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Diagnostic Study of Hydatidosis in Ruminants

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadissiya in partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ
إِلَيْكَ وَحْيُهُ ۖ وَقُل رَّبِّ زِدْنِي عِلْمًا ﴿١١٤﴾

صدق الله العظيم

من سورة طه

Certificate of Supervisor

I certify that the project entitled (Diagnostic Study of Hydatidosis in Ruminants) was prepared by **Mohammad Hassan Murad** under my supervision at the College of Veterinary Medicine / University of Al-Qadissiya.

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25 / 4 / 2016

Certificate of Department

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25 / 4 / 2016

Dedication

To

All People …

Who Love me…

Summary

This study was conducted to find out the diagnostic methods of hydatidosis that used to be suitable in camel. In addition, it was tried to make use of the SONAR to diagnose the pulmonary hydatidosis; the most properly infection in camel in Al-Qadissiya Province.

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Chapter One
Introduction

1-1 Introduction

Echinococcus granulosus is one of the most important zoonotic parasites that cause hydatid cysts (the larval stage) in human and domestic animals, which called "dog small tapeworm", it lives in the small intestine of dogs mainly. Hydatid cysts grow in the internal organs of human & other intermediate hosts in their livers & lungs probably (1; 2 & 3).

The adult worm required two hosts to complete its life cycle which are intermediate host like human & domestic animals and definitive host like canids (4). Hydatid cysts disease is caused by the larval stage (hydatid cyst) of *Echinococcus granulosus*, the hydatid cyst develops after ingestion of eggs containing oncosphere embryo that shown by (5), the cyst characterized by growth in the internal organs of intermediate host mainly liver and lungs of unilocular cysts filled with fluid surrounded by a two layered of hydatid cyst wall, nucleated inner germinal layer, where protoscoleces grow, and a cellular outer laminated layer surrounded by fibrous capsules of host (6 & 7).

General life cycle of *Echinococcus* spp. occur through passing of gravid segment or free eggs by adult parasite with feces of definitive host, where the intermediate host ingested the eggs with contaminated food lead to develop of metacestode stage and forming hydatid cyst containing protoscoleces. The cycle is completing if the definitive host eats the infected part of intermediate host then protoscoleces grow to adult cestode in definitive host small intestine (8).

As mentioned by (7, 9 & 10) the incubation period is very long and needs a long time to be discovered by the clinician, hydatid cyst may cause dissemination or anaphylaxis after cystic ruptures into biliary tract or peritoneum. Infection of the cyst enhance development of liver abscesses and local complications, like massive effect on bile ducts and vessels that lead to induce cholestasis & portal hypertension.

Clinical signs of hydatid disease depend on the size, localization, and relationships with the neighboring organs, it may occur after a highly variable incubation period of several months to years. Hepatic hydatid cyst may cause abdominal pain, hepatomegaly, cholestasis, biliary cirrhosis & ascites, while pulmonary hydatid cyst may cause chronic cough, dyspnea, expectoration, hemoptysis, lung abscesses & pleuritis.

Detection of echinococcal eggs in the feces of definitive host is difficult, because the eggs are morphologically indistinguishable from another *Taenia* spp., and small segments of *Echinococcus* spp. maybe not present in the feces. Diagnosis of the hydatid cyst in the infected animals do not explained, but rare cases have been detected by ultrasonography, serum antibody detection, ELISA as recorded by (8), But most dependent detections of hydatid cysts during carcass inspection and at post mortem examination (11 & 12).

Nowadays, more sensitive molecular techniques are used for determination species and strains of *Echinococcus granulosus*(13).There are ten distinct genotypes (G1-G10) have been recorded in the world based on nucleotide sequence analysis of the mitochondrial cytochrome oxidase subunit 1 (*cox1*) & dehydrogenase subunit 1 *(nad1*) genes. These genes have been related to intermediate hosts (14; 15 & 16).

(17) recorded the most common geographic distribution around the world is sheep strain (G1 genotype); it is also dominant in the Mediterranean area.

Chapter Two

Review of literature

2-1 Taxonomy

Echinococcosis is a zoonotic infection caused by adult or larval stages of cestode species belong to the genus *Echinococcus*, which is a member of the family Taeniidae (18), in the order Cyclophyllidea (19), subclass Eucestoda, class Cestoda and phylum Platyhelminthes (20).

Echinococcus is a genus named by (21), who claimed that parasitic organism is *Echinococcus granulosus* (22).

According to (23) and (24) the parasite was classified as following:

Kingdom *Animalia*
Subkingdom *Eumetazoa*
Phylum *Platyhelmynthes*
Subphylum *Cestodes*
Class *Cestoda*
Subclass *Eucestoda*
Order *Cyclophyllidea*
Family *Taeniidae*
Subfamily *Echinococcinae*
Genus *Echinococcus*
Species *Echinococcus granulosus*

2-2 Morphology

2-2-1 Adult

Echinococcus shows several characteristics that differentiate it from the other genus in the family *Taenia*. The adult tapeworm is only a few millimeters long, (usually 2-7 mm), whereas other species of *Taenia* can grow to several meters in length and consist of several thousand segments (8).

(25); (26) and (7) mentioned that the body consists of a scolex in the anterior end, followed by a neck and strobila. The scolex has four muscular suckers that

used to the fixation of body into the intestine wall of the host and has a rostellum which contains two rows of hooks about (25-50 hooks). The strobila consists of three to six proglottids. Proglottids mature gradually towards the posterior end of the worm and the last one is usually a gravid segment carrying eggs in uterus.

Echinococcus species lack digestive and respiratory systems, so the metabolic activities take place through the tegument. This structure covers the body and protects the parasite against host enzymes and immune responses (27).

The adult is hermaphrodite, reproductive ducts opening at a common lateral genital pore. The genital pore is proximal to mid body of the proglottid. The cirrus sac located horizontally or deviated interiorly, the vitellarium is globular. After fertilization uterus distended and occupies most of the terminal proglottids when the eggs are fully developed (8).

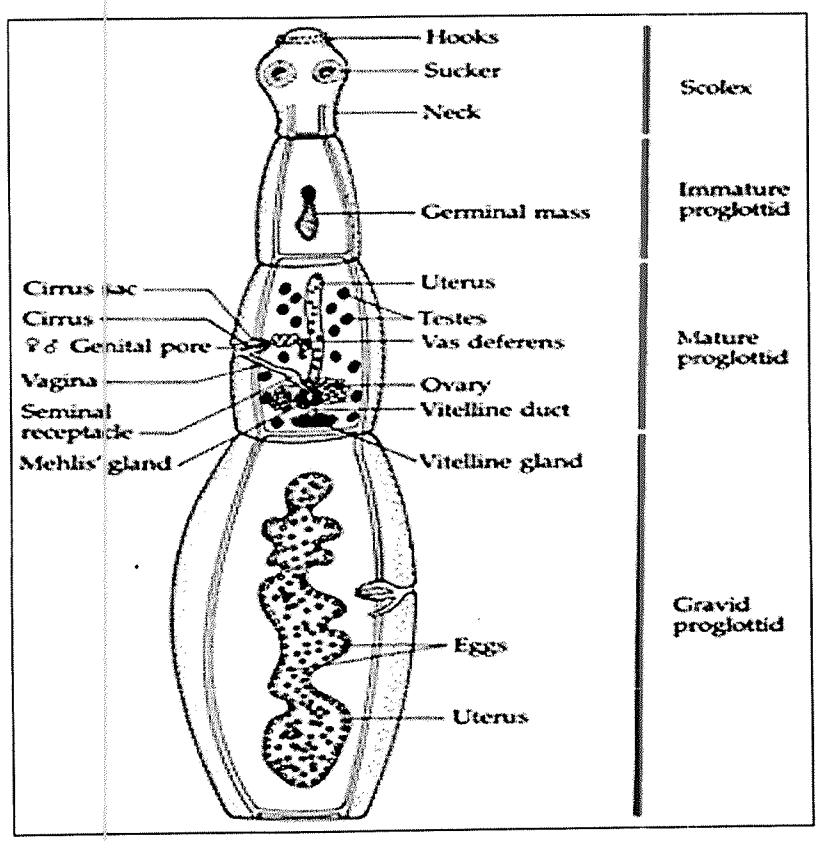

Fig. (2-1) *Echinococcus granulosus* adult tapeworm (28)

2-2-2 Eggs

The eggs of *Echinococcus* spp. are morphologically indistinguishable from other tapeworms of the taeniids family. They are ovoid, about 30-36 µm in diameter, containing the embryo which called oncosphere or hexacant embryo due to bearing three pairs of hooks (23).

The egg surrounded by several envelopes, the most clear one being the highly resistant keratinized embryophore, which gives the egg a dark brown striated appearance, once the eggs are liberated from the host the outer capsule quickly disappears, (8) (Fig.2).

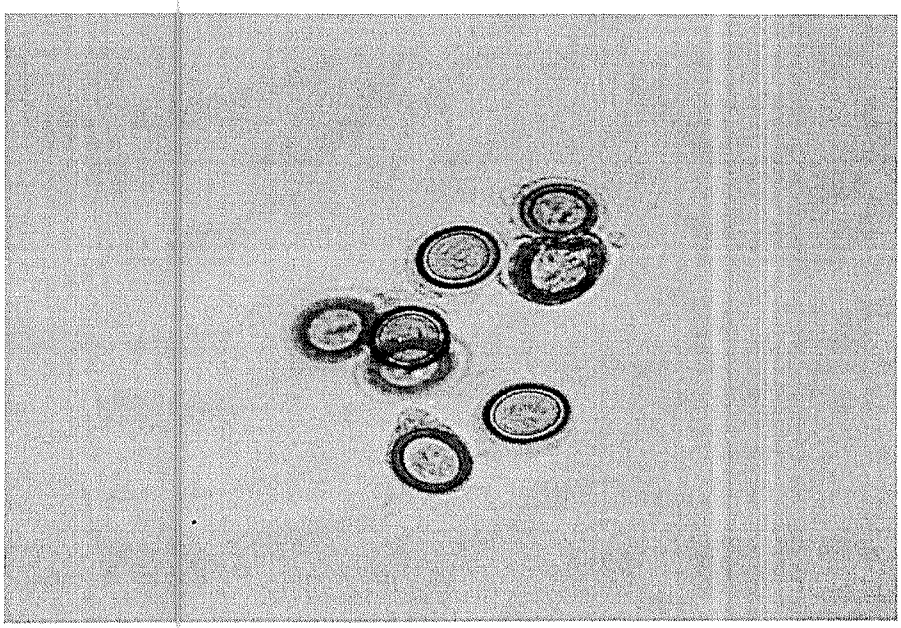

Fig. (2-2) *Echinococcus granulosus* eggs (29)

2-2-3 Metacestode (Hydatid Cyst)

Hydatid cyst is a bladder filled with much liters of fluid, when the cyst reaches to 1cm in diameter, it will consist of three layers:

a) The outer layer (pericyst), which composed of modified host cells that form a dense and fibrous protective band represented the response of the host to parasite as explained by (30), it consists of compressed host cell, fibroblast,

eosinophils, giant cells which packed together and form a rigid protective layer that have a few millimeters in thickness lead to prevent leakage of parasitic secretions which effect to host immune system (31).

b) The middle layer (laminated layer), is a thick, outer, noncellular, about 2mm in thickness which is formed by the parasite itself and allow crossing the nutrients. Laminated layer has a white color with chitin and formed by several packed layers of polysaccharide materials (32).

c) The inner layer (germinal layer), is a thin, nucleated layer about (20-25 micron in thickness) responsible for producing brood capsules, within which develop protoscolices that are infecting the definitive hosts. Brood capsules are small cysts, containing (10-30) protoscolices, development of protoscolices made by asexual reproduction until reaching high numbers as hundreds of thousands in a single hydatid cyst which usually are attached to the germinal layer by a slender stalk; they may separate and float within hydatid fluid. In addition to, individual protoscolices may separate from the germinal layer directly when being not surrounded by brood capsule (33).

(34) defined that ectocyst represented by laminated layer while endocyst is a germinal layer and both of them form the true wall of hydatid cyst.

As recorded by (5) hydatid cyst grows to about 5-10 cm at the first year and can be remained in development with in organs for several years or even decades.

The accurate time that required for protoscolices development within cysts thought to be more than ten months postinfection and protoscolices can be formed in cysts of 5-20 mm in diameter already. Hydatid cysts that results from infection with *Echinococcus granulosus* divided into: fertile, sterile and calcified. Fertile cysts can already produce protoscolices, but a rate of cysts does not produce protoscolices and remain sterile. Hosts who infected with calcified hydatid cysts typically do not induce major pathology, and may remain asymptomatic for years or permanently. Indeed, most of the cysts are unilocular but some of them, smaller

daughter cysts are formed within larger original cysts; however, the growth rates of cysts may differ between cysts in the same organ or in the same hosts and between hosts in various regions (7).

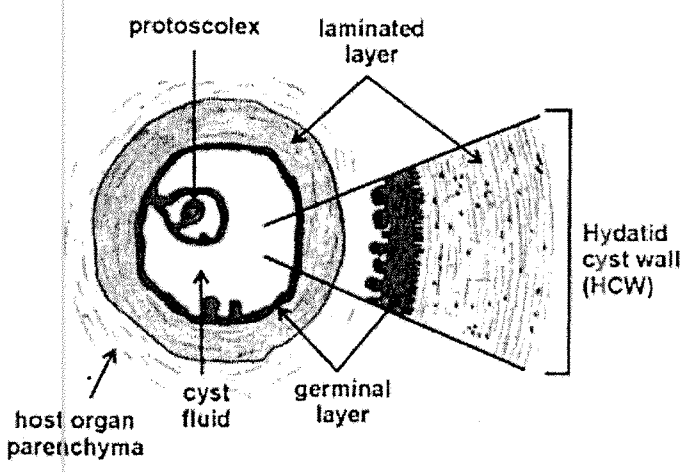

Fig. (2-3) Cross section in hydatid cyst (35)

2-2-3-1 Protoscolices

(36) has reported that the protoscolex plays a major role in life cycle, it`s being the only infective stage of the carnivores, the fertility of cysts responsible for maintenance of life cycle, while morbidity and pathological effects occur with all types of cysts.

There are a large number of protoscolices develop and begin in the formation of neck and strobilla whereas being inside the cyst (37).

Formation of the protoscolices starts when cellular buds formed by a clustering of cells emerge from germinal layer in hydatid cysts. The buds elongated and eliminated anterior (scolex) and (body) regions. The first fully differentiated structures formed at the scolex peak region of the budding scolex, the scolex shows circular projections and depressions that developed into suckers and the body is dilated and structured neck was visible between the scolex and body. At the

protoscolices development, this parasite form remains attached to germinal layer through a stalk. When the protoscolices fully differentiated, the stalk is cut off and the infective protoscolices being free in hydatid fluid (6).

Fully developed protoscolices have a two row of rostellar hooks (large and small hooks); these hooks have been arranged in alternative manner on protoscoleces. Total number of hooks range from (25-38) hooks as recorded by (38), the average length of large hooks was (21.0 - 21.5 μm) and the average length of small hooks was (16 -19 μm) (39).

The protoscolices contain a polysaccharide fraction in coming on acid hydrolysis galactose and glucosamine, but no uronic acid. The same compounds present in the hydatid cyst membrane which includes N-acetyl galagtosamine (in addition to galactose, galagtosamine) which are identified as hydrolysis product (40)

2-2-3-2 Hydatid Fluid

Cyst fluid is clear or pale yellow, has a neutral pH (7-7.2) and composed of sodium chloride, proteins, glucose, ions, polysaccharides and lipids. The fluid is antigenic and containing protoscolices and hooklets. In addition to it contains albumin, creatinine, urea and lecithin. The essential enzymes are diastases, but there are also proteolytic enzymes like: lipase, oxidase and protease enzymes (40).

The inorganic substances that present in the cyst fluid are: Potassium, Sodium, Calcium, Magnesium, Ferrous, Sulfur, Chlorine, Phosphor and Silicon while that present in the protoscolices are: Potassium, Sodium, Calcium, Magnesium, Ferrous, Zinc, Copper, Phosphor, Strontium, Silicon, Cobalt and Manganese. The reducing sugar present in the hydatid cyst fluid is variable; whereas the percentage of glycogen in protoscolices is 2.8% of net weight (8).

Biochemical materials within hydatid cysts play an essential role in the metabolism, physiology and immunology of cystic echinococcosis (41).

Variation in these parameters reflects strain variations in different hosts and also reflection the relation between intermediate hosts and parasite (42). There are vital and essential elements in the cyst fluid that are very significant to biological activities of the parasite. The composition of the hydatid cyst contents may vary in strains and different area. The composition of the hydatid cyst fluid about 90% the same host serum as recorded by (43), biochemeical analysis can provide much valuable data on identification of *Echinococcus granulosus* strains from different host origins which may related to their possible infectivity to host (44).

2-3 Transmission and Life Cycle

Cystic echinococcosis or hydatidosis is a parasitic zoonotic disease caused by metacestode of the tapeworm *Echinococcus granulosus* that mentioned by (45), globally sheep strain is the most important and prevalent strain of *Echinococcus granulosus* and founds in its domestic form (dog-sheep/goat) in many regions. The life cycles of *Echinococcus granulosus* strains can be classified into two types: **1)** domestic, including the domestic dog as the principal definitive host and different species of farm animals as intermediate hosts, and **2)** sylvatic, including wild carnivores and ungulates as definitive and intermediate hosts respectively (the wildlife cycle) (7).

Adult worms have been detected in various carnivores' particularly domestic dog (*Canis familiaris*), also wolf (*Canis lupus*), coyote (*Canis latrans*), dingo (*Canis lupus* f. dingo), silver-backed jackal (*Canis mesomelas*), golden jackal (*Canis aureus*), hunting dog (*Lycaon pictus*), red fox (*Vulpes vulpes*) and Felidae: lion (*Panthera leo*). In Africa the lion has been recorded as a definitive host of *Echinococcus granulosus* and this is the single record for a felid, whereas the domestic cat is not a suitable host for the adult cestode (26).

The domestic dog is considered as a principle definitive host in which the adult cestode attached to the intestinal epithelium and undergoes sexual reproduction and eggs development. The eggs contain an oncosphere were shedding with the feces of the definitive host into environment; when the infective eggs ingested by a suitable intermediate host like (sheep, cattle, goats, buffaloes, camels, horses, pigs, giraffes, hippos, elephants, primates, kangaroos and cervids in addition to human) with contaminated feed and water, as recorded by (30), following passage through stomach and intestine become activated, eggs affect by the action of gastrointestinal enzymes. Then the oncosphere released from its embryophore before passing through intestinal wall. This step is carried out by bile action, hooks movement and lytic secretions of onchosphere as said by (7), oncosphere reaches internal body organs through blood or lymphatic vessels, liver and lungs and are the most common organs of hydatid cysts development (8).

The metacestode stage followed eggs ingestion and growth by intermediate host, it may develop into fertile hydatid cysts (that may contain thousands of protoscolices) or sterile hydatid cyst (without protoscolices development) (30). Although there are different organs can be infected in addition to liver and lungs but in less proportion including spleen, kidneys, brain, bone, spleen, heart, ..., etc. Rupture of an initial cyst happens a new cyst develops in a new organs and this case called secondary cystic echinococcosis (8).

2-4 Clinical Signs

According to research of (7) the initial phase of the primary infection is mainly asymptomatic because of the cystic growth is ordinary slow.

The clinical signs may appear depending on the location of the cysts after a highly inconstant incubation period of several months or years. In hepatic cysts the

common clinical signs occur as hepatomegaly, pain in the upper abdominal region, cholestasis, biliary cirrhosis, portal hypertension, anemia, ascites, nausea, vomiting, indigestion and a variety of other manifestations. Sometimes cysts ruptured into the peritoneal cavity, causing anaphylaxis or secondary cystic echinococcosis, or into the biliary tree, leading to cholangitis and cholestasis. Secondary infection may result by bacteria which causing abscess formation (46).

The infected animal also suffers from weakness, stunted growth, loss of weight in addition to normal appetite , also wool dehydration and become brittle easily and little milk production. When the cyst grows on the liver may be observed a bloat with loss of rumen activity; in the liver palpation the animal feels with pain due to cystic growth on it (47).

In the pulmonary hydatid cysts, there are respiratory signs appear as chronic cough, expectoration, dyspnea, chest pain, pleuritis, pneumothorax, hemoptysis and lung abscess. There are neurological disorders when the hydatid cysts develop in the brain (8).

In addition development of hydatid cyst in bones can destroy bone structure and leading to spontaneous fracture. In the heart, the hydatid cyst can result in pericardial effusion, heart block, arrhythmias, and sudden death. Cysts in any location may subsequent by bacterial infected (46).

Cysts were usually well tolerated until they cause a pressure on surrounding tissues (48). Some of cysts become very large and may contain several liters of fluid. Where it`s being in the vital organ such as the brain, become symptomatic when they are still small (49).

Other specific symptoms may include anorexia, weight loss and weakness. When the cyst ruptured and leakage of cyst fluid secondary cystic echinococcosis may develop; dissemination is seen mainly in the abdominal cavity. Leakage of the cyst fluid may also cause allergic reactions, including shaking chills and or fever, asthma, urticarial, pruritis, or life-threatening anaphylaxis (50).

2-5 Diagnosis

A formal diagnose of any type of echinococcosis requires a combination of tools that involve imaging techniques, histopathology, or nucleic acid detection and serology. For cystic echinococcosis diagnosis, imaging is the main method—while serology tests (such as indirect hemogglutination, ELISA (enzyme linked immunosorbent assay), immunoblots or latex agglutination) that use antigens specific for E. granulosus verify the imaging results. The imaging technique of choice for cystic echinococcosis is ultrasonography, since it is not only able to visualize the cysts in the body's organs but it is also inexpensive, non-invasive and gives instant results. In addition to ultrasonography, both MRI and CT scans can and are often used although an MRI is often preferred to CT scans when diagnosing cystic echinococcosis since it gives better visualization of liquid areas within the tissue (52)

Diagnosis of the intermediate host infected with hydatid cysts based on clinical findings, imaging techniques, microscopic or histological examination of the cyst and its contents, serological and molecular techniques (51 and 52).

Hydatids are frequently found during slaughter or necropsy. Infected dogs pass eggs in their feces that cannot morphologically be distinguished from eggs of Taenia spp. The tapeworm may be demonstrated microscopically in the mucous portion of purged material. Immunodiagnostic tests using ELISA are used in human medicine. In addition, radiographic diagnosis is used. Recently, PCR techniques have been available which may identify antigenic material in feces (53).

2-5-1 **Serological Tests**

a) Enzyme Linked Immunosorbent Assay (ELISA): A test depends on using of Abs and changing color to detect the substance chiefly Abs in serum of intermediate host were shown to be detectable in the blood. Other major aspects to

diagnosis of infection in the definitive host by detection of adult tapeworm products in feces by using of the sandwich ELISA (54).

b) The monoclonal Abs specific for an Ags on the surface of *Echinococcus granulosus* onchosphere was used by (55) to distinguish *Echinococcus granulosus* eggs in the perineal swabs or samples from environmental sites.

c) Indirect Heamagglutination test (IHA): is a critical test to detect Abs in serum of animals infected with hydatid disease; sensitivity rates ranging between 60%- 90% depending on particularity of the cases. Crude hydatid cyst fluid is ordinary act as antigens. At the present time, the best obtainable serological diagnosis is taking out by using gathering of tests (54).

d) The latex agglutination test (56)

e) The complement fixation test (57)

f) Sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-Page) (58)

g) Western Blots: This test represented a definitive means to detect of Abs against the *Echinococcus granulosus.* Diagnosis may be enquire using the western blot assay to detect of IgG Abs in serum reactive with *Echinococcus granulosus* Ags which found on a membrane (59).

h) Casoni Test (60).

2-5-2 **Post-mortem examination**

After slaughtering of animal and post-mortem examination of the carcass to detect the cyst may be using ultrasonography particularly in sheep and goats; cyst fluid has been highlighted by microscope to look for the diacritical protoscolices. The viability of the protoscolices can be determined by adding of 1% of eosin stain to the fluid. Viable protoscolices eliminate the stain whilst non-viable protoscolices seize the stain (61).

2-5-3 **Histological examination**

The cyst wall can be examined histologically after surgical removal (62).

2-5-4 **Other methods may be used for diagnosing mostly in final hosts:**

a) Purgation with arecoline compounds which cause cleaning of whole intestinal contents when it's given to dog and lead to paralysis of the worm burdens then it can be collected and recognized; this technique considered as exhausted and not favorable technique but remained the unique quantitate method that used in the living final hosts (3).

b) Necropsy of the small intestine; it's the benefit method for dogs, foxes and other final hosts and the worms can be collected to identify (63).

2-5-5 Molecular methods

Polymerase Chain Reaction (PCR)

It is a dependable scientific technique used in molecular biology to amplify a single or few copies of a DNA fragment across several orders of magnitude generating thousand to millions of copies of a certain DNA sequence. This technique has been developed since 1984 by the American biochemist, Kary Mullis (64).

Principle of PCR technique based on turning of few molecules of particular target nucleic acid into much of microgram of DNA (too little have been analyzed and used in biochemical reactions). PCR has tight arrangement after the normal DNA replication process. Two of nucleotide primers restrict and recognize the target sequence to be amplified; these primers hybridize to adverse strands of DNA to act as initiation points for the synthesis of new strands of the DNA. The DNA synthesis made by thermostable DNA enzymes like Taq DNA polymerase and catalyzes. There are three steps involved in each cycle of PCR synthesis which are: denaturation, annealing and extension (65).

This technique used to be distinguished of *Echinococcus* species and strains; this was followed by restriction fragment length poly morphism analysis or sequencing method (66).

(67) used DNA extraction of *Echinococcus granulosus* mitochondrial genomes for the molecular analysis; he showed essential genetic diversity which has important modulation for resolution and development of diagnosis then treatment of the infection.

The pathological lesion of the causative agent is difficult to determine specially in aberrant hosts. Such lesions must be subjected to molecular analysis for species identification. Recently the clinical samples undergoes to PCR and the amplified fragment of mitochondrial and nuclear genome sequenced by gene sequencer technique (26).

Genotyping technique using DNA sequencer

DNA sequencing means a method to determine the sequence of nucleotides bases (adenine, guanine, cytosine and thymine) in a DNA molecule using specific dye (68).

PCR can determine strains of *Echinococcus granulosus* (G1→G10) depending on mitochondrial genes cytochrome C oxidase subunit1 (*cox1*) and NADH dehydrogenase subunit1 (*nad1*) (63).

The object to use of mtDNA is for getting useful genetic information and it is generally haploid, multicopy, and neutral provides entry to a set of genes with no recombination (69).

Genotyping variations based on analysis of mtDNA to detect strains of *Echinococcus* species and determine phylogenetic relationship and acquaint taxonomic species questions ().

DNA markers has been recorded that at least seven of this strains can infect human, but the most common infective strain that of sheep (26).

2-5-6 **Imaging technique**

The most dependent method that used in diagnosis of human cystic echinococcosis is imaging diagnosis which includes: X-ray and ultrasonography for recognition of daughter cysts and hydatid sand as reported by (71) and computed tomography for detection of hydatid cysts and determine the infected organ specially small extra-hepatic cysts; furthermore ability to differentiate the hydatid cystic infection that said by (72); (7); whereas Magnetic Resonance Imaging (MRI) used in pre-operative testing particularly to detection of returning hydatid cysts (73).

Chapter Three

Conclusions and recommendations

3-1 Conclusion

There are some points concluded in this study:

1. Iraq is endemic with Hydatidosis.
2. Diagnosis of Hydatidosis is relatively easy.
3. Different methods may be used in the diagnosis.
4. Imaging with confirmation by fecal analysis is the most recommended clinical way.
5. Molecular diagnosis is the most precise one, but it is expensive.

3-2 Recommendations

1. Public health education Program should be run according to different level of community like medical and veterinary services.
2. Advanced and clinical studies should be run in using the imaging technique in diagnosis.
3. Establishment of modern healthy abattoirs and prevent illegal (backyard) slaughtering.
4. Routinely treatment of animals with anti-parasitic medicines and prophylactic anthelminthic dosage four time yearly for all farm animals.
5. Using of recombinant vaccine (EG95) in farm animals to prevent growth of onchosphere into hydatid cysts.