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Molecular Weight Analysis of Adult *Echinococcus granulosus* Proteins from Dogs Using SDS-PAGE

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Abstract | *Echinococcus granulosus* is a parasitic tapeworm responsible for cystic echinococcosis (CE), a significant zoonotic disease affecting humans and animals worldwide. This study is the first in Iraq to investigate the molecular weights of adult *E. granulosus* proteins from dogs by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Experimental infection with viable protoscoleces obtained from naturally infected sheep livers was performed in three healthy domestic dogs. Seven months later, the dogs were euthanized, and adult worms were harvested from their small intestines. The stored worms were separated for protein extraction, and equal amounts of protein were separated under reducing conditions on a 12.5 % polyacrylamide gel by SDS-PAGE. Specific bands were observed at molecular weights of approximately 120 kDa, 45 kDa, 39 kDa, 30 kDa, and 25 kDa.The strongest banding pattern was obtained from the sample processed with RIPA lysis buffer, indicating that RIPA lysis buffer provided the most efficient protein extraction. Protein bands in the range of 25–120 kDa were identified, indicating the presence of several proteins that may have structural or functional roles in the parasite. Cystic echinococcosis is endemic in many parts of the world and is the focus of research aimed at discovering new diagnostic and treatment modalities.

Keywords | *E. granulosus*, SDS-PAGE, Molecular weight, Proteins, Cystic echinococcosis, Zoonotic disease

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INTRODUCTION

Hydatid disease, caused by tapeworms of the *Echinococcus* genus, is a parasitic infection with a global distribution. This primary helminthic disease primarily affects mammalian intermediate hosts through the larvae of the *E. granulosus* tapeworm, especially in areas where transmission occurs through close contact with dogs and ruminants. Recognized as a zoonotic disease, hydatid disease is included among the neglected tropical diseases in the WHO Strategic Plan. The pathogenic sequelae of this infection are not limited to cyst formation, as recent research has demonstrated a significant effect of cysts on host physiology. Hydatid cysts induce systemic biochemical changes consistent with the functional impairment of underlying organs. Based on (Liang *et al.*, 2020; Sabeeh et al., 2022, 2023; Farhood *et al.*, 2024). The transmission of cystic echinococcosis is most prevalent in

eastern Central Asia, southern South America, eastern and southern Europe, and northern Africa; however, it is present in pastoral and rangeland environments worldwide. There are at least 368 endemic counties in China (Qian *et al*., 2017).

Both definitive hosts (carnivores) and intermediate hosts (ungulates) are a part of *E. granulosus* life cycle. The mature worms shed their eggs through the developed proglottids they release in their feces. The small intestine of definitive hosts is where the worms reside. The illness is contracted by pigs, which act as intermediate hosts, when they eat adult proglottids, usually while grazing on pasture. Oncospheres enter the tissues after the eggs hatch and grow into metacestodes, which results in the formation of cysts (CE cysts) in internal organs. To complete the life cycle, final hosts consume the organs of intermediate hosts that are contaminated with the parasite (WHO, 2022).

The apparent molecular weight of proteins can be measured with high precision using SDS-PAGE, which analyzes the distance that proteins migrate in a polyacrylamide gel (Matsumoto *et al*., 2018; Ali *et al*., 2024; Al-Sailawi *et al*., 2024). From the recovered protein profiles of, for example, a parasite like *E. granulosus* in dogs, this method remains applicable. SDS-PAGE is based upon the gentle denaturation of proteins with the anionic detergent sodium dodecyl sulfate (SDS) and a reducing agent to give a charge-to-mass ratio that is proportional to the molecular weight of the polypeptide (Kielkopf *et al*., 2021; Qasim and Mohammed, 2024, 2025; Qasim *et al*., 2022, 2025; Al-Jassani *et al*., 2022; Mohammed and Mohammed, 2021; Al-Safi and Qasim, 2023).

This study was conducted to investigate the molecular weights of adult *E. granulosus* proteins from dogs using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and to offer an array of tools to characterize *E. granulosus* in pets.

MATERIALS AND METHODS

Experimental design and animal management

This experimental investigation aimed to determine, under controlled circumstances, the evolution of *E. granulosus* adult worms in definitive hosts. The study included three young, healthy household dogs (*Canis familiaris*). Throughout the experiment, the dogs were kept individually in well-ventilated, parasite-free kennels designed to prevent environmental contamination. They were provided with clean drinking water and a balanced diet. Every operation was conducted in accordance with institutional ethical standards and was approved by the relevant animal care and use committee.

This experimental study was conducted to investigate the development of *E. granulosus* adult worms in definitive hosts under control conditions. Three healthy, young domestic dogs (*Canis familiaris*) were selected for the study. The dogs were housed individually in well-ventilated, parasite-free kennels designed to prevent environmental contamination and were provided with clean drinking water and a balanced diet throughout the experiment. Every technique was approved by the relevant animal care and use committee and carried out in accordance with institutional ethical guidelines.

Source of infection and experimental infection

Naturally infected sheep livers harboring hydatid cysts were obtained from a local abattoir. Microscopic examination of cyst fluid for living protoscoleces (using 0.1% eosin staining) confirmed the fertility of the cysts. Under veterinary supervision, each dog ingested 50,000 viable protoscoleces mixed with liver tissue to ensure successful infection with *E. granulosus*. The dogs were then closely monitored regularly to ensure their health status, while being kept under strictly hygienic conditions to prevent secondary transmission or environmental contamination for a period of seven months. During this period, no protozoan anthelmintic was administered, allowing the adult stage of the parasite to develop fully. After the experimental period, the dogs were anesthetized in an approved manner (e.g., with intramuscular injection of ketamine and xylazine) and subsequently euthanized by humane endpoints. Adult *E. granulosus* worms were removed and examined in the small intestine.

Preservation of parasite specimens

Adult worms were carefully washed with sterile PBS to remove debris and intestinal contents. After that, they were then preserved in liquid nitrogen for molecular weight analysis of adult *E. granulosus* proteins.

SDS-PAGE analysis of protein extraction

Total protein extraction was conducted to study the molecular weight profile of proteins shed by adult *E. granulosus* worms. Worms were thawed on ice and homogenized in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% SDS, and a protease inhibitor cocktail). The mature worms (0.5 g) were suspended in 0.5 mL PBS and sonicated on ice for 5 seconds, three times. Soluble proteins were separated using a 12.5 % (w/v) acrylamide gel based on the methods of Walker (2002). With minor modifications, the homogenate was centrifuged at 12000 rpm for 20 minutes at 4°C, and the supernatant containing soluble proteins was collected.

Protein concentrations were ascertained using the bicinchoninic acid (BCA) test. After combining proteins

(30 µg per lane) with SDS loading buffer, they were boiled for 5 minutes at 95°C before being subjected to SDS-PAGE (12.5% polyacrylamide, reducing conditions). To identify the main protein using SDS-PAGE, the banding patterns were captured and examined.

RESULTS

The molecular weights of E. granulosus proteins isolated from dogs were measured by SDS-PAGE, as shown in Figure 1. The investigation included protein samples processed according to several procedures, in which proteins were isolated via centrifugation at various speeds (5000 × g and 10,000 × g), including crude samples and those treated with RIPA lysis buffer (Table 1, Figure 2). A molecular weight marker (10–250 kDa) is used as a reference for detecting protein sizes.

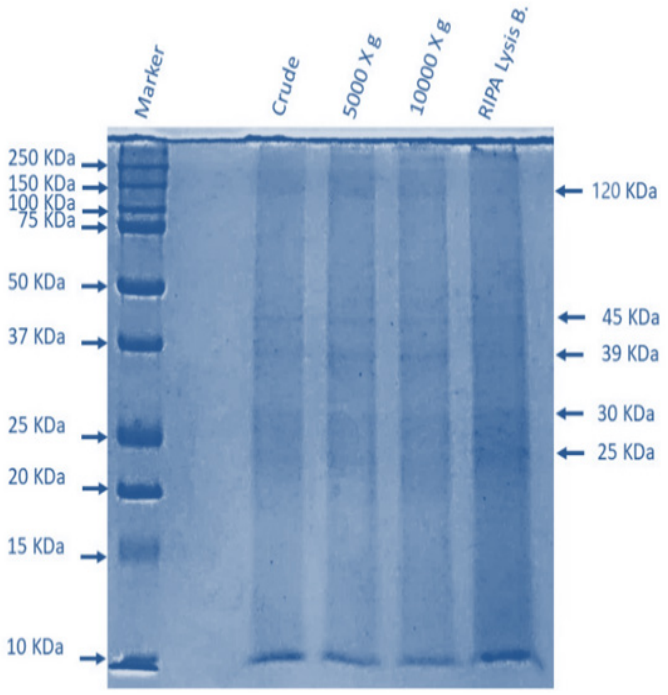

Figure 1: SDS-PAGE separation of adult worm of *E. granulosus*. Lane (crude): sonicated MHC in PBS. Land (5000 X g) and (10000 X g): crude samples centrifuged for 5 min at 5000 X g or 10000 X g (respectively). Lane (RIPA lysis B.): MHCs were sonicated in RIPA lysis buffer. 30 µg of protein was loaded on a 12.5% SDS-PAGE gel, 1 mm in thickness.

The study's findings revealed distinct protein bands with molecular weights of about 120 kDa, 45 kDa, 39 kDa, 30 kDa, and 25 kDa. All samples exhibited these bands, albeit at varying intensities, indicating changes in solubility or protein concentration. The sample subjected to RIPA lysis buffer had the most pronounced banding pattern, indicating the superior efficacy of this buffer in protein extraction relative to other techniques.

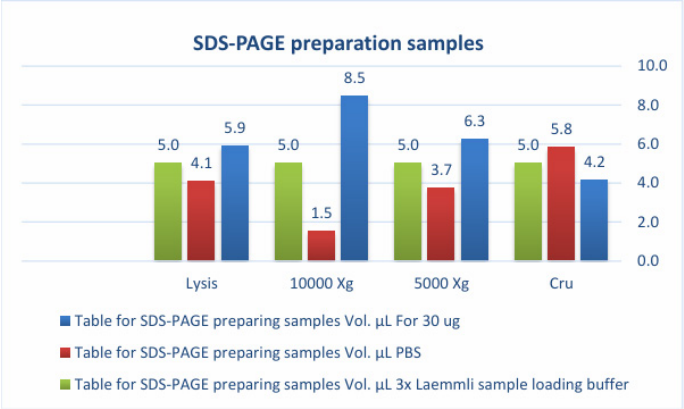

Figure 2: SDS-PAGE preparation samples.

Table 1: Quantification of homogenized sample protein concentrations by using Bicinchoninic acid assay for protein concentrations (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific Inc., USA). The absorbance wavelength is 562 nanometers (nm). Detection range of protein concentration was (20–2000 µg/mL).

Standard concentrations of BSA[1] (µg/mL)	[2]Reading data (replication) Absorbance unit (nm)		Sample types	[2] Reading data (replication) Absorbance unit (nm)	
2000	0.972	1.025	[3] Crude samples in PBS	0.622	0.873
1500	0.736	0.789	[4] 5000 g	0.533	0.557
1000	0.555	0.55	[5] 10000 g*	0.423	0.455
750	0.465	0.457	[6] Sample with Lysis buffer	0.592	0.546
500	0.357	0.348			
250	0.269	0.238			
125	0.195	0.183			
25	0.142	0.162			
0=Blank	0.14	0.127			

[1]Bicinchoninic acid assay. [2]Absorbance read with a spectrophotometer set to 562 nm. Three crude samples in PBS were sonicated twice, each for 30 seconds. Four crude samples in PBS were centrifuged at 5000 × g for 5 minutes. Five crude samples in PBS were centrifuged at 10000 × g for 5 minutes. 6 Crude samples in RIPA Lysis Buffer (Catalog number: E-BC-R327, Elabscience Biotechnology Inc., China).

The detection of protein bands in the 25–120 kDa range reveals several types of proteins that may have structural or functional roles in the parasite. Furthermore, discrepancies in band intensity across the samples indicate variations in band proteins within samples, providing information about the Molecular Features of this parasite.

DISCUSSION

This study aimed to analyze the protein profile of *E. granulosus* isolates obtained from dogs using SDS-PAGE,

with a particular focus on evaluating the effect of different protein extraction protocols on both the yield and the clarity of banding patterns. Various centrifugation speeds were applied in combination with RIPA lysis buffer, allowing for a comparative assessment of extraction efficiency. The results revealed apparent differences in protein banding patterns across a broad range of molecular weights, reflecting significant biochemical diversity in the parasite's composition. These findings underscore the importance of this molecular variation in understanding the structural organization of *E. granulosus* and provide a basis for more in-depth future studies in proteomics and parasitic immunology.

Comparison with other studies; the molecular weights of polypeptides differed by Burgu *et al.* (2000) found protein bands in the range of 38-116 kDa, while low molecular weight one, ranging from 12.5 to 17 kDa, was shown by Shapiro et al. (1992), but Hassanain *et al.* (2016) found protein bands of 20–100 kDa, and Köksal *et al.* (1995) found on 45-116 kDa, and Al-Olayan and Helmy (2012) low molecular weight found on 38–35 kDa, and Shambesh *et al.* (1995) low molecular weight two antigens of camel on 100 kDa and 130 kDa, and Maleki et al. (2023) found on 8-67 kDa, and Sbihi *et al.* (1996) low molecular weight found bands on 12-14, 20, and 34 kDa, and Keywanloo et al. (2011) found on 14-45 kDa, and Irshadullah and Rani (2011) found bands of buffalo on 14.4-116 kDa, and Latif et al. (2013) found band on 209, 138 and 63kDa, and Siddartha *et al.* (2022) found four bands on 72, 64, 48 and 24 kDa, and Ananda *et al.* (2024) found band on 16-114 kDa.

SDS-PAGE analysis of adult Echinococcus granuolosus revealed varying numbers and molecular weights of polypeptides in adult parasite. Identifying these proteins and understanding their functions could enhance our knowledge of the interaction between E. granulosus and its canine hosts ultimately aiding in developing effective control methods against echinococcosis. This study emphasizes the importance of proteomic analysis in parasitology and serves as a foundational step toward more thorough investigations into the biology of this significant, economically and health-related parasite

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NOVELTY STATEMENT

This study is the first to examine the molecular weight profile of adult *Echinococcus granulosus* proteins derived from experimentally infected dogs using SDS-PAGE. The novelty lies in identifying specific protein bands ranging from 25 to 120 kDa, with a pronounced yield from RIPA buffer treatment. These findings provide new insights into protein expression patterns of *E. granulosus* in definitive hosts and suggest extraction buffer type significantly influences protein band clarity an important consideration for downstream proteomic or immunodiagnostic applications.

AUTHOR'S CONTRIBUTION

Esraa Sabeeh: Conceptualization, experiment design, supervision, manuscript drafting, and correspondence. Alaa Ismail Saood: Conducted experimental infection and sampling, data analysis, contributed to writing and revision. Ameer Ibrahim Abdulzahra: Laboratory execution, SDS-PAGE processing, and result interpretation. Afaq Talib Farhood: Literature review, figure preparation, and referencing. Hayder Hussein Jalood: Statistical interpretation, discussion enhancement, and formatting. All authors reviewed and approved the final version of the manuscript.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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