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Morphological and Molecular Study of *Echinococcus granulosus* in Misan Province, South of Iraq

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By

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

وَمِنَ النَّاسِ وَالدَّوَابِّ وَالْأَنْعَامِ مُخْتَلِفٌ أَلْوَانُهُ كَذَٰلِكَ ۗ إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ ۗ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ

صدق الله العلي العظيم

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Dediction

To whom Allah sent as mercy to the Worlds prophet Mohammed

To my grandfather soul

The Martyr Razak Ibraheem Ahmed Al-Quzweeni

To the candle that melted to lighten my road My father

To the affectionate heart that filled me with love My mother

To the roses that perfumed my life My brothers

To who supported me My friends

With my Love

Hussein

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Summary

Hydatid cyst has serious impacts on the human health and his animals. It is leading to a significant public health and socio-economic problems in different parts of the world. This study includes a morphological and molecular characterization of *Echinococcus granulosus* in definitive and intermediate hosts including humans in Misan Province.

The survey for cystic echinococcosis was conducted during the period from December 2017 to October 2018. A 3287 cases were examined (922 sheep, 405 buffalo, 2 camels, 983 cow and 150 goats) from central slaughterhouse of Amara city (capital of Misan Province) and 819 of human from Al-Sader teaching hospital and Al-Zahrawi surgical hospital, the adult worms were obtained from 2stray dogs. The prevalence of *Echinococcus granulosus* in sheep, buffalo, camel, cow, goats, human and dog were found to be 2.17% (20/922), 2.22% (9/405), 0.00% (0/2), 3.05% (30/983), 0.00% (0/150), 1.71% (14/819) and 33.33% (2/6) respectively.

The morphological characterization of *E. granulosus* collected from different hosts which showed significant differences in some parameters of hooks and Protoscolices dimensions that measured in this study.

The genetic characterization of the *E. granulosus* complex in human and livestock population was described for the first time by using polymerase chain reaction (PCR) and DNA sequencing technology for two mitochondrial genes (*Cox1* and *Nad1*) in Misan Province. Genetic variation was detected in *E. granulosus* strains, sheep strain (G1), buffalo strain (G3) and SB041 strain. Those strains were recorded for some samples. Also (G1BC) genotype was recorded in sheep and buffalo hydatid cysts. The predominant strain causing cystic echinococcosis in humans and animals in Misan was sheep strain (G1).

This is the first record for the *Echinococcus granulosus* strains in Misan Province, were G1BC and SB041 strains recorded for the first time in Iraq.

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List of Abbreviations

ANOVA	Analysis of Variance
AαLH	Angle α of Large Hook
AαSH	Angle α of Short Hook
AβLH	Angle β of Large hook
AβSH	Angle β of Short Hook
AγLH	Angle γ of Large hook
AγSH	Angle γ of Short Hook
BC	Before Christ
BBL	Before blade length
BBLLH	Before Blade Length of Long Hook
BBLSH	Before Blade Length of Short Hook
BL	Blade length
BLLH	Blade length of long hook
BLSH	Blade Length of Short Hook
CE	Cystic Echinococcosis
CLH	Curved length of hook
CLLH	Curved Length of Long Hook
CLSH	Curved Length of Short Hook
Cox1	Cytochrome c oxidase subunit 1
CT	Computed Tomograph
EURLP	European Union Reference Laboratory for Parasites
HC	Hydatid cyst
HD	Hydatid disease
HL	Handle length
HLLH	Handle Length of long hook
HLSH	Handle Length of Short Hook
MEGA	Molecular Evolutionary Genetics Analysis
Nad1	NADH dehydrogenase subunit 1
NADH	Nicotinamide adenine dinucleotide
NCBI	National Centre for Biotechnology Information

NHDCC	National Hydatid Disease Center of China
OIE	Office International des Epizooties (World Organisation for Animal Health)
PCR	polymerase chain reaction
RW	Rostellum Width
SL	Sucker Length
SPSS	Statistical Package for Social Science
SW	Suckers width
THL	Total hook length
TLLH	Total Length of Long Hook
TLP	Total Length of Protoscolices
TLSH	Total Length of Short Hook
TWH	Total width of hook
TWLH	Total Width of Long Hook
TWP	Total Width of Protoscolices
TWSH	Total Width of Short Hook
UE	Unilocular Echinococcosis
UV	Ultra violate
WHO	World Health Organization

CHABTER ONE

Introduction

1:Introduction

Hydatid disease (HD) or Cystic Echinococcosis (CE) or hydatidosis refers to the disease caused by the larval stage of a zoonotic tapeworm *Echinococcus granulosus* which endemic in most regions of the world (WHO, 2014; Romig *et al*., 2015; Karamian *et al.,* 2017). The adult worm is living in the small intestine of canids as definitive host, mostly dogs and wolves (Karamian *et al.,* 2017; Chaudhari *et al*., 2017; Mulinge *et al.,* 2018).

The herbivores are infected with *E. granulosus* when grazing the contaminated herbage with eggs excreted with the canids feces. However, humans can be accidentally infected by swallowing eggs that contaminated food, water, directly from dog when contacted with it or by another route (Harandi *et al.*, 2002; Hammad *et al.*, 2018).

In livestock, the infection with *E. granulosus* caused a considerable economic loss in milk and meat production, edible organs, hide and fleece value and a decrease in fecundity (Polydorou, 1981; Romazanov, 1983; Hammad *et al.,* 2018).

The high infection rates and the global distribution of (CE) is return to the wide range species of infected intermediate hosts (Karamian *et al.,* 2017; Mulinge *et al.,* 2018).

However, the infection rate of human is related with the infection rate of domestic animals specially dogs and sheep (Khuroo, 2002; Ehsan *et al.*, 2017).

Cystic Echinococcosis (CE) has serious impacts on animals and human health (Chaudhari *et al*., 2017). It is leading to a significant public health and economic problem in many parts of the world particularly in rural communities where dogs and livestock are living together (Ekhnefer, 2012; Jenkins *et al.,* 2018).

More than one global study reported that *E. granulosus* had variable strains in different regions of the world (Ebrahimipour *et al.*, 2017; Jenkins *et al.*, 2018), which had different routes in epidemiology, pathology, control and prevention (Thompson and Lumbery,1988; Karamian *et al.*, 2017; Mulinge *et al.*, 2018).

Today, there are ten distinct strains or genotypes identification as G1, G2, G3……, G10. These genotypes are associated with distinct intermediate hosts like sheep, Buffalo, horses, cattle, camels, pigs, cervids, goats, and others (Sanchez *et al.*, 2010; Ebrahimipour *et al.*, 2017 Hodžić *et al.*, 2018).

In Iraq, hydatid cyst is one of the most endemic diseases in both humans and animals (Hammad *et al.*, 2018), which caused some significant human problems in health and economic activities (Hassoun and Al-Salihi, 1973; Molan and Saeed, 1990; Hammad *et al.*, 2018). In Iraq there are few studies on *E. granulosus* in the felid of epidemiology, biological study, molecular and genetic diversity and because the impact of *E. granulosus* on the human and his animal's health and economic activates which dependent on the parasite strains and because the lack of the same studies in Misan Province that conducted on distribution of *E. granulosus* we decide to carry out this study.

1:2: The aims of the study

The study aimed to characterize some evidence such as:

1. Identified the phenotypes of *E. granulosus* depending on some morphological characters like the shape and the measures of Protoscolices, scolex and hooks.

2. Identified the genotypes of *E. granulosus* by using polymerase chain reaction (PCR) analysis and DNA sequencing.

3. Estimate the genetic variation and identification the strains by using the gene sequencing depending on the mitochondrial Cytochrome c oxidase subunit 1 (*Cox1*) and NADH Dehydrogenase subunit 1(*Nad1*) genes.

CHAPTER TWO

Literature Review

2:1: History of Cystic Echinococcosis:

Echinococcosis is one of the parasitic diseases that has been recognized since immemorial time. Over four centuries BC, Hydatid cysts had been described by Hippocrates and other ancient physicians. Adult *E. granulosus* was described by Hartmann in the small intestine of dog in 1695 and the larval form hydatid cysts was recognized in 1782 by Goeze **(**Paniker and Ghosh, 2013).

2:2: Classification of genus *Echinococcus*:

The genus *Echinococcus* classify as: (Paniker, 2013).

Kingdom: Animalia
Phylum: Platyhelminthes.
Class: Cestoda
Sub class: Eucestoda
Order: Cyclophyllidea Ben; Braun, 1900.
Family: Taeniidae Ludwig, 1886.
Genus: *Echinococcus* Rud, 1801

There are many species of genus *Echinococcus* such as: *E. granulosus*; *E. multilocularis*; *Echinococcus oligarthrus* and *E. vogeli*; *Echinococcus shiquicus* (Xiao et al., 2005), which infected human and other intermediate hosts (Table, 1-2), these species are morphologically distinct in each adult and larval stages (Figure., 2-1)

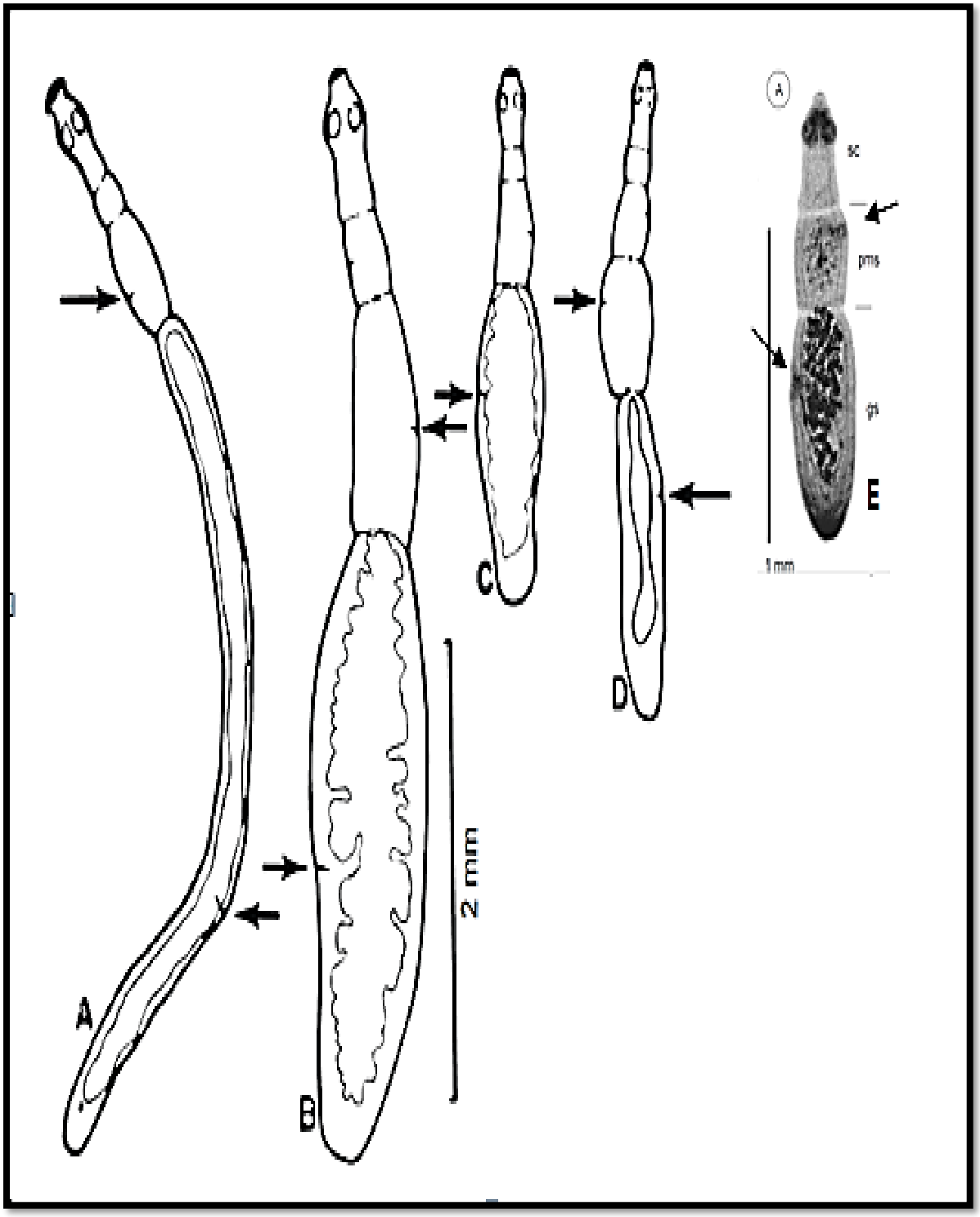

Figure (2-1): Comparative of general morphology for adult *Echinococcus* species A: *Echinococcus vogeli*, B: *Echinococcus granulosus*, C: *Echinococcus oligarthrus*, D: *Echinococcus multilocularis*, E: *Echinococcus shiquicus*, (←): genital pore ((Eckert *et al.*, 2001; Xiao *et al.*, 2005).

Table (2-1): The *Echinococcus* species main characterization.

***Echinococcus* species**	**Definitive host**	**Intermediate host**	**Adult length**	**Hooks measures**		**Proglottid number**
				Large	**Small**	
E.vogeli	bush dog *Speothus venaticus*	large rodents e.g. *Cuniculus paca*	3.9-5.5mm	30.4-43.9µm	19.1-36.5µm	usually has three
E.granulosus	Canids dogs and wolves	Herbivores such as sheep	2-11mm	25-49µm	17-31µm	two to six
E.oligarthrus	wild felids *Felis concolor and F. jaguarondi*	large rodents like *Dasyprocta sp., and Cuniculus paca*	2.2-2.9mm	25.9-37.9µm	22.6-29.5µm	three
E.multilocularis	wild canids *Vulpes, V. ferrilata, Alopex lagopus, Canis latrans*	small rodents such as voles and lemmings	1.2-4.5mm	25-34µm	20.4-31µm	two to six
E. shiquicus (Xiao *et al.*, 2005)	Fox *Vulpes ferrilata*	Pika *Ochotona curzoniae*	1.3–1.7mm	20–23µm	16–17µm	three

Based on mitochondrial DNA sequences molecular studies, have illustrate that *E. granulosus* is a complex of at least five species which differ in specificity to host, development rate, pathology and sensitivity to drugs. These species include *E. granulosus* sensu stricto (G1, G1CB, G2, and G3 genotypes), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus canadensis* (G6, G7, G8, and G10) and *Echinococcus felidis* (lion strain) (Thompson and McManus, 2001; EURLP, 2017; Hodžić *et al.*, 2018).

Echinococcus granulosus:

E. granulosus is the common species of all species of *Echinococcus*, this zonotic helminthes parasite was worldwide distribution. It is transmitted between the canids members (dogs and wolves) and some of domestic and wild herbivores species (Eckert *et al.*, 2001; Xiao *et al.*, 2006). The dog/sheep cycle (domestic cycle) is the most important in epidemiology of *E. granulosus*, Sylvatic cycle also occur, e.g. wolf/cervid cycle (Eckert *et al.*, 2001), Sylvatic cycles play only a minor role, if any, as reservoirs of infection for domestic cycles (Thompson and Lymbery, 1988).

The length of *E. granulosus* adult ranged between 2 to 11 mm and the number of proglottid ranged from two to six proglottid, the length of terminal gravid proglottid is usually more than half length of the mature worm and the position of genital pore usually in posterior to the middle in both mature and gravid proglottid. There are 26 to 40 hooks distributed around the rostellum in two rows, the size of first row hooks varies between 25 to 49μm, and between 17 and 31μm in the second row (Eckert *et al.*, 2001; Ekhnefer, 2012)

The gravid uterus has well-developed sacculation, the larval stage is developing to fluid filled bladder-worm commonly called hydatid cyst (HC) or Cystic Echinococcosis (CE). In species *E. granulosus,* (CE) called unilocular Echinococcosis (UE) in some time the connecting chambers may be occurred (Ekhnefer, 2012).

Growth is expansive, and the hydatid cyst may have produced endogenous daughter cysts, hydatid cyst may have reached to 30 cm in diameter. It occurs most frequently in liver and lungs. and may be developed in other organs like brain and bone marrow (Eckert *et al.*, 2001).

2:3: Life cycle of *E. granulosus*:

The eggs are spherical in shape (30-40μm in diameter), consisting of a hexacanth embryo called Oncosphere (Figure, 2-2) (Eckert *et al*., 2001), the eggs are surrounded by three covers, from the outside thin gelatinous which confused by time from exposed faecal eggs and thick yellow-brown shell which was provided with numerous tiny pores which giving the striated view and the third cover is egg cell membrane that lined inside the egg. The morphology of *E. granulosus* eggs are indistinguishable from other Taeniidae species (Eckert *et al.,* 2001).

The larvae penetrated the intestinal wall and mesenteric vessels of the intermediate host and then leave the blood circulation to the liver, lungs, or other organs tissues in some times (Eckert *et al.,* 2001).

The liver acts as the first barrier for the oncosphere penetrating the small intestinal mucosa to reach the portal circulation. Later, by blood stream the embryos are carried to all parts of body, due to the large size of oncospheres, most of them arrested and settled in the liver (Jarjees and Al-Bakri, 2012).

The metacestode are developing as bladder which coated with acellular layer comes from intermediate host activities as fibrous tissue around the bladder of metacestode (Hodžić *et al*., 2018), the inner germinal layer capable to produce Protoscolices (Eckert *et al*., 2001; Halajian *et al.,* 2017).

In liver or lungs, the larvae developed to encysted called hydatid cyst (HC) or Cystic Echinococcosis (CE) they developed to interior germinal membrane pudding to Produced daughter cysts which may give secondary and tertiary cyst (Eckert *et al.,* 2001).

when one canid's species (dog) take these cysts, the Protoscolices developed to adult worms in their small intestine (Huttner *et al.*, 2009; Ekhnefer, 2012; Lett, 2013).

The adults of *Echinococcus* sp. is about 2-7mm in length (rarely more than 7mm) in general it has no more than six proglottids, whereas other species of *Taenia* can reached to several meters in length and include several thousands of proglottids (Eckert *et al.*, 2001; Ekhnefer, 2012).

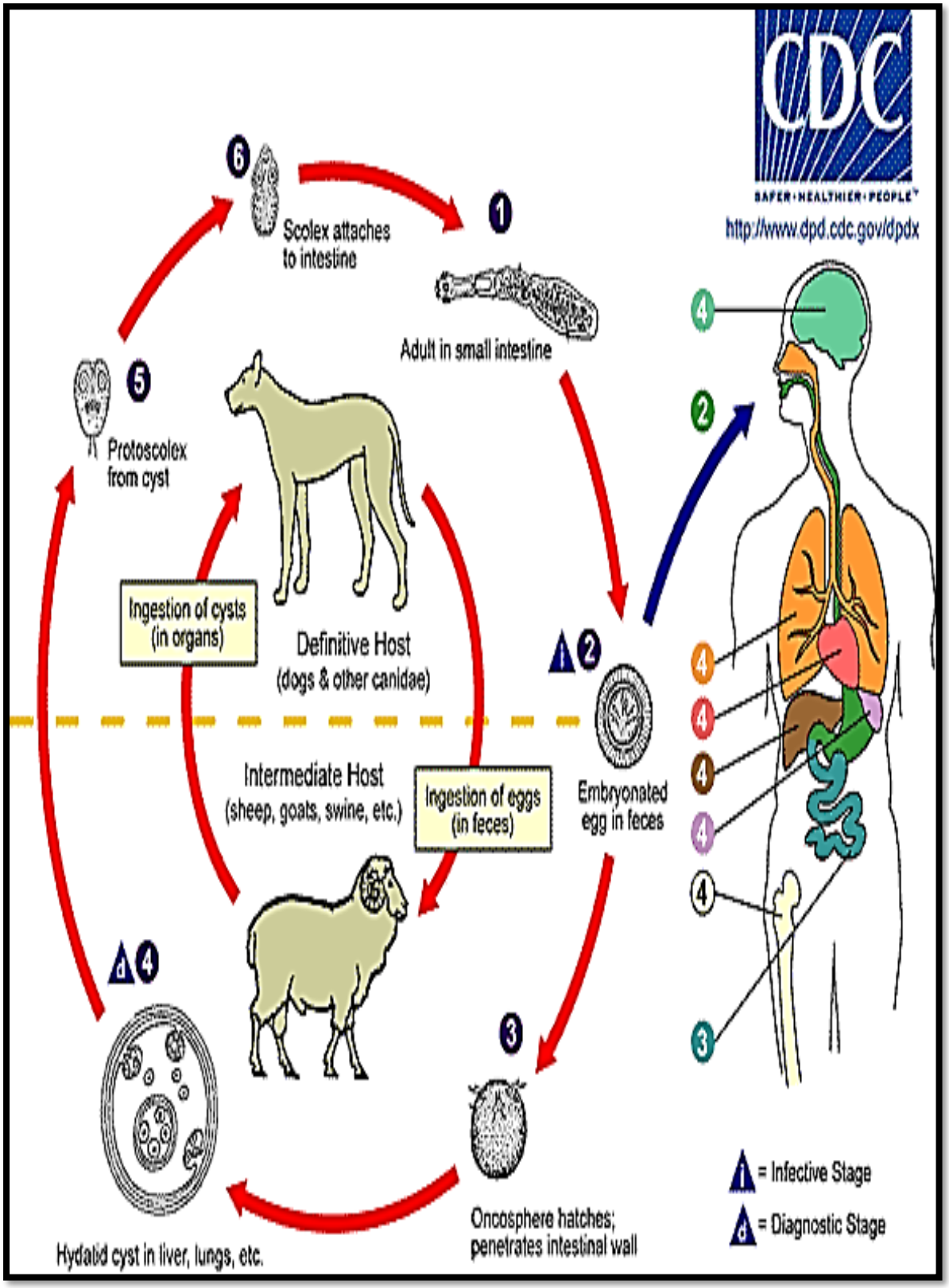

Figure (2-2): The life cycle of the *Echinococcus granulosus*. (cited by CDC) http://www.dpd.cdc.gov/dpdx

The adult worm of *E. granulosus* (Figure, 2-3) had a specialized adhering organ composed of four muscular suckers and two rows of hooks, placed on the rostellum. The body consists of variety of reproductive proglottids, which can range in number from two to six (Eckert *et al.*, 2001; Ekhnefer, 2012). like all tapeworms, *Echinococcus* lack the alimentary tract or gut canal, the feeding and defecation take place across tegument (Eckert *et al.*, 2001; Ekhnefer, 2012).

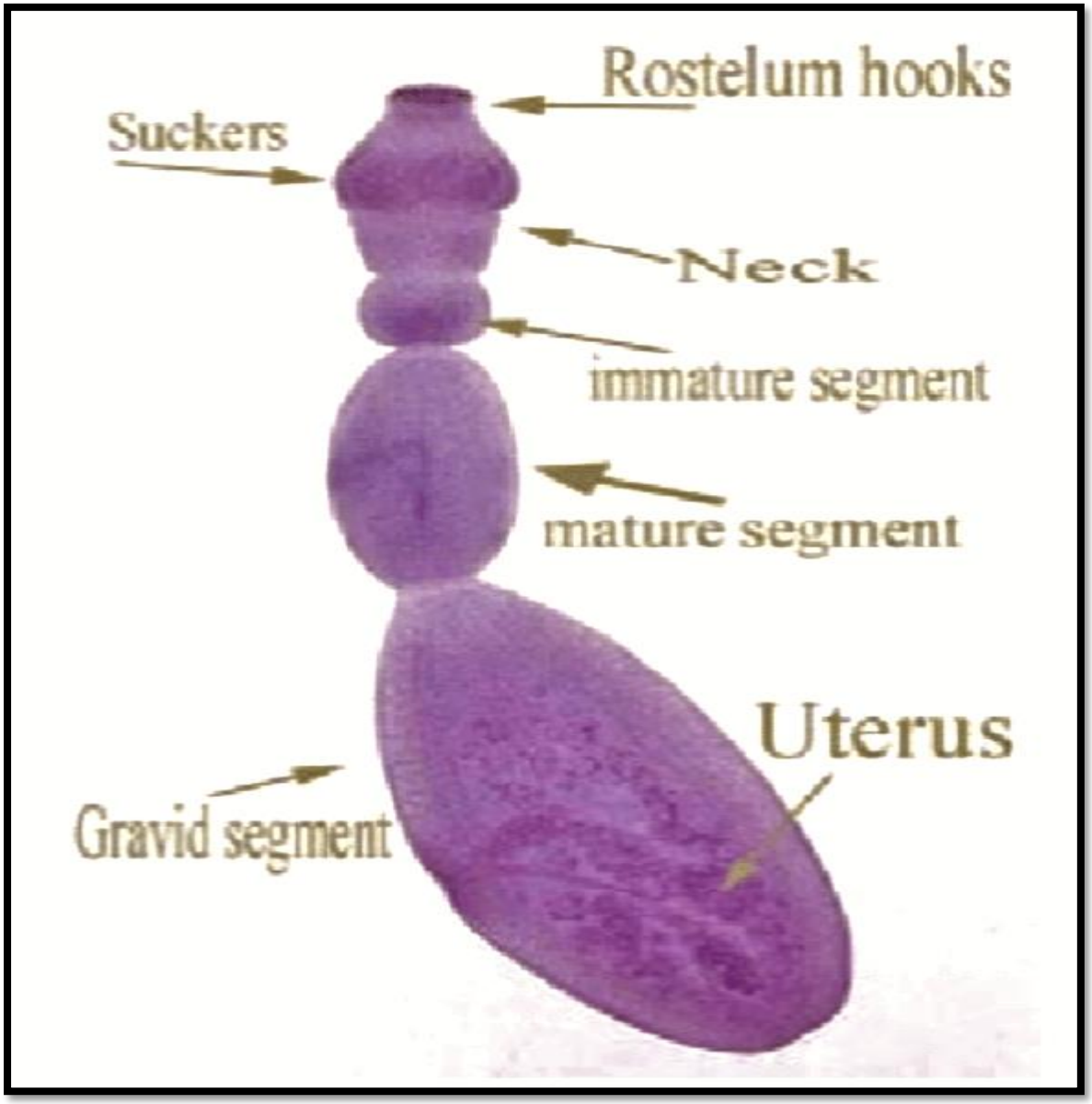

Figure (2-3): General structures of adult worm of *Echinococcus granulosus.*

https://www.researchgate.net/Figure/Morphology-of-Adult-Worm-of-E-granulosus_Figure2_318878214

2:4: Global distribution of *Echinococcus sp.*:

The *Echinococcus sp.* is worldwide distributed parasite **(**Figure. 2-4**)** found in **Africa**: Somalia, Mali, Chad, Niger, Nigeria, South Africa, Senegal, Mauritania, Algeria, Egypt, Libya, Tunisia, Morocco, Ethiopia, Kenya (Ekhnefer, 2012; WHO, 2014; Tigre *et al.,* 2016; Mulinge *et al.,* 2018). In **Asia**: India, Malaysia, Mongolia, Japan, Kazakhstan, Pakistan, China, Korea, Iran, Jordan, Iraq (WHO, 2014; Fadhil and A'aiz, 2016; Karamian *et al.*, 2017; Ehsan *et al.*, 2017; Chaudhari *et al.*, 2017; Ebrahimipour *et al*., 2017). In **North America**: USA (California, Texas), Mexico (Villalobos *et al.*, 2007; WHO, 2014 Massolo *et al.,* 2014). In **South America**: Argentina, Brazil (Fontanarrosa *et al*.,2006; WHO, 2014). In **Europe**: Sweden, Finland, Portugal, Italy, Spain, Greece (Busi *et al*., 2007; WHO, 2014; Roinioti *et al*., 2016). In **Eurasia**: Russia, Belarus, Turkey (Kul and Yildiz, 2010; Konyaev *et al.*, 2013; WHO, 2014), and **Australia** (WHO, 2014; Jenkins *et al.*, 2018).

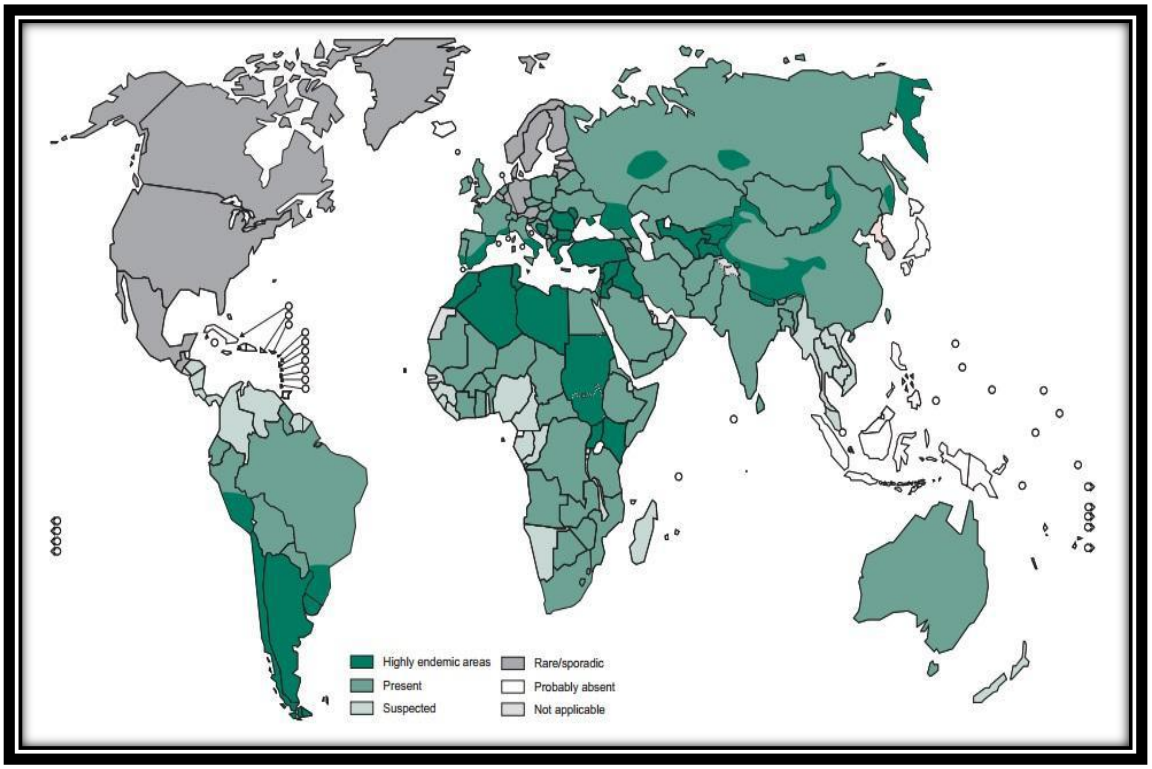

Figure (2-4): The Global distribution of *Echinococcus granulosus* (*WHO,* 2014).

2:5: The global Prevalence of *E. granulosus*:

2:5:1: The Prevalence of *E. granulosus* in definitive host (dog):

The dogs (all type and races) are susceptible for infection with *Echinococcus granulosus*, especially in poor region where domestic, stray, and feral dogs are spread in the roads and around slaughterhouse (Ekhnefer, 2012).

E. granulosus parasite is endemic a proximately in all countries of the world especially in Middle East, Africa, South America, Southern Europe, and Australia (Abu-Eshy, 1998; Al-Olayan and Helmy, 2012) there are some variation in distribution of *E. granulosus* from region to region, this variation depends on some factors like host species and the environmental factors such as humidity and temperature in the region where infection is spread (Eckert *et al.*, 2001; Xiao *et al.*, 2006).

Some studies reported that high infection rates with *E. granulosus* for dogs in many regions of the world such as: Iran (8-36.19%), (Shiraz) 36.19% (Mehrabani *et al.*, 1999); Iran (Khorasan) 22% (Razmi *et al*., 2006); Iran (Mashhad) 8% (Garedaghi and Safar, 2011); Turkey 24% (Utuk *et al.*,2008); Argentina 8.69% (Lavallén *et al*., 2011).

2:5:2: The Prevalence of *E. granulosus* in human population:

The human infection rates with *E. granulosus* parasite is related with contact with infected dogs especially in rural area (Ekhnefer, 2012).

Some studies reported that the infection rate of human (was calculated as case/100,000) in neighboring countries or our region was: in Jordan 1.5-6.5 (Kamhawi and Hijjawi, 1992). In Kuwait, 3.6 (Shaweiki *et al.*, 1990).

In Algeria about 3.4 to 4.6 (Larbaoui and Allyulya, 1979) In Turkey 0.87-6.6 (Altintas, 2003) In China (Xinjiang) 8.7 (NHDCC, 1993). High incidence rate of (HC) was recorded in Morocco 6.5-7.8% (Pandey *et al*., 1986).

2:5:3: The Prevalence of *E. granulosus* in intermediate hosts:

There are many studies on distribution of infection with *E. granulosus* in intermediate hosts around the world such as: Argentina 12.5% in sheep, 7% in cattle and 6.0% in goat (Kamenetzky *et al.,* 2002); Tunisia 16.4% in sheep, 8.5% in cattle, 5.9% in dromedaries, and 2.8% in goats (Lahmar *et al.*, 2013; Chaâbane-Banaoues *et al.,* 2015); in Kenya (Turkana) 3.6% in sheep, 19.4% in cattle, 61.4% in camels and 4.5% in goats (Mulinge *et al.*, 2018); Kenya (Meru and Isiolo) was 4.62% in sheep 1.92% in cattle, 6.94% in camels, and 0.37% in goats (Mulinge *et al.*, 2018); Kenya (Maasailand) was 16.5% in sheep, 25.8% in cattle and 10.8% in goats (Addy *et al.,* 2012; Mulinge *et al.,* 2018); Pakistan 6.35% in sheep, 13.46% in buffalo, 30.35% in cattle and 4.33% in goats (Hussain *et al.,* 2005; Ehsan *et al.,* 2017); Turkey 51.9% in sheep, 3.7% in buffalo, 39.7% in cattle and 2.0% in goats (Altintas, 2003; Utuk *et al.*, 2008); Iran (Ardebil) 33.8% in sheep, 1.6% in buffalo, 16.3% in cattle, and 5.8% in goat (Ahmadi and Dalimi, 2006); Iran (Lorestan, Ilam, Kermanshah and Azerbaijan) 11.1% in sheep, 12.4% in buffalo, 16.4% in cattle and 6.3% in goat (Mohammad *et al*., 2011; Hanifian *et al.,* 2013).

2:6: The Prevalence of *E. granulosus* in Iraq:

There are many Studies on *E. granulosus* and hydatid diseases in Iraq, but few in Misan Province, the hydatid cyst disease dispersal in all regions of Iraq and Some studies reported that a high infection rates in different regions of Iraq (Maktoof and Abu Tabeekh, 2015; Fadhil and A'aiz, 2016 and Hammad *et al.,* 2018).

2:6:1: Prevalence of *E. granulosus* in the dogs of Iraq:

The earliest report on the helminths of dogs in Iraq was held by Babero and Al-Dabagh (1963), In Baghdad the infection rate was 25% (Tarish *et al*., 1986); In Arbil the rate was 79.1% (Molan and Saida, 1989); in Theqar the infection rate was 56% (Molan, 1993); other study in Arbil reported the infection rate was 49.5% (Saeed *et al.*, 2000); in Basra the infection rate was 14.7% (Maktoof and Abu Tabeekh, 2015); in Misan % (Alsaady *et al.*, non-published).

2:6:2: Prevalence of *E. granulosus* in the Human in Iraq:

Some studies had been reported that the Prevalence of *E. granulosus* in Iraq, like in (Arbil) 2 per 100000 inhabitants (Saeed *et al.*, 2000**);** in (Basra) 3.2 cases per 100000 per year (Maktoof and Abu Tabeekh, 2015).

2:6:3: Prevalence of *E. granulosus* in the intermediate host in Iraq: -

The infection rate of *E. granulosus* in Baghdad was 11.93% in sheep and goats, 24.66% in cattle and one camel examined and found infested (Senekji and Beattie, 1940); In Basra was 22% in sheep (Maktoof and Abu Tabeekh, 2015); In Mosul was 2% in sheep, 0.55% in cattle and 0.52% in goats (Jarjees and Al-Bakri, 2012); In Arbil the infection rate was 15.0% in sheep, 10.9% in cattle, and 6.2% in goats (Saeed *et al*., 2000**)**.

2:7: Physical and chemical properties of the hydatid cyst fluid:

The properties of hydatid cyst fluid are illustrated in Table (2-2) and it contains Albumin, Creatinine, Lecithin, Urea, small amounts of Glucose, Sodium Chloride, Phosphates, Sodium sulfate, Sodium succinate and Calcium, it also contains some trace elements such as Iron, Copper, Zinc, Cadmium, Nickel, Chromium, Magnesium and Manganese (Erin, 2007; Ekhnefer, 2012).

Table (2-2): The Physical properties of hydatid cyst fluid.

Physical properties	
The Color	colorless to slightly yellow
Textures	liquid
specific gravity	1.012
pH	Neutral **(**7.2 - 7.6)
degree of freezing	0.53°C

2:8:The genetic diversity of *E. granulosus*:

Many genetic diversity studies found that the *E. granulosus* had ten strains, these strains was identified in different regions of the world (Karamian *et al.*, 2017; Hammad *et al.*, 2018), such as:

In, Iran: G1,G2,G3,G6 and G7strains (Ahmadi and Dalimi, 2006; Hanifian *et al.*, 2013; Ebrahimipour *et al.*, 2017; Karamian *et al.*, 2017), in Turkey: G1,G2 and G3 strains (Altintas, 2003; Utuk *et al.*, 2008; Kul and Yildiz, 2010), in Egypt: G6 and at least two distinct strains exist in this country (El Shazly *et al.*, 2007), in Sudan:G1 and G6 (Hamid, 2006), in Eastern Africa: G1, G5, G6 and G7 (Dinkel *et al.*, 2004), in Italy: G1, G2, G3, G4, and G7 (Busi *et al.*, 2007), in Romania: G1,G2 and G7(Bart, 2006), in Argentina: G1, G2, G6 and G7 (Kamenetzky *et al.*, 2002), in Russia (Yakutia): G6, G8 and G10 (Konyaev et al., 2013), in Poland: G9 (Scott *et al.*,1996), in Finland:G10 (Lavikainen *et al.*, 2003).

2:8:1: *Echinococcus granulosus* strains/genotypes identification:

Echinococcus granulosus genotypes or strain exhibit considerable levels of variation in biology, physiology and molecular genetics (Le *et al*., 2002; Karamian *et al.*, 2017; Hammad *et al.*, 2018).

These genotypes identified based on nucleotide sequence analysis of the mitochondrial cytochrome C oxidase subunit 1 (*Cox1*) gene and reduced Nicotinamide adenine dinucleotide (NADH) dehydrogenase 1 (*Nad1*) gene. The different genotypes of *E. granulosus* have been associated with distinct, intermediate hosts: sheep, buffalo, horses, cattle, camels, pigs, cervids, goats and others animals (Ebrahimipour *et al.*, 2017; Hammad *et al.*, 2018).

This categorization follows the pattern of strain variation emerging based on biological characteristics.

1. Cytochrome c oxidase 1 (*Cox1*), is a protein that encoded by the *Cox1* gene. Cytochrome c oxidase 1 is the main subunit of the cytochrome c oxidase complex. Cytochrome c oxidase subunit 1 (*Cox1*) is one of three mitochondrial encoded subunits (*Cox1*, *Cox2* and *Cox3*) of respiratory complex V, that considered as a third and final enzyme of the electron transport chain of mitochondrial oxidative phosphorylation (Baraak, 2014). Cytochrome c oxidase is a key enzyme in aerobic metabolism (Papa *et al.*, 1994). And every molecular study of animal species in the field involves mtDNA haplotyping at some stage (Ratnasingham and Hebert, 2007). a mitochondrial fragment, *Cox1*, as recently selected as the standardized tool for molecular taxonomy and identification (Ratnasingham and Hebert, 2007; Baraak, 2014).

***2*-NADH dehydrogenase subunit 1 (*Nad1*):**

This gene is mitochondrial encoded gene provides instructions for making a protein called dehydrogenase. This protein is a part of a large enzyme complex

known as complex I which is active in mitochondria (Lenaz *et al*., 2004; -Baraak, 2014).

2:8:2: The distribution of *E. granulosus* strains and hosts:

1- G1 or Sheep Strain *E. granulosus sensu stricto*: It is found in Australia, Europe, United States of America, Africa, China, Middle East, South America, and Russian (Kamenetzky *et al.*, 2002; Dinkel *et al.*, 2004; Hamid, 2006; Busi *et al.*, 2007; Kul and Yildiz, 2010; WHO, 2014; Karamian *et al.*, 2017; Hammad *et al.*, 2018). Dogs, fox, dingo, jackal, and hyena are definitive hosts, while sheep, goat, cattle, pig, camel, and man are intermediated hosts (Thompson and McManus, 2001; Baraak, 2014).

2- G2 or Tasmanian sheep strain *E. granulosus sensu stricto*: It was founded in Tasmania and Argentina (EURLP, 2017). Dog is a definitive host, the fox may play a role in some time as definitive host, sheep and man are intermediated hosts, no enough data on cattle as play a role as intermediated host (Thompson and McManus, 2001; Baraak, 2014).

3- G3 or Buffalo Strain *E. granulosus sensu stricto*: It is prevalent in Asia. Dog is definitive hosts and no enough data on fox as definitive hosts, Buffalo is intermediated host. cattle and man may play a role as intermediated hosts (Thompson and McManus, 2001; Baraak, 2014).

4- G4 or Horse strain which is identified as *E. equinus*: It is spreading in Europe, Middle East, but South Africa and USA may be concerned. Dog is the definitive host. The horse and other equine are intermediated hosts (Thompson and McManus, 2001; Baraak, 2014; EURLP, 2017).

5- G5 or Cattle strain which is identified as *E. ortleppi*: It is spreading in Europe, South America, India, and Russian. Dog is the definitive host. The

cattle and human are intermediated hosts (Thompson and McManus, 2001; Baraak, 2014; EURLP, 2017).

6- G6 or Camel strain which is identified as *E. canadensis*: It is spreading in the Middle East, Africa, China, Argentina, (Baraak, 2014) and Iran (Fasihi-Harandi *et al.,* 2002). The dog is a definitive host. The camel and goat are intermediated hosts (Thompson and McManus, 2001; El Shazly *et al.,* 2007; EURLP, 2017).

7- G7 or Pig strain which is identified as *E. canadensis*: It is distributing in Europe, Russia, and South America (Baraak, 2014; EURLP, 2017). The dog is a definitive host, pigs are intermediated hosts (Thompson and McManus, 2001; Busi *et al.*, 2007).

8- G8 or Cervid strain which is identified as *E. canadensis*: It is recorded in Eurasia and North America. The wolf and dog are the definitive hosts, where cervid and human are intermediated hosts (Thompson and McManus, 2001; Konyaev *et al.*, 2013; EURLP, 2017).

9- G9 or lion strain which is identified as *E. felidis*: It is distributing in Africa. The lion is the definitive host, where zebra, warthog, pig, buffalos and various antelope are intermediated hosts (Scott *et al.*,1996; Thompson and McManus, 2001; Baraak, 2014).

10- G10 or Human strain or *Fennoscandian cervid* strain which is identified as *E. Canadensis*: It is recorded in Finland and Russia. The wolf *Canis lupus* is the definitive host, where moose *Alces,* reindeer *Rangifer tarandus* and Human are intermediated hosts (Thompson and McManus, 2001; Lavikainen *et al*., 2003; Konyaev *et al*., 2013; EURLP, 2017).

The genotype G1 is worldwide distribution, it is responsible for the great majority of human cystic echinococcosis which consist about 88.44% of

human CE. It has the most cosmopolitan distribution and is often associated with transmission via sheep as intermediate host. The closely related genotypes G6 and G7 cause about 11.07% of human infection (Baraak, 2014).

CHABTER TREE

Materials & Methods

3: The Materials and methods.

3:1: The materials

3:1:1: Apparatus: they mentioned in Table (3-1).

Table (3-1): The Apparatus that used in current study.

Apparatus (model)	Manufactory
Centrifuge (UNIVERSAL 32)	Hettich / Japan
Centrifuge (EBA 20)	Hettich / Japan
Centrifuge refrigerated (5415 R)	eppendorf / Japan
DNA sequencer	**The test done by macrogen, korea**
E-Box UV Filter system (VX2)	Vilber / China
Electrophoreses apparatus	Biocom directcom / China
Freezer	Craft / China
Hot plate	Jlassco / China
Microscopy (CX21FS1)	Olympus / China
Safety Cabinet	Human Lab / China
Sensitive balance (BL210S)	Sartorius / Japan
Water bath (WNE 14)	Memmert / Germany
Thermal-cycler (Mastercycler personal)	Eppendorf / Japan
Transilluminator (UVIFOR)	Elettrofor / China
Ultrasound sonar (Voluson E6)	Voluson / USA
UPS (MAX3300)	Maxima / China
Vortex (REAX top)	Heidolph / UK
Water still (LWD-2008F)	LabTech / China
Microscope camera system	(Scope Image Dynamic pro)

3:1:2: The instruments: They mentioned in Table (3-2).

Table (3-2): The instruments that used in current study.

Instruments (Volume)
Micropipettes (1-10µL, 5-50µL, 20-200µL, 100-1000µL) / Japan
Micropipette Tip (100µL, 1000µL) / China
Conical flask (250ml, 500ml, 1000ml) / China
Cover slips / China
Cylinder (10ml, 250ml, 500ml) / China
Cup (60ml) / P.R.C
Flask (50ml, 500ml) / China
Forceps
Glass slides / China
Long Sleeve Gloves / China
Ocular micrometer
Safety white overall / P.R.C
Safety glasses / China
Scalpel
Scissors
Stage micrometer / USA
Syringe (20ml, 60ml) / Jordin
Tubes (1.5ml, 10ml, 13ml) / China
Gloves / China
Collection Tubes (2 ml) / China
GS Columns (1.5ml)

3:1:3: The reagents and chemicals: they mentioned in Table (3-3).

Table (3-3): The reagents and chemicals that used in current study.

REAGENTS AND CHEMICALS	
Distil water	Marker Ladder (L100)
Normal saline 0.9% NaCl	Proteinase K
Formalin solution 10%	PCR preMix
Ethanol Absolute, 96%, 70%	COX1.F and COX1.R primers
Iodine	Nad1.F and Nad1.R primers
Phosphate buffer saline	TE Buffer 10x solution
Kanda balsam	Oil immersion
Xylene	
Agarose	
Ethidium bromide solution	
Loading dye	
Nail polish	

3:1:4: The software programs: are mentioned in Table (3:4).

Table (3-4): The software programs used in this study.

Software Programs	
Morphological study	Molecular study
Scope Image Dynamic pro	MEGA-X
IC Measure	NETW5.0.10
IBM SPSS Statistics 24	DnaSP5.10

3:2: Methods

3:2:1: Epidemiological study

This study (Figure, 3-1) was carried out during the period from December 2017 to October 2018. The sample of hydatid cyst had been taken weekly from animals (sheep, buffalo, cow, goats and camel) from slaughterhouses of Amara city and human HC from Al-Sader and Al-Zhrawy hospitals, the adult worm was obtained from stray dogs (Figure, 3-2).

3:2:2: The study area

The present study was carried out in Amara city south of Iraq (Figure, 3-2) mainly in five locations (slaughterhouses of Amara, Al-Sader hospital, Al-Zahrawi hospital, near the old slaughterhouses in Al-Majdeea, near the new temporary slaughterhouses beside the main road of Amara - Al-Mashrah).

3:2:3: The samples collection

a- From livestock animals: The hydatid cysts samples were collected from infected organs of livestock animals (sheep, buffalo, cow, goat and camel) which were slaughtered in slaughterhouse. The samples were put in sealed and labeled polyethylene bags and then transferred directly to the laboratory of parasitology at Biology Department of science college.

b- From human: The hydatid cysts samples were collected from infected human whose had been previously diagnosed by ultrasound exam (sonar) or CT scan and undergo to surgical operations. Those samples were transferred in labeled container to the laboratory of parasitology immediately.

c- From stray dogs: The small intestine of hunted stray dog (shout by gun) was collected by necropsy. And then put in a labeled container with 70% ethanol. The sample was transferred immediately to the laboratory of parasitology.

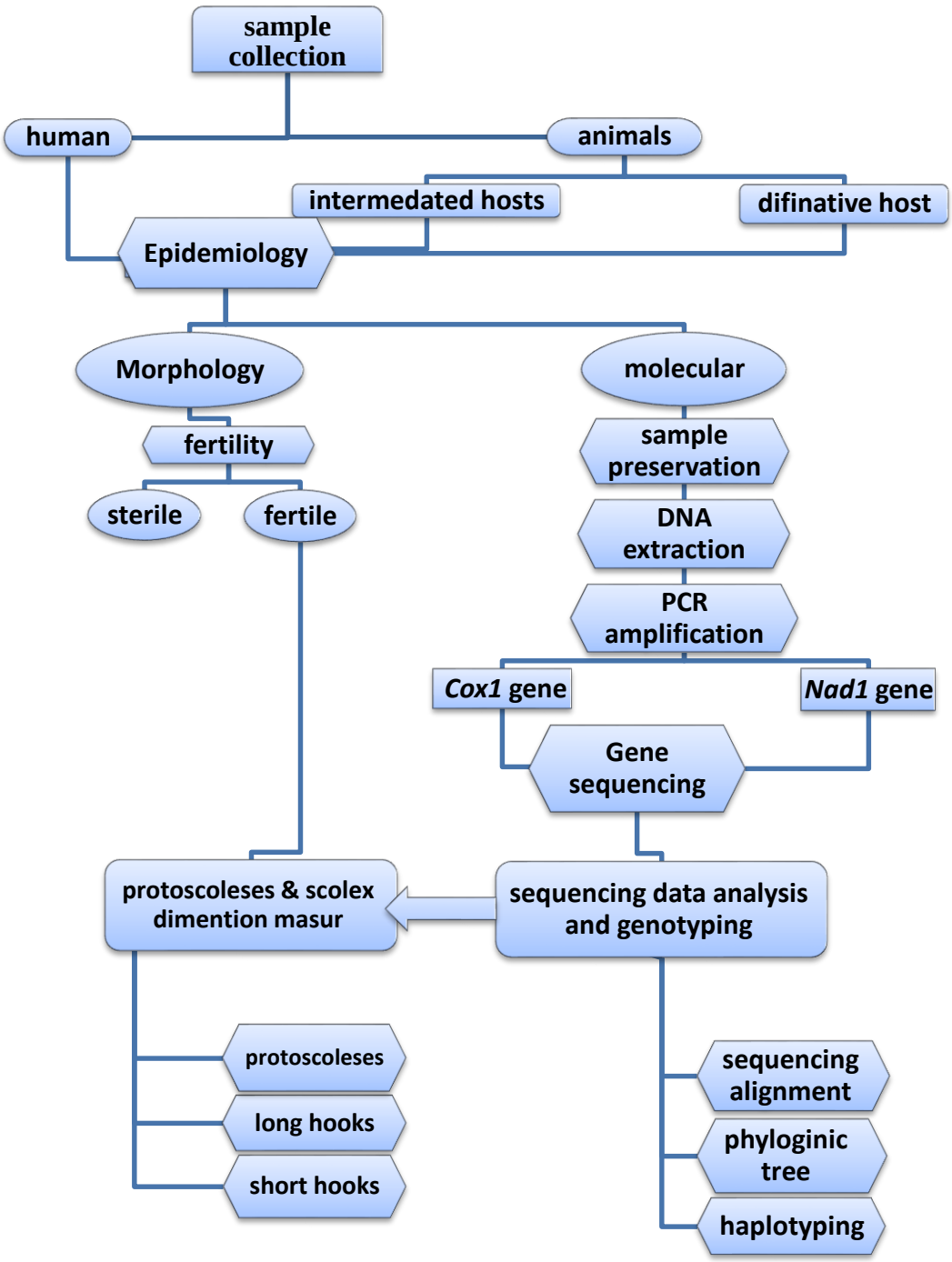

Figure (3-1): Scheme for the main steps in the current study.

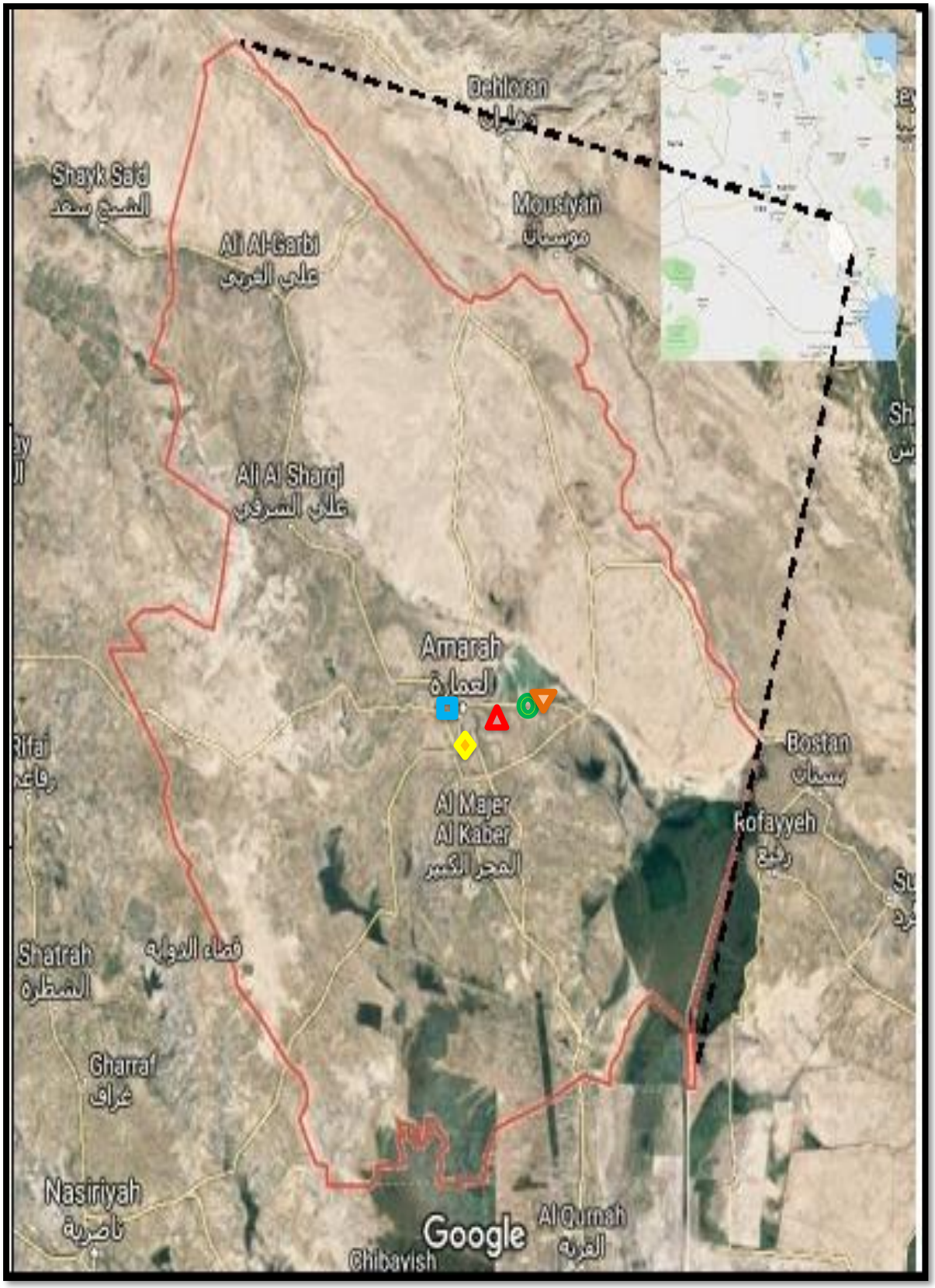

Figure (3-2): The study area and the locations of sample collection in Misan Province, ■: Al-Sader teaching hospital, ♦: Al-Zahrawi hospital, **o**: temporary slaughterhouse of Amara city, ▼: area around the temporary slaughterhouse, ▲: area around the old slaughterhouse of Amara city.

3:2:4: The Morphological study

3:2:4:1: The microscopic examination

3:2:4:1:1: The preparation of hydatid cyst samples.

The samples of hydatid cyst which collected previous step (3:2:3) were examined microscopically according to Lett (2013) as following steps:

1-The samples were placed in sealed polyethylene bags and then immediately transferred to the laboratory.

2-The samples were washed under tap water to get rid of the blood and left for two to five minutes to dry.

3- Samples were sprayed with 70% ethanol for surface sterilization and left to dry this step repeated three times.

4- The fluid of hydatid cyst was aspirated by 60 ml syringe until the cyst was completely emptied and the aspirated fluid transferred to a sterile 60 ml cups.

5- Take a piece of hydatid cyst germinal layer with sterile blade and transfer to a labeled tube, the tubes were preserved in 70% ethanol.

6-Determined the fertility of HC by microscopic examination, Fertility of the collected hydatid cysts was determined by detection of Protoscolices in aspirated fluid samples, calcified or Sterile cysts considered as infertile.

7- The HC content was centrifuged at 5000 rpm for five minute and the supernatant was discharge.

8- The precipitate Protoscolices washed by sterile normal saline and mix well then re-centrifuged and re-washed two time.

9- The Protoscolices and its hooks were imaged by using digital microscopical camera for later morphological parameter analysis.

10- Take specimen of HC then was washed with sterile distil water and centrifuged at 8000rpm / five minute for three time the precipitate was preserved in 70%ethanol for later molecular study.

3:2:4:1:2: Calibration of the microscopy.

The ocular micrometer (OM) is divided into ocular divisions (OD) (Figure,3-3a).

The stage micrometer (SM) has a calibrated scale which is divided into 0.1mm and 0.01mm units (Figure,3-3b,c).

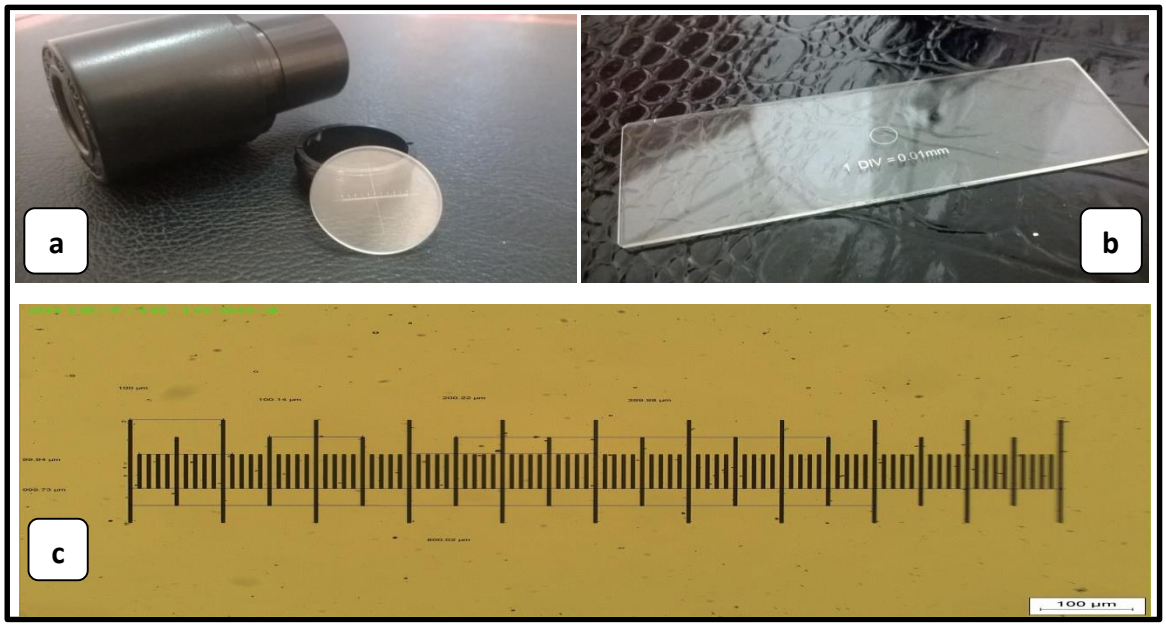

Figure (3-3): Microscopy calibration, (a): the stage micrometer (SM), (b): ocular micrometer (OM), (c): (SM) scale.

microscopy calibration steps:

1. Insert the (OM) into a 10X eyepiece.
2. Place the stage micrometer slide (SM) on the microscopy stage and focus on the scale.
3. Adjust the field so that the 0 line of the (OM) scale is exactly superimposed upon the 0.0 line of the (SM) scale.
4. locate the point at the extreme right as possible where any two lines are exactly superimposed on each other.
5. Count the number of divisions (mm) on the (SM)between the 0.0 line and the superimposed line.

6. Count the number of ocular divisions on the (OM) between the 0.0 line and the superimposed line.
7. Divide the distance of (SM) by the number of ocular divisions of (OM) and multiply by 1000 to give the ocular micrometer units in (μm).
8. Repeat the operation for each objective lens on the microscope.

3:2:4:2: Preservation of hydatid cysts and adult *E. granulosus* samples

samples of germinal layer were preserved in 70% ethanol for later DNA extraction Protoscolices or cyst contents as well as adult worms of *E. granulosus* were preserved in 70% ethanol and frizzing for further DNA extraction.

3:2:5: molecular study:

The genotype including sequencing of partial mitochondrial cytochrome c oxidase subunit 1 (*Cox1*) and of NADH dehydrogenase 1 (*Nad1*) genes (Bowles *et al*., 1992; Bowles and McManus, 1993).

3:2:5:1: Preparation of hydatid cyst contents for DNA extraction.

Preserved sample was centrifuged and discharge alcohol solution, then washed with sterile distil water, mixed by vortex thoroughly, then centrifuged at 13,200 rpm for three minute that repeated two times to remove the ethanol from the sample according to DNA extraction kit instructions.

3:2:5:2: DNA extraction

The ethanol preserved hydatid cyst samples was prepared for DNA extraction by fallowing protocol of DNA extraction kit (gSYNC^{TM} DNA Extraction Kit, geneaid, Korea) with some modification for some samples.

3:2:5:2:1: DNA Extraction procedure.

a. Tissue Sample Dissociation

1- Transfer (10-25 mg) of *E. granulosus* tissue to a 1.5 ml microcentrifuge tube.

2- Add 200µl of GST buffer and 20µl of Proteinase K then mixed thoroughly with vortex.

3- Incubate at 60ºC overnight or until the sample lysate becomes clear.

b. Cell Lysis

1- If insoluble material remains following incubation, centrifuge for 2 minutes at 13,200rpm then carefully transfer the supernatant to a new 1.5ml microcentrifuge tube.

2- Add 200µl of GSB Buffer and shake vigorously for 10 seconds.

c. DNA Binding

1-Add 200µl of absolute ethanol to the sample lysate and mix Immediately by shaking vigorously for 10 seconds.

2-Place a GS Column in a 2 ml collection tube. Transfer all of the mixture (including any insoluble precipitate) to the GS Column.

3-Centrifuge at 13,200rpm for 1 minute or until mixture passes completely.

4-Discard the 2ml collection tube containing the flow-through then transfer the GS column to a new 2 ml collection tube.

d. Washing

1- Add 400µl of W1 Buffer to the GS column.

2- Centrifuge at 13,200rpm for 30 seconds then discard the flow-through, Place the GS Column back in the 2 ml collection tube.

3-Add 600µl of Wash Buffer to the GS column. Centrifuge at 13,200rpm for 30 seconds then discard the flow-through.

4-Place the GS Column back in the 2 ml collection tube. Centrifuge again for 3minutes at 13,200rpm to dry the column matrix.

e. DNA Elution

1-Transfer the dried GS column to a clean 1.5 ml microcentrifuge tube.

2-Add 100µl of pre-heated elution buffer into the center of the column matrix.

3-Let stand for at least 3min to allow elution buffer to be completely absorbed.

4-Centrifuge at 13,200rpm for 30 seconds to elute purified DNA.

3:2:5:2:2: Visualization of extracted DNA by gel electrophoresis.

The electrophoresis tray and Agarose gel was done as fallowing:

a- Preparation of Agarose gel and electrophoresis tray.

Agarose gel was prepared by melting 1.0g of the agarose powder in 100 ml 1X TBE buffer (TBE Buffer (5X):108g Tris, 55g Boric acid, 40ml 0.5M EDTA, 2L H_2O) at 100°C until solution is completely clear. After cooling the agarose solution to about 50°C and then mixed with 3µl ethidium bromide and poured into electrophoresis tray sealed by its rubber, comb was placed and left to harden at room temperature or at 5^{o}C.

b- Preparation of DNA sample for gel electrophoresis:

Add 8µl of purified DNA on glass slide and mixed with 4µl of loading dye.

c- Gel Electrophoresis for extracted DNA:

1. The electrophoresis tray and agarose gel was prepared.
2. Carefully remove the comb from the agarose gel.
3. Load the DNA samples with running buffer to the clean wells, then chamber of Electrophoresis filled with 1X TBE buffer, tray supplied with the electrophoresis apparatus.
4. Connect to power supply at 100mV for 30 min.
5. Under ultraviolet (UV) transilluminator the gel was examined for the presence of DNA bands in darkness.
6. The gel was photographed directly by camera (E-Box UV Filter system).
7. Stored The DNA tubes in -20^{o}C.

3:2:5:3: The polymerase chain reaction (PCR):

The PCR was carried out for the purified DNA samples with Master mix (preMex), PCR preMex kit component illustrated in Table (3-5), Then DNA of *E. granulosus* was thawed and adequate cumulative volume of the amplification mix was prepared (Table, 3-6).

Table (3-5): The master mix (PreMix) components.

Component	For 50µl reaction volume
Taq DNA polymerase	2.5U
dNTP (dATP, dTTP, dGTP, dCTP)	Each 250 µM
Reaction Buffer, with1.5mM $Mgcl_2$	1X
Stabilizer and tracking dye	O

Table (3-6): The mixture of PCR amplification for 50µl reaction volume.

Compounds	Volumes
PCR preMix	Dried pellet
H2O	20µl
Foreword primer	5µl (10pmol)
Reverse primer	5µl (10pmol)
Template DNA	20µl
Total Reaction Volume	50µl

About 20µl of the DNA of *E. granulosus* were added to each tube, the tubes were closed and mixed by vortex then centrifugation at maximum speed for 10 sec.

3:2:5:3:1: Mitochondrial gene primers:

For mitochondrial genes (*Cox1* and *Nad1*) PCR amplification the fallowing primers (Table, 3-7) are used:

Table (3-7): Mitochondrial gene primers used in this study.

	Cox1 primers (Bowles *et al*., 1992)
F: JB3	′5 TTT TTT GGG CAT CCT GAG GTT TAT 3′
R: JB4.5	′5 TAA AGA AAG AAC ATA ATG AAA ATG 3′
	Nad1 primers (Bowles and McManus, 1993)
F: JB11	′5 AGA TTC GTA AGG GGC CTA ATA 3′
R: JB12	′5 ACC ACT AAC TAA TTC ACT TTC 3′

3:2:5:3:2: The polymerase chain reaction (PCR) amplification cycles:

The amplifying cycle for *Cox1* gene Table (3-8) and *Nad1* gene Table (3-9) was started on the thermo-cycler device.

Table (3-8): PCR condition for (*Cox1*) gene.

Step	Temperature °C	time	
Pre-Denaturation	95	5min	
Denaturation	94	45sec	40 cycle
Annealing	51	35sec	
Extension	72	45sec	
Final Extension	72	10min	

Table (3-9): PCR condition for (*Nad1*) gene.

Step	Temperature °C	time	
Pre-Denaturation	95	5min	
Denaturation	94	45sec	40 cycle
Annealing	58	35sec	
Extension	72	45sec	
Final Extension	72	10min	

When The PCR cycles completed the specimen tubes were kept on ice or refrigerated until running the electrophoresis.

3:2:5:3:3: Gel Electrophoresis for PCR products:

1. Electrophoresis apparatus was assembled.
2. One gram of agarose was added in 100ml of (1X) TBE in a glass beaker.

3. The agarose suspension boiled until the agarose became homogeneous with TBE, the agarose solution was allowed to cool and before it solidifies 3µl of Ethidium bromide solution was added carefully and mixed gently.
4. Rains the mixture to tray containing comb and left to solidify.
5. Removed the comb was gently and well loaded with PCR product, in each well 8µl and one of it by 5µl ladder marker (100bp ladder).
6. The tray with the gel was placed in the electrophoresis apparatus, the gel was covered with (1X) TBE buffer.
7. Connect the electrophoresis cell with power supply and run at 65mV for 65min.
8. switched off the power supply and transfer the gel for checking under UV illumination.
9. After the electrophoresis run, the gel was transferred to the imaging system (E-Box UV Filter system) and the results was pictured.

3:2:5:4: Sequencing of partial mitochondrial cytochrome c oxidase subunit 1 (*Cox1*):

Different isolates of *E. granulosus* were analyzed for sequence variation within a region of the mitochondrial cytochrome c oxidase subunit 1 (*Cox1*) gene. For each *E. granulosus* isolate examined, a double-stranded PCR product that has a size of 446bp was visualized on an ethidium bromide stained agarose gel (Bowles *et al*., 1992). 29 distinct partial *Cox1* sequences were detected amongst the examined *E. granulosus* isolates.

3:2:5:5: Mitochondrial gene sequence data analysis:

As a reference method, for verification of the PCR results the sequence of a part of the mitochondrial *Cox1* Nucleotide sequence analysis was made using the National Centre for Biotechnology Information BLAST programs and Databases, European Union Reference Laboratory for Parasites (EURLP)

method, DNasp5 program for haplotype the sequences, NETWORK5.0 Program for drawing the haplotype network diagram and Molecular Evolutionary Genetics Analysis (MEGA) program used for sequences alignment and phylogenic tree drawing.

3:2:6: Statistical Analysis:

Data were analyzed by **one-way ANOVA** (F-test) by general liner model using **Statistical Package for Social Science** program (**SPSS)** version 24. The comparisons between means were made using Duncan test. The difference was considered to be significant at $p<0.05$, The data are presented as mean ± Std. Deviation (Giolo-Ruiz, 2004).

CHABTER FOUR

The Results

4: The Results:

4:1: The Epidemiological study of *E. granulosus* in human and some livestock animals from Misan Province:

The study of *E. granulosus* was conducted in Amara city (31°51'49.0"N 47°08'51.1"E) Misan Province, south of Iraq, the present study was carried out on 3287 cases distributed as sheep:922, buffalo:405, camel:2, cow: 983, goat:150, human:819 and dog:6 (Table, 4-1), the present study was carried out during the period of December 2017 to October 2018, the results showed (Table,4-1) that the infection rates of *E. granulosus* in sheep, buffalo, camel, cow, goats, human and dog were found to be 2.16% (20/922), 2.20% (9/405), 0.00% (0/2), 3.05% (30/983), 0.00% (0/150), 1.70% (14/819) and 33.33% (2/6) respectively.

The fertility rates (Table, 4-2) computed as finding of Protoscolices in aspirated cystic fluid was found to be 65.00%, 11.12%, 20.00% and 60.00% in sheep, buffalo, cow and human respectively.

The statistical analysis (F-test) for the results of cyst fertility between different host showed **High significan**t differences ($F= 14.021$, $p< 0.001$).

Duncan test indicate that sheep cyst fertility was **significantly** different from that of buffalo, cow and **not significantly** with human cyst, were **no significant** differences between buffalo and cow cyst fertility (Table, 4-2).

Table (4-1): The sample distribution and infection rates in different host.

Host	Total Examinations	Total infection	Infection rate	Sterile cyst	Sterile cyst (%)
Sheep	922	20	2.16%	7	35.00%
Buffalo	405	9	2.20%	8	88.88%
Camel	2	0	0.00%	0	0.00%
Cow	983	30	3.05%	24	80.00%
Goat	150	0	0.00%	0	0.00%
Human	819	14(5)*	1.70%	2/(5)*	40.00%
Dog	6	2	33.33%	---	---
Total	3287	75	---	41	---
* five surgical sample obtains from human in Al-Sader teaching hospital.					

Table (4-2): The hydatid cyst fertility rates analysis in different hosts.

Host	N	Fertility %
sheep	20	65.00% [b]
buffalo	9	11.12% [a]
cow	30	20.00% [a]
human	5	60.00% [b]
F	14.021	
P	0.00000049	

4:2: The Morphological Study of *E. granulosus*:

The measures of some parameters of Protoscolices (Figure, 4-1) that obtain from hydatid cyst (Figure,4-2) and adults obtained from stray dogs (Figure, 4-4) such as: the total length of Protoscolices (TLP), total width of Protoscolices (TWP), rostellum length (RL), rostellum width (RW), suckers length (SL) and suckers width (SW) (Figure, 4-7).

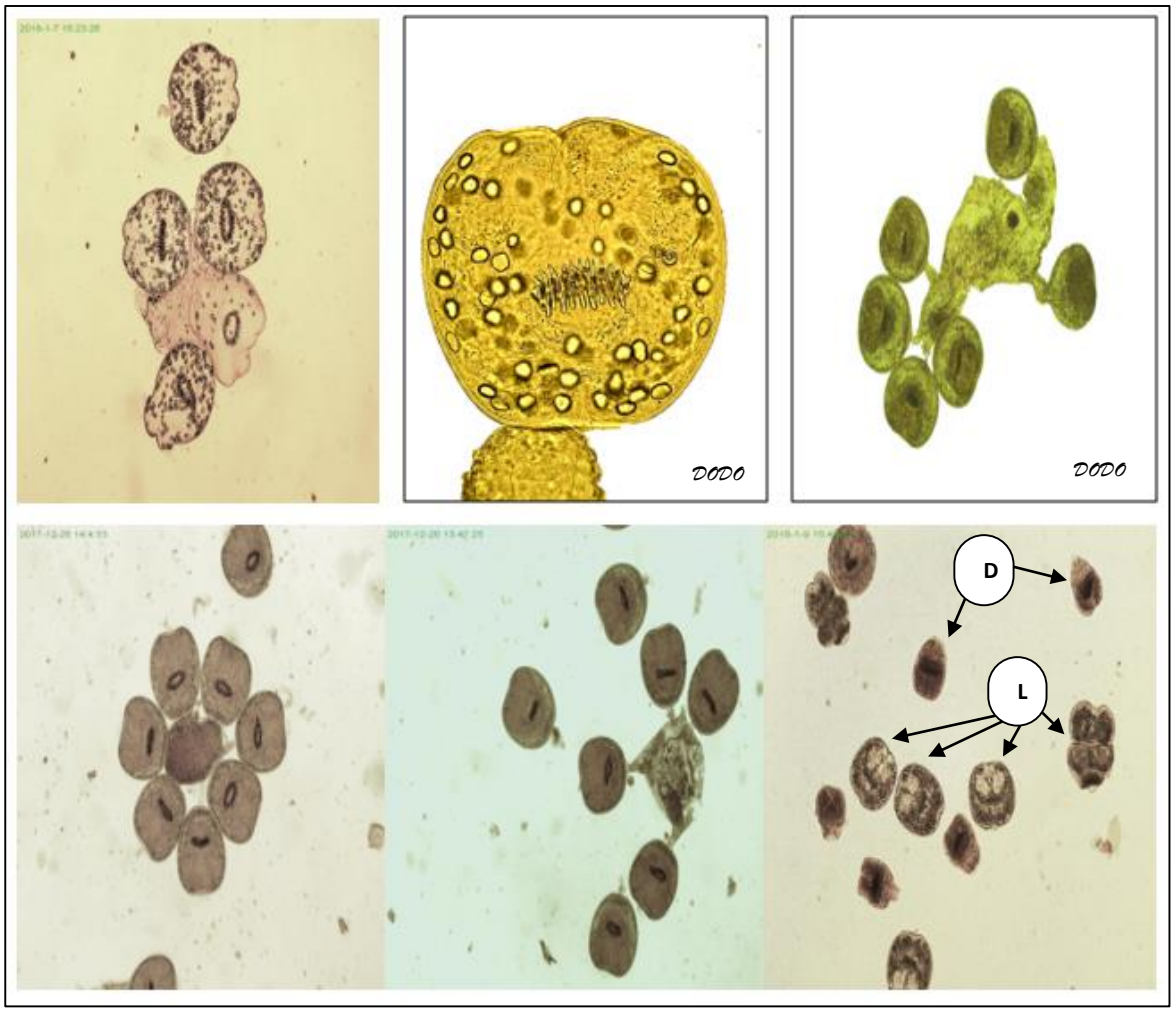

Figure (4-1): The Protoscolices of *E. granulosus*, L: live protoscolices, D: died Protoscolices (10X).

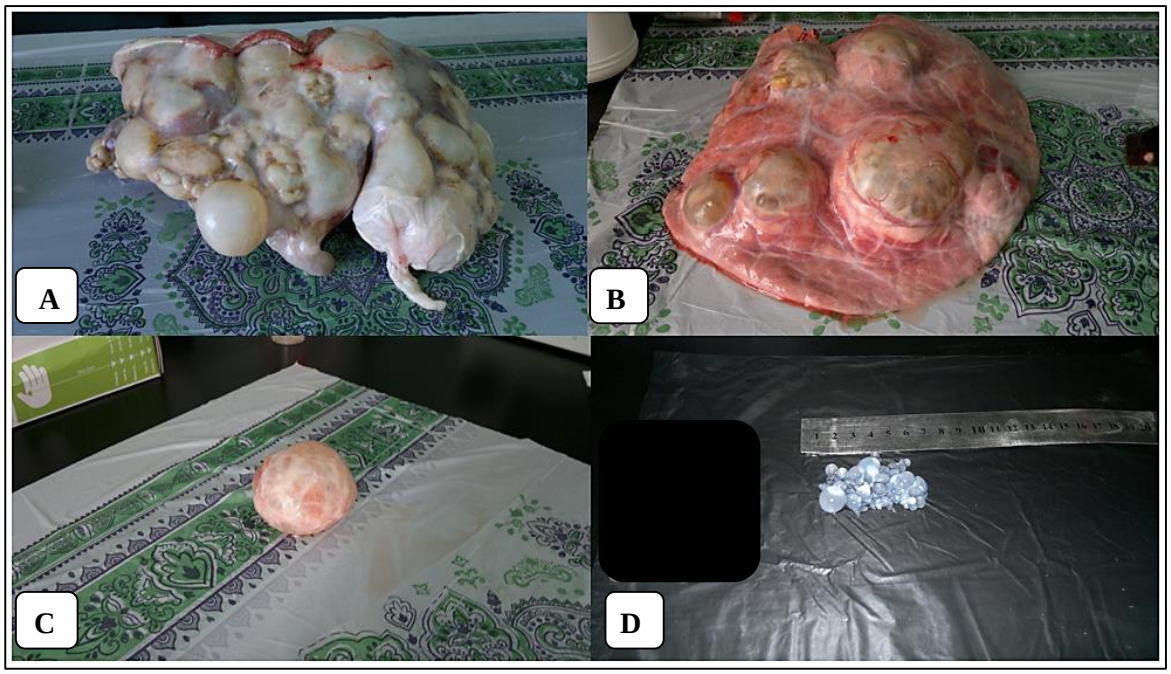

Figure (4-2): The hydatid cyst, A: hydatid cyst of sheep liver, B: hydatid cyst of cow lung, C: isolated cyst, D: daughter cyst.

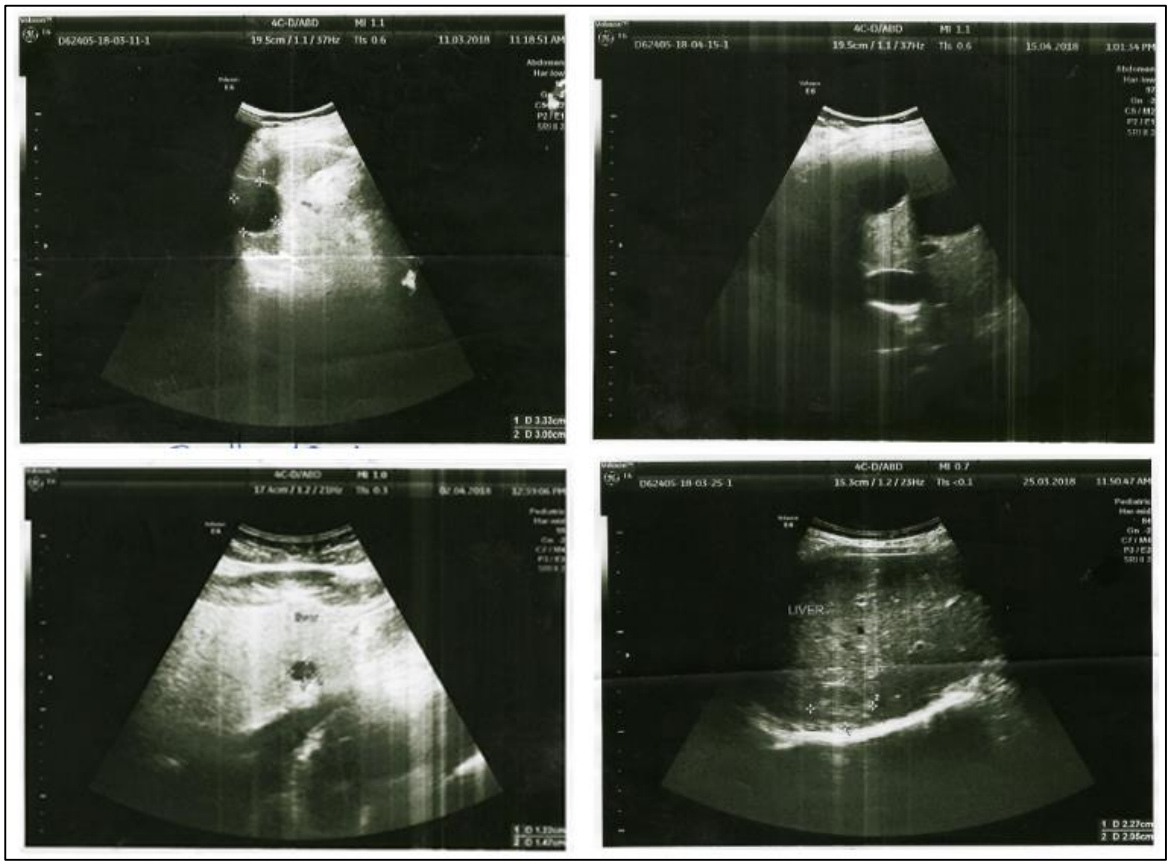

Figure (4-3): human hydatid cyst diagnosed by Altera sound in Al-sader teaching hospital.

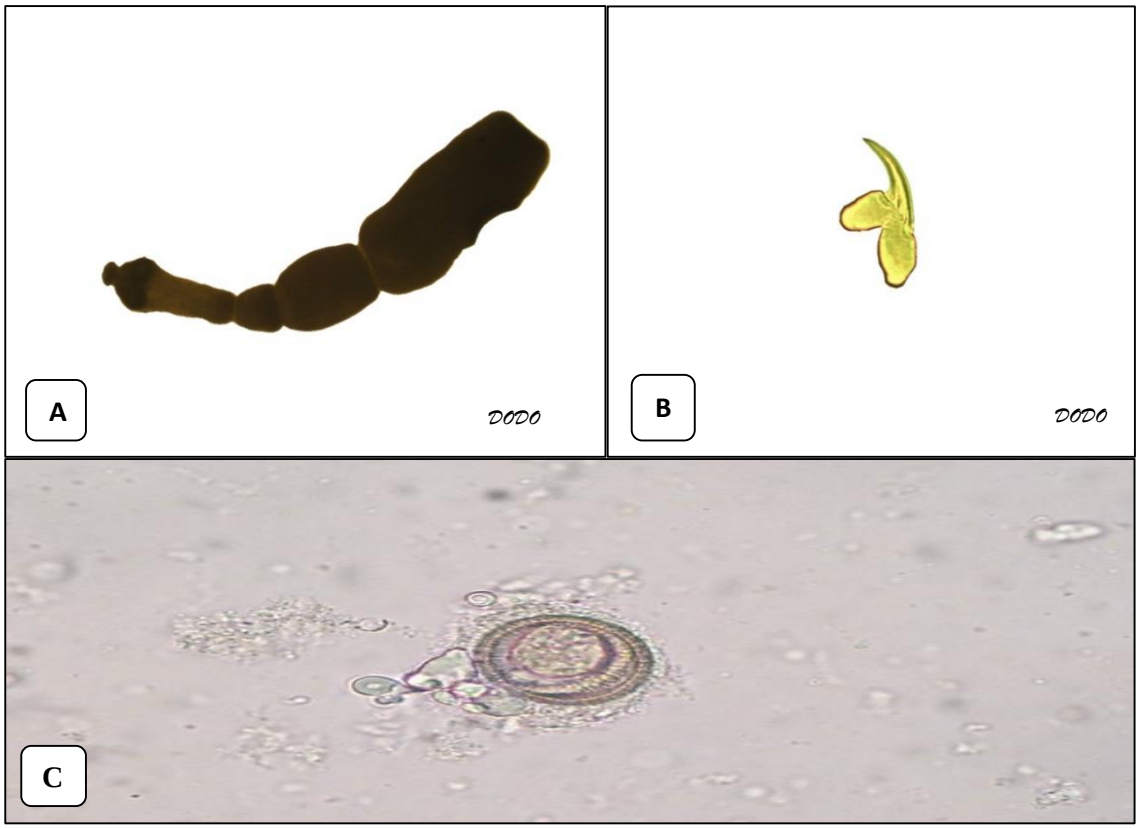

Figure (4-4): **adult worm of *E. granulosus***, A: the adult of *E. granulosus* (4X), B: adult worm hook (100X), C: *E. granulosus* egg (100X).

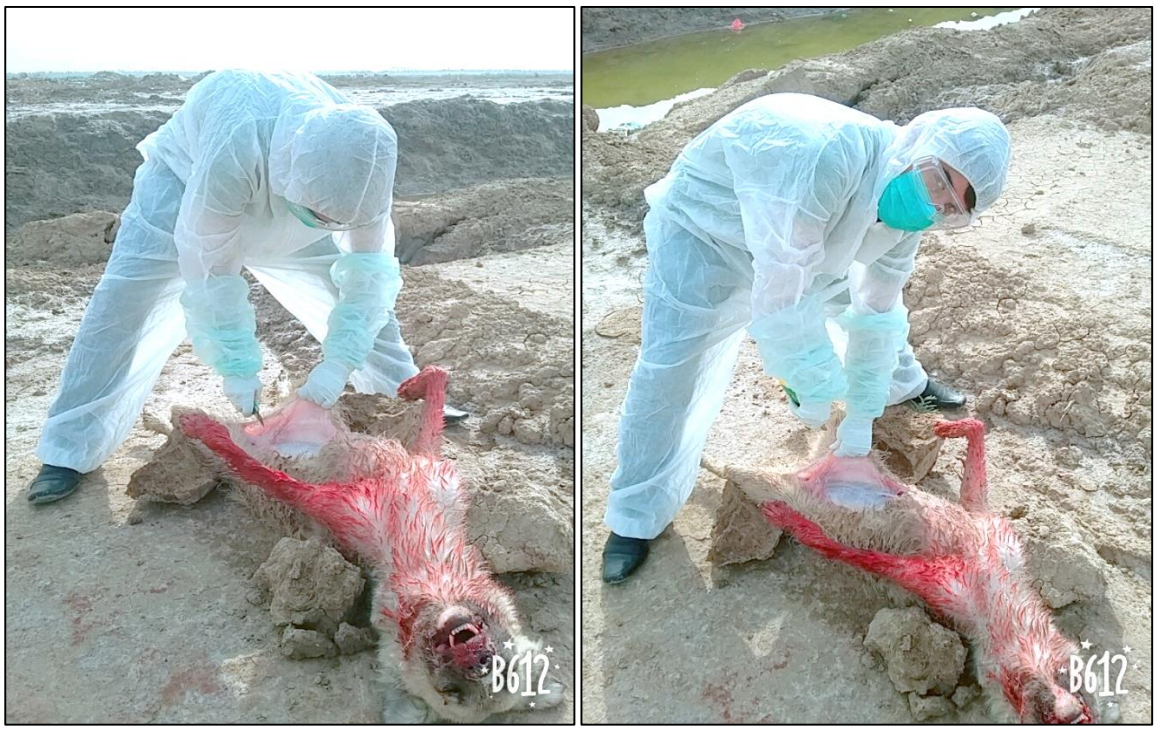

Figure (4-5): the hunted stray dog necropsy at the field.

The large and small hooks (10-15 hooks) (Figure, 4-6) were taken from every fertile hydatid cyst and measured of some parameters such as total hook length (THL), total width of hook (TWH), curved length of hook (CLH), before blade length (BBL), blade length (BL), handle length (HL) and three angles (α, β and γ) (Figures, 4-8, 4-9, 4-10, 4-11, 4-12, 4-13).

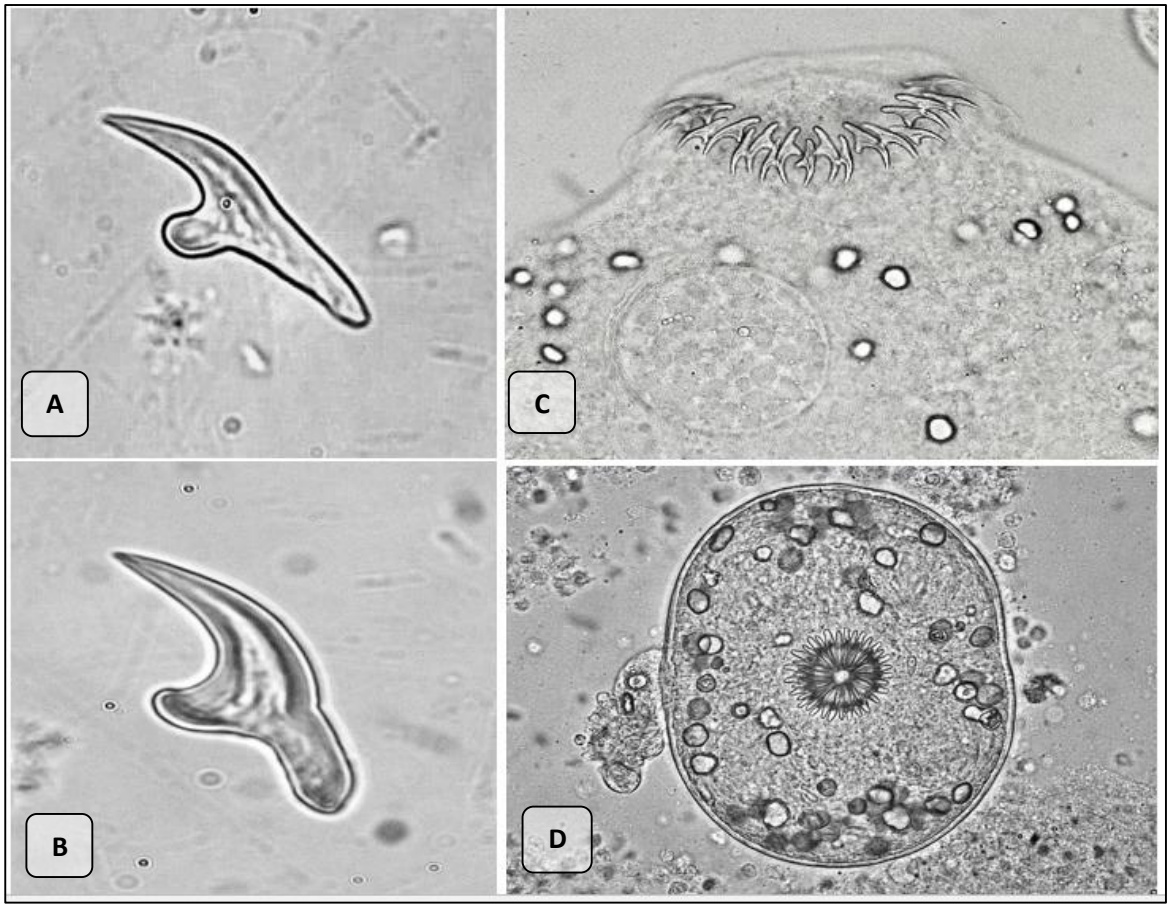

Figure (4-6): The hooks of *E. granulosus* Protoscolices, A: small hook (100X), B: large hook (100X), C: side view for rostellum hooks (40X), D: upper view for rostellum. Hooks (40X).

Also the adult worm (Figure, 4-4) that collected from stray dogs (Figure, 4-5) was measured and compared its scolex and hooks with that of Protoscolices obtained from intermediated hosts, adult total length was ranged between 2.23-2.85mm.

4:2:1: The Morphological characterization for Protoscolices parameters according to host origin of the specimen:

4:2:1:1: The Total Length of Protoscolices (TLP):

The results showed (Table, 4-3) that The **TLP** of *E. granulosus* from different host is 200.89, 119.09, 170.37, 189.64 and 561.10µm for sheep, buffalo, cow, human and dog respectively.

The highest mean of TL of scolex of adult worm and the lowest mean of TLP was received from buffalo.

The statistical analysis by using Analysis of Variance ANOVA /F-test of results showed high **significant** differences between the TLP obtained from varied hosts ($F=215.904$, $p<0.001$).

The Duncan test showed that the TL of scolex of adult was **significant**ly increased from the means of TLP of larval stage that obtained from intermediated hosts, in other hand the mean of TLP obtained from buffalo was significantly decreased from the mean of TLP collected from other intermediated hosts, the mean of TLP collected from sheep was **significantly** increased from that collected from buffalo, cow and **not significantly** increased from that of human , the mean of TLP from human **not significantly** increased from cow (Table,4-3).

4:2:1:2: Total Width of Protoscolices TWP:

The results showed (Table, 4-3) that The **TWP** of *E. granulosus* is 154.76, 102.05, 145.95, 130.51 and 197.55µm for sheep, buffalo, cow, human and dog respectively.

The highest mean of TW from adult worm scolex and the lowest mean of TWP was received from buffalo.

The statistical analysis by using ANOVA / F-test of results showed high **significant** differences between the TWP obtained from varied hosts ($F=18.260$, $p<0.001$).

Duncan test showed that the TW of scolex of adult was **significant**ly increased from the means of TWP of larval stage that obtained from intermediated hosts, in other hand the mean of TWP of buffalo was **significantly** decreased different from TWP obtain from intermediated hosts, and there are significant increased in the TWP of human with sheep, the TWP of cow was Not significant differences from sheep and human (Table, 4-3).

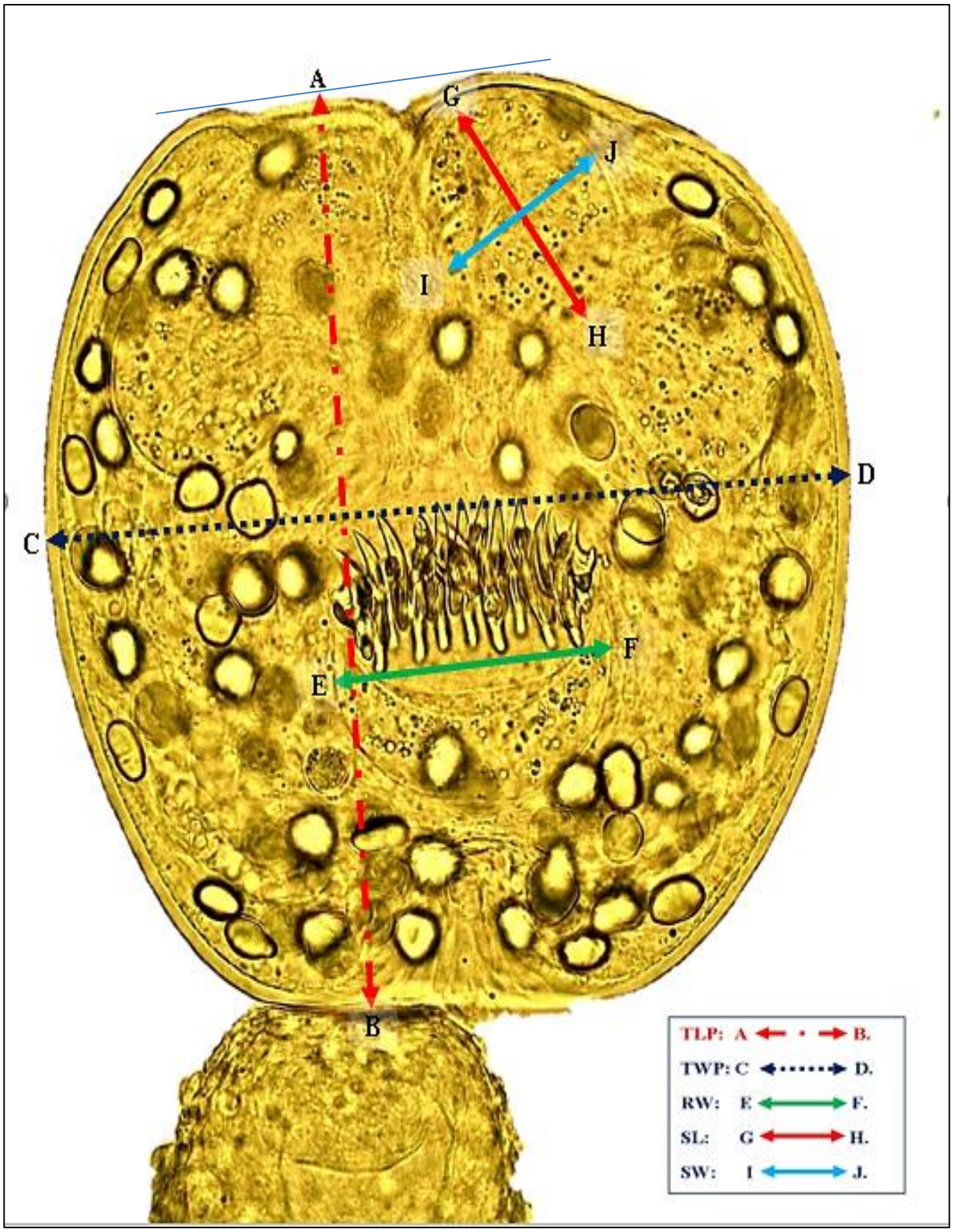

Figure (4-7): The Protoscolices parameters that measured in this study, **TLP**: total length of Protoscolices, **TWP**: total width of Protoscolices, **RW**: The Rostellum Width, **SL**: The Sucker Length, **SW**: The Sucker Width**.**

Table (4-3): The TLP and TWP parameters for *E. granulosus* in varied hosts.

Host	Protoscolices parameters (µm)			
	Total length (TLP)		Total width (TWP)	
	N	Mean ± S. D	N	Mean ± S. D
sheep	179	200.89 ± 57.16 ^{c}	179	154.76 ± 36.27 ^{c}
buffalo	14	119.09 ± 33.52 ^{a}	14	102.05 ± 33.09 ^{a}
cow	62	170.37 ± 25.23 ^{b}	60	145.95 ± 29.54 ^{bc}
human	44	189.64 ± 46.82 ^{bc}	44	130.51 ± 32.57 ^{b}
Dog*	16	561.10 ± 66.56 ^{d}	16	197.55 ± 47.61 ^{d}
F	215.904		18.260	
p	2.7897E-88		1.7864E-13	
* The measures for the adult worms was for scolex only				

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:2:1:3: The Rostellum Width (RW):

The results showed (Table, 4-4) that The (RW) of *E. granulosus* is 72.70, 73.13, 66.99, 68.21 and 131.66µm for sheep, buffalo, cow, human and dog respectively.

The highest mean of RW for adult worm obtained and the lowest mean of RW was received from buffalo.

The statistical analysis by using ANOVA / F-test of results showed **high significant** differences between the RW obtained from varied hosts ($F=94.745$, $p<0.001$).

Duncan test showed the mean of RW of adult was **significantly** increased from RW means of Protoscolices obtain from intermediated hosts, **no significant** differences between RW of Protoscolices collected from sheep, buffalo, cow and human (Table,4-4).

4:2:1:4: The Sucker Length SL:

The results showed (Table, 4-4) that The SL of *E. granulosus* is 58.47, 38.81, 60.47, 56.50 and 140.13μm for sheep, buffalo, cow, human and dog respectively.

The adult worm had the highest mean of SL and the lowest mean of SL was received from buffalo.

The statistical analysis by using ANOVA / F-test of results showed **high significant** differences between the SL obtained from varied hosts ($F=259.718$, $p<0.001$).

Duncan test showed that SL mean of adult worm was **significantly** increased from the means of SL of Protoscolices that obtained from intermediated hosts, also the mean of SL from buffalo was significantly decreased from Protoscolices SL means collected from sheep, cow and human, there **No significant** differences between SL of sheep, cow and human (Table,4-4).

4:2:1:5: The Sucker Width (SW):

The results showed (Table,4-4) that The SW of *E. granulosus* is 47.02, 29.17, 47.15, 43.70 and 110.24μm for sheep, buffalo, cow, human and dog respectively.

The highest mean of SW was of adult worm and the lowest mean of SW was from buffalo.

The statistical analysis (F-test) of results showed **high significant** differences between the SW obtained from varied hosts ($F=140.358$, $p<0.001$).

Duncan test showed that SW mean of adult worm was **significantly** increased from the means of SW of Protoscolices that obtained from intermediated hosts, also the mean of SW from buffalo was significantly decreased from

Protoscolices SW means collected from sheep, cow and human, there **no significant** differences between SW of sheep, cow and human (Table,4-4).

Table (4-4):The RW, SL and SW parameters of *E. granulosus* in varied host.

	Protoscolices parameters (µm)					
Host	Rostellum width (RW)		Sucker length (SL)		Sucker width (SW)	
	N	Mean ± S. D	N	Mean ± S. D	N	Mean ± S. D
sheep	94	72.70 ± 13.62 ^{a}	174	58.47 ± 9.04 ^{b}	168	47.02 ± 8.92 ^{b}
buffalo	6	73.13 ± 14.37 ^{a}	14	38.81 ± 13.41 ^{a}	14	29.17 ± 9.66 ^{a}
cow	40	66.99 ± 9.57 ^{a}	62	60.47 ± 9.24 ^{b}	58	47.15 ± 9.02 ^{b}
human	28	68.21 ± 6.09 ^{a}	44	56.50 ± 7.06 ^{b}	43	43.70 ± 6.34 ^{b}
dog*	15	131.66 ±11.19^{b}	13	140.13 ± 14.12 ^{c}	10	110.24 ±15.15^{c}
F	94.745		259.718		140.358	
p	4.9777E-43		2.0767E-96		2.1926E-66	

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:2:2: The Morphological characterization of hooks:

4:2:2:1: The morphological characterization for hooks number:

The results showed (Table,4-5) that the number of hooks are ranged between 26-40, 28-34, 30-36, 32-38 and 30-32hook in sheep, buffalo, cow, human and dog respectively.

The statistical analysis (F-test) for the number of hooks between different host show No significant differences (F=1.845, p> 0.05).

Table (4-5): The hooks number count for *E. granulosus* in varied host.

	Number of hooks			
host	N	Mean ± S. D	Minimum	Maximum
sheep	97	32.04 ± 2.85^{a}	26	40
buffalo	5	31.60 ± 2.60^{a}	28	34
cow	40	32.25 ± 1.98^{a}	30	36
human	8	34.50 ± 1.77^{a}	32	38
dog	2	31.00 ± 1.41^{a}	30	32
F=1.845, p= 0.123				

4:2:2:2: The morphological characterization for Long hooks parameters According to the host.

4:2:2:2:1: The Total Length of Long Hook (TLLH):

The results showed (Table,4-6) that The TLLH of *E. granulosus* is 25.56, 24.51, 23.62, 22.19 and 33.58µm for Protoscolices collected from sheep, buffalo, cow, human and adult worm of dog respectively.

The highest mean of TLLH was for adult worm and the lowest mean of TLLH was obtained from **human**.

The statistical analysis (F-test) of results showed **high significant** differences between the TLLH obtained from varied hosts ($F=129.473$, $p<0.001$).

Duncan test showed the mean of TLLH collected from adult worm was **significantly** increased with the TLLH means of larval stage (Protoscolices). In other hand the TLLH of sheep are **significant**ly increased from that of buffalo, cow and human, also the mean of TLLH from human was **significant**ly decreased from TLLH of sheep, buffalo and cow and **no significant** differences between TLLH of buffalo and cow (Table,4-6).

4:2:2:2:2 The Total Width of Long Hook (TWLH):

The results showed (Table, 4-6) that The TWLH of *E. granulosus* is 9.34, 9.15, 8.48, 7.82 and 16.11µm for Protoscolices collected from sheep, buffalo, cow, human and adult worm of dog respectively.

The highest mean of TWLH obtain from adult worm and the lowest mean of TWLH was received from **human**.

The statistical analysis (F-test) of results showed **high significant** differences between the TWLH obtained from varied hosts ($F=147.114$, $p<0.001$).

Duncan test (Table,4-6) showed that there are **significantly** decreased in mean of TWLH obtained from human with that of sheep, buffalo and cow, also that

there are **significant** decreased in the mean of TWLH obtained from cow with that of sheep, buffalo and human, **no significant** differences between TWLH of sheep and buffalo hydatid cyst.

4:2:2:2:3: The Curved Length of Long Hook (CLLH):

The results showed (Table,4-6) that The CLLH of *E. granulosus* is 28.94, 27.50, 26.54, 25.17 and 39.42μm for Protoscolices collected from sheep, buffalo, cow, human and adult worm of dog respectively.

The highest mean of CLLH received from adult worm and the lowest mean of CLLH was obtained from **human** HC.

The statistical analysis (F-test) of results showed **high significant** differences between the CLLH obtained from varied hosts ($F=167.608$, $p <0.001$).

Duncan test showed there are **significant** increased between the mean of CLLH obtained from sheep with that collected from buffalo, cow and human, also the mean of CLLH obtains from human was significantly decreased with CLLH means of sheep, buffalo and cow and **No significant** differences between CLLH of buffalo and cow (Table,4-6).

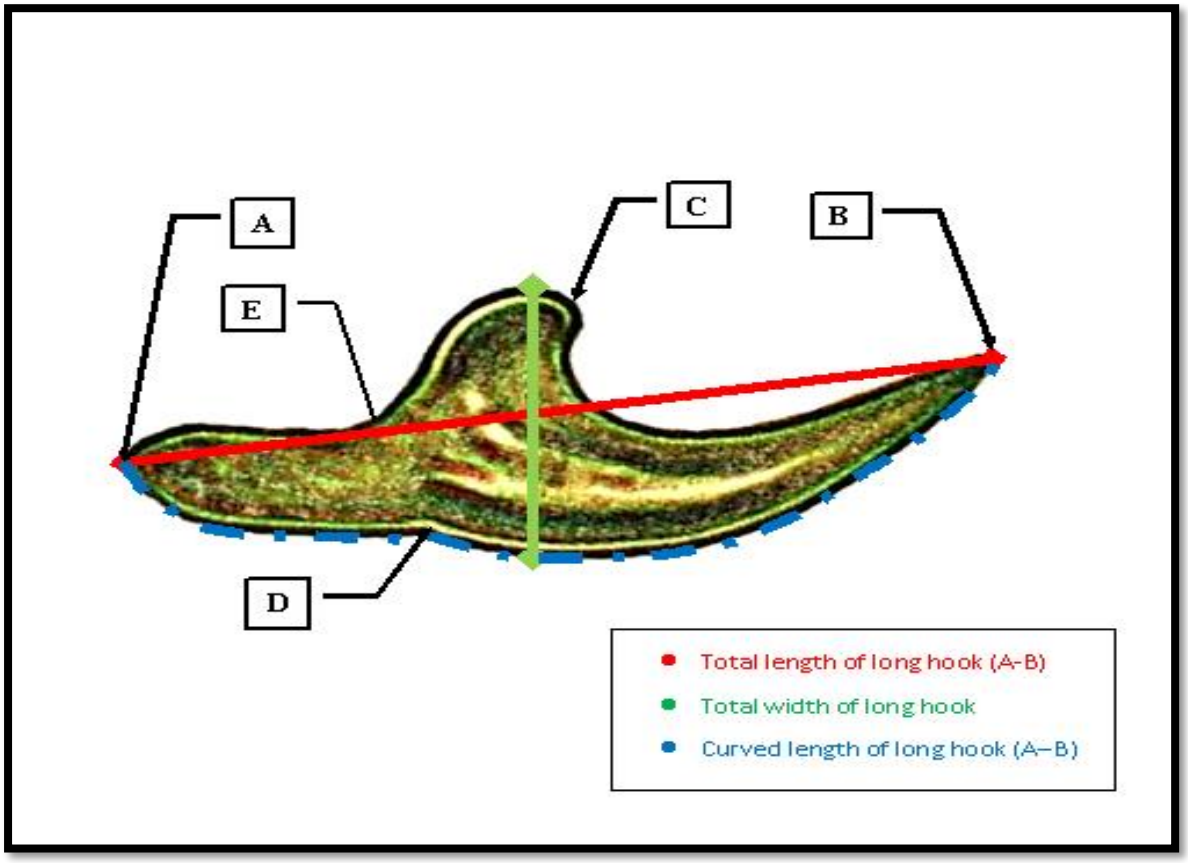

Figure (4-8): **The long hook parameters**, A-B: total length (TLLH) red line, total width (TWLH) green line, A~B: curved length (CLLH) blue line.

Table (4-6): The TL, TW and CL parameters for long hooks of *E. granulosus* in varied hosts.

	Long hooks Parameters (µm)			
Host	**N**	Total length TL	Total width TW	Curved length CL
		Mean ± S. D	Mean ± S. D	Mean ± S. D
sheep	103	25.56 ± 1.30 **c**	9.34 ± 0.79 **c**	28.94 ± 1.33 **c**
buffalo	8	24.51 ± 0.58 **b**	9.15 ± 0.48 **c**	27.50 ± 0.55 **b**
Cow	48	23.62 ± 1.43 **b**	8.48 ± 0.94 **b**	26.54 ± 1.69 **b**
Human	22	22.19 ± 1.11 **a**	7.82 ± 1.03 **a**	25.17 ± 1.30 **a**
dog	8	33.58 ± 1.54 **d**	16.11 ± 1.24 **d**	39.42 ± 2.06 **d**
F	129.473		147.114	167.608
P	0.0022E-49		0.0033E-53	0.0032E-57

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:2:2:2:4: Before Blade Length of Long Hook (BBLLH):

The results showed (Table, 4-7) that The BBLLH of *E. granulosus* is 13.08, 13.10, 11.83, 10.50 and 20.98μm for Protoscolices collected from sheep, buffalo, cow, human and adult worm of dog respectively.

The adult worm had the highest mean of BBLLH and the lowest mean of BBLLH was obtained from **human**.

The statistical analysis by using ANOVA Table / F-test of results showed **high significant** differences between the BBLLH obtained from varied hosts ($F=187.457$, $p<0.001$).

The Duncan test showed that the adult worm BBLLH mean was significantly increased with larval BBLLH means, the mean of BBLLH collected from human hydatid cyst was **significantly** decreased from that of sheep, buffalo and cow, also the mean BBLLH of cow hydatid cyst was significantly decreased from BBLLH means of sheep, buffalo and human Protoscolices, were **no significant** differences between BBLLH means of sheep HC and buffalo (Table, 4-7).

4:2:2:2:5: The blade length of long hook (BLLH):

The results showed (Table, 4-7) that The BLLH of *E. granulosus* is 12.47, 11.36, 11.74, 11.67 and 12.66μm for Protoscolices collected from sheep, buffalo, cow, human and adult worm obtained from dog respectively.

The highest mean of BLLH for adult worm and the lowest mean of (BLLH) was received from **human** HC.

The statistical analysis (F-test) of results showed **high significant** differences between the means of BLLH obtained from varied hosts ($F=7.811$, $p<0.001$).

Duncan test (Table, 4-7) showed there was **significant** increased between the mean of BLLH of adult worm and the mean of BLLH obtained from

intermediate hosts Protoscolices except of sheep, the mean of BLLH of sheep showed significant increased with that of buffalo, cow and human, were **no significant** differences between the means of BLLH of buffalo, cow and human.

4:2:2:2:6: The Handle Length of long hook (HLLH):

The results showed (Table, 4-7) that The HLLH of *E. granulosus* is 7.25, 6.63, 6.50, 5.64 and 12.10μm for Protoscolices collected from sheep, buffalo, cow, human and adult worm from dog respectively.

The highest mean of HLLH for adult worm and the lowest mean of HLLH was received from **human** HC.

The statistical analysis (F-test) of results showed **high significant** differences between the means of HLLH obtained from varied hosts ($F=65.603$, $p<0.001$).

Duncan test (Table, 4-7) showed there are **significant** increased between the mean of HLLH obtained from adult worm with that of Protoscolices collected from intermediated hosts, also there are **significant** decrased between the mean of HLLH obtained from human with that of sheep, buffalo and cow, were **no significant** differences between the HLLH of sheep, buffalo and cow.

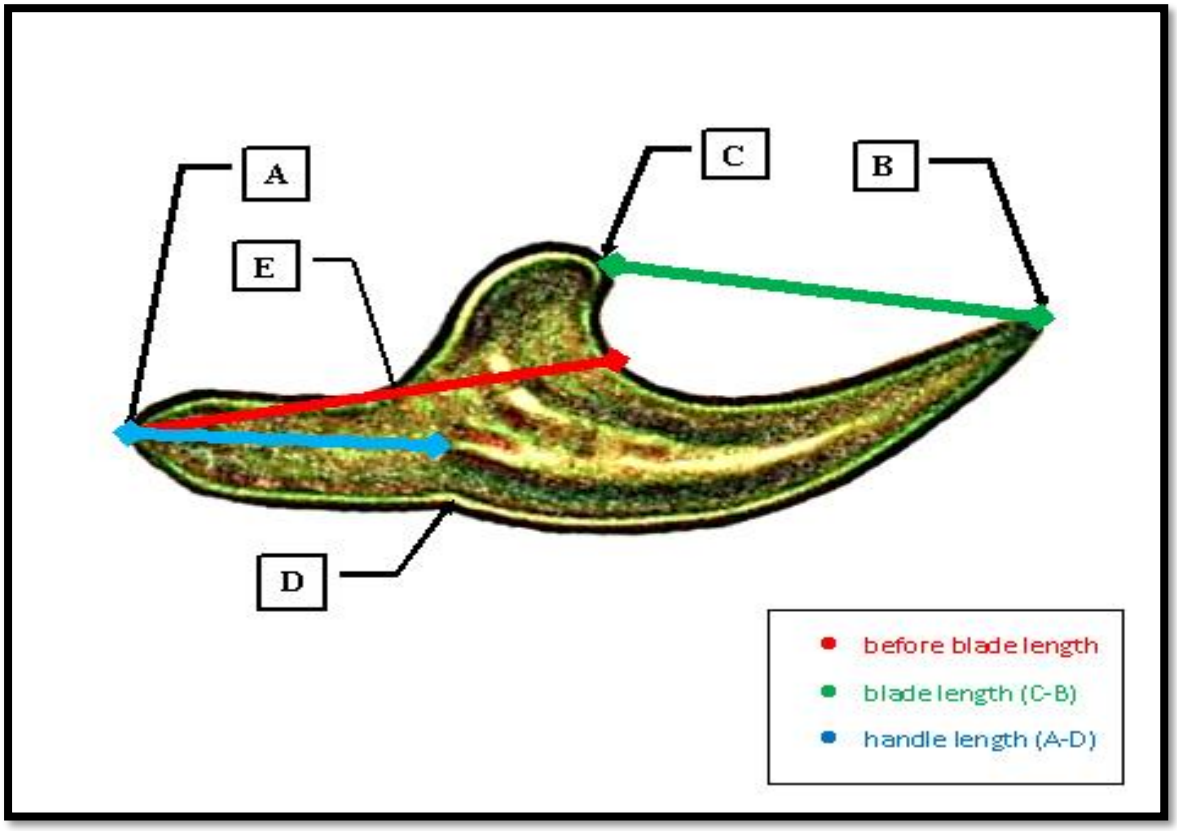

Figure (4-9): The long hook parameters, A-C: before blade length (BBLLH) red line, C-B: blade length (BLLH) green line, handle length (HLLH) blue line.

Table (4-7): The BBL, BL and HL parameters for long hooks of *E. granulosus* in varied hosts.

	Long hooks Parameters(µm)			
Host	N	before blade length BBL	blade length BL	handle length HL
		Mean ± S. D	Mean ± S. D	Mean ± S. D
sheep	103	13.08 ± 0.92 ^{c}	12.47 ± 0.98 ^{b}	7.25 ± 1.04 ^{b}
buffalo	8	13.10 ± 0.60 ^{c}	11.36 ± 0.59 ^{a}	6.63 ± 0.59 ^{b}
Cow	48	11.83 ± 1.12 ^{b}	11.74 ± 1.12 ^{a}	6.50 ± 0.99 ^{b}
Human	22	10.50 ± 0.95 ^{a}	11.67 ± 0.88 ^{a}	5.64 ± 0.76 ^{a}
dog	8	20.98 ± 0.89 ^{d}	12.66 ± 0.72 ^{b}	12.10 ± 1.39 ^{c}
F	187.457		7.811	65.603
p	0.0093E-61		0.008E-3	0.0021E-31

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:2:2:2:7: Angle α of Large Hook (AαLH):

The results showed (Table,4-8) that The measure of (AαLH) of *E. granulosus* is 149.75º, 146.83º, 151.15º, 151.62º and 124.40º for Protoscolices collected from sheep, buffalo, cow, human and adult worm obtained from dog respectively.

The adult worm had the lowest mean of AαLH and the highest mean of AαLH was received from **human** HC.

The statistical analysis by using ANOVA Table / (F-test) of results showed **high significant** differences between the AαLH obtained from varied hosts ($F=40.179$, $p<0.001$).

Duncan test (Table,4-8) showed that the mean of AαLH obtained from adult worm was significantly decreased with the means of AαLH of Protoscolices collected from intermediated hosts, also the AαLH obtained from buffalo was significantly decreased from the means of AαLH that collected from cow, human and not significantly decreased with sheep, were **no significant** differences between AαLH of sheep HC, buffalo and **human HC**.

4:2:2:2:8: Angle β of Large hook (AβLH):

The results showed (Table,4-8) that The measure of AβLH of *E. granulosus* are 38.14º, 34.60º, 39.00º, 38.77º and 39.05º for Protoscolices collected from sheep, buffalo, cow, human and adult worm obtained from dog respectively.

The highest mean of AβLH for **adult worm** and the lowest mean of AβLH was received from **buffalo** HC.

The statistical analysis (F-test) of results showed **no significant** differences between the AβLH obtained from varied hosts ($F=1.205$, $p>0.05$).

4:2:2:2:9: Angle γ of Large hook (AγLH):

The results showed (Table, 4-8) that The measure of AγLH of *E. granulosus* is 156.05º, 161.13º, 156.34º, 158.44º and 158.65º for Protoscolices collected from sheep, buffalo, cow, human and adult worm obtained from dog respectively.

The highest mean of AγLH was received from **buffalo** and the lowest mean of AγLH was received from **sheep** HC.

The statistical analysis (F-test) of results showed **no significant** differences between the AγLH obtained from varied hosts ($F=0.881$, $p>0.05$).

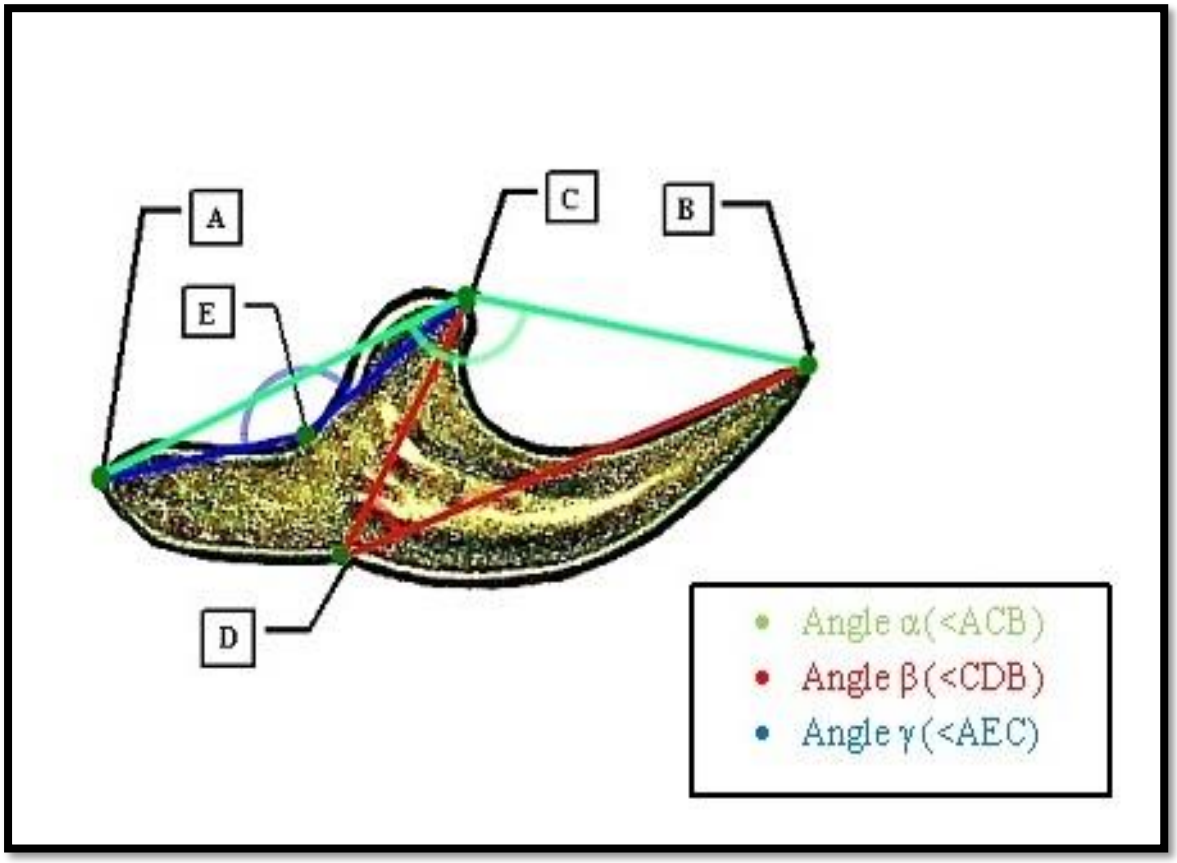

Figure (4-10): The long hook parameters, <ACB: Angle α (AαLH) green line, <CDB: Angle β (AβLH) red line, <AEC: Angle γ (AγLH) blue line.

Table (4-8): The Angle α, Angle β and Angle γ parameters for long hooks of *E. granulosus* in varied hosts.

	Long hooks Parameters (º)					
Host	Angle α (º) Aα		Angle β (º) Aβ		Angle γ (º) Aγ	
	N	Mean ± Std. D	N	Mean ± Std. D	N	Mean ± Std. D
sheep	103	149.75 ± 5.07 ^{bc}	99	38.14 ± 5.15^{a}	103	156.05 ± 7.96^{a}
buffalo	8	146.83 ± 4.45 ^{b}	8	34.60 ± 4.72^{a}	8	161.13 ± 5.80^{a}
Cow	48	151.15 ± 6.38 ^{c}	48	39.00 ± 6.48^{a}	48	156.34 ± 9.38^{a}
Human	22	151.62 ± 7.47 ^{c}	22	38.77 ± 5.24^{a}	22	158.44± 11.71^{a}
dog	8	124.40 ± 5.60 ^{a}	8	39.05 ± 3.72	8	158.65± 17.37^{a}
F	40.179		1.205		0.881	
p	0.0036E-21		0.31		0.47	

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:2:2:3: The Morphological characterization for the Short hooks parameters According to host.

4:2:2:3:1: The Total Length of Short Hook (TLSH):

The results showed (Table,4-9) that The TLSH of *E. granulosus* is 21.85, 20.04, 20.38, 19.69 and 21.24µm for Protoscolices collected from sheep, buffalo, cow, human and adult worm from dog respectively.

The sheep had the highest mean of TLSH and the lowest mean of TLSH was obtained from **human** HC.

The statistical analysis by using ANOVA Table / F-test of results showed **high significant** differences between the TLSH obtained from varied hosts ($F=11.216$, $p<0.001$).

The Duncan test showed that the mean of TLSH collected from sheep was **significantly** increased from TLSH obtained from buffalo, cow, human and not significantly increased with TLSH of adult worms, also human TLSH was significantly decreased from sheep and dog, where **no significant** differences between TLSH from buffalo, cow and human, also the different between buffalo, cow and dog are not significant (Table, 4-9).

4:2:2:3:2: The Total Width of Short Hook (TWSH):

The results showed (Table,4-9) that The TWSH of *E. granulosus* is 7.86, 7.23, 7.07, 6.90 and 13.45µm for Protoscolices collected from sheep, buffalo, cow, human and adult worms obtained from dog respectively.

The adult worm had the highest mean of TWSH and the lowest mean of TWSH was recorded from **human** HC.

The statistical analysis (F-test) of results showed **high significant** differences between the TWSH obtained from varied hosts ($F=114.151$, $P<0.001$).

The Duncan test showed that the mean of TWSH of adult worm was increased significantly with means of TWSH of larval stage collected from intermediated hosts, in other hand the results showed **significant** increased for the mean of TWSH obtained from sheep with buffalo, cow and human TWSH means, where **no significant** differences between TWSH of buffalo, cow and human (Table,4-9).

4:2:2:3:3: The Curved Length of Short Hook (CLSH):

The results showed (Table,4-9) that The CLSH of *E. granulosus* is 24.14, 21.91, 22.14, 21.95 and 24.22μm for larval stage Protoscolices collected from sheep, buffalo, cow, human and adult worms collected from dog respectively.

The highest mean of CLSH was for adult worm and the lowest mean of CLSH was recorded in **buffalo** HC.

The statistical analysis (F-test) of results showed **high significant** differences between the CLSH obtained from varied hosts ($F=16.710$, $P<0.001$).

The Duncan test (Table,4-9) showed that the adult worm mean of CLSH was increased **significant**ly from means of CLSH obtained from buffalo, cow and human and not significantly increased with that of sheep, the mean of CLSH of sheep was significantly increased with that of buffalo, cow and human, were **no significant** differences between CLSH of buffalo, cow and human.

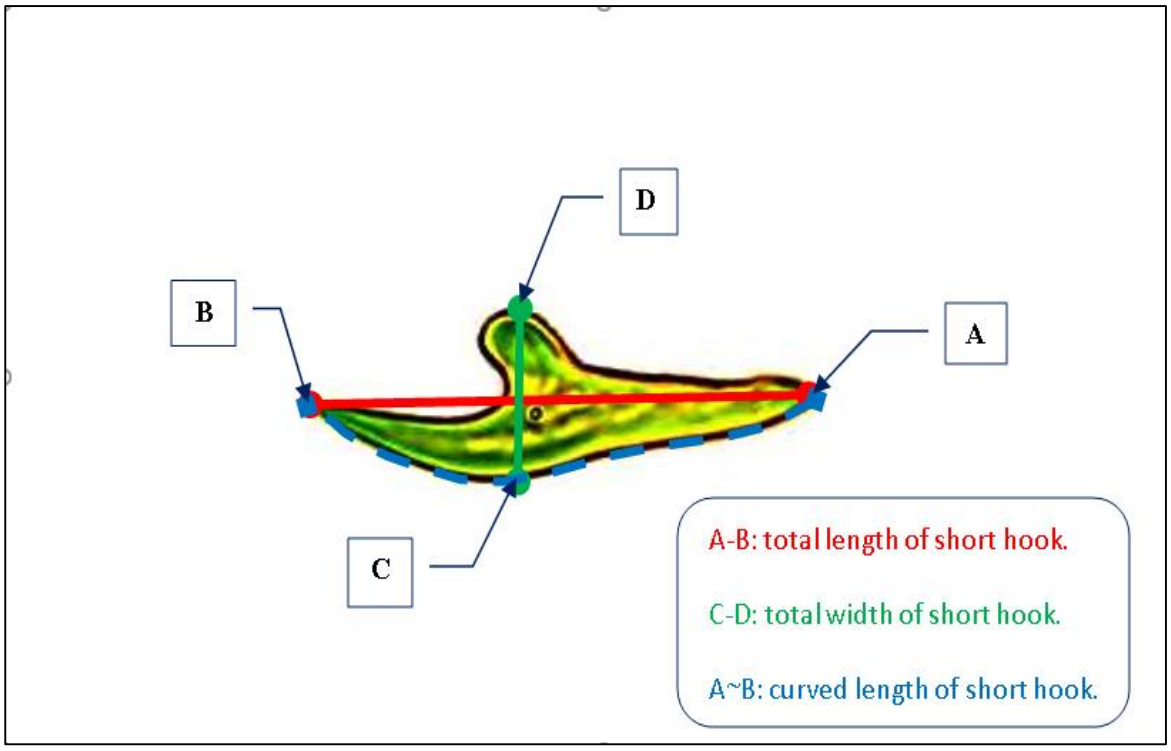

Figure (4-11): The short hook parameters, A-B: total length (TLSH) red line, C-D: total width (TWSH) green line, A~B: curved length (CLSH) blue line.

Table 4-9: The TL, TW and CL parameters for short hooks of *E. granulosus* in varied hosts.

		Short hooks Parameters (µm)		
Host	N	Total length TL	Total width TW	Curved length CL
		Mean ± S. D	Mean ± S. D	Mean ± S. D
sheep	84	21.85 ± 1.73 ^{c}	7.86 ± 0.66 ^{b}	24.14 ± 1.49 ^{b}
buffalo	6	20.04 ± 1.25 ^{ab}	7.23 ± 0.69 ^{a}	21.91 ± 1.18 ^{a}
Cow	35	20.38 ± 1.37 ^{ab}	7.07 ± 0.68 ^{a}	22.14 ± 1.61 ^{a}
Human	22	19.69 ± 1.44 ^{a}	6.90 ± 0.92 ^{a}	21.95 ± 1.56 ^{a}
dog	7	21.24 ± 1.88 ^{bc}	13.45 ± 1.44 ^{c}	24.22 ± 2.33 ^{b}
F		11.216	114.151	16.710
P		0.0055E-5	0.0024E-41	0.0024E-8

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:2:2:3:4: Before Blade Length of Short Hook (BBLSH):

The results showed (Table, 4-10) that The BBLSH of *E. granulosus* is 13.42, 12.30, 12.22, 11.03 and 12.65μm for Protoscolices obtained from sheep, buffalo, cow, human and adult worm of dog respectively.

The highest mean of BBLSH was from sheep and the lowest mean of (BBLSH) was that collected from **human** HC.

The statistical analysis by using ANOVA Table / (F-test) of results showed **high significant** differences between the BBLSH obtained from varied hosts ($F=22.098$, $P<0.001$).

The Duncan test showed that mean of BBLSH of human was **significantly** decreased with BBLSH obtained from sheep, buffalo, cow and dog, also there are significant increased between BBLSH of sheep with that of buffalo, cow, human and not significantly increased with BBLSH of adult worms, were **no significant** differences between (BBLSH) collected from buffalo, cow and dog (Table,4-10).

4:2:2:3:5: The Blade Length of Short Hook (BLSH):

The results showed (Table,4-10) that The BLSH of *E. granulosus* is 8.48, 7.68, 8.13, 8.60 and 8.57μm for Protoscolices collected from hydatid cyst of sheep, buffalo, cow, human and adult worms from dog respectively.

The highest mean of BLSH was for HC of **human** and the lowest mean of BLSH was received from **buffalo** HC.

The statistical analysis (F-test) of results showed **significant** differences between the BLSH obtained from varied hosts ($F=2.668$, $p<0.05$).

Duncan test showed that BLSH of buffalo was decreased significantly with BLSH of sheep, human and dog and not significantly decreased with that of

cow, were **No significant** differences between BLSH of sheep, cow, human and dog (Table, 4-10).

4:2:2:3:6: The Handle Length of Short Hook (HLSH):

The results showed (Table, 4-10) that The HLSH of *E. granulosus* is 8.40, 6.95, 7.12, 6.48 and 6.18μm for Protoscolices from sheep, buffalo, cow, human and adult worms from dog respectively.

The adult worm had the lowest mean of (HLSH) and the highest mean of HLSH was received from sheep HC.

The statistical analysis (F-test) of results showed high significant differences between the HLSH obtained from varied hosts ($F=24.063$, $p <0.001$).

Duncan test showed that mean of HLSH for sheep was significantly increased with that of other hosts, adult HLSH mean significantly decreased from HLSH means of cow and sheep, also cow HLSH mean was significantly decreased and increased with that of sheep and dog respectively, were no significant differences between HLSH of buffalo, cow and human, also no significant differences between HLSH of buffalo, human and dog (Table,4-10).

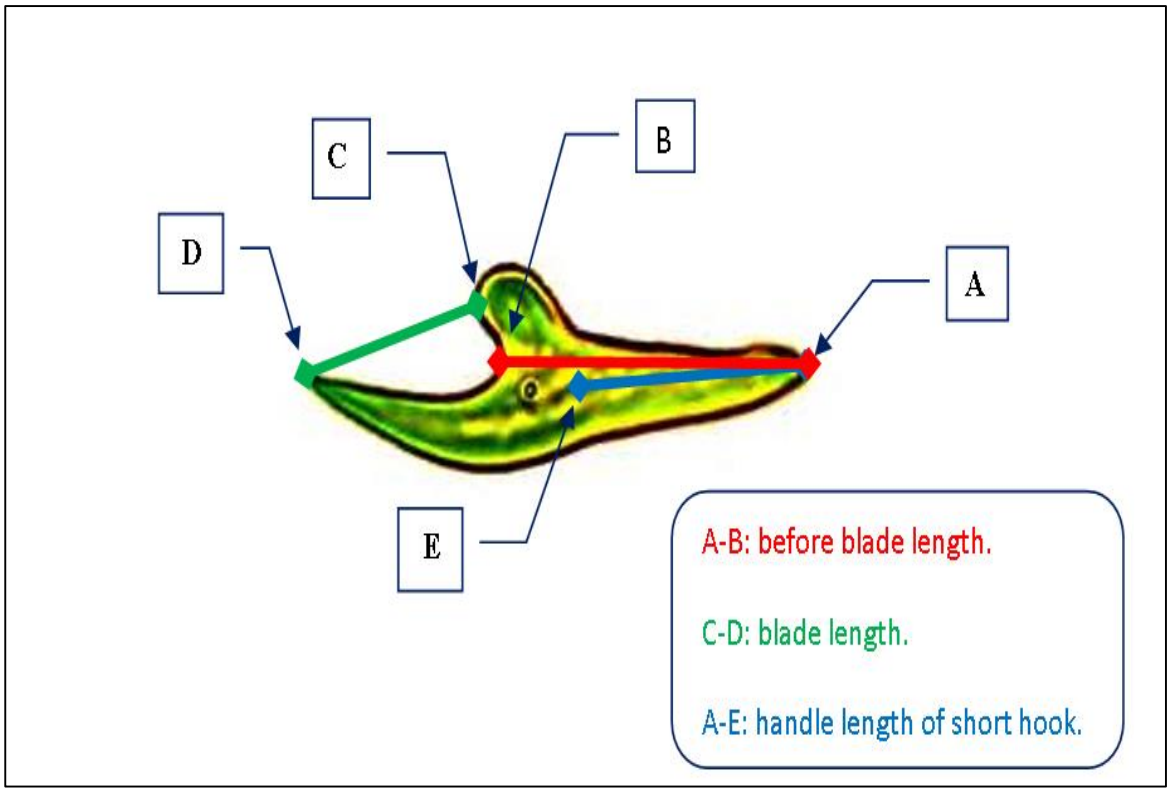

Figure (4-12): The short hook parameters, A-C: before blade length (BBLSH) red line, C-D: blade length (BLSH) green line, A-E: handle length (HLLH) blue line.

Table 4-10: The BBL, BL and HL parameters for short hooks of *E. granulosus* in varied hosts.

	Short hooks Parameters (µm)			
Host	N	before blade length	blade length	handle length
		Mean ± S. D	Mean ± S. D	Mean ± S. D
sheep	84	13.42 ± 1.04 ^{c}	8.48 ± 0.85 ^{b}	8.40 ± 0.95 ^{c}
buffalo	6	12.30 ± 0.69 ^{b}	7.68 ± 0.61 ^{a}	6.95 ± 0.57 ^{ab}
Cow	35	12.22 ± 1.00 ^{b}	8.13 ± 0.72 ^{ab}	7.12 ± 1.21 ^{b}
Human	22	11.03 ± 1.54 ^{a}	8.60 ± 0.90 ^{b}	6.48 ± 1.10 ^{ab}
dog	7	12.65 ± 1.52 ^{bc}	8.57 ± 0.65 ^{b}	6.18 ± 1.29 ^{a}
F	22.098		2.668	24.063
P	0.0024E-11		0.0345	0.0022E-12

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:2:2:3:7: Angle α of Short Hook (AαSH):

The results showed (Table, 4-11) that The measure of AαSH of *E. granulosus* is 144.70º, 140.40º, 148.21º, 147.67º and 99.12º for Protoscolices hooks obtained from sheep, buffalo, cow, human and adult worm hooks collected from dog respectively.

The adult worm had the lowest mean of AαSH and the highest mean of AαSH was recorded in **cow** hydatid cyst.

The statistical analysis by using ANOVA Table / F-test of results showed **high significant** differences between the AαSH obtained from varied hosts ($F=80.646$, $p<0.001$).

Duncan test showed there are **significant** decreased in the mean of AαSH obtained from dog with AαSH means of intermediated hosts, also there are **significant** decreased in the mean of AαSH obtained from buffalo with that of cow and human, were **no significant** differences between AαSH of sheep, cow and human, also **no significant** differences between (AαSH) means of sheep and buffalo (Table, 4-11).

4:2:2:3:8: Angle β of Short Hook (AβSH):

The results showed (Table,4-11) that The measure of AβSH of *E. granulosus* is 37.06º, 37.31º, 34.10º, 41.69º and 54.25º for Protoscolices of sheep, buffalo, cow, human and adult worms from dog respectively.

The adult worm had the highest mean of AβSH and the lowest mean of AβSH was received from **cow** HC.

The statistical analysis (F-test) of results showed **high significant** differences between the AβSH obtained from varied hosts ($F=25.869$, $p<0.001$).

Duncan test showed there are **significant** increased for the mean of AβSH obtained from dog with that of other intermediated hosts, also there are

significant increased between the mean of AβSH obtained from human with that of sheep, buffalo and cow, were **no significant** differences between AβSH of sheep, buffalo and cow (Table,4-11).

4:2:2:3:9: Angle γ of Short Hook (AγSH):

The results showed (Table, 4-11) that The measure of (AγSH) of *E. granulosus* is 168.88º, 167.55º, 174.55º, 174.57º and 145.74º for Protoscolices that collected from sheep, buffalo, cow, human and adult worm from dog respectively.

The lowest mean of AγSH was for adult worm and the highest mean of AγSH was received from **human** HC.

The statistical analysis (F-test) of results showed **high significant** differences between the AγSH obtained from varied hosts ($F=25.058$, $p<0.001$).

Duncan test showed **significant** decreased in the mean of AγSH obtained from dog with that of other intermediated hosts, also the mean of AγSH of buffalo was **significant**ly decreased from that of cow, human and not significantly decreased sheep, were **no significant** differences between AγSH means of sheep and cow and human (Table, 4-11).

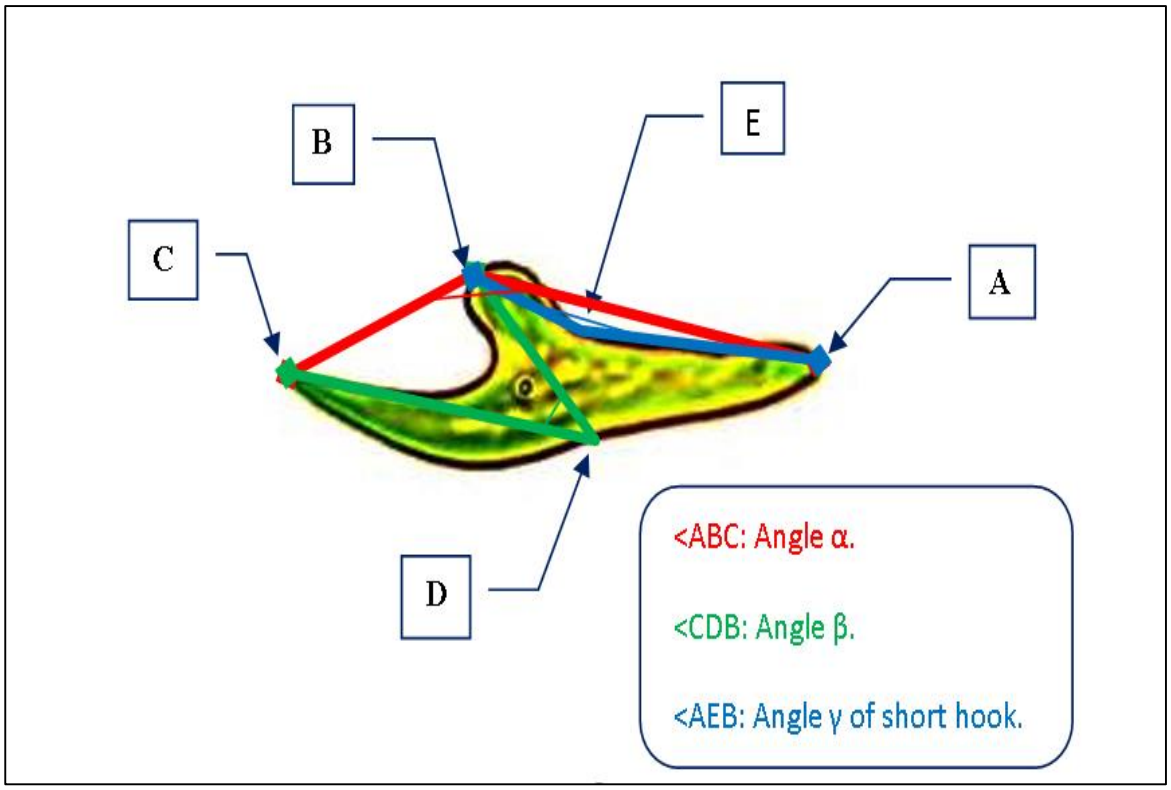

Figure (4-13): The short hook parameters, <ABC: Angle α (AαSH) red line, <CDB: Angle β (AβSH) green line, <AEB: Angle γ (AγSH) blue line.

Table (4-11) The Angle α, Angle β and Angle γ parameters for short hooks of *E. granulosus* in varied hosts.

	Short hooks Parameters (º)					
Host	Angle α		Angle β		Angle γ	
	N	Mean ± S. D	N	Mean ± S. D	N	Mean ± S. D
sheep	84	144.70±6.67^{bc}	83	37.06 ± 4.08 ^{a}	84	168.88 ± 6.38^{bc}
buffalo	6	140.40 ±5.42^{b}	6	37.31 ± 2.99 ^{a}	6	167.55 ± 4.50 ^{b}
Cow	35	148.21 ±5.95^{c}	35	34.10 ± 5.71 ^{a}	34	174.55 ± 6.61 ^{c}
Human	22	147.67 ±8.72^{c}	22	41.69 ± 5.50 ^{b}	22	174.57 ± 8.25 ^{c}
dog	7	99.12 ± 7.44 ^{a}	7	54.25 ±11.40^{c}	7	145.74 ± 16.56^{a}
F	80.646		25.869		25.058	
P	0.0027E-33		0.0028E-13		0.0074E-13	

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:3: The Molecular Study of *E. granulosus in* Human and Animals:

4:3:1: The DNA extraction

The DNA extraction was carried out on (32) *E. granulosus* specimen collected from (13) sheep, (5) buffalo, (8) cow, (4) human as intermediated hosts and (2) dog as definitive host.

4:3:2: The Polymer Chain Reaction (PCR):

The extracted DNA was used for improvement the identification of *E. granulosus* by PCR amplification for two mitochondrial genes *Cox1* and *Nad1*, the PCR products was view by gel electrophoresis 29 of *Cox1* gene (Figure, 4-14) and 18 of *Nad1* (Figure, 4-15) was successfully collected for later gene sequencing.

4:3:3: DNA Sequencing for *Cox1* and *Nad1* gens

The gene sequencing of 29 *Cox1* and 18 *Nad1* PCR product was showed that 22/29 (75.86%) of samples are comparable with the NCBI reference gene of Genbank (Table, 4-12) and this results demonstrate that isolated specimens and extracted DNA are belong to *E. granulosus*.

4:3:4: Genotyping and genetic analysis

By using the NCBI and Genbank data bases and some molecular programs (MEGA, DNasp, NETWORK) we detect sheep strain (G1) of *E. granulosus* in 16/22(72.72%) samples (Table, 4-12) (Figure, 4-17), also 2/22(9.09%) samples (sheep and buffalo origin) was matched with (G1BC) genotype (Table, 4-12) which is variant from G1according to **EURLP** method that depending on five codons (16, 18, 20,85,87codon) variability to determine the ten genotypes of *E. granulosus* the sheep s.14 and buffalo s.46 samples coding to Valine (GTG) amino acid instead of Alanine (GCG) of G1in codon 20 (Figure, 4-16), in 3/22(13.63%) of the samples the buffalo strain (G3) of *E. granulosus* were recorded (Table, 4-12) (Figure, 4-18,19,20) and only 1/22(4.54%) of sample was matched with strain (SB041) (Table, 4-12).

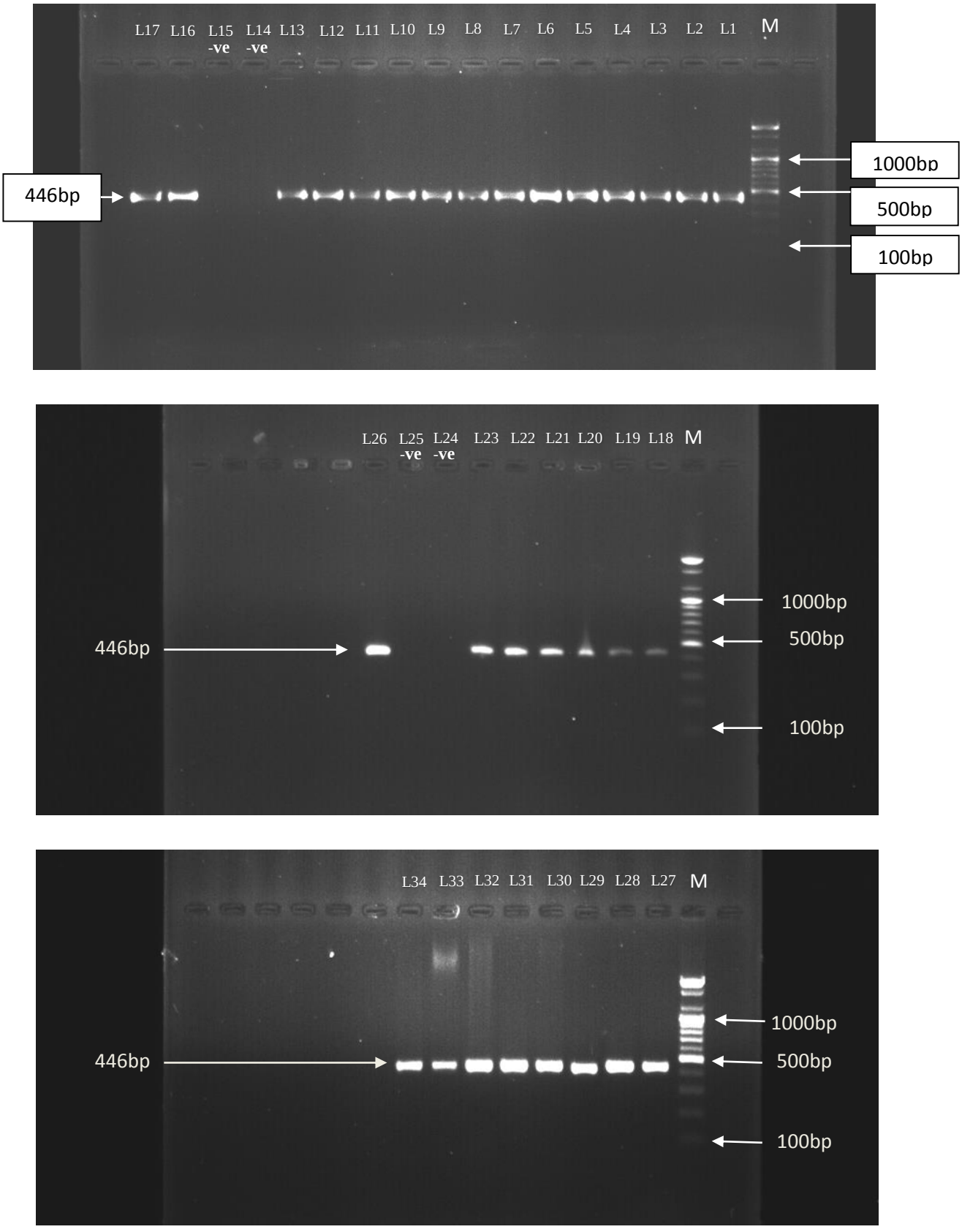

Figure (4-14): Electrophoreses pattern of PCR product for *Cox1* gene 446bp, 1% Agarose , 65mV, 60min, M: DNA marker ladder 100bp.sheep: L4-L7, L19-L23, L30-L34. buffalo: L8-L10,L33. cow: L1-L3, L18, L27-L29. human: L11-L15, dog: L16, L17, L26.

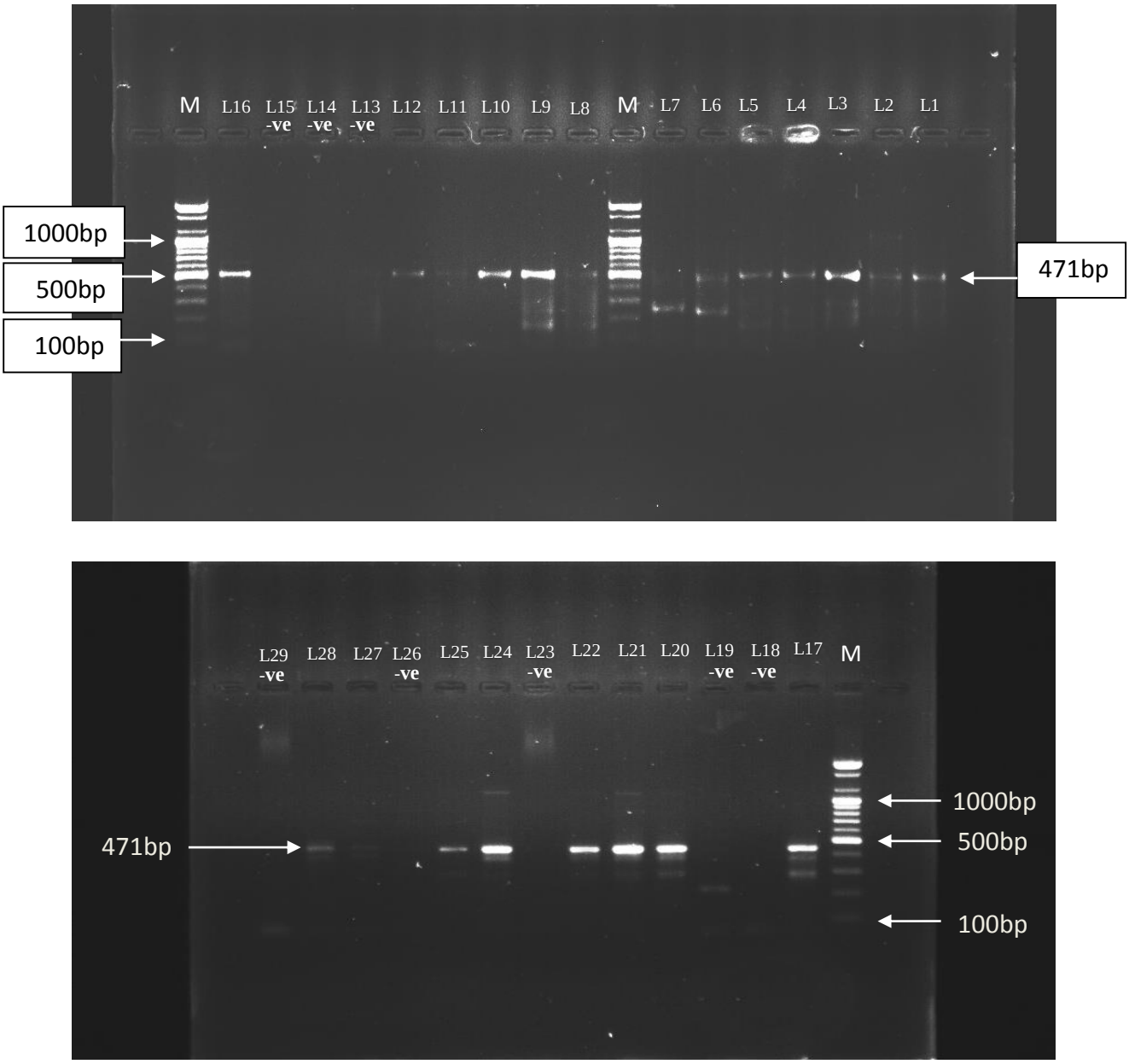

Figure (4-15): Electrophoreses pattern of PCR product for *Nad1* gene 471bp,1% Agarose , 65mV, 60min, sheep samples: L4, L5, L6,L7,L17,L19,L20,L21,L22. Buffalo: L8,L9,L10,L18,L23,L28. cow: L1,L2,L3 human: L11,L12,L13,L14,L15,L24,L25,L26. Dog: L16,L27. M: DNA marker ladder 100bp.

Table (4-12): The sequence of PCR products of *Cox1*gene identification with NCBI databases and Genbank reference genes.

Sample	host	genotype	Ident %	Reference Accession in Genbank
S.8	Sheep	G1	94%	KM014634.1 - MH025946.1
S.9	Sheep	G3*	96%	GU984809.1*
		G1	96%	JF513064.1
S.10	Buffalo	G1	92%	KJ628331.1 - KT254125.1
		G1*****	96%	(KT200217.1 - KT200212.1)*****
S.11	Buffalo	G1	100%	MH025946.1 - MG808349.1 - MG808348.1
S.12	Cow	G1	100%	MH025946.1 - MG808349.1 - MG808348.1
S.14	sheep	G1	100%	MG808347.1 - MG808322.1 - MH050611.1
		G1BC***		
S.16	Cow	G1	100%	MG672210.1 - KX039952.1
S.17	sheep	G1	99%	MH025946.1 - MH050617.1
		SB041**		HF947592.1**
S.19	sheep	G1	96%	MK214421.1 - MH025947.1 - MH025946.1
S.22	sheep	G1	99%	MH025946.1 - MG808348.1
		G3*	99%	GU984809*
S.25	sheep	G1	100%	MH025946.1 - MG808349.1 - MG808348.1
S.26	sheep	G1	100%	MH025946.1 - MG808349.1 - MG808348.1
S.35	sheep	G1	100%	MH025946.1 - MG808349.1 - MG808348.1
S.36	sheep	G1	100%	MH025946.1 - MH050615.1 - MG672293.1
S.37	cow	G1	77%	MG672157.1 - MG674403.1 - MG548790.1
S.46	Buffalo	G1	100%	MH050611.1 - MG808347.1 - MG672290.1
		G1BC***		
S.51	Buffalo	G1	100%	AB688592.1 - MG792555.1 - KX269862.1
S.59	Cow	G1	100%	MG792563.1 - MG672286.1
		G3****	99%	M84663.1****
S.60L	Human	G1	100%	MH025946.1 - MH050608.1 - MG808349.1
S.60G	Human	G1	100%	MH025946.1 - MH050608.1 - MG808349.1
S.61	Human	G1	84%	DQ104330.1 - GQ502208.1
S.70	Dog	G1	98%	MH025946.1 - MG808349.1 - MH050608.1

note: The COX1 gene sequences are used to this identification.

* Genotype: G3, cytochrome oxidase subunit I-like gene recorded by (Simsek *et al.*, 2010).
** strain: SB041 according to (Beato *et al.*, 2013)
*** Genotype: G1BC according to European Union Reference Laboratory for Parasites (EURLP). http://www.iss.it/binary/crlp/cont/MI_05_METHOD_WEB_SITE.pdf .
**** Genotype: G3 according to (Bowles *et al.*, 1992; Busi *et al*., 2007; Hammad *et al*., 2018).
***** strain: G1 by NADH dehydrogenase subunit 1 gene sequence.

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AB033407_G1  LPGFGIISHICLSISANFDAFGFYGLLFAMFSIVCLGSRVWGHHMFTVGLDVKTAVFFSSVTMIIGVPTGIKVFTWLYML
Sheep_s.8    ----.............................G...T.L..P..C.....A....C----------------------
Sheep_s.9    -A.-..-.....................................N.....................NK.........G....I..
Sheep_s.14   ----..............V.....................................................................
Sheep_s.17   ---L..............................................................................
Sheep_s.22   ----..............................................................................
Sheep_s.25   ----..............................................................................
Sheep_s.26   ----..............................................................................
Sheep_s.35   ----..............................................................................
Sheep_s.36   ----..............................................................................
Buffalo_s.10 I.R-...-.....NF.S.............K...M....M........D.......D.YI.L.........L....GI...
Buffalo_s.11 ----..............................................................................
Buffalo_s.46 ----..............V...............................................................
Buffalo_s.51 ----..............................................................................
Caw_s.12     ----..............................................................................
Caw_s.16     ----.........V....................................................................
Caw_s.59     ----..............................................................................
Human_s.60G  ----..............................................................................
Human_s.60L  ----..............................................................................
Human_s.61   ----..-.......G.....................................TF.LL.L...M.LRGLPGYILW
Dog_s.70     AL.....................----------------------------------------------

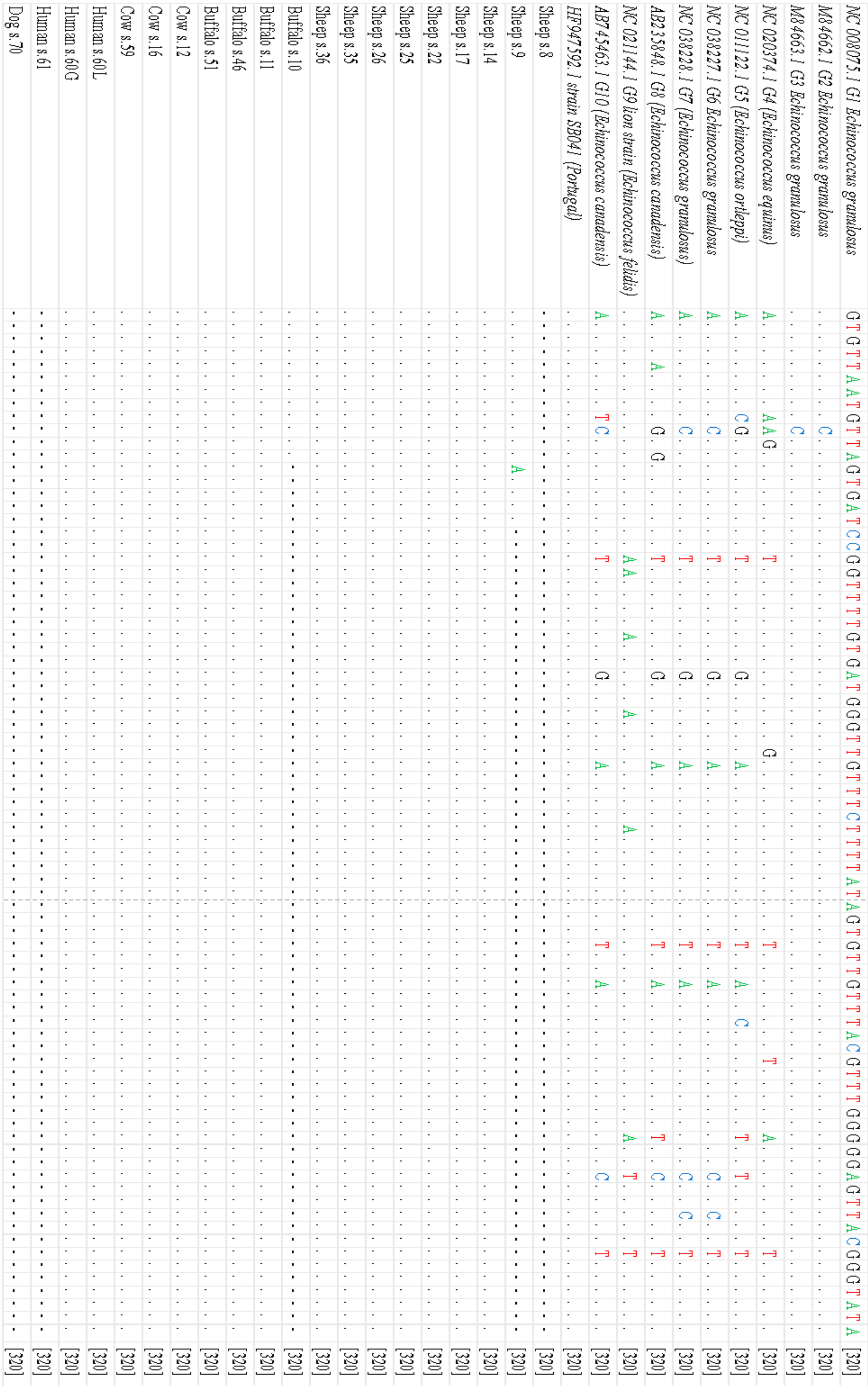
[                                 1111111111111111111111111111111]
[           8888888889999999990000000000111111111122222222223]
[           1234567890123456789012345678901234567890123456789]
AB033407_G1  LNSSVNVSDPVL*WVVSFIVLFTFGGVTGIVLSACVL-------------
Sheep_s.8    ---------------------------------------------
Sheep_s.9    .......N.---------------------------------------------
Sheep_s.14   .....................................DNILHDTWFVVAH
Sheep_s.17   .....................................DNILHDTWFVVAH
Sheep_s.22   .....................................NNILHDTWFVVAH
Sheep_s.25   .....................................DNILHDTWFVVAH
Sheep_s.26   .....................................DNILHDTWFVVAH
Sheep_s.35   .....................................DNILHDTWFVVAH
Sheep_s.36   .....................................DNILHDTWFVVAH
Buffalo_s.10 .......---------------------------------------
Buffalo_s.11 .....................................DNILHDTWFVVAH
Buffalo_s.46 .....................................DNILHDTWFVVAH
Buffalo_s.51 .....................................DNILHDTWFVVAH
Caw_s.12     .....................................DNILHDTWFVVAH
Caw_s.16     .....................................DNILHDTWFVVAH
Caw_s.59     .....................................DNILHDTWFVVAH
Human_s.60G  .....................................DNILHDTWFVVAH
Human_s.60L  .....................................DNILHDTWFVVAH
Human_s.61   *.PR.M.M...WEG..PLLGWLPL.E.P.LF.PVGG*NKILQKPGLGGVH
Dog_s.70     ---------------------------------------------
;
end;
```

Figure (4-16): The alignment of translated mitochondrial cox1 gene according to the EURLP method to identify the *E. granulosus* genotypes depending on the 5 codons (16, 18, 20, 85, 87codon) the codon surrounded by red square show that S.14 and S.46 are translated differently to Valine and that make them recorded as G1BC genotype were the other sequence are considered as G1.

NC 008075.1 G1 Echinococcus granulosus	TTGGTATAATTAGTCATATTTGTTTGAGTATTAGTGCTAATTTTGATGCGTTTGGGTTCTATGGGTTGTTGTTTGCTATG	[80]
M84662.1 G2 Echinococcus granulosus	. . . . . T . . . . . T	[80]
M84663.1 G3 Echinococcus granulosus	. . . . . T	[80]
NC 020374.1 G4 (Echinococcus equinus)	. . . . . A. . G. . . . . G. . . T . . . . . T	[80]
NC 011122.1 G5 (Echinococcus ortleppi)	. . . . . G. T . . . . . C. . . . . G. . . TT . . . . . T . . . . . A	[80]
NC 038227.1 G6 Echinococcus granulosus	. . . . . G. T . . . . . G. . . . . T . . . . . G. . . TT . . . . . T	[80]
NC 038228.1 G7 (Echinococcus granulosus)	. . . . . G. T . . . . . G. . . . . T . . . . . G. . . TT . . . . . T	[80]
AB235848.1 G8 (Echinococcus canadensis)	. . . . . G. T . . . . . G. . . . . G. . . TT . . . . . T	[80]
NC 021144.1 G9 lion strain (Echinococcus felidis)	. . . . . G. . . . . A. . . . . T . . . . A	[80]
AB745463.1 G10 (Echinococcus canadensis)	. . . . . G. T . . . . . G. . . . . T . . . . . G. . . TT . . . . . T	[80]
HF947592.1 strain SB041 (Portugal)	. GT	[80]
Sheep s.8	. . . . .	[80]
Sheep s.9	. . . . . -	[80]
Sheep s.14	. . . . . T	[80]
Sheep s.17	. G.	[80]
Sheep s.22	. . . . .	[80]
Sheep s.25	. . . . .	[80]
Sheep s.26	. . . . .	[80]
Sheep s.35	. . . . .	[80]
Sheep s.36	. . . . .	[80]
Buffalo s.10	. . . . . - . . . . . A. TT . . . . GC . . . . . A.	[80]
Buffalo s.11	. . . . .	[80]
Buffalo s.46	. . . . . T	[80]
Buffalo s.51	. . . . .	[80]
Cow s.12	. . . . .	[80]
Cow s.16	. . . . . G.	[80]
Cow s.59	. . . . . T	[80]
Human s.60L	. . . . .	[80]
Human s.60G	-	[80]
Human s.61	. . . . . - . . . . . G. . . . . T	[80]
Dog s.70	. . . . .	[80]

NC 008075.1 G1 Echinococcus granulosus	TTTTCTATAGTGTTTTGGGTAGTAGGGTTTGGGGTCATCATATGTTTACTGTTGGGTTGGATGTGAAGACGGCTGTTTT	[160]
M84662.1 G2 Echinococcus granulosus	..C.....	[160]
M84663.1 G3 Echinococcus granulosus	..C.....	[160]
NC 020374.1 G4 (Echinococcus equinus)	..A..A.....	[160]
NC 011122.1 G5 (Echinococcus ortleppi)	..A.....	[160]
NC 038227.1 G6 Echinococcus granulosus	..A.....	[160]
NC 038228.1 G7 (Echinococcus granulosus)	..A.....	[160]
AB235848.1 G8 (Echinococcus canadensis)	..A.....	[160]
NC 021144.1 G9 lion strain (Echinococcus felidis)	..A.....	[160]
AB745463.1 G10 (Echinococcus canadensis)	..A.....	[160]
HF947592.1 strain SB041 (Portugal)	..C.....	[160]
Sheep s.8	.G..C..CC..C..C..G..C..C.....	[160]
Sheep s.9	..AC.....	[160]
Sheep s.14	..C.....	[160]
Sheep s.17	..C.....	[160]
Sheep s.22	..C.....	[160]
Sheep s.25	..C.....	[160]
Sheep s.26	..C.....	[160]
Sheep s.35	..C.....	[160]
Sheep s.36	..C.....	[160]
Buffalo s.10	...A.....CT.....A.....	[160]
Buffalo s.11	..C.....	[160]
Buffalo s.46	..C.....	[160]
Buffalo s.51	..C.....	[160]
Cow s.12	..C.....	[160]
Cow s.16	..C.....	[160]
Cow s.59	..C.....	[160]
Human s.60L	..C.....	[160]
Human s.60G	..C.....	[160]
Human s.61	..C.....	[160]
Dog s.70	.....	[160]

NC 008075.1 G1 Echinococcus granulosus	TTTTAGCTCTGTTACTATGATTATAGGGGTTCCTACTGGTATAAAGGTGTTTACTTGGTTATATATGTTGTTGAATTCGA	[240]
M84662.1 G2 Echinococcus granulosus	.....	[240]
M84663.1 G3 Echinococcus granulosus	.....	[240]
NC 020374.1 G4 (Echinococcus equinus)	T..... A..... T..... G..... A.....	[240]
NC 011122.1 G5 (Echinococcus ortleppi)	T..... T..... G..... A..... T.....	[240]
NC 038227.1 G6 Echinococcus granulosus	T..... T..... G..... A..... T.....	[240]
NC 038228.1 G7 (Echinococcus granulosus)	T..... T..... G..... A..... T.....	[240]
AB235848.1 G8 (Echinococcus canadensis)	T..... T. C..... A..... C..... G. C..... A..... T.....	[240]
NC 021144.1 G9 lion strain (Echinococcus felidis)	T..... T..... A. G..... A. A..... T.....	[240]
AB745463.1 G10 (Echinococcus canadensis)	T..... T..... G..... A..... T.....	[240]
HF947592.1 strain SB041 (Portugal)	.....	[240]
Sheep s.8	----------.....----------	[240]
Sheep s.9	..... A. A..... C..... G..... A T.....	[240]
Sheep s.14	.....	[240]
Sheep s.17	.....	[240]
Sheep s.22	.....	[240]
Sheep s.25	.....	[240]
Sheep s.26	.....	[240]
Sheep s.35	.....	[240]
Sheep s.36	.....	[240]
Buffalo s.10	..... T. C..... G..... T..... G. A..... A..... C.	[240]
Buffalo s.11	.....	[240]
Buffalo s.46	.....	[240]
Buffalo s.51	..... C.	[240]
Cow s.12	.....	[240]
Cow s.16	.....	[240]
Cow s.59	.....	[240]
Human s.60L	.....	[240]
Human s.60G	.....	[240]
Human s.61	.....	[240]
Dog s.70	----------.....----------	[240]


NC 008075.1 G1 Echinococcus granulosus
M84662.1 G2 Echinococcus granulosus
M84663.1 G3 Echinococcus granulosus
NC 020374.1 G4 (Echinococcus equinus)
NC 011122.1 G5 (Echinococcus ortleppi)
NC 038227.1 G6 Echinococcus granulosus
NC 038228.1 G7 (Echinococcus granulosus)
AB235848.1 G8 (Echinococcus canadensis)
NC 021144.1 G9 lion strain (Echinococcus felidis)
AB745463.1 G10 (Echinococcus canadensis)
HF947592.1 strain SB041 (Portugal)
Sheep s.8
Sheep s.9
Sheep s.14
Sheep s.17
Sheep s.22
Sheep s.25
Sheep s.26
Sheep s.35
Sheep s.36
Buffalo s.10
Buffalo s.11
Buffalo s.46
Buffalo s.51
Cow s.12
Cow s.16
Cow s.59
Human s.60L
Human s.60G
Human s.61
Dog s.70
[320]

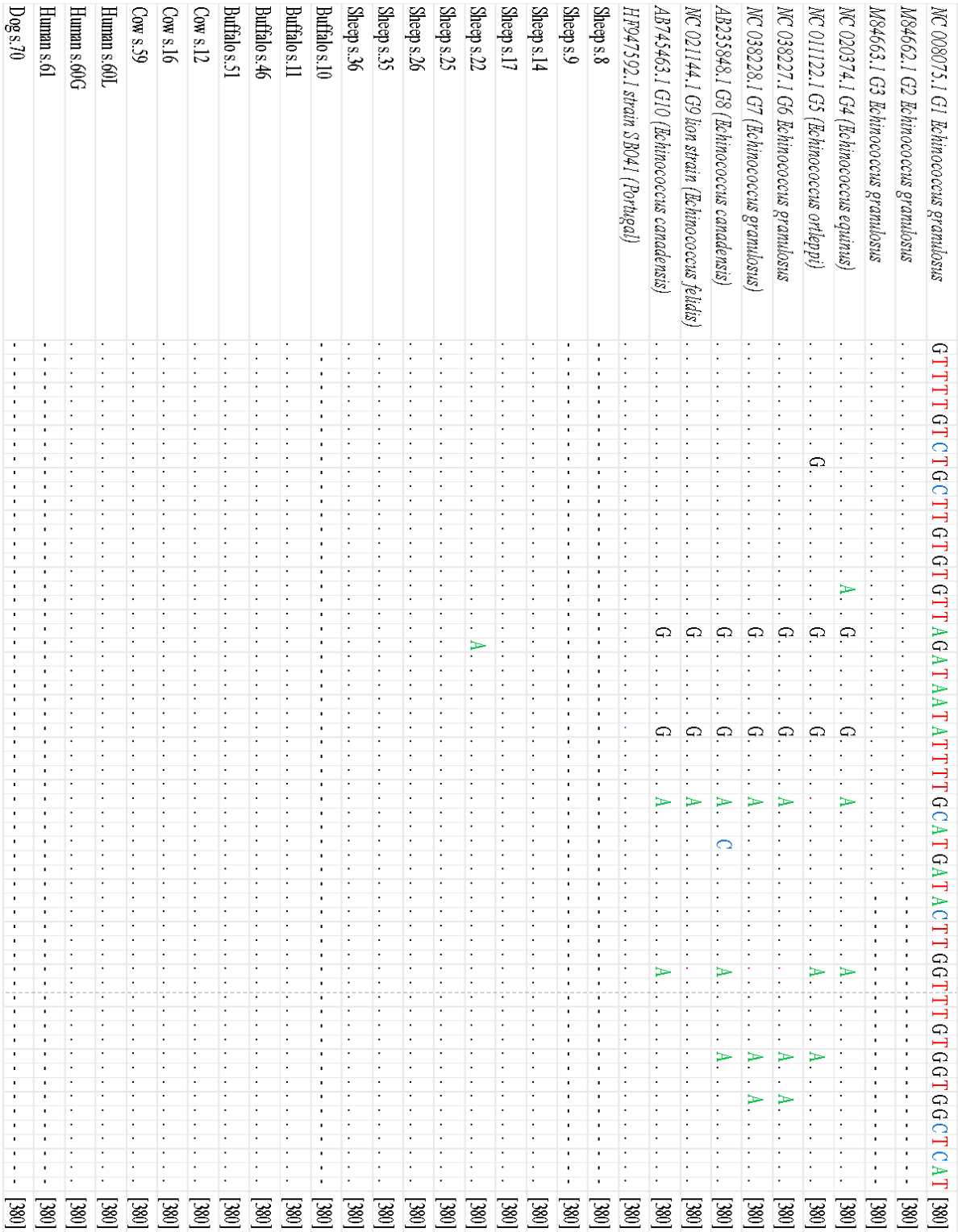


Figure (4-17): The sequences alignment of PCR products for mitochondrial *Cox1*gene with NCBI reference gene sequences of *E. granulosus* varied genotypes.

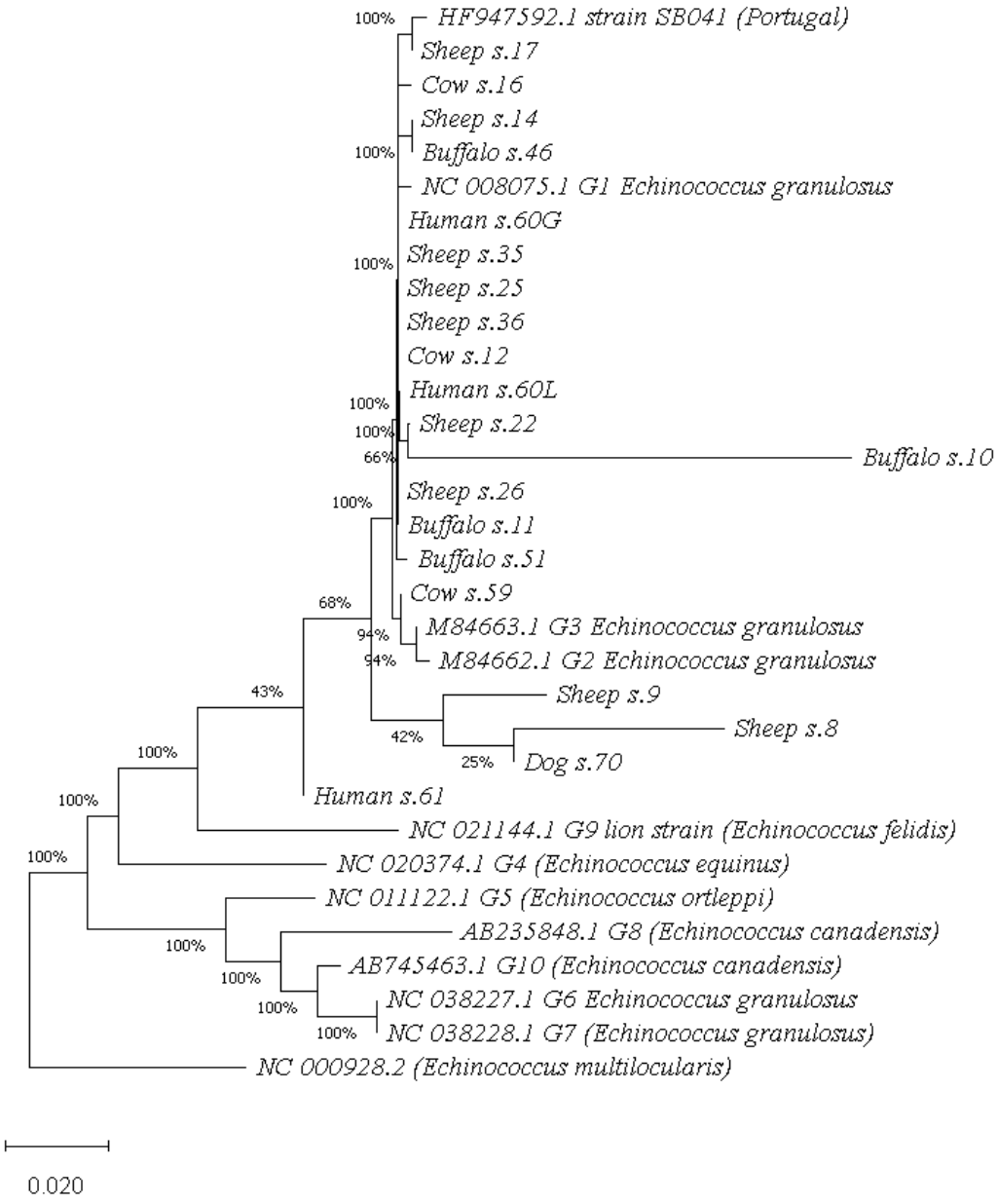


Figure (4-18): **The evolutionary history was inferred using the Neighbor-Joining method**. The optimal tree with the sum of branch length = 0.50691062 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 32 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 380 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

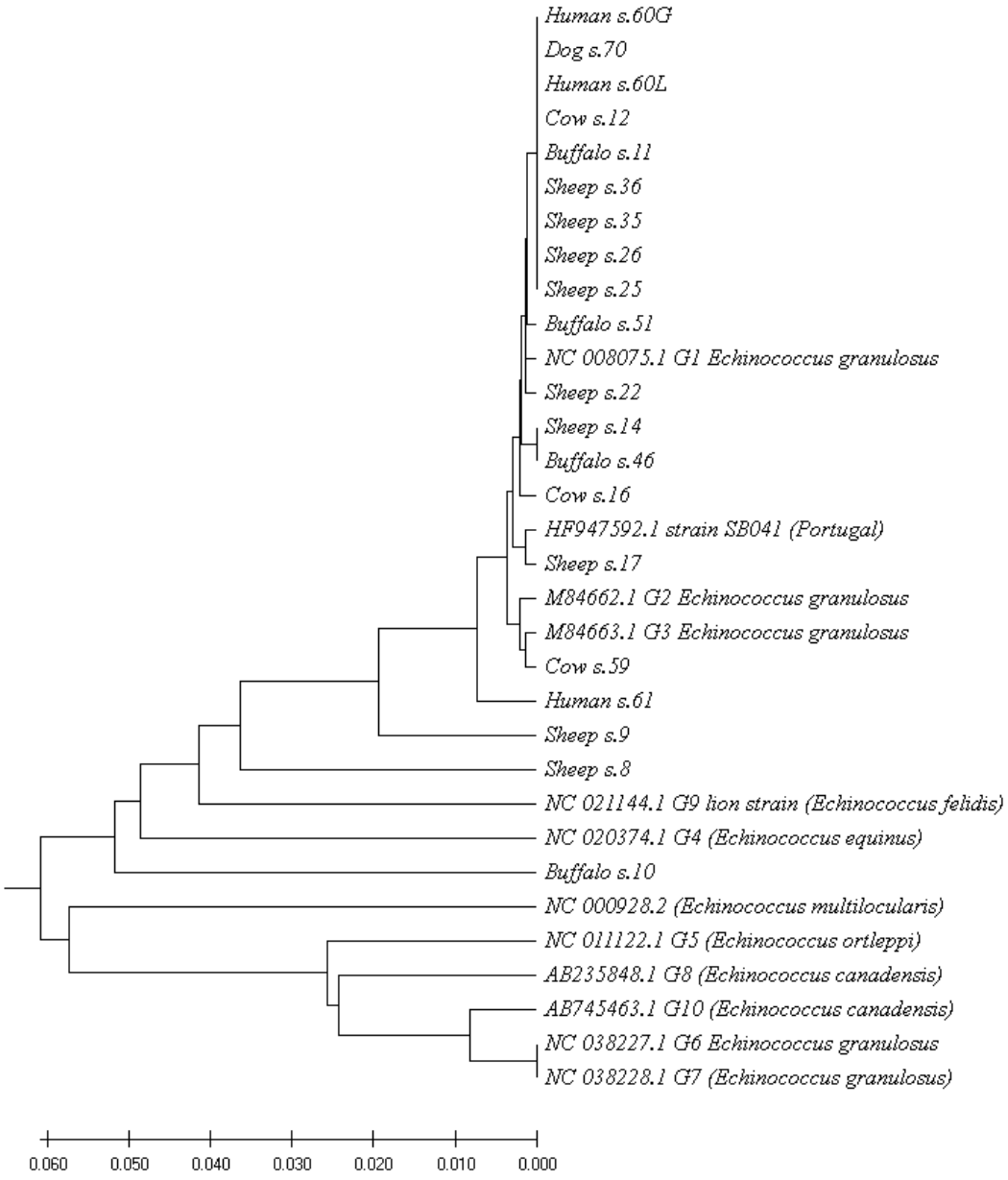
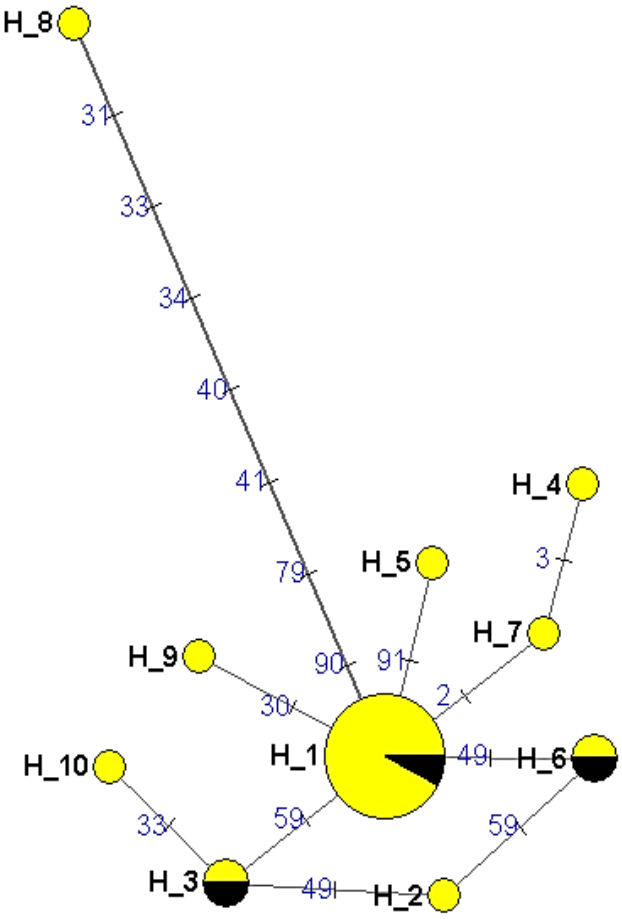


Figure (4-19): **The Evolutionary relationships of *E. granulosus*: -** The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 0.46113100 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 32 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 380 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.



Hap_1: 14 [NC_008075.1_G1_ GU984809.1_G3__Turke Sheep_s.9 Sheep_s.22 Sheep_s.25 Sheep_s.26 Sheep_s.35 Sheep_s.36 Buffalo_s.11 Buffalo_s.51 Cow_s.12 Human_s.60L Human_s.60G Dog_s.70]
Hap_2: 1 [M84662.1_G2]
Hap_3: 2 [M84663.1_G3_ Cow_s.59]
Hap_4: 1 [HF947592.1__strain_SB041]
Hap_5: 1 [Sheep_s.8]
Hap_6: 2 [Sheep_s.14 Buffalo_s.46]
Hap_7: 1 [Sheep_s.17]
Hap_8: 1 [Buffalo_s.10]
Hap_9: 1 [Cow_s.16]
Hap_10:1 [Human_s.61]

Figure (4-20): Haplotype network generated using partial cytochrome c oxidase subunit 1 mitochondrial nucleotide sequences of *E. granulosus* from Misan Province together with GenBank retrieved reference gene of *E. granulosus*. Circle size is proportional to number of individuals, Transversal lines indicate single nucleotide polymorphisms.

4:4: The Morphological Study of *E. granulosus* according to genotype of sheep hydatid cyst:

The molecular study for *E. granulosus* represent some variation in the genotype of the sample that collected from different hosts of *E. granulosus* especially in sheep hydatid cyst and to study the effect of this genetic variation on the morphological characterization of *E. granulosus* the morphological parameters reanalysis depends on the genotype of sheep hydatid cyst samples.

4:4:1: Morphological characterization of Protoscolices parameters according to the genotype of sheep hydatid cyst:

4:4:1:1: The Total length of Protoscolices (TLP):

The results (Table, 4-13) showed that The **TLP** of *E. granulosus* that collected from sheep is 203.27, 213.75, 192.88, and 172.05μm for G1, G1BC, G3 and SB041genotype respectively.

The TLP of G1BC had the highest mean of TLP and the lowest mean of TLP was of SB041Genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **no significant** differences between the TLP obtained from varied genotype ($F=1.559$, $p >0.05$) (Table, 4-13).

4:4:1:2: The Total Width of Protoscolices (TWP):

The results (Table, 4-13) showed that The **TWP** of *E. granulosus* that collected from sheep are 150.76, 189.84, 156.39 and 153.59μm for G1, G1BC, G3 and SB041 respectively.

The highest mean of TWP was of G1BC the lowest mean of TWP was of G1 genotype.

The statistical analysis (F-test) of results showed **significant** differences between the (TWP) of varied genotype ($F=5.637$, $p <0.01$).

Duncan test showed there are **significantly** increased between the mean of (TWP) obtained from G1BC with G1, G3 and SB041genotype and there **no significant** differences between G1, G3, SB041 genotype (Table, 4-13).

Table (4-13): The Protoscolices TL and TW of *E. granulosus* of varied genotypes.

	Protoscolices parameters (µm)		
Genotype	N	Total Length TL	Total Width TW
		Mean ± S. D	Mean ± S. D
G1	134	203.27 ± 60.75[a]	150.76 ± 36.22 [a]
G1BC	15	213.75 ± 42.41[a]	189.84 ± 22.93 [b]
G3	17	192.88 ± 51.12[a]	156.39 ± 39.40 [a]
SB041	13	172.05 ± 26.67[a]	153.59 ± 23.92 [a]
F	1.559		5.637
p	0.201		0.001

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:4:1:3: The Rostellum width (RW):

The results (Table, 4-14) showed that The RW of *E. granulosus* that collected from sheep is 73.02, 76.83, 68.79 and 63.96µm for G1, G1BC, G3 and SB041genotype respectively.

The RW of G1BC genotype had the highest mean of RW and the lowest mean of RW was received from SB041 genotype.

The statistical analysis (Table, 4-14) by using ANOVA Table (F-test) of results showed **no significant** differences between the RW obtained from varied genotype (F=0.887, p >0.05).

4:4:1:4: The Sucker Length (SL):

The results (Table, 4-14) showed that The SL of *E. granulosus* from different genotype that infect the sheep is 58.92, 56.60, 55.46 and 60.14μm for G1, G1BC, G3 and SB041genotype respectively.

The SL of SB041genotype had the highest mean of SL and the lowest mean of SL was received from G3 genotype.

The statistical analysis (Table, 4-14) by using ANOVA Table (F-test) of results showed **no significant** differences between the SL obtained from varied genotype ($F=1.096$, $p >0.05$).

4:4:1:5: The Sucker width (SW):

The results (Table, 4-14) showed that The SW of *E. granulosus* from different genotype that infect the sheep is 47.84, 46.48, 43.86 and 43.68μm for G1, G1BC, G3 and SB041genotype respectively.

The SW of G1genotype had the highest mean of SW and the lowest mean of SW was received from SB041 genotype.

The statistical analysis (Table, 4-14) by using ANOVA Table (F-test) of results showed that there are **no significant** differences between the SW obtained from varied genotype ($F=1.661$, $p >0.05$).

Table (4-14): The RW, SL and SW parameters for Protoscolices of *E. granulosus* in varied genotypes.

	Protoscolices parameters (µm)					
Genotype	Rostellum width RW		Sucker length SL		Sucker width SW	
	N	Mean ± S. D	N	Mean ± S. D	N	Mean ± S. D
G1	75	73.02 ± 14.27[a]	129	58.92 ± 8.44[a]	124	47.84 ± 9.28[a]
G1BC	8	76.83 ± 9.90[a]	15	56.60 ± 6.28[a]	15	46.48 ± 7.17[a]
G3	8	68.79± 11.89[a]	17	55.46± 14.83[a]	16	43.86 ± 9.74[a]
SB041	3	63.96 ± 2.65[a]	13	60.14 ± 7.52[a]	13	43.68 ± 3.79[a]
F	0.887		1.096		1.661	
p	0.451		0.352		0.177	

4:4:2: The Morphological analysis for the long hooks parameters according to the genotype of sheep hydatid cyst samples.

4:4:2:1: - Total Length of Long Hook (TLLH):

The results (Table, 4-15) showed that The TLLH of *E. granulosus* from different genotype that infect the sheep is 25.39, 25.25, 25.85 and 26.96µm for G1, G1BC, G3 and SB041genotype respectively.

The TLLH of SB041genotype had the highest mean of TLLH and the lowest mean of TLLH was received from **G1BC** genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **significant** differences between the TLLH obtained from varied genotype ($F=3.788$, $p<0.05$).

Duncan test showed there are **significantly** increased between the mean of TLLH obtained from SB041genotype with that of G1, G1BC and G3genotype, were **no significant** differences between TLLH of G1, G1BC and G3genotype (Table, 4-15).

4:4:2:2: -The Total Width of Long Hook (TWLH):

The results (Table, 4-15) showed that The TWLH of *E. granulosus* from different genotype that infect the sheep is 9.17, 8.97, 10.34 and 9.05μm for G1, G1BC, G3 and SB041 genotype respectively.

The TWLH of G3 genotype had the highest mean of TWLH and the lowest mean of TWLH was received from **G1BC** genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **high significant** differences between the TWLH obtained from varied genotype ($F=15.858$, $p <0.001$).

Duncan test showed there are **significantly** increased between the mean of TWLH obtained from G3genotype with that of G1, G1BC and SB041genotype and **no significant** differences between TWLH of G1, G1BC and SB041 genotype (Table, 4-15).

4:4:2:3: The Curved Length of Long Hook (CLLH):

The results (Table, 4-15) showed that The CLLH of *E. granulosus* from different genotype that infect the sheep is 28.83, 28.07, 29.49 and 29.88μm for G1, G1BC, G3 and SB041 genotype respectively.

The CLLH of SB041genotype had the highest mean of CLLH and the lowest mean of CLLH was received from **G1BC** genotype.

The statistical analysis (Table, 4-15) by using ANOVA Table (F-test) of results showed **significant** differences between the CLLH obtained from varied genotype ($F=3.853$, $p <0.05$).

Duncan test showed there are **non significant** differences between the mean of (CLLH) obtained from **G1** genotype with that of **G1BC**, **G3** and **SB041**genotype, were there **significantly** decreased between CLLH of **G1BC**

genotype with that of **G3** and **SB041,** were **no significant** differences between G3 and SB041 genotype (Table, 4-15).

Table (4-15): The TL, TW and CL parameters of long hooks of *E. granulosus* in varied genotypes.

	long hooks Parameters (µm)					
Genotype	Total length TL		Total width TW		Curved length CL	
	N	Mean ± S. D	N	Mean ± S. D	N	Mean ± S. D
G1	70	25.39 ± 1.28 **a**	70	9.17 ± 0.67 **a**	70	28.83 ± 1.28 **ab**
G1BC	9	25.25 ± 1.16 **a**	9	8.97 ± 0.78 **a**	9	28.07 ± 1.27 **a**
G3	17	25.85 ± 1.34 **a**	17	10.34 ± 0.49 **b**	17	29.49 ± 1.36 **b**
SB041	7	26.96 ± 0.62 **b**	7	9.05 ± 0.68 **a**	7	29.88 ± 0.98 **b**
F	3.788		15.858		3.853	
p	0.013		0.001703E-5		0.012	

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:4:2:4: Before Blade Length of Long Hook (BBLLH):

The results (Table,4-16) showed that The BBLLH of *E. granulosus* from different genotype that infect the sheep is 12.98, 13.13, 13.65, and 12.60µm for G1, G1BC, G3 and SB041 genotype respectively.

The BBLLH of G3genotype had the highest mean of BBLLH and the lowest mean of BBLLH was received from **SB041** genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **high significant** differences between the BBLLH obtained from varied genotype ($F=3.302$, $p<0.05$).

Duncan test showed there are **significant** increased between the mean of BBLLH obtained from G3 genotype with SB041 genotype, were **no significant** differences between BBLLH of G1 with G1BC, G3, and **no**

significant differences between G1BC with G1, G3 and SB041genotype (Table, 4-16).

4:4:2:5: The Blade Length of Long Hook (BLLH):

The results (Table, 4-16) showed that The BLLH of *E. granulosus* from different genotype that infect the sheep is 12.35, 12.08, 12.41 and 14.25µm for G1, G1BC, G3 and SB041 genotype respectively.

The BLLH of SB041genotype had the highest mean of BLLH and the lowest mean of BLLH was received from **G1BC** genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **high significant** differences between the (BLLH) obtained from varied hosts ($F=10.788$, $p <0.001$).

Duncan test showed there are **significant** increased between the mean of BLLH obtained from SB041 with that of G1, G1BC, G3 genotype, were **no significant** differences between BLLH of G1, G1BC and G3 genotype (Table, 4-16).

4:4:2:6: The Handle Length of Long Hook (HLLH):

The results (Table, 4-16) showed that The HLLH of *E. granulosus* from different genotype that infect the sheep is 7.33, 7.24, 7.18 and 6.57 for G1, G1BC, G3, SB041 genotype respectively.

The HLLH of G1genotype had the highest mean of HLLH and the lowest mean of HLLH was received from **SB041** genotype.

The statistical analysis (Table, 4-16) by using ANOVA Table (F-test) of results showed **no significant** differences between the HLLH obtained from varied genotype ($F=1.156$, $p >0.05$).

Table 4-16: The BBL, BL and HL parameters for long hooks of E. granulosus in varied genotypes.

	long hooks Parameters (µm)			
Genotype	N	Before blade length BBL	Blade length BL	Handle length HL
		Mean ± S. D	Mean ± S. D	Mean ± S. D
G1	70	12.98 ± 0.91 **ab**	12.35 ± 0.81 **a**	7.33 ± 1.04**a**
G1BC	9	13.13 ± 1.03 **ab**	12.08 ± 0.68 **a**	7.24 ± 1.29**a**
G3	17	13.65 ± 0.79 **b**	12.41 ± 1.21 **a**	7.18 ± 1.06**a**
SB041	7	12.60 ± 0.53 **a**	14.25 ± 0.49 **b**	6.57 ± 0.47**a**
F	3.302		10.788	1.156
p	0.023		0.000003	0.331

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:4:2:7: Angle α of Large Hook (AαLH)

The results (Table, 4-17) showed that The measure of AαLH of *E. granulosus* from different Genotype that infect the sheep is 149.69º, 150.72º, 146.55º and 156.90º for G1, G1BC, G3 and SB041 genotype respectively.

The SB041genotype had the highest mean of AαLH and the lowest mean of AαLH was received from **G3** genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **high significant** differences between the AαLH obtained from varied genotype ($F=40.179$, $p<0.001$).

Duncan test showed there are **significant** increased between the mean of AαLH obtained from SB041 genotype with that of G1, G1BC and G3 genotype, also there are **significant** increased between the mean of AαLH obtained from G1BC with that of G3 genotype, were **no significant** differences between AαLH of G1 with G1BC and G3 genotype (Table, 4-17).

4:4:2:8: Angle β of Large hook (AβLH)

The results (Table, 4-17) showed that The measure of AβLH of *E. granulosus* from different Genotype that infect the sheep is 38.99º, 39.30º, 37.01º and 31.47º for G1, G1BC, G3 and SB041 genotype respectively.

The G1BC genotype had the highest mean of AβLH and the lowest mean of AβLH was received from SB041 genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **high significant** differences between the AβLH obtained from varied genotype ($F=5.613$, $P<0.01$).

Duncan test showed there are **significantly** decreased between the mean of AβLH obtained from SB041 genotype with that of G1, G1BC and G3 genotype, were **no significant** differences between AβLH of G1 with G1BC and G3 genotype (Table, 4-17).

4:4:2:9: Angle γ of Large hook (AγLH):

The results (Table, 4-17) showed that The measure of (AγLH) of *E. granulosus* from different Genotype that infect the sheep is 155.78º, 159.63º, 152.83º and 162.02º for G1, G1BC, G3 and SB041 genotype respectively.

The **SB041**genotype had the highest mean of AγLH and the lowest mean of AγLH was received from G3 genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **significant** differences between the AγLH obtained from varied genotype ($F=3.047$, $p<0.05$).

Duncan test showed there are **significantly** decreased between the mean of AγLH obtained from G1 genotype with that of G1BC, G3 and SB041 genotype and there are **significantly** decreased between the mean of AγLH obtained

from G3 genotype with that of G1BC and **SB041** genotype, were **no significant** differences between AγLH of G1BC and SB041 (Table, 4-17).

Table 4-17: The Angle α, Angle β and Angle γ parameters for long hooks of *E. granulosus* in varied genotype.

	long hooks Parameters (°)					
Genotype	Angle α Aα		Angle β Aβ		Angle γ Aγ	
	N	Mean ± S. D	N	Mean ± S. D	N	Mean ± S. D
G1	70	149.69 ± 4.80^{ab}	66	38.99 ± 5.20 ^{b}	70	155.78 ± 8.19^{ab}
G1BC	9	150.72 ± 3.15 ^{b}	9	39.30 ± 3.70 ^{b}	9	159.63 ± 4.47 ^{b}
G3	17	146.55 ± 4.95 ^{a}	17	37.01 ± 4.37 ^{b}	17	152.83 ± 7.93 ^{a}
SB041	7	156.90 ± 2.03 ^{c}	7	31.47 ± 2.41 ^{a}	7	162.02 ± 4.42 ^{b}
F	8.529		5.613		3.047	
p	0.000043		0.001		0.032	

The different letters refer to significant difference among group. The same letters refer to non significant difference among group.

4:4:3: The Morphological analysis for the Short hooks parameters according to the genotype of sheep hydatid cyst samples:

4:4:3:1: The Total length of Short Hook (TLSH):

The results (Table, 4-18) showed that The TLSH of *E. granulosus* from different genotype that infect the sheep is 22.01, 20.48, 21.21 and 22.39µm for G1, G1BC, G3 and SB041 genotype respectively.

The TLSH of SB041genotype had the highest mean of TLSH and the lowest mean of TLSH was received from **G1BC** genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **no significant** differences between the TLSH obtained from varied genotypes (F=2.070, p>0.05).

4:4:3:2: The Total Width of Short Hook (TWSH):

The results (Table,4-18) showed that The TWSH of *E. granulosus* from different genotype that infect the sheep is 7.85, 7.76, 8.13 and 7.57μm for G1, G1BC, G3 and SB041 genotype respectively.

The TWSH of G3 genotype had the highest mean of TWSH and the lowest mean of TWSH was received from **SB041** genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **no significant** differences between the TWSH obtained from varied genotypes ($F=1.178$, $P>0.05$).

4:4:3:3: The Curved Length of Short Hook (CLSH):

The results (Table, 4-18) showed that The CLSH of *E. granulosus* from different genotype that infect the sheep is 24.33, 22.48, 23.59 and 24.54μm for G1, G1BC, G3 and SB041 genotype respectively.

The CLSH of SB041 genotype had the highest mean of CLSH and the lowest mean of CLSH was received from **G1BC** genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **significant** differences between the CLSH obtained from varied genotypes ($F=3.306$, $p<0.05$).

Duncan test showed there are **significantly** decreased between the mean of CLSH obtained from G1BC genotype with G1, SB041 and **not significantly** with G3 genotype, were **no significant** differences between CLSH of G1, G3 and SB041 genotype (Table, 4-18).

Table 4-18: The TL, TW and CL for the short hooks of *E. granulosus* in varied genotypes.

Short hooks Parameters(µm)				
Genotype	N	Total length TL	Total width TW	Curved length CL
		Mean ± S. D.	Mean ± S. D.	Mean ± S. D.
G1	60	22.01 ± 1.78 **a**	7.85 ± 0.66 **a**	24.33 ± 1.44 **b**
G1BC	5	20.48 ± 1.64 **a**	7.76 ± 0.32 **a**	22.48 ± 1.67 **a**
G3	11	21.21 ± 1.48 **a**	8.13 ± 0.77 **a**	23.59 ± 1.48 **ab**
SB041	8	22.39 ± 1.23 **a**	7.57 ± 0.58 **a**	24.54 ± 1.48 **b**
F		2.070	1.178	3.306
p		0.110	0.323	0.024

The different letters refer to significant difference among group.

The same letters refer to non significant difference among group.

4:4:3:4: Before Blade Length of Short Hook (BBLSH):

The results (Table, 4-19) showed that The BBLSH of *E. granulosus* from different genotype that infect the sheep is 13.59, 12.77, 13.21 and 12.81µm for G1, G1BC, G3 and SB041 genotype respectively.

The BBLSH of G1genotype had the highest mean of BBLSH and the lowest mean of BBLSH was received from **G1BC genotype**.

The statistical analysis (Table, 4-19) by using ANOVA Table (F-test) of results showed **no significant** differences between the BBLSH obtained from varied genotypes (F=2.373, p >0.05).

4:4:3:5: The Blade Length of Short Hook (BLSH):

The results (Table, 4-19) showed that The BLSH of *E. granulosus* from different genotype that infect the sheep is 8.51, 7.65, 7.98 and 9.51µm for G1, G1BC, G3 and SB041 genotype respectively.

The BLSH of SB041 genotype had the highest mean of BLSH and the lowest mean of BLSH was received from G1BC genotype.

The statistical analysis (F-test) of results showed **significant** differences between the BLSH obtained from varied genotypes ($F=8.415$, $p<0.001$).

Duncan test showed there **significant** increased between the mean of BLSH obtained from SB041 with G1, G1BC, G3 genotype, also there are **significantly** decreased between the mean of BLSH obtained from G1BC with G1, SB041, were **no significant** differences between BLSH of G1 and G3 genotype (Table, 4-19).

4:4:3:6: The Handle Length of Short Hook (HLSH):

The results (Table, 4-19) showed that The (HLSH) of *E. granulosus* from different genotype that infect the sheep is 8.57, 7.54, 8.28 and 7.85μm for G1, G1BC, G3 and SB041 genotype respectively.

The HLSH of G1 genotype had the highest mean of HLSH and the lowest mean of HLSH was received from **G1BC genotype**.

The statistical analysis by using ANOVA Table (F-test) of results showed **significant** differences between the HLSH obtained from varied hosts ($F=3.169$, $p<0.05$).

Duncan test (Table, 4-19) showed there **significant** increased between the mean of HLSH obtained from G1 with HLSH of G1BC, were **no significant** differences between HLSH of G1, G3 and SB041 genotype and **no significant** differences between HLSH of G1BC, G3 and SB041 genotype.

Table 4-19: The BBL, BL and HL parameter for short hooks of *E. granulosus* in varied genotypes.

	Short hooks Parameters (µm)			
Genotype	N	Before blade length BBL	Blade length BL	Handle length HL
		Mean ± S. D.	Mean ± S. D.	Mean ± S. D.
G1	60	13.59 ± 0.94^{a}	8.51 ± 0.74 ^{b}	8.57 ± 0.94 ^{b}
G1BC	5	12.77 ± 1.34^{a}	7.65 ± 0.70 ^{a}	7.54 ± 1.55 ^{a}
G3	11	13.21 ± 1.33^{a}	7.98 ± 0.65 ^{ab}	8.28 ± 0.62 ^{ab}
SB041	8	12.81 ± 0.89^{a}	9.51 ± 0.99 ^{c}	7.85 ± 0.52 ^{ab}
F	2.373		8.415	3.169
p	0.076		0.00006	0.028

The different letters refer to significant difference among group.

The same letters refer to non significant difference among group.

4:4:3:7: Angle α of Short Hook (AαSH)

The results (Table, 4-20) showed that The measure of **AαSH** of *E. granulosus* from different Genotype that infect the sheep is 144.76º, 143.32º, 138.52º and 153.58º for G1, G1BC, G3 and SB041 genotype respectively.

The SB041genotype had the highest mean of AαSH and the lowest mean of AαSH was received from **G3** genotype.

The statistical analysis (F-test) of results showed **high significant** differences between the AαSH obtained from varied genotype (F=10.724, P <0.001).

Duncan test showed there **significant** increased between the mean of AαSH obtained from SB041 genotype with that of G1, G1BC and G3 genotype, also there are **significant** increased between the mean of AαSH obtained from G1 with that of G3and not significantly with G1BC genotype, were **no significant** differences between AαSH of G1BC and G3 genotype (Table, 4-20).

4:4:3:8: - Angle β of Short hook (AβSH)

The results (Table, 4-20) showed that The measure of AβSH of *E. granulosus* from different Genotype that infect the sheep is 36.73º, 38.96º, 39.16º and 35.36º for G1, G1BC, G3 and SB041 genotype respectively.

The G3 genotype had the highest mean of AβSH and the lowest mean of (AβSH) was received from **SB041** genotype.

The statistical analysis (F-test) of results showed **non significant** differences between the AβSH obtained from varied genotype (F=1.987, p >0.05) (Table, 4-20).

4:4:3:9: - Angle γ of Short hook (AγSH):

The results (Table, 4-20) showed that The measure of AγSH of *E. granulosus* from different Genotype that infect the sheep is 168.80º, 172.00º, 164.98º and 172.93º for G1, G1BC, G3 and SB041 genotype respectively.

The **SB041**genotype had the highest mean of AγSH and the lowest mean of AγSH was received from G3 genotype.

The statistical analysis by using ANOVA Table (F-test) of results showed **significant** differences between the AγSH obtained from varied genotype (F=3.053, p <0.05).

Duncan test showed **significantly** decreased between the mean of AγSH obtained from G3 genotype with that of G1BC and SB041 genotype, were **no significant** differences between AγSH of G1, G1BC and SB041genotype (Table, 4-20).

Table 4-20: The Angle α, Angle β and Angle γ parameter for short hooks of *E. granulosus* in varied genotypes.

	Short hooks Parameters (°)					
Genotype	Angle α Aα		Angle β Aβ		Angle γ Aγ	
	N	Mean ± S. D	N	Mean ± S. D	N	Mean ± S. D
G1	60	144.76 ± 5.31 **b**	59	36.73 ± 3.88[a]	60	168.80 ± 5.45 **ab**
G1BC	5	143.32 ± 2.56**ab**	5	38.96 ± 2.98[a]	5	172.00 ± 4.96 **b**
G3	11	138.52 ± 7.61 **a**	11	39.16 ± 4.69[a]	11	164.98 ± 9.59 **a**
SB041	8	153.58 ± 7.22 **c**	8	35.36 ± 4.44[a]	8	172.93 ± 6.10 **b**
F	10.724		1.987		3.053	
p	0.000005		0.122		0.033	

The different letters refer to significant difference among group.

The same letters refer to non significant difference among group.

CHAPTER FIVE

DISCUSSION

5: DISCUSSION

It is necessary to mention that there was one study which carried on the epidemiology of *E. granulosus* in definitive host (stray dogs) in peripheral region of Amara city in 2016 by (Alsaady *et al*, non-published), No anther study was conducted on definitive host in this region and no study was carried on intermediated hosts.

5:1: The infection rates of *E. granulosus*:

The present study states that the infection rate of *E. granulosus* in definitive host is 33,33%, this finding is lower than those recorded previously in Iraq by Molan and Saida (1989) in Arbil the rate was 79.1% ; Molan (1993) in Theqar was 56% and Saeed *et al.* (2000) in Arbil was 49.5%, but it is higher than that recorded by Tarish *et al.* (1986) in Baghdad 25% and by Maktoof and Abu Tabeekh (2015) in Al-Basra infection rate was 14.7%, also it is higher than that recorded in Turkey 24% by Utuk *et al.* (2008) and in Iran 22% by Razmi *et al.* (2006), these variation in the infection rats may be return to some factors such as the diversity of intermediate hosts, stray dogs distribution in studied area, the present of random slaughterhouses and applied of stray dog control programs (Ekhnefer, 2012). .

The infection rate of *E. granulosus* in sheep, buffalo, camel, cow, goats and human are 2.16%, 2.20%, 0.00%, 3.05%, 0.00% and 1.70% respectively, are higher than that recorded by Jarjees and Al-Bakri (2012) in Mosul 2% in sheep, 0.55% in cattle and 0.52% in goats and lower than that of Senekji and Beattie (1940) in Baghdad, 11.93% for sheep-goats, 24.66% in cow and one camel was examined and was found infested and that recorded by Maktoof and Abu Tabeekh (2015) in Al-Basra province 22% of sheep, recorded by Saeed *et al.* (2000), In Arbil (15.0%) in sheep, (10.9%) in cow and (6.2%) in goats. This study results were lower than those reported in neighboring countries like in Turkey 51.9% in sheep, 3.7% in buffalo, 39.7% in cow and

2.0% in goats by Altintas (2003); Utuk *et al.* (2008), were in Iran recorded as 11.1-33.8% in sheep, 1.6-12.4% in buffalo, 16.3-16.4% in cow, 5.8-6.3% in goats by Ahmadi and Dalimi (2006); Mohammad *et al.* (2011); Hanifian *et al.* (2013).

In other hand, some studies reported that some breeds of goat such as local Iraqi black breed may be have a kind of resistance to *E. granulosus* parasite compared with other susceptible breeds in some regions of the world, like African, Masailand goats breed (Macpherson,1985; Jarjees and Al-Bakri, 2012; Jenkins *et al*., 2018).

Prevalence of Echinococcosis is varying between individual group, years of study, region investigated, source of animals and their contact with dogs and other factors (Ekhnefer, 2012).

5:2: The fertility rate of hydatid cyst (FR):

The results showed that the fertility rates of hydatid cyst are 65.00, 11.12, 20.00, 60.00% in sheep, buffalo, cow, human respectively.

The present study showed that sheep had the highest FR among other intermediated hosts, this finding was agreement with other studies conducted in some region of Iraq in sheep 64%, goats 35.7% and cow 29.8% by Saeed *et al.*, (2000); (Jarjees and Al-Bakri, 2012), fertility rate (FR) playing an important role in the spreading of HC in the world (Macpherson, 1985), for this results can have concluded that the sheep acted a significant role in spreading the *E. granulosus* parasite in our region.

A large number of sheep are slaughtering out slaughterhouses in some religions and social ceremonies compared with other livestock animals (Jarjees and Al-Bakri, 2012), as well as they are slaughtering in traditional activities and festivals, these activities may be increase the potential role of sheep in spreading and distribution the *E. granulosus* in different region, this

phenomena (fertility) of HC had been pointed out in sheep from more than four decades (Soulsby, 1982; Jarjees and Al-Bakri, 2012).

There is low request on goat and camel meat in Misan's market so that the number of examination for them was lower than other livestock.

5:3: The morphological study:

The characteristic of some morphological parameters of *E. granulosus* to recognize the differences and the impact of the wide range of host for *E. granulosus* on the morphology and properties of the larval stage Protoscolices and its hooks parameter, also the impact of genetic variation on this morphological characters represent many variations on the *E. granulosus* in different hosts and genotypes, this may be useful for morphological differentiation between the strains of *E. granulosus* (Ahmadi and Dalimi, 2006).

This study showed significant differences among some morphological parameters of Protoscolices in intermediated hosts or scolex of adult in definitive host.

These results agreed with some study in other region in the world, these variation may return to the variation of hosts, in this point, the body internal the environment of the deferent hosts like physiological factors play a role in this variations of morphological parameters of *E. granulosus* (Ibrahim *et al*., 2009), for this we can say that the host had the main impact factors on *E. granulosus* morphological properties of larval stage such as Protoscolices, hooks, suckers and rostellum dimensions, that agree Harandi *et al.* (2002) that There is high impact of host on the morphological variation of *E. granulosus*.

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5:3:1: Effect of host variation on morphological parameters *E. granulosus*:

5:3:1:1: Protoscolices parameters:

The TLP, TWP, RW, SL and SW measurement (Tables, 4-3, 4-4) were significantly differing between *E. granulosus* in varied hosts, this results indicate on the hosts ability to manipulate *E. granulosus* Protoscolices morphology, this effects are noted by other researchers as Ahmadi and Dalimi (2006) in Iran and disagree with Hussain *et al.* (2005) in Pakistan.

5:3:1:2: Long hooks parameters:

The TLLH, TWLH, CLLH, BBLLH, BLLH, HLLH and AαLH (Table, 4-6, 4-7, 4-8) were significantly deffeince in varied hosts , this variations are recognized by Ahmadi and Dalimi (2006), Angles were measured for first time by current study.

5:3:1:3: The short hook parameters:

The TLSH, TWSH, CLSH, BBLSH, BLSH, HLSH, AαSH, AβLH and AγLH (Tables 4-9, 4-10, 4-11) have significant differences between short hooks collected from varied hosts of *E. granulosus*, this results agree with Ahmadi and Dalimi (2006) and disagree with Hussain *et al.* (2005), were no significant difference was found between the total numbers of hooks of *E. granulosus* that collected from different hosts, this result agrees with finding of Hussain *et al.* (2005).

5:4: The molecular study:

The strain characterization of *E. granulosus* in human and livestock population are described as the first time in Misan Province by using polymerase chain reaction and gene sequencing technology.

In Iraq few studies were conducted in molecular field, some studies agreed with our findings in different species host, like Hama *et al.* (2015); Hassan *et al.* (2016) and Hassan *et al.* (2017).

5:4:1: The genotyping of *E. granulosus*:

The results of molecular study by gene sequencing found that G1 (sheep strain) was the dominant strain of *E. granulosus* it is consisted about 72.72% of testing samples. G1 strain was distributed as: sheep (66.66%), buffalo (75%), cow (75%), human (100%) and Dog (100%), this results agreement with relatively high infection rate 2.16% and high fertility 65.00% of sheep hydatid cysts, this domination of G1 was reported by other studies in some areas of Iraq such as: Baraak (2014) in human from different provinces; Hassan *et al.* (2016) and Hassan *et al.* (2017) Hammad *et al.* (2018) that reported sheep strain (G1) as the most prevalent strain in Kirkuk , Iraqi Kurdistan and Kirkuk - Sulaimania respectively.

The present results confirm previous evidences from molecular genotyping surveys described high prevalence for sheep strain (G1) in Iraq (Hama *et al.*, 2015; Hassan *et al.*, 2016).

The buffalo strain G3 was recorded in this study with low frequency 13.63% since it has been observed in *E. granulosus* specimens distributed between sheep (22.22%) and cow (25%) of hydatid cysts, this genotype was detected previously in some regions of Iraq like in Al-Qadisiyah province it recorded as dominant genotype (Fadhil and A'aiz, 2016), also recorded in Kirkuk province and Sulaimania province by Hammad *et al.* (2018).

The G1BC was recorded in 9.09% of *E. granulosus* specimens, this genotype recorded in sheep (11.11%) and buffalo (25%) of hydatid cysts. This genotype was identified according to EURLP method (Figure 4-16). The morphological parameters of G1BC was significantly different with other

genotypes. The buffalo hydatid cyst with this genotype was the only fertile buffalo's HC sample, where buffalo HC with G1 was sterile this may indicate that buffalo of Misan region is not suitable host for G1, this is the first record for G1BC genotype in Iraq.

Only one (4.54%) of *E. granulosus* specimens was recorded as SB041 strain (recorded by Beato *et al.* (2013)) this genotype recorded in sheep (11.11%) hydatid cyst. The variations of morphological features of SB041 (Tables, 4-15, 4-16, 4-17, 4-19, 4-20) when compared with G1 and bosting with gene sequencing results and result of analysis with haplotype network (Figure,4-20) exhibited that our sheep isolate (S.17) is likely to be SB041.

5:4:2: The phylogenetic tree:

The two methods of phylogenetic analysis for mitochondrial *Cox1*: first the evolutionary history (Neighbor-Joining method) (Figure, 4-18) and second the evolutionary relationships (UPGMA method) (Figure, 4-19), they showed that the majority of *E. granulosus* samples in various hosts aligned with G1 genotype. It also showed the origin similarity of some *E. granulosus* samples (s.14-s.46, s.17-SB041) and (G2-G3-s.59, G6-G7).

5:4:3: The Haplotype network:

The haplotype network (Figure, 4-20) analysis for partial *Cox1* sequences distribute *E. granulosus* samples and reference gene sequences on ten distinct haplotypes, this makes easy to show the genetic variations and relationships between different sequence.

5:4:4: Effect of genotype variation on morphological parameters of *E. granulosus* of sheep:

5:4:4:1: The Protoscolices parameters:

The TWP (Table 4-13) was significantly different between G1BC and other three genotypes of *E. granulosus*, where the TLP, RW, SL and SW were non significantly different, this results make the Protoscolices are not characteristic features between these four genotypes.

5:4:4:2: The long hooks parameters:

the TLLH, TWLH, CLLH, BBLLH, BLLH, AαLH, AβLH and AγLH (Tables, 4-15, 4-16, 4-17) are significantly differing between G1, G1BC, G3 and SB041genotypes of *E. granulosus*, these variations in the morphological parameters improve the genetic variations and give an evidence on genotyping results accuracy. This finding agreement with Ahmadi and Dalimi (2006) finding.

5:4:4:3: The short hooks parameters:

The CLSH, BLSH, HLSH, AαSH and AγSH (Tables, 4-18, 4-19, 4-20) were significantly differenced between varied genotypes of *E. granulosus* which are recorded in this study (G1, G1BC, G3, SB041), where the TLSH, TWSH, BBLSH and AβSH are non significantly differenced in all four genotypes, these results encourage for more intense studies for morphological characters on other genotypes of *E. granulosus*.

This information on the strain which endemic in the area supported the observation of G3 strain in sheep and cow HC in Iraq (Fadhil and A'aiz, 2016; Hammad *et al.,* 2018), this results are agree with our finding from molecular study which showed different genotypes in some intermediated hosts.

CONCLUSIONS & RECOMMENDATIONS

Conclusions

- The sheep and cow have the highest infection rates percentage with *E. granulosus* in Misan.
- The goats and buffalo are not suitable host for *E. granulosus* in Misan especially for G1genotype.
- In this study the genotype G1 is recorded as predominant in all *E. granulosus* hosts.
- There are four genotypes of *E. granulosus* (G1, G1BC, G3, SB041) spread in Misan.
- The statistical analysis for morphological parameters for *E. granulosus* collected from different host indicated that there are high significant differences between them that may return to the impact of the host on the *E. granulosus*.
- buffalo strain is recorded in both sheep and cow hydatid cyst for first time in Misan.
- G1BC and SB041 genotypes are recorded for the first time in Iraq
- Buffalo, goat, and camel hydatid cyst is Not the main source for spreading *E. granulosus* infections in Misan.

Recommendations

- Apply the laws by preventing the randomly slaughtering outer slaughterhouses.
- Apply control program on stray dogs spread and reproduction and tray to treat the farm dogs against intestinal parasitic worms especially *E. granulosus*.
- Spread the knowledge in the community about the hydatid disease and between the butchers about how dealing with infected organs and cut the life cycle of *E. granulosus*.
- Build a modern slaughterhouse containing a proper cremator.
- Future study on *E. granulosus* to determination the physiological factors that impacting on hydatid cyst fertility in different hosts.
- Future molecular studies on both sterile and fertile hydatid cysts to more acute determination for genetic diversity of *E. granulosus*.

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APPENDIX

Table of samples				
Date of collection	Cyst nature	Site of infection	The host	label
2017/12/10	Sterile	liver	cow	S1
2017/12/10	Sterile	lung	cow	S2
2017/12/11	fertile	lung	cow	S3
2018/12/11	Sterile	lung	cow	S4
2017/12/14	fertile	lung	cow	S5
2017/12/18	fertile	liver	cow	S6
2017/12/21	Sterile	liver	cow	S7
2017/12/25	fertile	liver	sheep	S8
2017/12/25	fertile	liver	sheep	S9
2017/12/26	Sterile	liver	buffalo	S10
2017/12/26	Sterile	lung	buffalo	S11
2017/12/27	fertile	liver	cow	S12
2017/12/28	Sterile	lung	buffalo	S13
2018/1/2	fertile	liver	sheep	S14
2018/1/2	Sterile	liver	sheep	S15
2018/1/2	Sterile	lung	cow	S16
2018/1/4	fertile	lung	sheep	S17
2018/1/7	Sterile	lung	cow	S18
2018/1/7	fertile	liver	sheep	S19
2018/1/7	fertile	liver	sheep	S20
2018/1/7	fertile	liver	sheep	S21
2018/1/9	fertile	liver	sheep	S22
2018/1/13	calcified	liver	cow	S23
2018/1/13	Sterile	lung	cow	S24
2018/1/13	fertile	liver	sheep	S25
2018/1/13	Sterile	liver	sheep	S26
2018/1/18	fertile	lung	sheep	S27
2018/1/23	Sterile	lung	cow	S28
2018/1/23	Sterile	liver	cow	S29
2018/1/23	calcified	liver	sheep	S30
2018/1/23	calcified	liver	sheep	S31
2018/1/26	Sterile	liver	cow	S32
2018/1/29	Abscess	liver	cow	S33
2018/2/1	Abscess	liver	cow	S34
2018/2/1	fertile	liver	sheep	S35
2018/2/1	fertile	lung	sheep	S36
2018/2/4	fertile	liver	cow	S37
2018/2/6	fertile	liver	sheep	S38
2018/2/6	Small cyst	liver	sheep	S39
2018/2/11	Sterile	lung	buffalo	S40
2018/2/11	calcified	liver	cow	S41
2018/2/12	Sterile	lung	buffalo	S42
2018/2/13	Sterile	liver	cow	S43
2018/2/13	Sterile	liver	buffalo	S44
2018/2/13	Sterile	lung	cow	S45
2018/2/20	fertile	lung	buffalo	S46

2018/2/20	Sterile	liver	sheep	S47
2018/2/24	Sterile	lung	cow	S48
2018/2/24	Sterile	liver	buffalo	S49
2018/2/24	Sterile	liver	sheep	S50
2018/2/26	Sterile	liver	buffalo	S51
2018/2/26	Sterile	lung	cow	S52
2018/2/26	Sterile	liver	cow	S53
2018/2/27	Sterile	liver	cow	S54
2018/3/1	Sterile	lung	cow	S55
2018/3/1	Sterile	liver	cow	S56
2018/3/3	Sterile	liver	cow	S57
2018/3/4	Sterile	liver	cow	S58
2018/3/8	fertile	liver	cow	S59
2018/3/26	fertile	lung/liver	human	S60
2018/4/1	fertile	liver	human	S61
2018/4/10	Sterile	lung	human	S62
2018/5/13	Sterile	lung	human	S63
2018/5/21	Not infected	Small intestine	dog	S64
2018/5/24	infected	Small intestine	dog	S65
2018/5/28	Not infected	Small intestine	dog	S66
2018/5/31	fertile	liver	human	S67
2018/6/10	Not infected	Small intestine	dog	S68
2018/6/18	Not infected	Small intestine	dog	S69
2018/7/4	infected	Small intestine	dog	S70

الخلاصة

ان لداء الأكياس المائية العذرية تأثيراً خطيراً على صحة الانسان وسلامة حيواناته، يسبب هذا المرض مشاكل صحية ومجتمعية في مختلف بلدان العالم.

تضمنت هذه الدراسة وصفاً مظهرياً وجزيئياً للمشوكة الحبيبية *Echinococcous granulosus* في كل من العائل النهائي (الكلاب) ومجموعة من العوائل الوسطية (المواشي) فضلاً عن الانسان ضمن محافظة ميسان، تضمنت الدراسة الحالية مسحا وبائيا للإصابة بالأكياس المائية العذرية للحيوانات الداخلة الى مجزرة العمارة المركزية خلال الفترة الممتدة من شهر كانون الاول لعام 2017 ولغاية شهر تشرين الاول من عام 2018، تم خلالها فحص 3287 حاله توزعت بين العوائل المختلفة للطفيلي حيث اشتملت على 922 رأسا من الغنم و405 رأسا من الجاموس واثنين من الجمال و983 رأسا من البقر و150 رأسا من المعز تم فحصها في المسلخ المركزي لمدينة العمارة إضافة الى 819 حالة سريرية للإنسان فحصت في كل من مستشفى الصدر التعليمي ومستشفى الزهراوي الجراحي، اما بالنسبة لكاملات الدودة المشوكة الحبيبية *E. granulosus* فقد جمعت من اثنين(2\6) من الكلاب السائبة. كانت نسب انتشار الإصابة لكل من الأغنام، الجاموس، الجمال، الابقار، الماعز، الانسان، الكلاب هي 2.16%، 2.20%، 0.0%، 3.05%، 0.0%، 1.70%، 33.33% على التوالي.

ان التفريق المظهري لطفيلي المشوكة الحبيبية *E. granulosus* في عوائله المختلفة اظهر اختلافات معنوية لأغلب المؤشرات التي تم قياسها كأبعاد الاشواك والرؤيسات.

تم في هذه الدراسة التشخيص الجزيئي لطفيلي المشوكة الحبيبية *E. granulosus* لإصابات الانسان وباقي حيوانات المزرعة لأول مرة باستخدام تقنيتي التفاعل البلمرة المتسلسل PCR و تحليل تسلسل القواعد النيتروجينية DNA sequencing الخاصة بجيني *Cox1* و*Nad1* في محافظة ميسان، أظهرت النتائج وجود تنوع جيني لطفيلي الاكياس المائية العذرية حيث تم تسجيل كل من عترة الأغنام sheep strain (G1) و buffalo strain (G3) و العترة SB041 إضافة الى العترة G1BC لمختلف العوائل، وبينت النتائج ان العترة G1 هي العترة الأكثر انتشارا في كل من الانسان وحيوانات المزرعة التي تم دراستها. ان نتائج هذه الدراسة تعتبر الأولى فيما يخص التنوع الجيني لطفيلي المشوكة الحبيبية *E. granulosus* في محافظة ميسان جنوب العراق، وقد تم خلال هذه الدراسة تسجيل العترتين G1BC, SB041 لأول مرة في العراق.

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

قسم علوم الحياة

دراسة مظهرية وجزيئية للمشوكة الحبيبية *Echinococcus granulosus* في محافظة ميسان جنوب العراق

رسالة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

حسين علي نعيم القزويني

بكالوريوس علوم - علوم الحياة / كلية العلوم - جامعة ميسان (2013)

بأشراف

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