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Identification of protein extracts from *cysticercus tenuicollis* using SDS–PAGE method collected from sheep in slaughterhouse, Kerbala Provenance, Iraq

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Abstract
Cysticercosis is a disease cause by the *cysticercus tenuicollis*, An infection caused by metacestodes that impacts a broad range of ruminant animals. When dogs and wild dogs contract the intestinal tapeworm *Taenia hydatigena's* larval stages, it happens. The adult cysticercus larvae found in the intermediate host's omentum, mesentery, and peritoneum are usually the mature cysticerci's typical sites; the pleura and pericardium are less frequently found. The migratory larvae, which are mostly found in the parenchyma of the liver, can cause traumatic hepatitis overall the young animals. Most infections are found in the slaughterhouse and are chronic and asymptomatic. Acute infection in sheep is rare, and reports of lamb fatalities are rare as well. The *Taenia hydatigena larva Cysticercus tenuicollis* is frequently seen in Iraqi sheep that have been slaughtered. 25 specimen cysts of *Cysticercus tenuicollis* were taken from the slaughtered sheep (two cysts per animal) in slaughterhouses in the province of Kerbala during October and November of 2024 for this study. In this investigation, samples of liver from sheep have been slaughtered at the Karbala Abattoir in Iraq were used to acquire fluid from cysts of larval stage (N = 25). The SDS-PAGE method (Sodium Dodecy Sulfate-Polyacrelamide Gel Electrophoresis) was used to test different proteins in the fluids extracted from cysts in order to discover reactive proteins. The results of fluid antigens' SDS PAGE analysis findings showed 73, 51, 45, and 8 kDa. In conclusion, Identifying antigens in helminthes will enhance diagnostic techniques and control them, while investigating the host-parasite connection is crucial for disease control.

Introduction

Goats and sheep have an impact on the economical viability of rural people in tropical nations, although their low output is mostly caused by parasite infection and *Cysticercus tenuicollis*, the prevalence of stray dogs in the grazing area, which are essential to the

parasite's life cycle, and improper disposal of infected and confiscated offal, organs, and corpses have been linked to the incidence of Cysticercus tenuicollis in the majority of afflicted places worldwide (Lawan et al., 2024). According to Scala et al. (2015) and Muku et al. (2020), cysticercosis has veterinary implications and causes significant social losses to subsistence farming groups as well as significant financial losses when contaminated meat and offal are rejected during slaughter inspections. Livestock with Cysticercus tenuicollis infections have a high rate of mortality and morbidity. (Wondimu et al., 2011) (Murrell et al., 2013). A parasite called *Taenia hydatigena* is common in many countries worldwide, as adults, they reside in dogs' and other canines' small intestine, including wolves and foxes. Since ruminants make excellent intermediate hosts, *Cysticercus tenuicollis*, its larval stage, may infect a wide variety of herbivores. Sheep, goats, deer, and pigs are all susceptible to infections. It is not common in herds of cows or other ruminants, though. Additionally, it may infect a variety of animals, including rats, monkeys, and camels. (Jenkins *et al*., 2014; Scala *et al*., 2015; Foroutan *et al*., 2022; Wakid &Alsulami, 2022). Since adult worms reside in the small intestine of definitive hosts, like dogs, they get the infection when they consume the parasite's larval stage present within the infected organs, carcass or remnants of deceased of sheep which consider as intermediate hosts, since the pregnant proglottid, each carrying 6000–43000 eggs, are expelled with around four proglottid of excrement through one day, the pre-patent period is estimated to be between 7 and 9 weeks, therefore, the average number of eggs that a single worm excretes each day is about 100,000 (Forbes, 2021). When the intermediate hosts ingested eggs from feed and grass tainted with infected dogs' excrement, they contracted the larval stage of the infection, following the membrane of egg digestion, the embryo passes through intestinal tract wall and either migrates to liver and settles, once there, it connects to the mesentery or omentum and develops into the *cysticercus tenuicollis*, a fluid-filled sac, only when many larvae enter the body as a result of ingesting multiple eggs is the illness considered serious, it is important to note that the disease has no known cure and that treating and avoiding contact with affected dogs is crucial to its prevention (Miller *et al*., 2012). Geographic regions affect the occurrence of the *Cysticercus tenuicollis* infection, with greater rates seen in nations with inadequate sanitary management and unchecked population migrations of wild animals. (Samuel and Zewde . 2010) (Yalelet etal., 2018) A localized disease, cysticercosis primarily affects impoverished people with inadequate hygiene measures, large numbers of stray dogs raise the likelihood of the disease spreading since they are significant reservoirs for the spread of zoonotic helminths, such as cysticercosis (Mohammed and Kadir .2020). The primary source of metacestodes is rural people' close interactions with dogs and other household animals (Murrell et al., 2013). According to Hama-Soor et al. (2021), these tapeworm is regarded as one among the most significant and commercially significant veterinary tapeworm in sheep in Iraq. It's vital to note that the proteins can be identified in the parasite's larval stage will serve as a crucial guide to figuring out their function in the parasite's biological traits (Cai et al., 2021). Sodium dodecyl-sulfate polyacrylamide gel electrophoresis, or SDS-PAGE, is widely employed to achieve high-resolution protein mixture separation, the process initially denatures the proteins that will be

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electrophoresed, even while SDS-PAGE may detect the covalent structural properties of resolved proteins, functional aspects that have been used in the investigation of the Determination of Immune Reactive Proteins of Cysticercus Tenuicollis are lost, such as the presence of non-covalently bound metal ions. (Nowakowski et al., 2014; Al-Rishawi and Al-Mayali, 2023).

Materials and methods

Cysticercus tenuicollis collection

Cysticercus tenuicollis samples were obtained from the livers and omentum of around 25 cysts that were infected with the parasite during the examination of the sheep carcass at the slaughterhouses (Kerbalaa). When analyzing the internal viscera of the slaughter animal, the cysts were discovered in the liver, mesentery, and omentum since they are easily identified by their size, form, and preferred sites (Arunkumar et al., 2014). A cooling box was then used to carry these samples into the lab for analysis. The cysts of *C. tenuicollis* were gathered at random during the examination of the sheep corpses in the slaughterhouse for this investigation. *Cysticercus tenui*collis cysts were first recognized by their characteristics, which included a single, long-necked scolex, nearly transparent fluid of cyst, and hooks shape Figure (1) (Essa and Al-Azziz, 2011).

Collection of Fluids from *C. tenuicollis*

Cysts were cleaned with distilled water, and the fluid was then collected by making punctures. The fluids were filtered following a 10-minute centrifugation at 13,000 rpm and 4°C, use a Millipore filter (0.45 µm) to remove the supernatant, then store it at 4°C(Kara and Doðanay, 2005).

2-Detection of reactive proteins in fluids by (SDS-PAGE):

A. The protein precipitation methodology for A-Trichloroactetic acid (TCA) was developed by (Koontz. 2014).
B. The Resolving and Stacking Gels recipe, which calls for 5 milliliters of 10% polyacrylamide Gel Mix (Hashim et al., 2019)
C. Gel staining technique in accordance with C-Silver staining (Blum et al., 1987).

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Figure (1): gross lesions of A sheep with an infected omentum showed a free bladder-like cyst filled with fluid, each of which had a slender neck and one infectious tape worm head

Results

The Protein Ladder with molecular weights 6, 12, 17, 20, 24, 35, 49, 64, 76, and 100 KDa used to analyze Cystic fluid's active proteins extracted from the viscera of sheep infected by *C. tenuicollis* use the SDS-PAGE technique. the outcomes *Cysticercus tenuicollis* fluid's reactive proteins were identified using SDS-PAGE.The *Cysticercus tenuicollis* fluid included four distinct protein bands, ranging in size from 73 to 8 kDa (Table 1) and (Fig. 2).

(Table -1). Show the bands of protein of *Cysticercus tenuicollis* separated using SDS-PAGE.

Band	Molecular weight KD of *Cysticercus tenuicollis*
1	73
2	51
3	45
4	8

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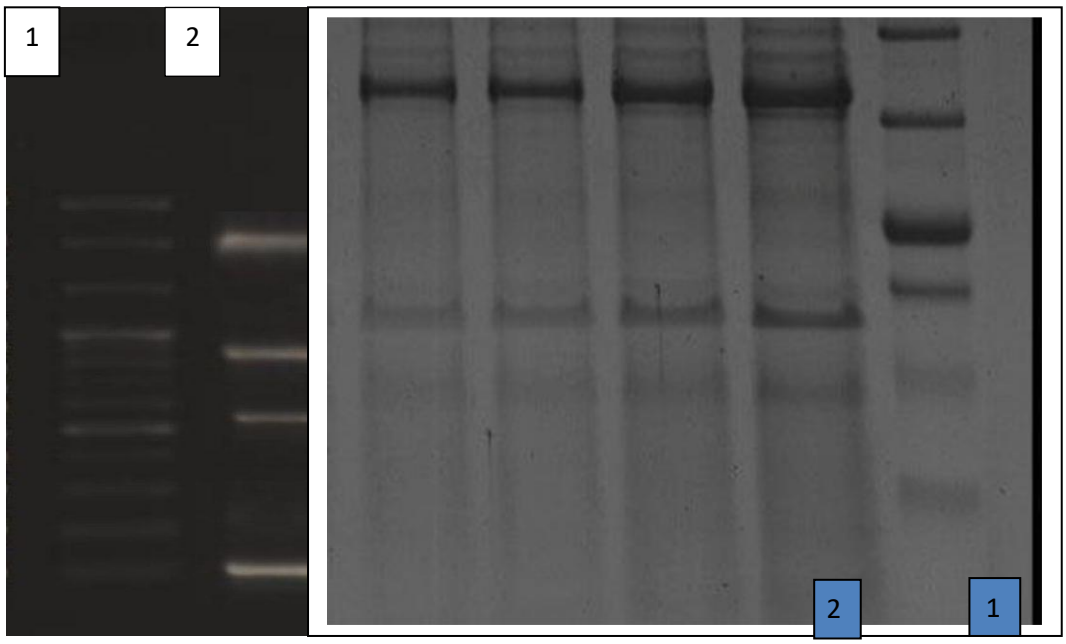

Figure (2):. Polypeptide profiles of cystic fluid that prepared from Hydatid cyst, resolved on SDS-PAGE and stained with silver stain. The Sample (2) *C. tenuicollis* (1) The BLUslf prestained protein

Discussion

SDS-PAGE has been employed extensively in tapeworm serologic investigations in recent years, generally speaking, tapeworms are beneficial as immune-stimulating agents against other parasites (Al-Rishawi and Al-Mayali, 2023). Furthermore, precise identification of the antigens in these helminthes would undoubtedly enhance diagnostic methods, which is the initial stage of control. (Abuseir et al., 2018). A common characteristic of both phylogenetically related and unrelated parasites is cross-reactivity, given that these parasites had a common ancestor at the time of their development, the prevalence of common antigens suggests that antigenic continuity is the norm rather than the exception (El-Moghazy & Abdel-Rahman 2012). Finding the antigens in these helminthes accurately would undoubtedly enhance diagnostic methods, which is the first step in controlling them, it's also important to research the host-parasite relationship during the disease.I concur with (Abuseir et al., 2018). Since a cross-reactive antigen may occasionally be useful to protect against multiple infestations, immunodiagnosis, it frequently allows the use of a diagnostic antigen from one species to possibly protect against another, based on the general similarities in antigenic structure among parasites (El-Moghazy & Abdel- Rahman 2012). Reactive proteins in Cysticercus tenuicollis cyst fluids are also identified using this method, this method relies on parasite proteins, which can be comparable among parasites, all of these proteins might be regarded as main polypeptides from each sample, with just slight changes. (Kara and Doðanay, 2005; Dirwal et al.,2020). In the tested *T. hydatigena*, certain protein bands were quite obvious and showed up in large amounts, while others showed up faintly.

Notably, cysticercus tenuicollis cysts included the 73-, 50-, 45-, and 8-kDa proteins, the current study's findings concur with those of past research projects carried out in Iraq (Dirwal et al., 2020). In line with a study carried out in Iran by Kordafshari et al. (2010), Eight proteins with a molecular weight range of 12-57 kDa, eleven proteins with a molecular weight range of 12-100 kDa, and fourteen protein bands from the scolex, cyst fluid, and cyst wall, respectively, are found in the cysts from infected sheep, this study was somewhat different from that of Al-Rishawi and Al-Mayali (2023), whose SDS-PAGE results revealed the present of seven bands of protein.The molecular weights of these bands ranged from 120 to 27 kDa, and they were 120 to 90 to 70 to 60 to 47 to 38 to 27 kDa, the discrepancy can be explained by the fact that complexes of large molecular weight proteins in some tapeworms may dissolve under extreme circumstances, resulting in two or more subunits being decreased in size (McManus, 2014). This might also explain why some samples had tiny proteins that showed up on SDS-PAGE, whereas other samples had none at all or very little of them. Furthermore, as noted by Miquel et al. (2015), in specific tapeworm infections, distinct protein components may contribute to the larvae's future growth within the host animal's body (Abuseir et al., 2018). Relied on analyzing the findings and comparing them to another study that described the similarities between the proteins extracted from various tapeworm that could be useful in the diagnosis of such helminthes by serodiagnosis, this is because serological cross-reactivity with other Taenia or Echinococcus species sometimes makes reliable serological diagnosis in animals difficult due to antigenic similarities across cestodes (Mcmanus 2014; Abuseir et al., 2018). This might also help to explain why some samples had smaller proteins that showed up on SDS-PAGE, whereas other samples had either no tiny proteins at all or very little of them. Furthermore, it is agreed upon by Miguel et al. (2015) that the development of some cestode infestations in the animal body is influenced by the location of particular proteins.

Conclusion

Accurately identifying the antigens in these helminthes will surely improve diagnostic techniques, which is the first step in controlling them. Investigating the host-parasite connection throughout the disease is also crucial.

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