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## MORPHOLOGICAL CHARACTERIZATION OF *ECHINOCOCCUS GRANULOSUS* ISOLATED FROM HUMAN AND SHEEP IN EUPHRATES REGION OF IRAQ

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### ABSTRACT

The study included examine of 25 samples of hydatid cysts of *Echinococcus granulosus* (20 human samples and 5sheep samples) During the study period from January 2019 - December 2019, Human hydatid cysts samples were collected from some hospitals in the middle Euphrates provinces, which are the hospitals of Al-Muthanna, Al-Diwaniyah, Al-Najafalashraf and Babil province hospitals, Sheep hydatid cysts samples from the animals abattoirs in the same provinces. The study aimed to determine the morphological characters of hydatid cysts and protoscolexes and differences between strains by studying the numbers of number,shape, Size and color of germinal layer of hydatid cysts also number of rostellar hooks, arrangement, measuring the total length and blade length (BL) of hooks, Size of smallhooks (SH) and large hooks(LH) for protoscolexes its shapes in studied hydatidcysts.

The highest percentage of organ infection in humans was in the liver by 75%, then the lungs by 25%,no cysts the spleen and other organs was recorded. While in sheep, it was 80% in the liver and spleen by 20%, and no cysts in lung was recorded, As for the colors of the germinal layers, 60% was recorded for white color and 40% for yellowish white, and the predominant color is white in humans. And when studying the diameters cysts In the affected organs, it was found that the diameter of the cysts ranged between 5 - 65 mm, No significant differences In the number and size of cysts in both hosts, the numbers of cysts ranged from 1 to more than 10 cysts.

There are significant differences between the number of hooks, as it appeared that the average number of small hooks (SH) in a human is 16 hooks, While the average number of large hooks (LH) was 13.4, the total number was 29.4, While In sheep the average number of small hooks was 17.2, and the average number of large hooks (LH) was 14.6, and the total number of hooks was 32 hooks.

This results may be refers to presence different of *E. granulosus* in human than the sheep.

**Keywords:** Morphological, *Echinococcus granulosus*, human, sheep, Euphrates region.

### Introduction

Echinococcosis or Hydatidosis is a parasitic disease which is caused by the larval stage of metacestode *E. granulosus* is one of the most important zoonotic diseases in the world.It is one of the most important zoonotic diseases in humans and domestic animals (Hama *et al.*, 2015). This disease leads to many medical, veterinary and economic problems, and it represents a public health problem, especially that affects rural and poor communities.

Many authors has shown the existence of more than one strains of *E. granulosus*, which differ from each other in some phenotypic, genetic, physiological and immunological aspects, As the diversification process in *E. granulosus* is an important issue that has been clarified by many studies; Thus strains are expressed as groups that differ from each other in characteristics, or it is expressed as local communities that do differ in morphological and biological characteristics and include a geographical definition and have a geographical basis and that have significant differences in epidemiology, growth rate, antigens, pathogenicity to humans, or sensitivity (Eckert & Thompson, 1988).

The phenotype of *E. granulosus* is an important criteria in taxonomic studies. The studies differed in terms of the morphological features chosen in the study, however recent studies have focused on rostellar hooks in both larvae and adults, as well as ovarian shape and testicular distribution of granulomatous echinococcosis to distinguish between strains collected from different intermediate hosts in Europe (Eckert *et al.*, 1993) and Spain (Gordo & Bandera, 1997).

### Materials and methods

#### Samples collection

25 cysts of humans and sheep (20 from human and 5 from sheep) were collected from January 2019 to December 2019 from the regions of the Middle Euphrates / Iraq. Human hydatid cysts samples were collected after surgical operations from some hospitals in the middle Euphrates provinces, which are the hospitals of Al-Muthanna, Al-Diwaniyah, Al-Najafalashraf and Babilprovince hospitals, Sheep hydatid cysts samples from the animals abattoirs in the same provinces .The organs examined were the liver, lungs, and spleen for the presence of hydatid cysts.

Hydatid cysts were identified in the organs of infected sheep, either from the presence of a white or yellowish white layer on the surface of the external organ, which resembles a bubble, especially in the liver as it is very clear. These samples were transferred to the parasitology laboratory in the College of Education / University of Al-Qadisiyah by refrigerated plastic boxes or directly from hospitals or the slaughterhouse to the laboratory.

**Hydatid cysts prevalence and it’s features in human and slaughtered sheep**

Certain biological aspects were recorded during samples collection and the isolation of the hydatid cysts components of the animals. These aspects included disease prevalence and characterization of affected organs and the cyst such as color, shape, and diameter.

**Morphological study**

Protoscoleces are preserved in polyvinyl lactophenol solution and placed on a glass slide and covered with a slide cover with appropriate pressure to make it flat. The components of the hooks (Hobbs, Lymbery, & Thompson, 1990) are then measured by measuring the total length and blade length (BL), Size of small hooks (SH) and large hooks (LH) for protoscoleces of two hooks from each rostellum. For at least ten Protoscoleces using a 100x objective lens under a microscope to demonstrate the differences between strains (Zar, 1984).

**Results**

Human samples were obtained in this study from the affected organs, which included the liver and lungs, while the sheep included the liver and spleen figures (1-3).

It appeared that the highest percentage of organs infection in humans was in the liver at 75%, and in the lungs 25%, and no cysts in the spleen was recorded. While in sheep, it was 80% in the liver and 20% in the spleen, as shown in Table (1)

**Table 1 :** Illustrates distribution of Hydatid Cysts According to infected Organs.

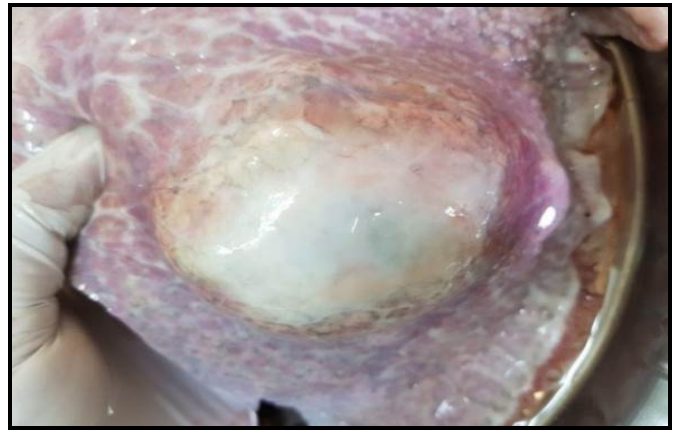
Host infected Organs	Human		Sheep		Total	
	No.	%	No.	%	No.	%
Liver	15	75	4	80	19	76
Lung	5	25	—	—	5	20
Spleen	—	—	1	20	1	4
Total.	20		5		25	



**Fig. 1:** Represents a hydatid cysts infection in the liver isolated from sheep



**Fig. 2 :** Represents an infection of the hydatid cysts in the lungs isolated from sheep



**Fig. 3:** Represents an infection of the hydatid cysts in the spleen

Two colors of the inner germinal layers of hydatid cysts were observed in the present study which are white and yellowish-white figures (4) (5).

The inner layers of the white color hydatid cysts were 60% and 40% yellowish white color, and the white color was predominant in both hosts As in Table (2).

**Table 2 :** The color of the inner layers in the hydatid cysts of the infected human and sheep organs.

Color	Human	Sheep	No.	%
white	10	5	15	60
yellowish white	10	—	10	40
Total	20	5	25	100



**Fig. 4 :** The inner layers of hydatid cysts isolated from infected sheep.



**Fig. 5 :** The inner layers of white hydatid cysts are isolated from an infected human

The results of this study showed that the higher infections were in the liver in humans and sheep, with varying numbers, followed by lung infection. No infection to the spleen was observed in humans, while there was one in the spleen of sheep, it was found that the numbers of cysts ranged from 1 to more than 10 cysts in both hosts, Table (3).

**Table 3 :** Shows the number of cysts in different organs of human and sheep.

Cysts number	Liver				Lung				Spleen			
	Human		Sheep		Human		Sheep		Human		Sheep	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1-4	—	—	—	—	5	100	—	—	—	—	1	100
5-7	5	33.3	—	—	—	—	—	—	—	—	—	—
8-10	5	33.3	2	50	—	—	—	—	—	—	—	—
10 <	5	33.3	2	50	—	—	—	—	—	—	—	—
Total	15		4		5						1	

Diameters of the hydatid cysts have been studied in humans and sheep collected during the present study. Cyst diameters ranged between 5 - 65 mm, and no significant differences in size were observed in both hosts, as in table (4).

**Table 4 :** Shows the diameters of the hydatid cysts in the affected organs of humans and sheep

Hydatid Cysts diameters (mm)	Human	Sheep	No.	%
5 – 25	3	1	4	16
26 – 45	5	4	9	36
46 – 65	10	—	10	40
65 <	2	—	2	8
Total	20	5	25	

The current study showed that the average number of SH in humans is 16, while the average number of LH is 13.4, and the total number of hooks is 29.4. In sheep, the average number of SH was 17.2, while the average number of LH was 14.6 and the total number of hooks was 32. table (5).

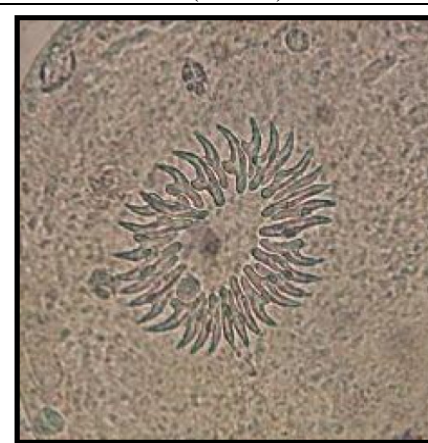
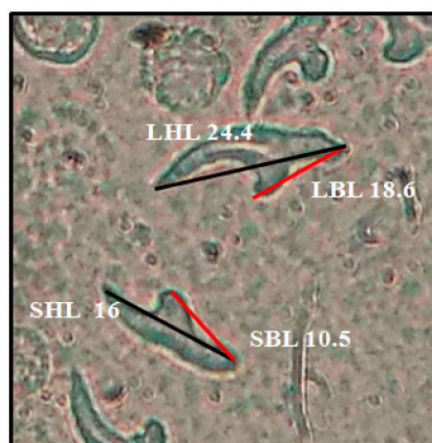
**Table 5 :** Shows the number of total hooks and the number of small and LH.

Host	Mean ± SEM		
	The number of SH	The number of LH	Total number of hooks
Human	16 ± 0.858 E	13.4 ± 1.146 D	29.4 ± 1.964 D
Sheep	17.2 ± 1.404 C	14.6 ± 0.665 C	32 ± 2.166 B
Significant difference (P<0.05)			

The current study also showed in figure (7) that the average length of the SH in humans is 16 microns and the average of BL is 10.5 microns, while the LH, the average length of the hook is 24.4 microns and the BL is 18.6 microns. While the average length of the SH in the sheep was 19.8 microns and BL is 10.9 microns. For LH, the average hook length is 24.1 microns and the average of BL is 14.4 microns. table (6) and figures(6 A,B,C).

**Table 6 :** Shows the lengths of LH and SH and the lengths of their blades

Host	Mean ± SEM		
	Small hook length	The length of her blade	Big hook length
Human	16 ± 1.736 E	10.5 ± 1.003 D	24.4 ± 0.882 D
Sheep	19.8 ± 1.111 C	10.9 ± 0.759 C	24.1 ± 1.086 E
Significant difference (P<0.05)			



**Fig. 6 A,B,C :** Shows the hooks under the microscope (10x and 40X magnification power)

## Discussion

The current study indicates that the liver and lungs in humans, and the liver and spleen in sheep are the most susceptible to infection with hydatid cysts.

The study showed that the rate of liver infection in humans is 75% and in the lungs is 25%. In sheep, the percentage in the liver was 80%, and in the spleen 20%.

In human the current study is agreement with previous studies on hydatid cysts disease, such as the study (Al-Khafaji, (2006)), (Al-Ghezi, 2008) and (Taher, (2009)) which recorded rates of 61.8%, 55.6% and 76.67%, respectively, and it also agrees with (Baraak, 2014) that the liver is The organ most exposed to infection, as it was found that 50% of the infections were in the liver, 30% in the lungs, and 20% in other organs. It also agreed with (Agha, 2015) in the province of Diwaniyah, where liver infections were recorded at 91.66%.

While this study differed from what was recorded (Ahmed, Mero, & Salih, 2012) , as most human lung infections were 58.82%, and liver infections formed the lowest rate 41.18%.

In sheep, the results of the current study are in agreement with previous study conducted in Najaf, where the liver infection rates of sheep were 76.92% (Al-dujaily & Al-mialy, 2017). And in Basra (Murtaza, Al-Azizz, Abdulhameed, & Kadhim, 2017) that the liver is the most affected organ in sheep, as it constituted 61.6%.

It was found in some neighboring countries, such as Iran, in Lorestan province, (Parsa, Haghpanah, Pestechian, & Salehi, 2011) That the rates of infection in sheep liver is 57%, and this is consistent with the current study. In a study conducted in Saudi Arabia, (Toulah, El Shafi, Alsolami, & Wakid, 2017) it was mentioned that the prevalence of infection in sheep liver is 73.89%.

The current study differs from what recorded with (Azami, Anvarinejad, Ezatpour, & Alirezaei, 2013) in sheep as lung infections were the highest.

The liver is primarily affected by hydatid cysts, as it is released hexacanthembryo that penetrates the intestinal mucosa. And its migrate passively through the blood in the portal vein to the liver. One (or more) embryos develop into a hydatid cyst containing the cyst fluid and protoscoleces (Zhang, Chen, & Wen, 2017).

The human liver has a larger right lobe and a large right portal vein compared to the left side. When the superior mesenteric vein and the splenic vein meet in the portal vein, most of the blood goes to the right portal vein, thus carrying more parasites into the right lobe .The majority of hydatid cysts human liver (60% -80%) are found in the right lobe (R.-Q. Zhang *et al.*, 2017) While other studies reported that the lung of the intermediate host is the place of infection of the hydatid cysts, and this is due to the large lymph vessels, which provide an opportunity for the fetus to reach the lymphatic capillaries, which travel through the lymphatic vessels to the lungs before being transported in the veins to the liver (Elmajdoub & Rahman, 2015).

The current study showed that there are two colors for the inner layers of the hydatid cysts, which are white and yellowish white color, the inner layers of the hydatid cysts

were white by 60% and the yellowish white by 40%, the largest percentage of the layers are white.

This is consistent with what was mentioned (Hammad, 2017) in Kirkuk and Sulaymaniyah, where the white layers were the layers with the largest percentage, as it was in sheep at a rate of 95%, this is in Kirkuk, but in Sulaymaniyah, it also agrees with the current study, in sheep it was 64.71 white and 29.41% yellowish white.

It also agrees with a study (Al-Rishawi & Al-Mayali, 2019) that the colors of the inner layers of the hydatid cyst ranged from white to yellowish white, as it was 100% in sheep. The difference in the layers surrounding the hydatid cysts between different hosts is due to several reasons, some of which may be related to the host and others due to the parasite itself. The outer laminated layer is a fibrous capsule made up of white or collagenous fibers, which are strong, duct-resistant fibers formed by fibroblasts, which are connective tissue cells that are scattered around that layer, and this procedure is part of the body's resistance to the hydatid cysts, and this is supported by the researcher (Zhang, Li, & McManus, 2003) The metabolism of the host may be related to the color of the inner layers of the hydatid cysts. As for the reasons related to the parasite, it may be due to the different strains of the parasite, its fertility and infertility, as well as on the chemical components of the cysts and the hydrophobic fluid. The results of the current study showed that the percentage of cysts in human liver is 33.3% for each of (5-10) and (8-10) and greater than 10, and its percentage in sheep liver is 50% for each of (8-10) and greater than 10. While for the human lung, the percentage was 100% (5 infections). No infection was observed in sheep lungs. The rate of infection to the spleen of sheep was 100% (one infection), and no infection to the human spleen was observed.

The largest percentage of livers containing 8-10 hydatid cysts , at a rate of 25%, followed by those with 5-7 and more than 10 hydatid cysts, at 17.5%, and the lowest percentage was in the group that containing of 1-4 cysts at 7.5%.

In a study conducted in the Al-Qadisiyah province (Agha, 2015), which recorded a rate of 75% for those infected with cysts 3-1, and 25% for those with more than 4 cysts. The researcher attributed the reason for this to the fact that human food is usually clean and washed well, which leads to disposal of most eggs if the food was contaminated with them.

In a study conducted in the city of Kut (Rahi & Ali, 2016) all of the infected had one cysts in the organ for humans.

The current study disagreed with (Taha & Hassen, 2018) the number of hydatid cysts in any of the organs ranged from one to about 20 for each lung and two for each liver. The total number of organs containing a hydatid cyst or more was 58 (78.38%) for the lungs and 14 (18.92%). for the liver, 1 (0.8%) for the spleen, and 1 (0.8%) for the heart.

The reason for the variation in the number of cysts in the organs may be due to the number of embryos that reach the affected organs, and this may be due to the repeated eating of food contaminated with the parasite eggs as a result of the spread of the final hosts (for example, infected dogs) in the pastures in which these animals graze. Infested with eggs

of the *E. granulosus*. Or the cause may be attributed to variation in strains and geographic regions, different environmental conditions, or host immunity.

Cattle herders tend to sell older livestock to butchers and keep younger livestock; As well as the fact that these older animals contain previous and old infections, which may explain the increase in the number of cysts in the target organ, and this corresponds to (Ibrahim, 2010)

The diameters of the hydatid cysts in humans and sheep were studied in the current study, and the cysts' diameters ranged between 5 - 65 mm and the size of the largest cysts was 46 - 65 mm by 40% in humans, followed by cysts with a size larger than 65 mm by 8% in humans.

The present study agreed with a study conducted in China where it was found (Mao *et al.*, 2017) that the diameter of the hydatid cysts in sheep's liver ranges in diameter from 1 to 36 mm, followed by the hydatid cysts of the sheep lung from 1 to 28 mm.

In another study (McManus, Zhang, Li, & Bartley, 2003), it was reported that the size of the hydatid cysts varies from a few millimeters to a large diameter of 150-200 mm.

The size of the hydatid cysts depends on the rate of its growth, which may be influenced by the age of the affected host, the place of the infection and the host's immune response.

The size of the hydatid cysts increases with the age of the affected animal. Several cases of hepatocellular cyst infection have shown that the liver tissue is resistant and surrounding the cyst, determining slow growth or even avoiding growth for many years. Therefore, this explains the small size of the cysts in the liver. Whereas, the lungs show low resistance to the growth of hydatid cysts due to their elasticity. This statement allows for a proportional increase in the size of the hydatid cyst in the lungs (Pednekar, Gatne, Thompson, & Traub, 2009) and (Dopchiz *et al.*, 2011).

As for the lengths of hooks, the current study showed that the average number of small hooks in human is 16 hook, while the average number of large hooks is 13.4 hook and the total number of hooks is 29.4. hook In sheep, the average number of small hooks was 17.2 hook, while the average number of large hooks was 14.6 hook, and the total number of hooks was 32 hook.

As for the lengths of the hooks, the current study showed that the average lengths of the SH in humans are 16 microns, the average of blades is 10.5 microns, for the LH, the average length of the hooks is 24.4 microns and the is 18.6 microns. As for sheep, the average lengths of the SH are 19.8 microns and the blades is 10.9. For large hooks, the average of hook length is 24.1 microns and the average blades is 14.4 microns.

The results of the current study are consistent with a study (Hama & Shareef, 2016) in Sulaymaniyah city, where it found that the average total length of the LH isolated from sheep was  $22.24 \pm 1.300 \mu\text{m}$ , and the BL measurement of the LH which are isolated from sheep was  $10.55 \pm 1.700 \mu\text{m}$ . This study also recorded that the total length of the SH was  $0.900 \pm 17.60 \mu\text{m}$ , and the BL of the SH measured  $1.00 \pm 8.10 \mu\text{m}$ .

Our study is in agreement with a study (Fadakar, Tabatabaei, Borji, & Naghibi, 2015) in Iran which reported that the average total length of LH isolated from sheep was  $23.43 \pm 0.600 \mu\text{m}$ , BL  $12.14 \pm 1.000 \mu\text{m}$ .

The study under investigation was also in agreement with what was recorded (Latif, Tanveer, Riaz-Ud-Din, Maqbool, & Qureshi, 2009), as the total number of hooks in sheep was  $1,270 \pm 31.40$ .

The current study disagreed with results (Mustafa *et al.*, 2015) in Pakistan, who found a large significant differences in the total number of hooks in sheep of  $28.68 \pm 3.800$  hook.

The arrangement of the hooks of the protoscoleces in the *E. granulosus* in this study agreed with other studies. These studies reported that these hooks are arranged in two alternating rows of LH (upper row) and SH (lower row) (Almeida *et al.*, 2009; Andresiuk *et al.*, 2009; Calderini, Gabrielli, & Cancrini, 2012; Gordo & Bandera, 1997; Hussain, Maqbool, Tanveer, & Anees, 2005; Latif *et al.*, 2009; Lymbery, 1998; Singh *et al.*, 2014)

They also mentioned (Gholami, Irshadullah, & Mobedi, 2011) that the hooks in all Protoscoleces collected from different livestock (sheep, cows, and camels) contain two rows of LH and SH alternately. While they found that about 25% of the hydatid cysts samples isolated from buffalo contain either two SH between the LH or two LH between the small. The reason for the variation in the measurements of the hooks in the heads may be due to the variation in the host, as the internal environment of the host bodies, such as physiological factors, play a role in the differences in the morphological characteristics of the *E. granulosus* parasite, and this supports what it's mentioned (Z. A. Ibrahim, Muhsin, & Risan, 2009) and the place of infection in the host's body also has an important role in determining. Many morphological characteristics, in addition to the difference in strains between *E. granulosus* are supported by (Salam Jumaah Hammad, Cavallero, Milardi, Gabrielli, & Al-Nasiri, 2018).

This differences in number and size of large and small hooks and total length of blades may be due to differences in strains of *E. granulosus* infected the different hosts in other countries and regions.

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