumonia	15	34. 9%
Fibrinous pneumonia	6	13. 9 %
Granulmatous pneumonia	3	7 %
Total	43	100%

**Histopathological Study** : Tissues of infected lung's sheep were examined in laboratory of histopathology , after microbiological isolation performed , results sowed different type of pneumonia **1- interstitial pneumonia**:there isextensive thickening of alveolar walls and interstitial tissue by fibroblast proliferation

withmononuclear cell infiltrations and congested blood vesselin alveolar capillary leading to narrowing of alveolar lumen Figure (1).

**Acute suppurative bronchopneumonia**: there is cellular infiltrate in the bronchial wall epithelia causing sloughing of some epithelial lining, also observed as in Figure(2).

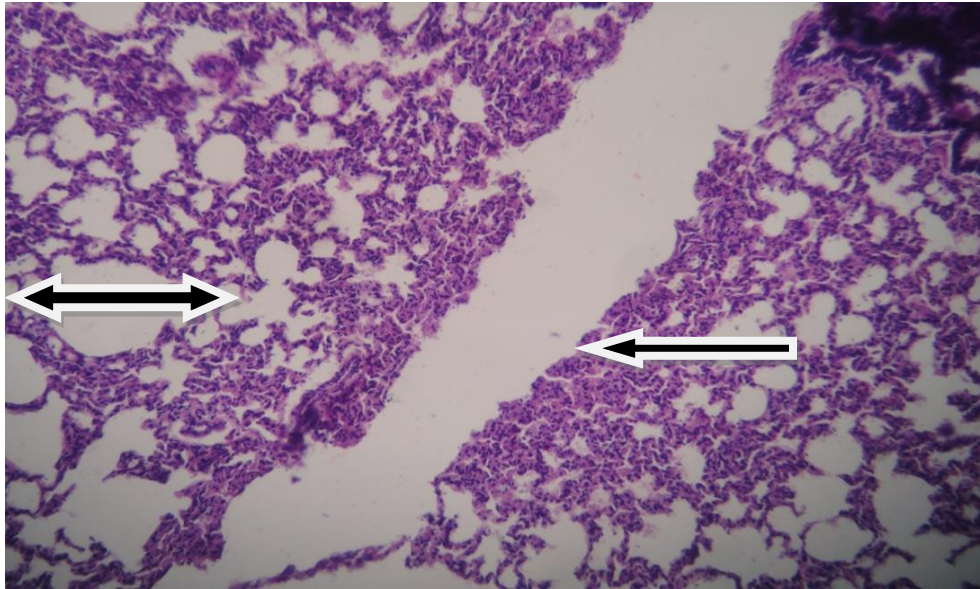
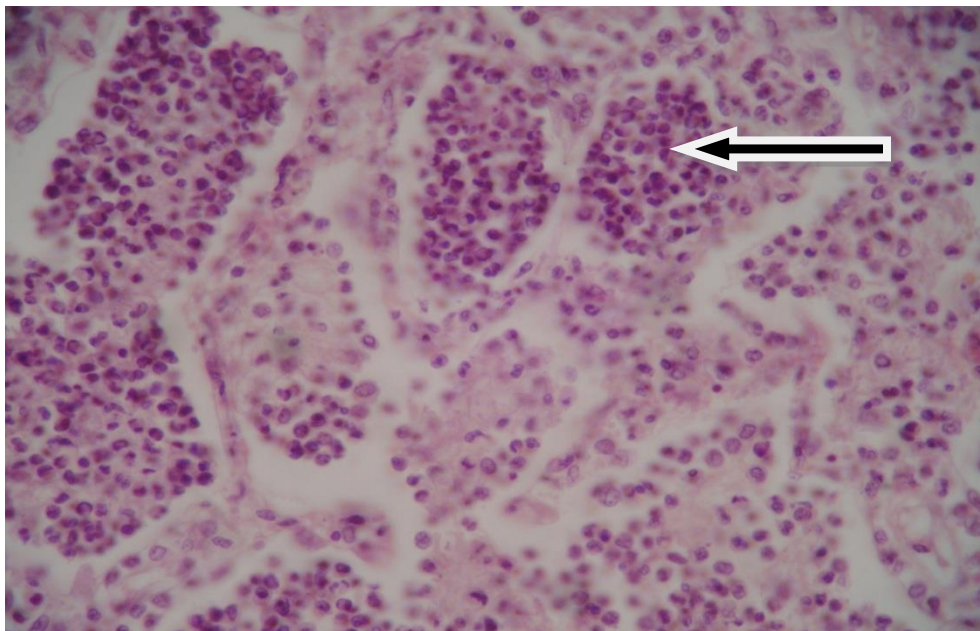


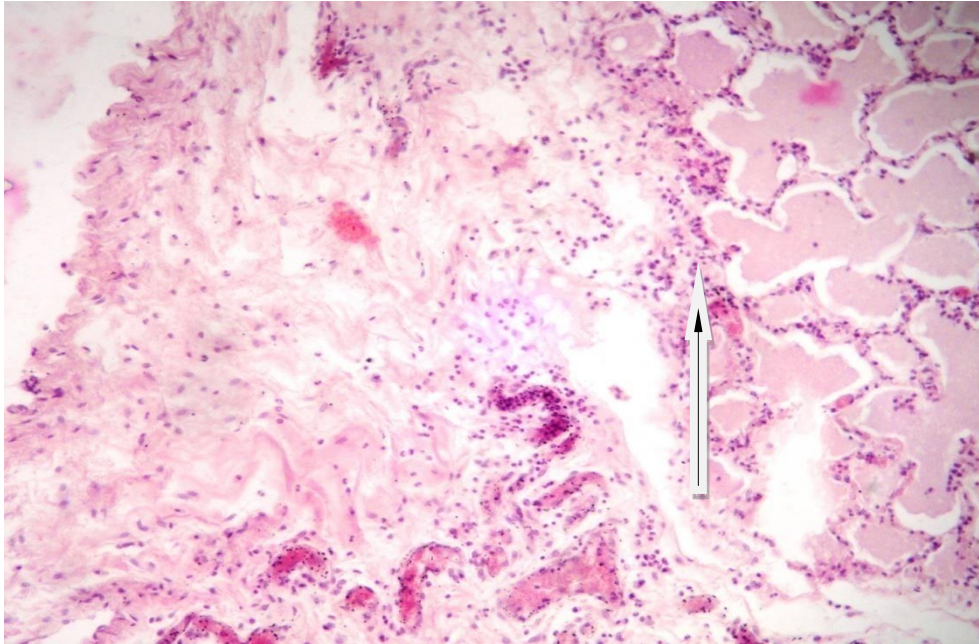
Figure (1): Histopathological section of the lung of sheep shows interstitial Pneumonia with moderate thickness of interalveolar septa due to replacement by inflammatory cell with emptyalveolar space. (H&E stain 100X).



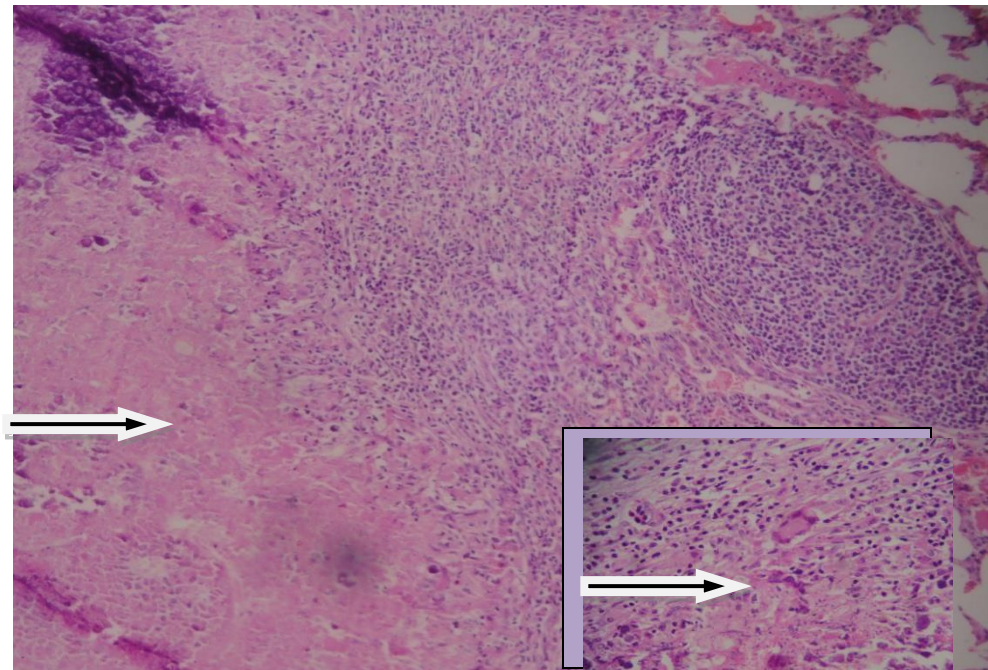
Figure(2): Histopathological section of lung of sheep showing alveolar spaces filled polymorphonucleus with edema (H&E)X400.

**Fibrinouspleuro pneumonia:** There was extensive fibrinous exudates together with neutrophils, RBCs deposition in pleura, pulmonary alveoli and bronchiole. There was congestion of alveolar wall and pulmonary tissue, Figure (3).

**Granulomatous pneumonia:** There is many granulomatous lesions within pulmonary tissue had central pale pink coloured necrotic area surrounded by inflammatory cells 'principally with epithelioid and giant cells and areas of fibrosis, Figure (4).



Figure(3): Histopathological section of lung of sheep shows fibrinous exudates in the pleura with neutrophils infiltration. (H&E stain 100X).



Figure(4): Histopathological section of lung of sheep shows granulomatous inflammatory reaction which consist of central caseous necrosiswith giant cell formation (H&E stain 100X).

This study showed percentage of pulmonary lesions in sheep through examination of 100 samples. It was found that most hydatid cysts were observed in (52%). The prevalence of hydatid cysts were higher than those noted in a retrospective survey conducted in Kerbala abattoirs (Al-Nassir *et al.*, 2012). There are many factors behind the high prevalence of hydatidiosis in Kerbala province, such as presence of many stray dog population particularly around abattoirs plays an important role in transmission of the hydatid cyst (*Echinococcus granulosus*) because infected dogs are able to excrete a great number of segments full of egg with their feces containing the soil and spreading the infection and illegal killing of food animals outside slaughterhouses usually without veterinary inspection, as well as improper disposal of affected organs (Al-Nassir *et al.*, 2012). In Iraq and elsewhere, ascertainment rate of pathological lesions may be affected by season, rainfall level, climate, feeding regimen, route of husbandry as well as variable numbers of animals slaughtered due to local tradition and religious feasts and socio-economic and culture background (Mahmoud, 1980; Ghaffer, 2008). Apart from environmental factors, other important agents play major roles such as types and strain of bacteria and their virulence, the immunological status of the body, the nutritional content of the ration and animal management followed by consequently, hydatidosis is dependent on the age and immune response of the host (Soluly, 1982). The histopathological findings showed in this study were in agreement with those described in several scientific literatures (George, 1972; Jones *et al.*, 1997). The alveolar emphysema and pulmonary atelectasis usually accompany pneumonic lesions (Jones *et al.*, 1997). The microscopically changes of the lung lesions indicated high occurrences of different types of pneumonia include in: interstitial pneumonia, suppurative bronchopneumonia, Fibrinous pleuro pneumonia, granulomatous pneumonia, parasitic pneumonia and this variation in the prevalence rates of different types of pneumonia in sheep in different studies also may be attributed to the factors variation such as nutritional status, breed, nature of country and environmental condition, also the effect of stressors involving transporting and overcrowding, all these factors could possibly play a constructive role in predisposing factors for development of different types of pneumonia (Ertan, 2006; Ezzi *et al.*, 2007; Mohammed *et al.*, 2012). This phenomenon confirmed other interpretations that lung infections are common in

ruminants, especially in herbivores are conducive to adverse environmental conditions (Edwards *et al.*, 1999). The frequency rate of this disease varies from one place to another. In Iraq mainly in Kirkuk slaughterhouse the frequency rate of pneumonia showed (8.4%) from total examined cases (Kadiret *et al.*, 2012). In Khanqeen abattoir the frequency rate of bacterial pneumonia represents (6.4%) from total examined (Mahmoud, 2017) while in India the frequency rate varies from 15 to 27% and the reference the difference in the ratio of infection in the same country. In New Zealand the frequency rate was 3.4% (Goodin-Ray, 2006). In the Benjaf the frequency rate was 6% and in the Tara city was 2.4% (Symbyal *et al.* 1980). The interstitial pneumonia represents (47.4%) of the total cases of pneumonia more isolates were *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pasteurella multocida*, *Streptococcus pneumonia*. This is in agreement with Sharma (1977) and Abubaker (1980), who recorded interstitial pulmonary response in the infected sheep. Also, in this study there are bacteria recorded in the interstitial pneumonia and these bacteria were *Pasteurella multocida*, *Staphylococcus aureus*, *Streptococcus pneumonia* which is similar to other researchers isolated these bacteria from the interstitial pneumonia (Mahmoud *et al.*, 2017). There are other bacteria recorded in the interstitial pneumonia and these bacteria were *Klebsiella pneumonia* similar with other researchers isolated this bacteria from interstitial pneumonia (Abbas, H and Zainab, W., 2016). The pathological changes as well as macroscopic and their distribution in the lungs reported in this study it was similar to (Bhagwan & Singh 1972; Abubaker *et al.*, 1980). The suppurative pneumonia represents (28.9%) from the total cases of pneumonia. There were two types of it, firstly the suppurative bronchopneumonia which represents 18.4% and second type the pulmonary abscess which represents 15.5%, the frequency rate of occurrence registered by other researchers, such as (Ozyildiz *et al.* 2013), Sheehan *et al.* (2007) who registered respectively occurrence rates: 43%, 36%, 37%. Other researchers isolated both types of bacteria (*Staph.* & *Strept.*) from naturally affected animal and revealed the ability of this bacteria induce the suppurative pneumonia (Hazirolu *et al.* 1994). The other bacteria were isolated from suppurative pneumonia are *Klebsiella pneumonia* and the number of the isolates are 2, *Proteus* spp and the number of the isolates are 2, *E. coli* and the number of isolates 2, these bacterial agents were isolated from pneumonia (Boljihad *et al.*, 1995). The

fibrinous pleuro pneumonia represent (15. 8%) from total pneumonia types. The bacteria which isolated from this type were *Pasturella multocida* and this type of pneumonia is reported conveniently and caused by this bacteria in experimental and natural pneumonia in lamb and sheep (Aziz *et al.*,2015). The other bacteria isolated from this type of pneumonia are *Echerichia coli* and *Enterobactorspp.* The granulomatous pneumonia represent (7. 9%) from total pneumonia types. The bacteria which isolated from this pneumonia were *Corynebacterium pseudotuberculosis*. Consequently granulomatous lesions similar those of tuberculosis with the typical histopathological observation as demonstrated by (Mahdi *et al.*,2015).

### Conclusion

Data reports shows that examined lungs samples from different cities in Kerbala prevalence were infected with parasites and bacterial causes which associated with histopathological lesion mainly granulomatous and fibrinous and suppurative pneumonia that considers the main cause for delectation and economic loses of animals

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