

# Isolation Of Bacteria Associated With Hepatic Hydatid Cyst Of Iraqi Sheep

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## Abstract

The goal of the current study was to investigate the incidence of liver hydatid cyst in apparently healthy sheep, for such objective a survey from November 2019 until April 2020 was performed in AL-Shoula slaughterhouse/ Baghdad province to find out most frequent hepatic hydatid cyst lesions and the associated pathogen. Out of 2196 slaughtered sheep, 50 livers showed a detectable hydatid cyst, these lesions were sampled and transported directly to the laboratory for bacteriological examination. The content was inoculated into cultures media. The isolates were identified according to Gram's stain, as well as biochemical characters for final bacterial identification.

The prevalence of hydatid cyst were (2.28%). In total, 54 of bacteria isolate were identified, these bacteria represented 10 species. All samples were subjected for primary bacterial isolation and the following bacterial species were isolated, *Staphylococcus aureus* 4(7.41%), *E.coli* 17(31.48%), *Pseudomonas auroginosa* 4(7.41%), *Klebsiella pneumonia* 8(14.82%), *Listeria grayi* 5(9.26%), *Proteus vulgaris* 4(7.41%), *Salmonella typhi* 2(3.70%), *Streptococcus pyogenes* 2(3.70%), *Enterococcus faecalis* 5(9.26%), *Shigella flexneri* 3(5.55%). This study recorded that the percentage of *E.coli* (31.48%) is significantly higher ( $P<0.01$ ) as compared to the lower percentage as in *Salmonella typhi* and *Streptococcus pyogenes* (3.70%). In conclusion, the prevalence of liver hydatid cyst were low in Baghdad city and the *E.coli* was the predominant bacteria isolated.

**Key words:** sheep, liver, hydatid cyst, Baghdad city

## Introduction:

The liver could be considered the major vital Known biological structure in mammalian metabolism, since it the main site for regulation of carbohydrate, protein and lipid metabolism through the synthesis of albumin, fibrinogen, prothrombin and the essential role that plays in fat digestion by the secretion of bile (Huntington, 1990; Danfaer, 1994). Also, this organ acts as a storage compartment for many vitamins, trace elements, glycogens as well as its contribution in immune regulation via the action of Kupffer cells (Jones *et al.*, 1997; Kelly, 2007).

Thus, any disease condition of liver influences on one or more of the mentioned vital functions that will reflect on animal growth, wool or meat production, general health status, and disease tolerance (Constable *et al.*, 2016). Millions of dollars are the estimated economic losses annually due to the condemnation of whole infected livers or trimming of a portion at slaughtering inspection (Khaniki *et al.*, 2013; Liba *et al.*, 2017).

Hydatid cysts, the larval stage of the dog tapeworm *Echinococcus granulosus* that usually localized in sheep liver have a major zoonotic impact on human health (Khajuria *et al.*, 2013). The liver examination is a fundamental practice in meat inspection since liver lesions may indicate the presence of many diseases in other organs since liver acts as a catchment for the vast absorptive area of the gut, with all its resident microorganisms (Kelly, 2007). Many previous studies have linked such lesions to many microbes including *Staphylococcus aureus*, *Corynebacterium pyogenes*, *Escherichia coli*,

*Corynebacterium ovis*, *Fusobacterium necrophorum* and *Clostridium* species (Ghadrdan-Mashhadi and Yosefi, 2004; El-Shahawy and Abdel-Gaied, 2006; Abo El Fetouh *et al.*, 2010; Tehrani *et al.*, 2012). This Study aimed to investigate the incidence of hydatid cyst affecting the liver of sheep slaughtered in Baghdad province, Isolation and identification of some bacterial agents associated with detected liver lesion.

## Material and methods

### 1. Animals

Sheep at 1.5 – 3 years of age, local breed and both sexes were presented for slaughter in Al-Shoula slaughter house/ Baghdad province; The study extended from November 2019 until April 2020.

### 2. Sample collection

A one visit a week was conducted periodically to Al-Shoula abattoir during which post-mortem examination of the livers of slaughtered sheep. A total of fifty livers showed pathological lesions (hydatid cyst) from sheep which were collected and thoroughly examined. The livers showed visible lesion of hydatid cyst that were collected into plastic bags, described and labeled in place and transferred in an ice box and under aseptic condition to the laboratory for bacteriological and parasitic examination.

### 3. Macroscopic examination technique:

It was done according to Herenda *et al.* (2000) mainly to identify the parasitic causes of liver condemnation. This included description of the hepatic lesions due to parasitic infestations (shape, size and consistence) of the lesion.

### 4. Culturing, identification and preservation:

Samples Surfaces were sterilized by a hot spatula and incised by using a sterile scalpel. By using a sterilized loop, the exudates or the pus were inoculated into a nutrient agar, were then incubated aerobically at 37°C and anaerobically using an aerobic jar for 24 hours, different colonies were selected and transferred to a new nutrient agar, blood agar and macConkey agar plate and incubated at 37°C for 24 hours (Quinn *et al.*, 2004). The existence of similar distinct colonies along the streak line and their pureness was microscopically demonstrated by Gram's stain reaction were obtained and macroscopically described (Markey *et al.*, 2013).

The definitive diagnostic isolates were cultured on brain heart infusion agar and glycerol 20% and incubated at 37 °C for 24 hours, stored in a deep freezing and re-cultured monthly to maintain their viability and activity. Gram's staining Performed according to procedure described by Quinn *et al.*, (2004). Catalase test and Oxidase test Performed according to procedure described by Quinn *et al.*, (2004) and Markey *et al.*, (2013). And KOH test and slide coagulase test Performed according to procedure described by Quinn *et al.* (2004)

## Results:

### 1. The incidence of liver diseases:

In the present investigation, out of 2196 sheep slaughtered at Baghdad city/ Iraq (both sex with a different age), only 50 animals revealed definite lesions of hydatid cyst in their livers on gross examination with an overall incidence of 2.28% (Table 1).

**Table (1): Incidence of gross liver hydatid cyst in sheep.**

Number of sheep examined	Number of sheep with gross Liver hydatid cyst lesions (%)
2196	50(2.28%)

## 2. Bacterial isolates:

In the present study, 50 samples of sheep livers were subjected for primary bacterial isolation. A total of 54 isolates of bacteria were identified, these bacteria represented 10 species and the following bacterial species were isolated; *Staphylococcus aureus* 4(7.41%), *E.coli* 17(31.48%), *Pseudomonas auroginosa* 4(7.41%), *Klebsiella pneumonia* 8(14.82%), *Listeria grayi* 5(9.26%), *Proteus vulgaris* 4(7.41%), *Salmonella typhi* 2 (3.70%), *Streptococcus pyogenes* 2(3.70%), *Enterococcus faecalis* 5(9.26%), *Shigella flexneri* 3(5.55%). This study recorded that the percentage of *E.coli* (31.48%) is significantly higher (P<0.01) as compared to the lower percentage as in *Salmonella typhi* and *Streptococcus pyogenes* (3.70%) ( Table 2).

**Table (2): Various Bacteria Isolated from Liver Hydatid Cyst in Sheep.**

No.	Isolated bacteria	No. of isolates	Percentage %
1.	<i>Staphylococcus aureus</i>	4	7.41
2.	<i>E.coli</i>	17	31.48
3.	<i>Klebsiella pneumoniae</i>	8	14.82
4.	<i>Listeria grayi</i>	5	9.26
5.	<i>Proteus vulgaris</i>	4	7.41
6.	<i>Salmonella typhi</i>	2	3.70
7.	<i>Streptococcus pyogenes</i>	2	3.70
8.	<i>Pseudomonas auroginosa</i>	4	7.41
9.	<i>Enterococcus faecalis</i>	5	9.26
10.	<i>Shigella flexneri</i>	3	5.55
	TOTAL	54	100
	Chi-square ( $\chi^2$ )		15.73 **
	** (P<0.01)		

## Discussion:

In Iraq, the shortage in the supervisory role of veterinary services as one of many outcomes to frequent militia conflicts makes the slaughterhouse the ideal site for studying the subclinical and chronic animal diseases because butchers commonly slaughteres their diseased animals outside the slaughterhouse to avoid the condemnation of the affected organs and that was the reason beyond the preferring abattoirs for the current study since it aims to reveals the sub-clinical and chronic diseases of the liver.

The incidence of Echinococcosis in Baghdad city that recorded in the current study was quite low 50(2.28%), yet it matches some record in other parts of Iraq such as Khalil (2010) who found the same record in Al-Najaf city; Also Al-dujaily and Al-mialy (2017) who affirmed the low prevalence of this parasite in sheep(1.04%) in this city , further, Kadir *et al.* (2012) had a close record in Kirkuk province when they recorded that 0.77% of sheep were infected by this parasite. The low record could be one of many consequences of drought that takes place in the studied areas as an outcome of blocking and destroying of several dams during the military conflicts with ISIS prior to the time of current study. However, the prevalence of this parasite was significantly higher in another area, for instance Duhok province in the north of Iraq when Sargali and Mero (2013) found that 12.3% of sheep were infected, and in Al- Basrah province in the extreme south also close record was reported by Murtaza *et al.* (2017) when found that 14.75 % of sheep where infected; This confirms the influence of climate variability on the incidence of this parasite.

In this study, 10 different bacterial causes of liver hydatid cyst were isolated at different percentage as in (Table 2), many researchers have isolated multiple causes of hydatid cyst such as Al-Ani (2012) at Al-Shuala and Al-Karkh abattoirs from sheep liver in Baghdad city/ Iraq, and Fallah *et al.* (2014) reported bacterial isolates from Hamadan and Boroujerd slaughterhouse in Midwest of Iran from sheep liver have hydatid cyst.

Carpenter (1998) recorded that due to continuous bile flow, the bacteriostatic properties of the bile, the blocking of the biliary system and its stagnation, a connection between bacterial and parasitic infection was recorded due to parasitic infections, then bacteria gain entry to the biliary system via either papilla or portal circulation.

On the other hand, If the scale of the hydatid cyst grows with time, the permeability of the adventitious capsule, laminated layer and germ layer of hydatid cyst increases, contributing to the entry of several varieties of bacteria into hydatid fluid. The hydatid fluid structure can also promote the survival bacteria, the fluid has a pH similar to 7 and is richer than serum in amino acids and rich in lipids and sugars (Shaafie *et al.*, 1999, Celik *et al.*, 2001, Radfar and Iranyar, 2004).

In conclusion, the prevalence of liver hydatid cyst were low in Baghdad city and the *E.coli* was the predominant bacteria isolated.

### References

- Abo El Fetouh, E. H.; Kadry, M. B. and Alam, T. H. (2010).** Bacteriological, parasitological and pathological studies on some liver affections of buffaloes in Sharkia governorate. *Zag. Vet. J*, 38(4): 61-75.
- Al-Ani, W. A. (2012).** Hydatidosis of slaughtered sheep in Baghdad City; bacteriological study of infected hydatid cyst fluid. *Mustansiriyah Medical Journal*, 11(2): 45-48.
- Al-dujaily, A. H. and Al-mialy, A. J. (2017).** Study the Rate of Hydatid Cysts, Liver Fluke, Pneumonia and Hepatitis in Al-Najaf Slaughter House, Al-Najaf, Iraq. *Kufa Journal For Veterinary Medical Sciences*, 8(2): 137-142.
- Carpenter, H. A. (1998).** Bacterial and parasitic cholangitis. In *Mayo Clinic Proceedings* (Vol. 73, No. 5, pp: 473-478). Elsevier.
- Celik, C.; Amanvermez, R. and Özkan, K. (2001).** Free amino acid concentration in hydatid cyst fluids from fertile and infertile human and animal *Echinococcus granulosus*. *Parasite*, 8(4): 343-348.
- Constable, P. D.; Hinchcliff, K. W.; Done, S. H. and Grünberg, W. (2016).** *Veterinary medicine-e-book: a textbook of the diseases of cattle, horses, sheep, pigs and goats*. 11ed. Elsevier Health Sciences. p. 622.
- Danfaer, A. (1994).** Nutrient metabolism and utilization in the liver. *Livestock Production Science*, 39(1): 115-127.
- El-Shahawy, H. S. and Abdel-Gaied, S. S. (2006).** Histopathological studies in young diarrheic calves due to some anaerobic bacteria. *J. Egypt. Vet. Med. Assoc*, 66(4): 51-66.
- Fallah, M.; Kavand, A. and Mashouf, R. Y. (2014).** Infected hydatid cysts bacteria in slaughtered livestock and their effects on protoscolex degeneration. *Jundishapur journal of microbiology*, 7(6).
- Ghadrdan-Mashhadi, A. and Yosefi, H. (2004).** A survey on some bacteriological and pathological aspects of sheep liver abscesses, *International Society for Animal Hygiene* citsteer: 455.
- Herenda, D.; Chambers, P. G.; Ettriqui, A.; Seneviratna, P. and Dasilva, P. (2000).** A Manual on Meat Inspection for Developing Countries. FAO Animal Production and Health paper 119.
- Huntington, G. B. (1990).** Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition. *Reproduction Nutrition Development*, 30(1): 35-47.
- Jones, T. C.; Hunt, R. D. and King, N. W. (1997).** Cerebrospinal nematodiasis. *Veterinary Pathology*, 6th ed. Williams and Wilkins, Philadelphia, London, Paris: 626-628.
- Kadir, M. A.; Ali, N. H. and Ridha, R. G. M. (2012).** Prevalence of helminthes, pneumonia and hepatitis in Kirkuk slaughter house, Kirkuk, Iraq. *Iraqi J. Vet. Sci*, 26(83): e88.
- Kelly, W. R. (2007).** The liver and biliary system. In: K. V.F. Jubb, P. C. Kennedy, and N. Palmer, editors, *Pathology of domestic animals*. 5th ed. Academic Press, San Diego, CA. p: 370–371.

- Khajuria, J. K.; Katoch, R.; Yadav, A.; Godara, R.; Gupta, S. K. and Singh, A. (2013).** Seasonal prevalence of gastrointestinal helminths in sheep and goats of middle agro-climatic zone of Jammu province. *Journal of parasitic diseases*, 37(1): 21-25.
- Khalil, K. Z. (2010).** Prevalence of Hydatidosis among slaughtered ruminants in Al-Najaf slaughter house, Al-Najaf, Iraq. *Kufa Journal For Veterinary Medical Sciences*, 1(2).
- Khaniki, G. R. J.; Kia, E. B. and Raei, M. (2013).** Liver condemnation and economic losses due to parasitic infections in slaughtered animals in Iran. *Journal of parasitic diseases*, 37(2): 240-244.
- Liba, J. W.; Atsanda, N. N. and Francis, M. I. (2017).** Economic loss from liver condemnation due to fasciolosis in slaughtered ruminants in Maiduguri abattoir, Borno State, Nigeria. *Journal of Advanced Veterinary and Animal Research*, 4(1): 65-70.
- Markey, B.; Leonard, F.; Archambault, M.; Cullinane, A. and Maguire, D. (2013).** *Clinical Veterinary Microbiology E-Book*. Elsevier Health Sciences.
- Murtaza, M.; Al-Azizz, S. A.; Abdulhameed, F. M. and Kadhim, L. (2017).** Active survey of hydatid cysts in slaughtered sheep at Basrah abattoirs, Basrah province, Iraq. *Journal of Entomology and Zoology Studies* 2017; 5(5): 951-954.
- Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J. and Leonard, F.C.(2004).** *Veterinary Microbiology and Microbial Diseases*. 1<sup>st</sup> Ed.; Blackwell Science Ltd.; pp: 327.
- Radfar, M. H. and Iranyar, N. (2004).** Biochemical profiles of hydatid cyst fluids of *Echinococcus granulosus* of human and animal origin in Iran. *J. Vet*, 4(6): 435-442.
- Sargali, A. M. and Mero, W. M. (2013).** Epidemiological Study of Hydatid Cyst of *Echinococcus Granulosus* Isolated From Sheep and Goats in Duhok Province, Kurdistan Region of Iraq. *Science Journal of University of Zakho*, 1(1): 38-43.
- Shaafie, I. A.; Khan, A. H. and Rambabu, K. (1999).** Biochemical profiles of hydatid cyst fluids of *Echinococcus granulosus* of human and animal origin in Libya. *Journal of helminthology*, 73(3): 255-258.
- Tehrani, A.; Javanbakht, J.; Hassan, M. A. M. H.; Zamani, M.; Rajabian, M.; Akbari, H. and Shafe, R. (2012).** Histopathological and bacteriological study on hepatic abscesses of Herrik sheep. *J. Med. Microb. Diagn*, 1(4).