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Morphological and morphometric study of *Echinococcus granulosus* (metacestode) in Sulaimani Province/ Kurdistan Region, Iraq

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Abstract

Hydatid disease (echinococcosis) is a parasitic infection caused by the larval stage of *Echinococcus granulosus with the global* distribution. This disease is endemic in Iraq and Kurdistan Region with no strategy and long-term control plan. The strain identification of this parasite is essential to improve both monitoring and prevention of the disease. For this reason, the present on the morphological characterization of the larval stage of *Echinococcus granulosus* isolated from sheep, cattle, and human beings, was carried out in Sulaimani as an atempt to determine the polymorphism among echinococcus hooks isolated from diferent hosts. The morphometric of hooks were evaluated by (computerized morphometric) software.

A significant difference was found in the blade length and width (guard) of the hook isolated from a different intermediate host of *Echinococcus*. This finding suggested that different strains of *E. granulosus* are responsible for animal and human infection in Kurdistan-Iraq.

Key words: Echinococcus granulosus, hook, hydatid cyst, strain.

Introduction

Hydatid disease is a silent zoonotic infection caused by the larval stages of *Echinococcus granulosus* with global distribution (Mero *et al*., 2013). Cystic echinococcus poses a significant economic and public-health concern in Iraq and Kurdistan (Hama *et al*., 2012). Humans become infected accidentally by ingesting food or water contaminated with fecal material containing *E. granulosus* eggs passed from infected carnivores, or during handling pet or infected dogs (Eckert *et al*., 2002; WHO 2006; Satoskar, *et al*., 2009). The larval stage (metacestode) of Echinococcus can grow in various body organs, the commonest sites of infection are the liver and lungs (Markell *et al*., 1999), whereas, the transmission of the disease from human to human and between other intermediate host are not recorded (Rood and Kelly, 2009).

The strains of *E. granulosus* were adapted with different intermediate hosts *such as* sheep, pigs, cattle, horses, camels, goats and cervid (Sánchez *et al*., 2010).

There are ten distinct genotypes (G1-G10) based on the molecular tool (Rinaldi et al., 2008), also morphological and biochemical criteria were studied for strain identification by many researchers (Kumaratilake and Thompson, 1983), (Latif *et al*., 2010). The strains of *E. granulosus* were adapted with different intermediate hosts such as sheep, pigs, cattle, horses, camels, goats and cervid (Sánchez *et al*., 2010), at least, seven of these strains were infective to human (Eckert *et al*., 2002).

The morphological and biochemical characterization were reliable methods for recognizing of *E. granulosu* strains (*Harandi et al*. 2002), identification of the strains which are responsible for human echinococcosis is a significant point for control and the means of diagnosis (Hama *et al*., 2012).

Echinococcosis is considered a major endemic disease in Iraq and Kurdistan and its seriousness in humans and animals have been recognized by many epidemiological and biological studies (Magid, 2008, Abdullah, 2010). Although there are no adequate molecular, morphological and biochemical studies of *E. granulosis.*

The morphological and molecular characterization will open a new clue in strain identification and will be of great help in building effective control measures and predictive epidemiology (Rahimi *et al*., 2007).

Materials and Methods

The hydatid cysts isolated from sheep (n=15) and cattle (n=5) collected from new Sulaimani abattoir, and the human liver hydatid cysts (n=6) collected from Sulaimani scientific hospital after the surgical operation. The fluid and a germinal layer of each cyst were collected in the sterile tube individually, then transferred to laboratory directly. The cyst fluid and germinal layer were washed three times with phosphate buffer solution (PBS) (Latif *et al*., 2010). The protoscoleces were separated using centrifugation and preserved in 10% formalin. The protoscoleces were gently crushed under cover slip in a drop of normal saline then 188 hooks from different protoscolex (125 large hooks and 63 small hooks) were measured for total, blade, width (guard) and hand.

A procedure described by Almeida *et al*., (2015) was followed for hook mesuerment using a microscope (Olympus, UK) supported with a camera and morphometric software (Micam). Statistical analysis was carried out using one-way ANOVA, Chi-square test to determine the probability value (p-value) by using SPSS (version 17).

Results

The microscopic examination shows morphological aspects of protoscoleces and hook. There are two rows of hooks on the rostellum with a large hook intercalated by a small hook (Figure 1A), both large and small hooks vary morphologically, although all hooks possess a handle, blade, and width (Figure 1B). The result of this study confirmed morphologically there are different strains of *Echinococcus granulosus* in Kurdistan (Figure 2).

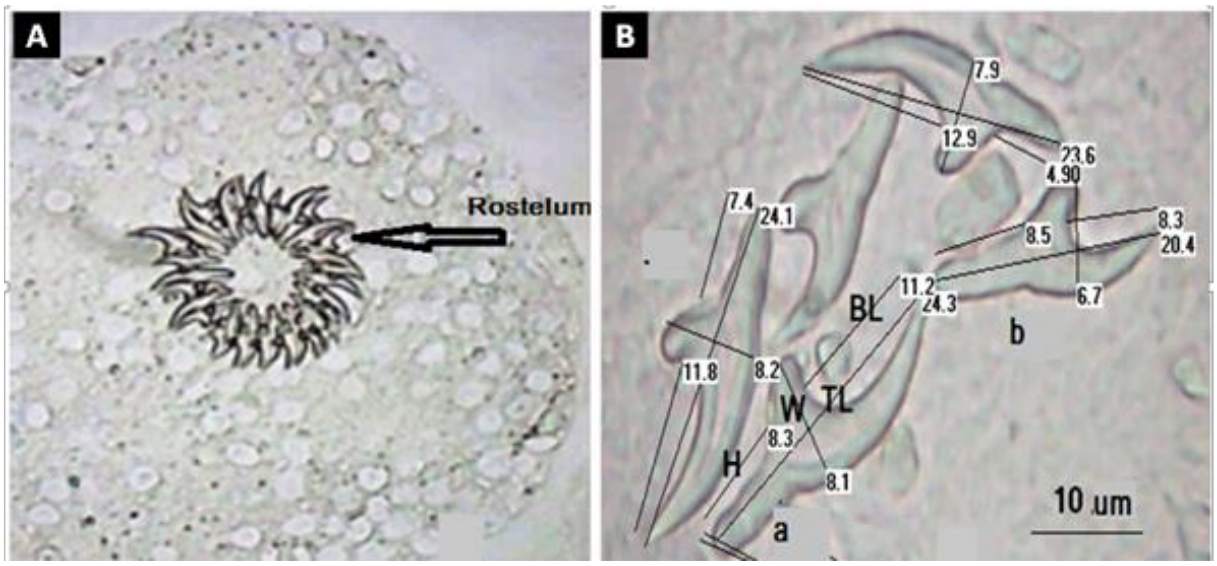

Figure 1: Photometric graph of Echinococcus granulosus metacestodes isolated from sheep. **A)** Represent rostellum of protoscoleces double row of hook 10x. **B)** Represent large and small hook of protoscoleces 40X, a= large hook, b= small hook. H=Hand, W=Width (guard), BL=Blade, TL=Total length.

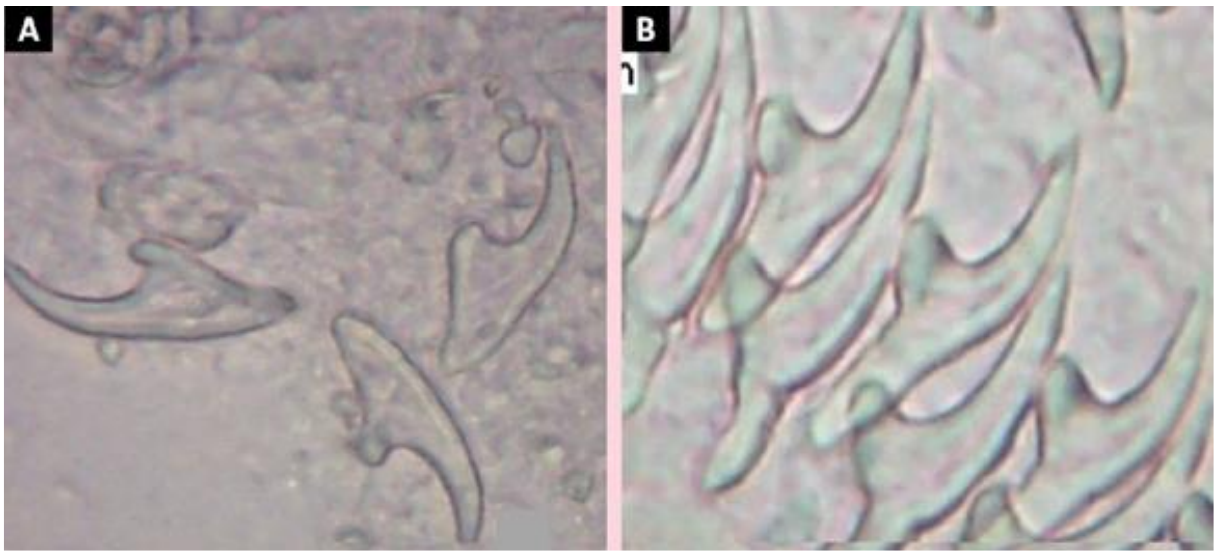

Figure 2: Photometric graph of Echinococcus granulosus metacestodes. The rostellum hooks from different intermediate hosts. **A)** Isolated from human liver cyst 40x. **B)** Isolated form sheep liver cyst 40x.

Table (1) shows the morphological structure of small hook, the mean of the total length of the small hook (TL) of human, sheep and cattle isolates were 17.6 µm, 17.6 µm, and 18.6 µm respectively. The significant differences were shown in hand and width (guard) region of the hook isolated from human, sheep and cattle.

Table (2) shows morphometric of the large hook of the rostellum from the different intermediate host. The total length of large hooks of human, sheep and cattle isolated were 22.3 µm, 22.24 µm, and 22.17 µm respectively, the statistically significant difference ($P \leq 0.05$) were recorded only in the blade region of the hook of a different source (intermediate hosts).

Table 1: The mean±SD of the total length, blade, width (guard) and hand of Small hooks.

Intermediate host	Human	Sheep	Cattle	total	P-value
Total number of the hook (n)	20	39	4	63	
Total length (µm)	17.6±1.3	17.6±0.9	18.6±0.8	17.7±1	0.28
Blade (µm)	8.7±1.1	8.1±1	6.8±1	8.1±1	0.6
Hand (µm)	6±1.4	7±0.4	9.5±0.8	8.88±1	0.00
Width (guard) µm	5.2±0.6	6±0.7	6.4±0. 8	5.78±0.7	0.00

Table 2: The mean±SD of the total length, blade, width (guard) and hand of large hooks.

Intermediate host	Human	Sheep	Cattle	Total	P-value
Total number of the hook (n)	22	67	36	125	
Total length (µm)	22.3±1	22.24±1.3	22.17±1	22.23±1.3	0.9
Blade (µm)	11.5±1.4	10.55±1.7	10.65±1.8	10.7±1.6	0.03
Hand (µm)	6.89±1	7.26±14	7/54±0. 8	7.27±1	0.08
Width (guard) (µm)	6.69±1.1	6.7±0.7	7.1±0.7	6.82±0.8	0.06

Discussion

The rostellum hooks morphology and size have been used for identification of species and strain in *Echinococcus* and other cestodes by Hobbs *et al*. (1990). Many researchers have suggested that the hook shape is influenced by the host (Sweatman and Williams, 1963, Hobbs *et al*., 1990). While the blade lengths of the large and small hook were not affected by the host, that make it more reliable aspect than hook number and total hook length for separation of different species and strains of *Echinococcus*.

This conclusion will decrease their precision as taxonomic tools (Lymbery, 1998). A similar morphological study in Peru (Almeida et al., 2009) agreed with the finding of the present study for the presence of polymorphism among rostella hooks. Similarly, Antoniou and Tselentis (1993) suggested that width (guard) of large hooks was more slender than the guard of the small hooks.

The present study has confirmed the existence of different strains of *Echinococcus granulosus* in Kurdistan-Iraq, this result agree with (Hama *et al*., 2012) study that identified sheep and cattle strain in Kurdistan-Iraq using a molecular marker. Also, this study aimed to discriminate strains of *Echinococcus granulosus* morphologically, a result which is in agreement with the previous study in Kurdistan carried out by Hama *et al*., (2015). The accurate diagnosis of *Echinococcus* species and strains is valuable from an epidemiological and control point of view.

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