



MANIFESTATION OF *CYSTICERCUS TENUICOLLIS* CYST INFECTED ORGANS OF SLAUGHTERED SHEEP WITH DETERMINATION OF REACTIVE PROTEINS OF CYST FLUIDS BY SDS-PAGE

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Abstract

The *Cysticercus tenuicollis* cyst : A high prevalence of infection in sheep slaughtered in Iraq and most of the cysts were attached to the liver and omentum. In this study, determination of total Protein of cyst fluid (10.98 ± 0.02 mg/ml). And also determination of protein bands of *Cysticercus tenuicollis* cyst fluid, by using [SDS-PAGE] that showed three polypeptides were recognized in cystic fluid antigens of *Cysticercus tenuicollis* it is 63kDa, 48 kDa and 70 kDa as polypeptides.

Key words : SDS-PAGE, Iraq, sheep, *Cysticercus tenuicollis*.

Introduction

Cysticercus tenuicollis cyst is a larval stage of canine tapeworm *T. hydatigena*. The sheep and others domestic and wild herbivores, including horse and pig, act as intermediate hosts. Wolf, dogs, hyena and other wild carnivores are the final hosts (Taylor *et al.*, 2007; Smith and Sherman, 2011; Oryan *et al.*, 2012).

Adult *T. hydatigena* tapeworms in the final hosts are found in the intestine, which can harbour tapeworms from several months to a year or more (Smith and Sherman, 2011; Taylor *et al.*, 2007). Tapeworm segments, containing eggs, which pass into the external environment through the feces. After disintegration of segments, eggs are disseminated in pastures by insects and wind (Hansen, 1995). Intermediate host animals, it is infected through the ingestion of the hatch eggs of tapeworm in the intestine. The oncospheres of parasites are carried to the live through the blood which they migrate to the surface of the organ and attach to the peritoneum. And develops into the large metacestode, *Cysticercus tenuicollis* (Hansen, 1995; Taylor *et al.*, 2007) found attached to the greater omentum, mesentery and liver (Kaufmann, 1996).

Aberrant migrations sometimes occur with *Cysticercus* found in the other organs like the lungs (Smith and Sherman, 2011). *Cysticercus tenuicollis* have diameter about 5-8 cm, within a single invaginated scolex (bladder worms) and have a long neck (Hansen, 1995; Taylor *et al.*, 2007).

Immune reactive proteins of cystic fluid antigens and whole cyst lysate antigens of *Cysticercus tenuicollis*, are eight major polypeptides of molecular weight (Mr) 9.6 kDa, 15.7 kDa 23.9 kDa, 36.2 kDa, 63.5 kDa, 74.2 kDa, 92.9 kDa and 149.4 kDa were resolved by SDS-PAGE with minor variations. Out of these, three polypeptides of Mr 9.6 kDa, 36.2 kDa and 23.9 kDa were recognized as immunodominant polypeptides (Goswami *et al.*, 2013).

Examination, of *C. tenuicollis* infected sheep were also seen protein bands are 6.1, 14, 16, 23, 27.5, 36, 45, 55, 66, 116 kDa in the gel had a strong appearance and also appearance weak bands (Kara, 2003). Determining immunogenic protein band for *Cysticercus tenuicollis* in sheep by cyst fluid by using SDS-PAGE may have importance role in the vaccination and diagnostic kit studies for *Taenia hydatigena* or its larva for dog, sheep and other animals parasite.

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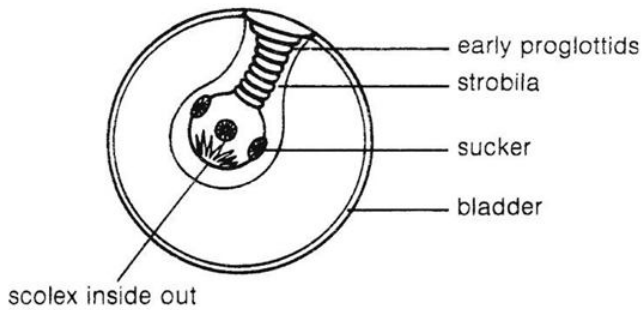


Fig. 1 : *Cysticercus tenuicollis* structure (Hale *et al.*, 2005).

Materials and Methods

The collection of the cysts of *Cysticercus tenuicollis*

In the present study, the cysts of *C. tenuicollis* were collected randomly during inspection of the sheep carcasses in the abattoir and transferred into the laboratory for examination and checking. *Cysticercus tenuicollis* cysts initially identified according to their feature such as a long-necked single scolex, virtually translucent cyst fluid and hook morphology (Essa and Al-Azziz, 2011).

Collection *Cysticercus tenuicollis* cyst fluid

By distilled water washed cysts than collected the fluid by puncturing.

The fluid was centrifuged for 10 minutes at 13.000 rpm at 4°C and filtered The supernatant by use Millipore filter (0.45 µm) and keep at 4°C (Kara and Dođanay, 2005).

Determination of reactive proteins by SDS-PAGE

A- 5ml of 10% polyacrylamide Gel Mix- Separating Gel and Stacking Gel.

Materials needed

5 ml of 10% separating gel

2 ml ddH₂O

1.25 ml 1.5M Tris pH 8,8

1.7 ml 30% Acrylamide

50 ml 10% APS

50 ml 10% SDS

5 ml TEMED

Making the separating gel

1. Begin with the 2 ml of ddH₂O
2. Add 1.67 ml of the 30% Acrylamide - bis acrylamide solution.
3. Add 125 ml of 1.5 M Tris solution pH 8.8 and mix.
4. Mix in 50 ml 10% SDS.

5. When ready to use, add 5 ml of TEMED and mix goods.

6. Immediately before pouring the gel, add 50 ml of 10% APS and mix.

Making the stacking gel

1- Begin with 1.1 ml of ddH₂O.

2- Add 0.4 ml of 30% Acrylamide/bis-acrylamide solution.

3- Add 0.3 ml of 0.5 M Tris pH 6.8 and mix.

4- Mix in 20 ml 10% SDS.

5- When ready to use, add 5 ml of TEMED and mix.

6- Immediately before pouring the gel, add 50 ml of 10% APS and mix.

The solution can be used immediately, or it can be stored for 1 week at 4°C.

A small amount of bromophenol blue can be added to aid In observing the dye. Front while the gel is running.

The solution should be pouted right away as it will begin to polymerize after the of APS

B- Coomassie staining of protein gels (Brian, 2016).

The most common methods of staining protein gels using Coomassie:

✓ 0.1% w/v Coomassie Blue R-250

✓ 50% v/v Methanol

✓ 10% v/v acetic acid

- After the gel has finished running, remove it from the tank and remove the gel from the glass/plastic plates
- Place the gel in a container and briefly rinse in H₂O.
- Add enough Coomassie to cover the gel and either put a lid on the container or cover it in cling film to prevent the Coomassie from evaporating
- Place the container on a rocker for overnight
- Once the gel has stained sufficiently, poor the used Coomassie off into the used Coomassie container and rinse the gel briefly in H₂O.
- To de-stain, role up some tissue paper and place in the dish with the gel, but make sure the gel doesn't stick to the paper or it will rip – this step is optional
- Then cover the gel in de-stain solution and place on a rocker for 30 minutes
- Next, remove the de-stain solution and replace with fresh and replace the tissue
- Return to the rocker until the gel reaches a satisfactory clarity

- Rinse the gel in H₂O and image and/or store the gel
- **The destain solution for Coomassie can be made up as follows:**
 - 50% v/v Methanol
 - 10% v/v acetic acid

Determination of Total Protein by biuret method

Principle (Gornall *et al.*, 1949; Tietz, 1995)

Colorimetric method, The peptide bonds of Cu₂⁺ proteins react with alkaline solution to form complex a coloured that which absorbance, at 550 nm.

Statistical analysis

Statistical analysis was applied by using statistical package for social sciences (SPSS).

Results

Cysticercus tenuicollis cyst manifestation

A high prevalence of infection with *C. tenuicollis* was demonstrated in sheep. The results showed that most of the cysts were attached to the liver and Omentum. And take the Cysts from liver and omentum.

Table 1 : Percentages of infected organs of slaughtered animals with *Cysticercus tenuicollis* cyst.

No. of animals	Infected animals (%)	Liver	Omentum
220	(23) 11.5%	14%	86%

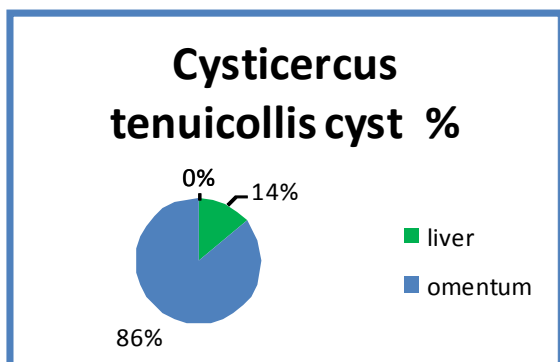


Fig. 2 : Percentages of infected organs of slaughtered animals with *Cysticercus tenuicollis*.

The external cyst wall was semi-transparent, white in color, some with yellowish. The wall of the bladder was transparent, faint and contained clear watery fluid. The cysts was bulging with a thin, long neck that hanging in the cyst fluid, which isolated from slaughtered sheep, The cysts consisting of one an invaginated scolex surrounded by a transparent membrane with clear fluid filed cyst cavity. Also, the present results showed that the size of the cysts was different between slaughter

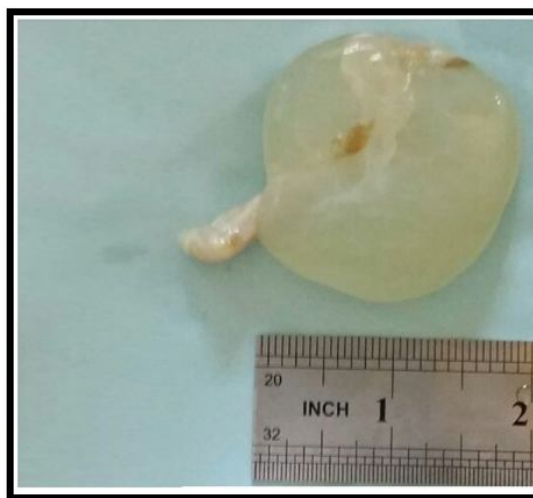


Fig. 3 : Morphological appearance of *Cysticercus tenuicollis* cyst that attached to the omentum. The cysts appears as bladder cyst filled with clear fluid and elongated neck.



Fig. 4 : Morphological appearance of *Cysticercus tenuicollis* cysts found in the liver of infected sheep. The cysts content of one an invaginated scolex surrounded by a transparent membrane with clear fluid filed cyst cavity.

sheep in diameter (figs. 3 and 4).

Determination of total protein of cyst fluid

The ten cysts were selected for Biochemical analyses to Determination of Total Protein of cyst fluid by biuret method was observed the ratio of total protein, it is 10.98 ± 0.02 mg/ml.

Determination of proteins in *Cysticercus tenuicollis* cyst fluids of by SDS-PAGE

The BLUslf prestaine Protein Ladder of SDS-PAGE consist of eight major polypeptides of molecular weight 35 kDa, 48 kDa, 63 kDa, 75 kDa, 100 kDa, 135 kDa, 180 kDa and 245 kDa. Out of these, three polypeptides were recognized in cystic fluid antigens *Cysticercus tenuicollis*



Fig. 5 : *Cysticercus tenuicollis* cyst of infected sheep attached to the omentum. The cysts look as long necked bladder worm, consisting of a transparent membrane with clear fluid filled cyst cavity.

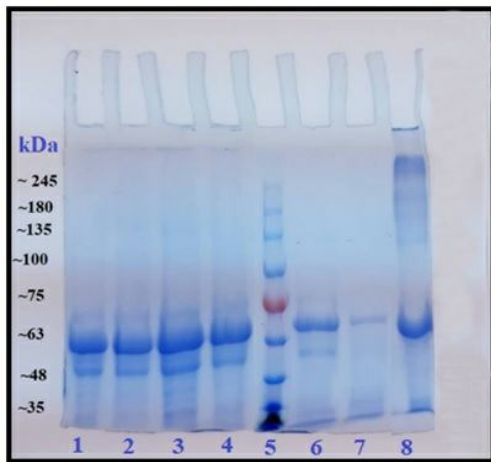


Fig. 6 : Polypeptide profiles of cystic fluid antigens that prepared from *Cysticercus tenuicollis*, resolved in SDS-PAGE and stained with Coomassie. The sample (1,2,3,4) cystic fluid antigens of *C. tenuicollis*, (5) The BLUSlf prestained Protein Ladder (6, 7) cystic fluid antigens of *C. tenuicollis*.

it is 63kDa, 48 kDa and 70 kDa (fig. 6) as polypeptides by SDS-PAGE. All of them could be considered as major polypeptides from each sample with minor variations.

Discussion

Cysticercus tenuicollis cyst manifestation

The result shows *C. tenuicollis* cysts of *Taenia hydatigena* is prevalence in sheep of Iraq that most of the live cysts were bulged that attached to the liver and great omentum. This result agreement with Kara and Doganay (2005). That report *C. tenuicollis* of *T.*

hydatigena, it is a high degree of mortality and morbidity in livestock, sheep intermediate host animals it is infected through the ingestion of the hatch eggs of tapeworm in the intestine. The oncospheres of parasites are carried to the live through the blood which they migrate to the surface of the organ and attach to the peritoneum. And develops into the large metacestode, *Cysticercus tenuicollis* (Hansen, 1995; Taylor *et al.*, 2007)

On the other hand the morphological of *Cysticercus tenuicollis* have diameter about 5-8 cm, within a single invaginated scolex (bladder worms) and have a long neck (Hansen, 1995; Taylor *et al.*, 2007).

Surrounded by a transparent membrane with clear fluid filled cyst cavity (Athmar, 2017) and this identical with our result.

Total Proteins of *Cysticercus tenuicollis* cyst fluid

The concentration of total protein in the cyst fluid of *C. tenuicollis* was 10.98 ± 0.02 mg/ml. In the present study, which agree with the result of Arunkumar and Prakash Krupakaran (2014).

Depends on studies, confirmed the presence of several parasite and host proteins, including serum albumin and immunoglobulin in cyst fluid. Host protein may enter the cyst by by endocytosis or by specific filter or transport mechanisms (Nazifi *et al.*, 2011). And increasing protein reflects its importance in the catabolism and anabolism (growth) activities and mixed production of its by host and parasite (Hustead and Williams, 1977).

Proteins of *Cysticercus tenuicollis* cyst fluids (SDS-PAGE)

In recent years, SDS-PAGE widely used in the serologic studies of cestods. Generally, focused on cestods which are useful as protective immune against other parasite.

In our attempt to determine reactive proteins of *Cysticercus tenuicollis* cyst fluids. this attempt depends on the protein of parasite that can be similar in both parasites. Therefore, in our study, the protein bands appeared is 63kDa, 48 kDa and 70 kDa were recognized in cystic fluid antigens as polypeptides by SDS-PAGE. And this agree with (Kara and Dođanay, 2005). And all of them could be considered as major polypeptides from each sample with minor variations.

Parasites present a mosaic of immunogenic epitopes to their hosts and evoke complex humoral and cellular responses. Some of these epitopes are exclusive to each species, while others epitopes are common (Harrison *et al.*, 2002).

The antigen complexity is the intriguing phenomenon of serological cross-reactivity among parasites. This phenomenon emerged from antigen conservancy or the existence of common components among parasites. It is also important in the development, cross-protective immunity in hosts against multiple parasitic infections using cross-reactive components (Zhou *et al.*, 2002).

The most famous example of heterologous resistance was that observed between the antigenic profiles of *Pneumocystis carinii* trophozoites cysts and *Cysticercus tenuicollis* cyst fluids (Chatterton *et al.*, 1995).

A low molecular weight common component of 63-kDa was found in trophozoite antigen.

Collectively, cross-reaction examples between tissue parasites in farm animals with emphasis on cross-protective components, which raised the possibility of developing multipurpose vaccine for veterinary use.

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