

HISTOPATHOMICROBIAL EXAMINATION OF *ECHINOCOCCOSIS* IN LUNG AND LIVER INFECTED WITH *KLEBSIELLA PNEUMONIA* IN SHEEP OF IRAQ.

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ABSTRACT

Investigation about hydatid cyst infection in lungs and liver of sheep and its association with *Klebsiella pneumonia* was done ,specimens were collected from sick and healthy sheep at different slaughter houses in Iraq Cities, each specimens which contained cystic lesions were divided to three parts , 1st part was sent to microbial laboratory ,2nd part was sent to parasitology laboratory, 3rd part was sent to histopathology laboratory . A number (100) of infected specimens lung and liver were examined grossly in order to look for any cystic lesions, as well as each specimen was examined microbiologically, and were positive for both *Klebsiella pneumonia* and parasitism. Histopathological examination grossly showed cystic lesions on surfaces of lungs and livers with paleness appearance, microscopically pathological lesion mainly were edematous and hemorrhagic changes inside and outside alveoli in lung, with sever destructive and necrotic with hemolytic changes in livers .Reported results' concluded that most cystic lesions' specimens were presented in lung , liver which were infected with bacteria *Klebsiella pneumonia*.

Key word: respiratory, digestive, parasitic, bacterial, animals

هاشم

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تشخيص نسيجي مرضي مايكروبي لداء المشوكات في رئة وكبد الاغنام المصابة بالكليبيسيلا الرئوية في العراق

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المستخلص

تم التقصي عن الاصابة بالاكياس المائية في رئات واكباد الاغنام وعلاقتها بالكليبيسيلا الرئوية، العينات جمعت من حيوانات مريضة وصحيحة في مجازمختلفة لمدن العراق، كل عينة كانت تحتوي آفات تكيسية قسمت الى ثلاث اجزاء، الجزء الاول ارسل الى مختبرالمايكروبيولوجي، الجزء الثاني ارسل الى مختبرالطفيليات، الجزء الثالث ارسل الى مختبرالهستوباثولوجي. عدد100من عينات الرئات والاكباد شخضت عيانيا، وكذلك تم تشخيص العينات في المختبر. أظهرت النتائج انه اكثر من 50%من العينات الكبدية والرئوية كانت مصابة بالاكياس المائية وكانت موجبة للاصابة بالكليبيسيلا الرئوية.التشخيص المرضي أظهرآفات تكيسية على سطح الرئة والكبد مع مظهرشاحب. الآفات المرضية المجهرية بصورة رئيسية كانت تغيرات وذمية ونزفية داخل وخارج الحويصلات الرئوية، مع تغيرات تحطمية وتنخرية قاسية وتحلل دموي في الاكباد. استنتاج النتائج المسجلة الاصابة الطفيلية بالاكياس المائية كانت واضحة الوجود في الرئات والاكباد المصابة بالكليبيسيلا الرئوية.

الكلمات المفتاحية: تنفسي، هضمي، طفيلي، بكتيري، حيوانات.

INTRODUCTION

The first discovery of infection with *Klebsiella pneumonia* was in Taiwan at 1980 in liver abscess biopsies of diabetic person in spite of their no biliary tract disorders. (9; 35) . As sequel community's infection develops in other part of word and reach to Asia, North America, Europe and Australasia (10; 31). Virulence strains of *klebsiella pneumonia* have serotype K1 or K2 which are hyper mucoid strains (28).infection's spreads and results endo- phthalmitis's and meningitis's inflammations (29).Main routs is hematological via portal vein (18).Other rout for infection through biliary rout. (21; 16; 22).Which associated with malignancy and bacteria's proliferation's within bile ducts in cases of ascending's cholangitis' invasive liver's lobs. (3; 24). Experimental researching on mouse's tissues found that *K. pneumoniae* passing intestine's barrier to producing liver's abscess's (8).Echinococcosis's term refer to parasiting disease's causing by *Echinococcus sp.*(7;11). Which related to family's Taeniidae , tapeworm's diseases the proper and zoon tic's host is Dog, others intermediate host Hyenas and Cats carry's the adults parasitic cyst's as sub clinic infection's factors asymptotically , vitals' organs lung and liver are the target sites' for invasion , maturation of larvae and growing mainly in body of infected individuals maturity phase contained internal or germinal's layer, then middle or lamina's layer and last's 3rd one adventitia's layer.(11; 14) About two or three million cases with *E. granulosus* reported of world life.(14; 15) Mostly in human's and animal's. Tearing effects led to irreversible injury punctuating cysts can released larval seeding within different organ's producing anaphylactic shock .(15).Slaughtering animals debris contained many cysts but they not mature enough to cause systemic distress , when these cysts feed's by dog's , they become mature and discrete within fecal materials and contaminated grass and plants and be effective for causing disease. (15;

34)Straining of Echinococcosis's from g1 to g10, g1 is sheep strain, g2 Tasmanian's sheep strain's, g3 is buffalo's ,all them beyond to *Echinococcus granulosus* ,g4 strain special of equine which are intermediates host these strain is un mature and known *Echinococcus equine's* ,g5 is bovine strain's known *Echinococcus ortleppi*, g6 is camel's strains , g7 pig's strain's . g8, g9 and g10 named *E. Canadensis's* had been reported in human(6;27)

MATERIALS AND METHODS

Survey samples

A – Infected lung's and liver's samples (100) were collected randomly from sheep in Iraqi Cities slaughter's housing.

B- Special cleaned containers; test's tubes and without fixatives' and another's with fixatives.

Experiment's sampling

1- lungs and livers piece's about 1cm³ size taken from enclosed lesions tissues t and fixed with 10% neutral formalin solution for , after few days all pieces were processing according to(19)(30).

2- Cystic lesions fluids were taken in special test tubes and centrifuged, supernatant's drop were examined on ordinary glass slide to see protoscolex under microscope (23)

Microbial test's API-20E

Biochemical examining collecting system constituting number of twenty biochemical tests which they are specificity to *K. pneumonia*. (12).

Examinations tests' hydatid cyst

Cystic fluids must be examining as following:

1-Put in tests tubes (6 ml).

2-Must is separated by centrifugation

3- Fluid droplet put on glass slides for showing protoscolex under light microscope. (25).

RESULTS AND DISCUSSION

Examined samples shows presence of cystic lesion in livers and lungs, indicated for existence of parasitism infection with hydatid cysts as well as the same samples were positive for bacterial infection with *K. pneumonia*. As shows in Table 1 and Figure 1.

Table 1 . Infection rate of sheep lungs at slaughter house

Samples	Total	%
Lungs	100	52(52%)*
Livers	100	42(42%)*
None infected	6	(6%)

Survey showing highly incidences and significantly at *: P 0. 50

Microbiological results of abarotory analysis for examination of *Klebsiella pneumonia* were specific positive results as shows in Figure 1.



Figure 1. Results of the specific analysis of differentiated positive testes for diagnosis of *Klebsiella pneumonia* isolated from lungs and livers

Gross appearance: grossly appearance shows cystic structures scattered out of liver's and lungs' surfaces. Infected lungs and livers appears paleness with cystic lesions scattered within external or internal surface. Figure 2 A&B and C .

Microscopic appearance

On microscopically appearance hydatid cysts appeared have thick wall consists of three structural details: cellular laminated membrane; germinal membrane and protoscolex. Figure 3(A, B, C).

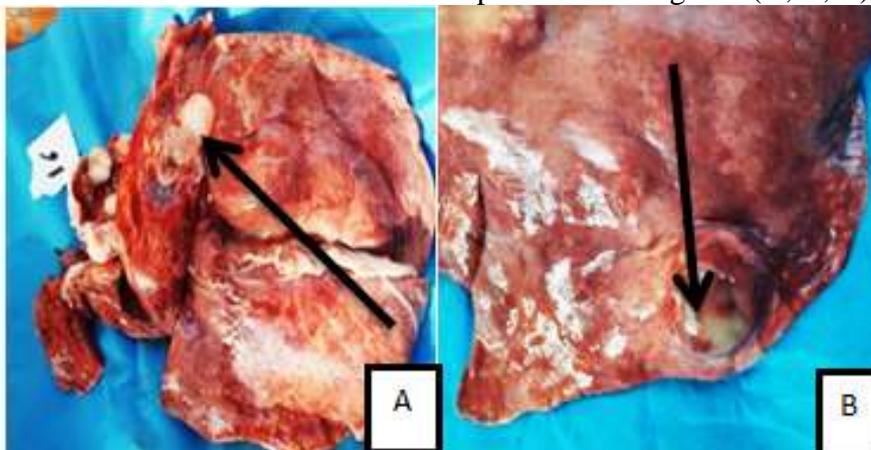


Figure 2. A&B liver. Gross appearance of hydatid cysts in examined samples. (arrow)

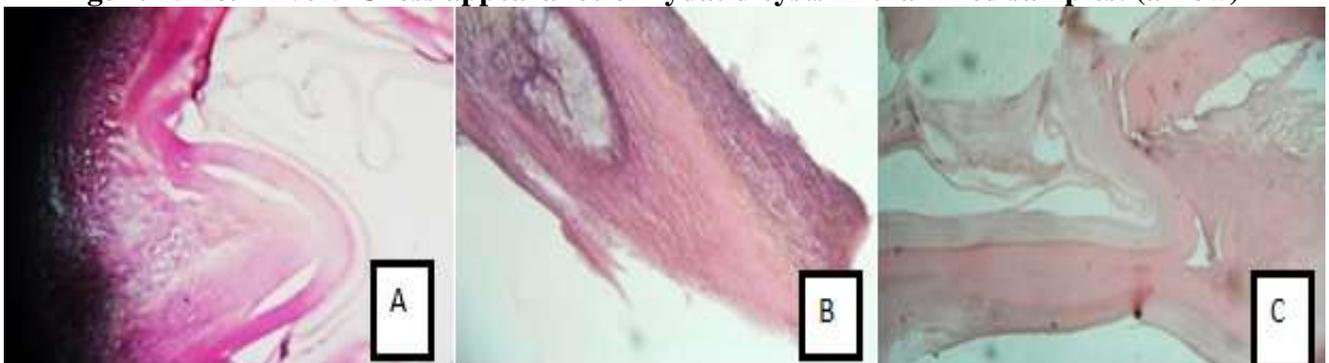


Figure3. A, B and C : Examined Hydatid cysts showed their structure : A : protoscolex B: cellular laminated membrane C: germinal membrane and protoscolex

Affected lungs and livers, shows severe pathological changes closed to cystic lesion characterized by acute phase represented by heavy inflammatory response mainly neutrophils and eosinophils with fluid oozing appear as edematous lesion, lung's alveoli

appears emphysematous and others sections of affected lungs have thick inter alveolar septa which refers to interstitial pneumonia occurrence. Figures 4.

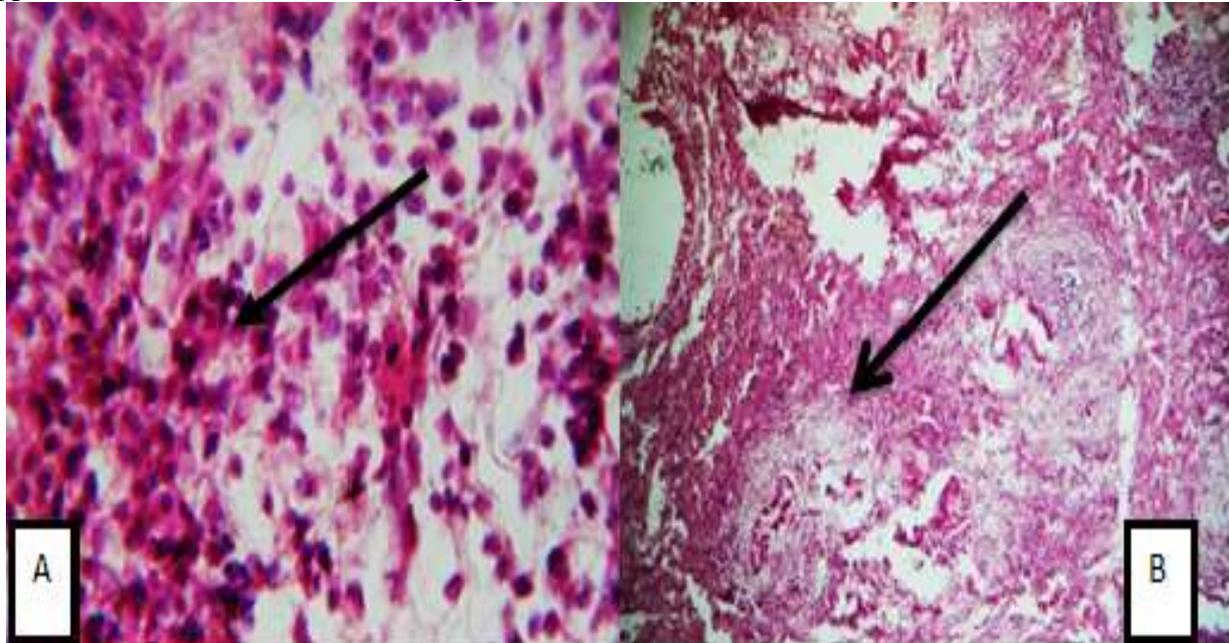


Figure 4 A. Lung of sheep infected with hydatid cysts and *Klebsiella pneumonia* shows eosinophil's inflammatory reaction due to aggregations of eosinophil's (black arrow); B: neutrophils infiltration with necrotic debris;

Other sections of examined lungs tissues and livers shows chronic inflammatory response represented by interstitial Pneumonias that's refers to inflammation of inter alveolar area within lungs parenchyma, in liver histopathological examination observations mainly granulomatous and fibrotic changes

which resulting from chronic parasitic infection and harmful effects of worm' migration through blood vessels until reach to hepatic tissues accompanied by inflammatory reaction against parasitic and bacterial infection, as shows in Figure 5.

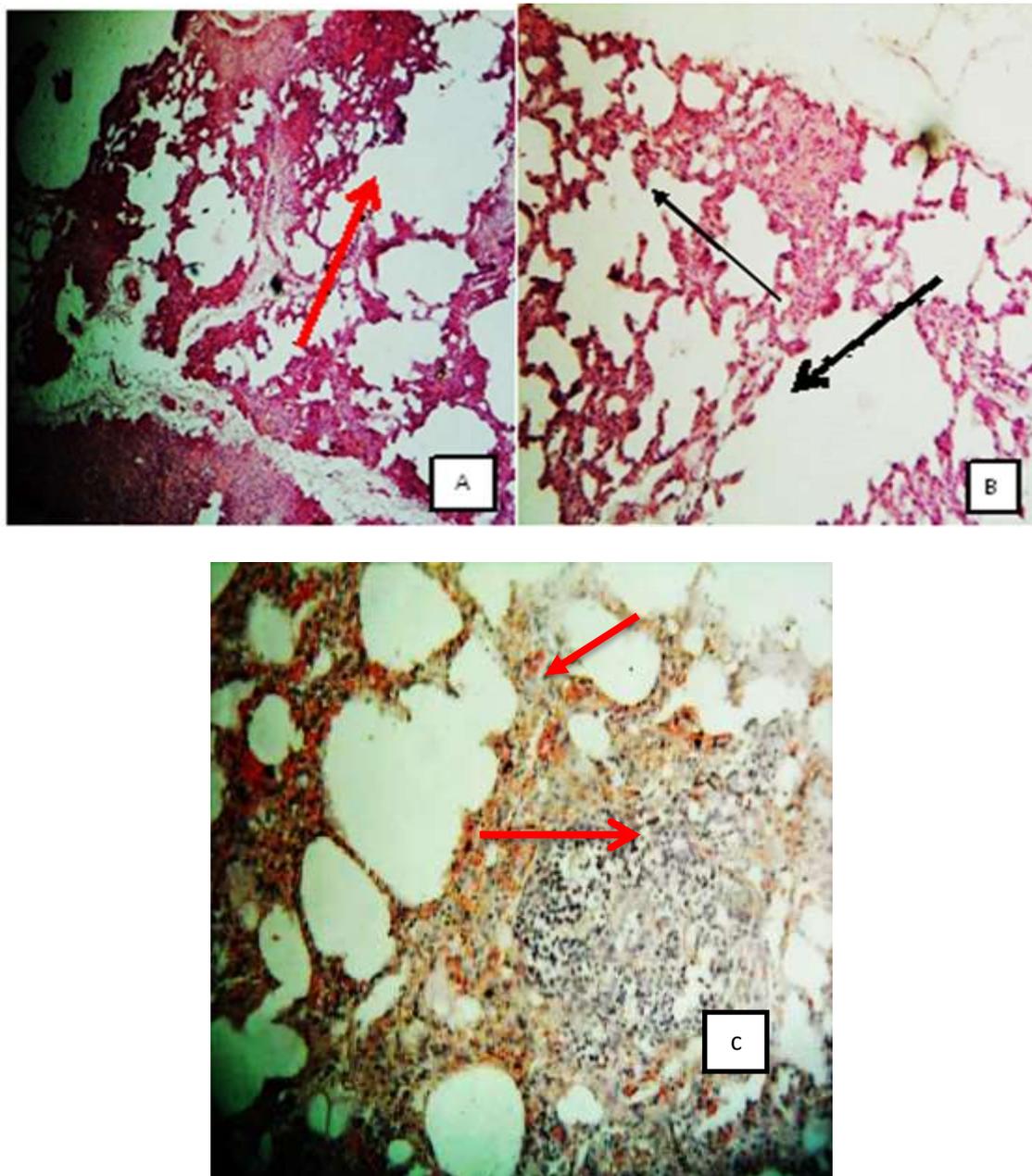


Figure 5. Lung of sheep infected with Hydatid cysts and *Klebsiella pneumonia* affected lungs showed A ;B and C: interstitial Pneumonia with emphysema appearance as branching's from destructing parasitic effects and enclosed to heavy inflammatory cell infiltrations (red arrow) .

Histopathological examined specimen 's lunges and livers shows acute phase of parasitic and bacterial infection appear as focal aggregation of neutrophil's and eosinophil's and fibrin us exudation and inflammation gradually converted to fibrous tissue at chronic phase of parasitic associated with bacterial infection, other Iraqi authors report's revealed similar pathogens (25). *Klebsiella pneumonia* caused severe infection and to highly mortality and food industrial losses , many researchers found that infectious Pneumonia in sheep can caused lower immune status which led to attacking of virulent parasitic infection as

Echinococcus granulose (33) Different pathogens with cystic lesions were detected by other authors in sheep of Iraq as well as associated with *Klebsiella pneumonia* (36,12) .Paleness anemia and blood losses as a results of sharpness of protoscolices invasions intercellular space represents inflammatory response and tearing effects of parasites are agrees with (1). Loss of alveolar wall and dilation produced emphysematous changes were enclosed with reports of author.(32). *K. pneumonia* utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane

proteins and determinants for iron acquisition and nitrogen source utilization's, for survival and immune evasion during infection (26). Examined lung's samples showed highly incidence of bacterial infection with *K. pneumoniae* in Iraqi cities and this agreed with some authors, who isolated the same bacteria from nose of infected sheep in Iraq. (2). Results showed presence of infection with two type of pathogen which is parasitic infection as well as bacterial infection and these results agreed with authors (2, 36.5). We found that examined samples of livers and lungs were infected and reported at significance's value (100%) occurrence for hydatid cysts as well as for *Klebsiella pneumoniae*, sudden death occurs mainly without sureness diagnosis of parasitic infection in most cases, as reporting in other's researches. (13,17, 20,36).

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