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# Gene Polymorphism of Antigen B Subunit 2 and Pathogenesis of Cystic Echinococcosis in Murine Model

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***Abstract*—A complex genetic diversity among the causative agent, *Echinococcus granulosus*, is documented. Antigen B (AgB) is a major antigenic fraction of hydatid fluid and hydatid cyst tissues. This study aims to investigate the role of antigen B subunit 2 (*AgB2*) gene polymorphism in the pathogenesis of cystic echinococcosis (CE) in murine model. Ovine liver hydatid cysts are obtained from Erbil Slaughterhouse. Protoscoleces from each isolate are separated into two batches. First preserved at −20°C for molecular analysis whereas the second is used for experimental infection in mice. Parasite DNA was extracted, and *AgB2* genome was amplified and sequenced. The sequencing profile of six of the isolates (1, 2, 3, 5, 8, and 11) revealed a 100% analogy with *AgB2* gene of *E. granulosus* genotype G2. Minor sequence polymorphisms, 1.67%, are observed in one of the isolates, whereas remarkable DNA sequence polymorphisms are noticed in three of the isolates. The polymerase chain reaction (PCR) products sequencing profiles revealed 100% polymorphisms in four of the isolates in comparison with the source gene (AY569356.1), instead, those isolates reveal various degrees of analogy, 80.33%, 80.87–89.05%, and 89.36% to G1, G3, and G6, respectively. Polymorphic sequencing profile of the PCR-amplified product (250 bp) of *E. granulosus* clone EgB2G2v13 *AgB2* gene (Accession no.: AY569356.1) has no significant impact on the pathogenicity of the CE in murine model. To upgrade the diagnostic sensitivity rates of the immunological techniques, a mixture of native hydatid antigens containing AgB is recommended to be used in the ser-diagnosis of this infection.**

***Index Terms*—Antigen B subunit 2, cystic echinococcosis, *Echinococcus granulosus*, genotype G1, genotype G2, Tasmanian sheep strain.**

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## I. Introduction

Cystic echinococcosis (CE) is a public health challenge caused by the larval stage of *Echinococcus granulosus* (Elmajdoub and Rahman, 2015) (Shirazi, et al., 2016) (Alsakee, 2023). Annual incidence is estimated to be approximately 188,000 new cases worldwide and contributes to 1,097,000 disability-adjusted life years (Shao, et al., 2023). The disease is cycled among carnivorous animals such as dogs, coyotes, and jackals, acting as definitive hosts, and herbivorous animals such as sheep, goats, cattle, camels, and deer, serving as intermediate hosts and harboring a cyst-forming stage, hydatid cyst. Man is accidentally infected following ingestion of *E. granulosus* eggs that shed with the infected dog's excretions, and this later host is considered a dead end for the parasite (Patra, et al., 2021) (Hogea, et al., 2024).

Many intraspecific variations or strains within *E. granulosus* sensu lato have been recognized (Pal and BDutta, 2013) (Abbas, Al-Kappany and Al-Araby, 2016) (Babaei, et al., 2021). Those strain variations have been critically impacted upon the epidemiology of the disease, parasite transmission dynamics, life cycle pattern, host specificity, hydatid cyst growth rate within the intermediate hosts, pathogenicity, immunogenicity, and responsiveness to anthelmintics treatment, and even upon the diagnostic efficiency of the routinely used serological tests (Siyadatpanah, et al., 2019) (Macin, et al., 2021). So far, and based on the mitochondrial DNA, 10 strains (genotypes), namely G1-G10 and line strain of *E. granulosus* sensu lato, have been recognized. G1 is the common sheep strain, which is responsible for the majority of human cases worldwide; G2 (Tasmanian sheep strain), G3 (buffalo strain), G4 (horse strain), G5 (cattle strain), G6 (African camel strain), G7 (swine strain), G8 (cervid strain), G9 (Poland swine/human strain), and G10 (Eurasian reindeer strain), and recently another novel species, *E. felidis* isolated from lions, have also been added to those strains (Alvarez Rojas, Romig and Lightowlers, 2014) (Shariatzadeh, et al.,

2015) (Shahbazi, et al., 2020) (Mahdi, Al-Hamairy and Al-Rubaiey, 2020). Of those well-recognized strains of *E. granulosus*, G1, G2, G3, G5, G6, G7, G8, G9, and G10 are known to be infective to human beings. However, human susceptibility to the genotypes, G4, and lion strain has not been proven yet (Shahbazi, et al., 2020) (Utuk and Simsek, 2012). The predominant genotypes causing the bulk of the global burden of human infestation are *E. granulosus* s. (G1-G3) (Macin, et al., 2021) (Casulli, et al., 2022).

Humans get infected through ingestion of the parasite eggs either through direct contact with dogs and dog feces or consumption of vegetables or food and water contaminated with infected dog feces. Once ingested, the released oncospheres that emerge from the eggs will penetrate the small intestinal mucosa mechanically and by lytic action, getting access to the portal venous circulation, which provides access to the liver, lungs, and various other organs (Ün, Yaman and Erbaş, 2020).

Antigen B (AgB) is a major *E. granulosus* native antigenic fraction of hydatid fluid and hydatid cyst tissues. It is a substance of lipoprotein nature with a molecular weight of 60–120 kDa and resists temperatures of 100°C for 15 min without great alteration of its antigenicity. Genotype-specific AgB is likely to develop different immune responses in the host (da Silva, et al., 2018). Furthermore, (Lorenzo, et al., 2005) demonstrated that antigen B is a thermostable heteropolymorphic protein composed of several distinct but related 8 kDa subunits, encoded by a multigenic family, and it binds strongly to lipids and other hydrophobic compounds, suggesting its possible involvement in the process of lipid uptake or detoxification. (Sadjjadi, et al., 2022) revealed that the application of AgB extracted from only one genotype is not adequate to be used in the serodiagnosis of human CE. The cytokine expression in a host is significantly varied and could lead to both protective and/or immunopathological reactions. As revealed previously, at the time of cyst development, TH1 response, which is associated with increased levels of interferon-gamma (IFN-γ), could be detected, but if TH1 responses begin to damage the parasite, then the parasite starts to dominate TH2 response through the release of antigens that induce TH2-promoting cytokines such as interleukin (IL)-4 and IL-13 (Beyhan, Albayrak and Guven, 2022). TH1 is associated with protective immunity and produces IL-2, IL-12, tumor necrosis factor, and IFN-γ, whereas TH2 is associated with susceptibility to infection and pathogenicity and ensures the survival of the parasite within host tissues through the expression of the cytokines IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (Zhang, et al., 2012) (Li, et al., 2019). Antigen B is thought to contribute to the switching of the immune response toward non-protective mechanisms as a part of the survival strategies of the parasite (Riganò, et al., 2001) (Riganò, et al., 2007). The application of antigen B, which originated from different strains of *E. granulosus* sensu stricto, individually or in combination, revealed various responses in the serodiagnosis of human CE that could be questionable for the presence of polymorphic genes that encode for the AgB fraction (Sarkari Shahriari, et al., 2013).

To the best of our knowledge so far, no studies concerning the role of antigen B subunit 2 (*AgB2*) gene polymorphisms in the pathogenesis of CE have been conducted. The current study aimed to investigate the association of *AgB2* gene polymorphisms from different isolates of *E. granulosus* metacestode and the pathogenesis of the infection in a murine model.

## II. Materials and Methods

### *A. Source of Protoscoleces*

Ovine liver hydatid cysts were collected from Erbil Governmental Slaughterhouse over a period from January 2024 to April 2024. The cysts were immediately delivered to the microbiology laboratory at the International University of Erbil for processing and separation of the protoscoleces in accordance with (Hassan, AL-Hadithi and Al-Sakee, 2016). Briefly, under aseptic conditions, the cysts were dissected, and the contents (hydatid fluid and hydatid sand) were aspirated and dispensed in a clean, sterile screw-capped test tube. Hydatid sand was allowed to stand for 20 min at room temperature, and the fluid was carefully removed. The precipitated protoscoleces were washed thrice with 0.15 M phosphate-buffered saline (pH 7.2) and assessed for viability by motility of flame cells and eosin (0.1%) exclusion test as described by Khoshnaw and Al-Sakee (Khoshnaw and Al-Sakee, 2022). For each isolate, two batches of protoscoleces were collected. First preserved in an equal volume of ethanol (70%) at −20°C for molecular analysis, whereas the second batch was used immediately in the *in vivo* study.

### *B. Molecular Analysis*

#### *Genomic DNA extraction*

A commercially available kit from GeneAll® Exgene™ (South Korea) was used for the extraction of genomic DNA as recommended by the leaflet that was provided with the kit. Frozen, ethanol-fixed hydatid cyst tissue samples were allowed to thaw at room temperature (25°C–27°C) for 30 min before processing. Ethanol was removed by rinsing the hydatid tissue samples thrice with an equal volume of sterile distilled water in a 10 mL capacity sterile screw-capped centrifuge test tube. The concentration and purity of extracted genomic DNA were assessed by a Nano-Drop spectrophotometer. The samples that yield a purity of 1.8–2.0 were considered for amplification.

#### *Polymerase chain reaction (PCR) and sequence analysis*

A region of 250 bp of the *E. granulosus* clone EgB2G2v13 *AgB2* gene (Accession no.: AY569356.1), that was characterized previously by (Kamenetzky, et al., 2005) in a human hydatid cyst sample of G2 strain, was amplified using the following primers: F-5’ TCTCTCTTGCTCTCGTGGCT and R-5’ TTGCAGCTTCTGGCAAATGG 3’. The amplification protocol was carried out in accordance with (Sarkari Shahriari, et al., 2013). Briefly, the reaction was performed using mixtures of 0.3 μL of Taq polymerase (Ampliqon, Denmark) with 2.5 μL of 10X PCR buffer, 17.5 μL of deionized distilled water, 0.6 μL of $MgCl_2$,

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0.7 µL (10 mM) of dNTP, 25 pmol of each primer, and 2 µL of DNA template, in a total volume of 25 µL. The PCR reactions involved an initial 3 min at 95°C denaturation step, followed by 35 cycles of 60 s at 95°C, 60 s at 56°C, and 90 s at 72°C, with a final incubation at 72°C for 3 min in a PCR ALPHA MAX Thermal Cycler (Alpha, United Kingdom). The PCR yields were subjected to agarose gel electrophoresis and ethidium bromide (BioBasic Canada INC, Canada) staining. The results were visualized by ultraviolet (UV) illumination at (240–366 nm) wavelength on a UV transilluminator, and the gel was documented with a Polaroid photo documentation camera. Amplified DNA bands were purified from the gel using the QIAquick Gel Extraction Kit (Qiagen, Germany), following the manufacturer's instructions. The purified amplicons were sequenced in both directions using forward and reverse primers by the ABI Prism Terminator Sequencing Kit (Applied Biosystem) at Humanizing Genomics Macrogen Inc., Seoul, South Korea. Sequence data were obtained from the National Center for Biotechnology Information (NCBI) database to characterize the parasite isolates AgB2. The AgB2 sequencing results were analyzed and compared with the relevant gene sequences deposited in GenBank using the basic local alignment search tool (BLAST). Chromatograms of the amplified region of *E. granulosus* clone EgB2G2v13 *AgB2* gene (Accession no.: AY569356.1) were interpreted using Finch TV software, while MEGA X software was used to edit the PCR product sequences.

### *C. In vivo Experiments*

*Experimental infection in murine model*

Fourteen groups of Albino (BALB)/c mice (male and female) weighing 22–25 g were used in the *in vivo* experiments. Each group included six animals and was infected experimentally with the protoscoleces obtained from 14 isolated sheep liver hydatid cysts. Each mouse was injected with 2000 protoscoleces (viability >90%) in 200 µL of Phosphate-buffered saline pH 7.2. Ninety days later, all mice were anesthetized with 12.5 mg/kg xylazine (Interchemie, Netherlands) and 87.5 mg/kg ketamine (Cluj-Napoca, Romania) and dissected. Internal organs were inspected for developed hydatid cysts (Ma, et al., 2007) (Hassan, AL-Hadithi and Al-Sakee, 2016).

### *D. Ethical Consecrations*

The study protocols were approved by the Research Ethics Committee in the College of Science, International University of Erbil (meeting code: 1, paper code: 1 on September 2nd, 2024), and the statement of the World Medical Association for animal use in biomedical research (https://www.wma.net/policies-post/wma-statement-on-animal-use-in-biomedical-research/) was followed for mice handling and management.

### *E. Statistical Analysis*

The obtained data were interpreted by Statistical Package for the Social Sciences (version 25.0). The correlation coefficient (r) as well as analysis of the variance of two factors without replication was calculated between the size and number of the developed secondary hydatid cysts and *E. granulosus AgB2* gene. $p \leq 0.05$ was considered statistically significant.

## III. Results

Sixteen isolates of hydatid cysts (containing protoscoleces with viability >90%) that were obtained from infected sheep livers were subjected to genomic DNA extraction and PCR, targeting a region of 250 bp of the *E. granulosus* clone EgB2G2v13 *AgB2* gene, and sequenced (Fig. 1). The sequencing profile of the isolates revealed no polymorphisms (100% analogy) in the isolates 1, 2, 3, 5, 8, and 11, in comparison with the source gene (Genotype G2, AgB2 [Accession no.: AY569356.1]). To the best of our knowledge, this is considered the first record instance of *E. granulosus* genotype G2 in Erbil province. Minor sequence polymorphisms, 1.67%, were observed in isolate 9, whereas remarkable DNA sequence polymorphisms, 4.49%, 4.76%, and 6.41%, in isolates 7, 10, and 14, respectively, were observed. Furthermore, the PCR products sequencing profiles revealed 100% polymorphisms in four of the isolates (4, 6, 12, and 13) in comparison with the amplified region of the source (Accession no.: AY569356.1) (Table I); instead, those isolates revealed various degrees of identity to the previously published *E. granulosus* AgB genes on NCBI (Table II). Two of the 16 isolates revealed sequence profiles that did not match any previously published genes on the *NCBI* gene bank; therefore, those two isolates were excluded from the *in vivo* experiments.The results of the *in vivo* experiments revealed that the fourteen isolates that were used in the experimental infection of the mice exhibited various degrees of pathogenesis as reflected by the number and size of the developed hydatid cysts (Table I). The number of the developed hydatid cysts ranged from 12 ± 7.54 to 59.5 ± 67.71, with cyst size ranging from 18.67 ± 5.61 mm to 68 ± 23.27 mm. The highest number and largest size of cysts were detected in the mice group that was infected with isolate 10, in turn, exhibited 95.24% identity to the studied source (AY569356.1) (Table I). However, there was no significant ($p = 0.6073$, $p = 0.7122$) association between *E. granulosus* clone EgB2G2v13 *AgB2* gene polymorphisms and the number and size of the developed hydatid cysts.

Four of the studied isolates (4, 6, 12, and 13) revealed 100% sequence polymorphisms compared with the source

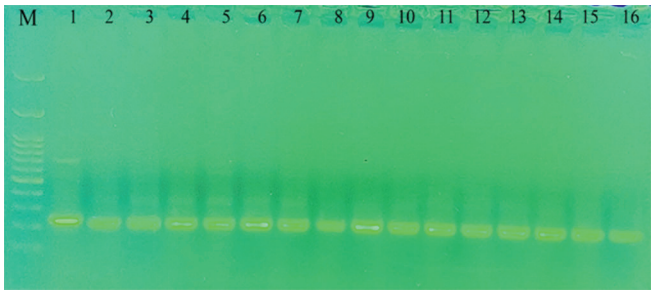


Fig. 1. Polymerase chain reaction-amplified *E. granulosus* antigen B subunit 2 gene fragments (250 bp) from 16 isolates (lanes 1–16). M: 100 bp DNA molecular weight ladder.

TABLE I
CORRELATION OF PARASITE ISOLATE PATHOGENESIS AND ANTIGEN B SUBUNIT 2 (AGB2) GENE POLYMORPHISM IN EXPERIMENTALLY INFECTED MURINE MODEL

| Mice Group | Parasite Isolate | No. of Secondary Hydatid Cysts Mean±SD# | Size of Secondary Hydatid Cysts (mm) Mean±SD* | Identity to the Source (AY569356.1) (%) | Polymorphism (%)*# |
|---|---|---|---|---|---|
| 1 | 1 | 12±7.54 | 19.2±9.73 | 100 | 0 |
| 2 | 2 | 20±12.84 | 21.33±13.89 | 100 | 0 |
| 3 | 3 | 25.75±8.95 | 29±8.9 | 100 | 0 |
| 4 | 4 | 15.5±10.55 | 18.67±5.61 | 0 | 100 |
| 5 | 5 | 25.5±10.55 | 34±8.58 | 100 | 0 |
| 6 | 6 | 28.75±12.36 | 35±6.22 | 0 | 100 |
| 7 | 7 | 25.6±15.58 | 36±5.83 | 95.51 | 4.49 |
| 8 | 8 | 28.75±14.0 | 43±11.01 | 100 | 0 |
| 9 | 9 | 33.5±30.59 | 47±7.02 | 98.33 | 1.67 |
| 10 | 10 | 59.5±67.71 | 68±23.27 | 95.24 | 4.76 |
| 11 | 11 | 27.83±14.0 | 35.67±28.93 | 100 | 0 |
| 12 | 12 | 25.6±8.08 | 25.33±13.31 | 0 | 100 |
| 13 | 13 | 26.5±9.32 | 32±13.57 | 0 | 100 |
| 14 | 14 | 31±18.57 | 35.33±14.79 | 93.59 | 6.41 |
| | | p=0.6073 | p=0.7122 | | |
| | | F=0.8571 | F=0.7281 | | |
| | | F crit=2.576 | F crit=2.576 | | |

*Correlation coefficient = −0.1155774 (association of the number of developed hydatid cyst and *AgB2*) gene polymorphism. #Correlation coefficient = −0.2962189 (association of size of developed hydatid cyst and *AgB2*) gene polymorphism

TABLE II
BASIC LOCAL ALIGNMENT SEARCH TOOL OF THE ISOLATES THAT REVEALED 100% POLYMORPHISMS COMPARING WITH THE STUDIED SOURCE (AY569356.1)

| Isolate | Polymorphisms (%) | Identity to genes other than the source on NCBI | | | Isolate country | Isolate strain | Host |
|---|---|---|---|---|---|---|---|
| | | % | Accession no. (NCBI) | Gene | | | |
| 4 | 100 | 89.36 | MH243705.1 | *E. canadensis* voucher C5B2 antigen B subunit 4 (*AgB/4*) gene | Iran | G6 | Camel |
| | | 89.05 | AY357112.1 | *E. granulosus* clone EgB4.3 antigen B subunit 4 gene, exons I and II, and partial cds | Brazil | G3 | Cow |
| | | 88.73 | AY357111.1 | *E. granulosus* clone *EgB4.2* antigen B subunit 4 gene, exons I and II, and partial cds | Brazil | G3 | Cow |
| | | 88.69 | LC781357.1 | *E. granulosus* B4H2 EgAgB4 gene for antigen B subunit 4, partial cds | Turkey | ? | Human |
| 6 | 100 | 85.07 | AY357111.1 | *E. granulosus* clone *EgB4.2* antigen B subunit 4 gene, exons I and II, and partial cds | Brazil | G3 | Cow |
| 12 | 100 | 77.4 | LC781357.1 | *E. granulosus* B4H2 *EgAgB4* gene for antigen B subunit 4, partial cds | Turkey | ? | Human |
| | | 77.05 | LC781358.1 | *E. granulosus* B4H3 EgAgB4 gene for antigen B subunit 4, partial cds | Turkey | ? | Human |
| 13 | 100 | 80.87 | AY871034.1 | *Echinococcus granulosus* antigen B2 (*AgB*) gene, AgB-K4 allele, and partial cds | Brazil | G3 | Cow |
| | | 80.65 | GU166201.1 | *Echinococcus granulosus* isolate ZGA2-4 antigen B 2/1 (*AgB2/1*) gene, partial cds | Australia | ? | Dog |
| | | 80.33 | AY569344.1 | *Echinococcus granulosus* clone EgB2G1v4 isolation-source cyst 2 antigen B subunit 2 (*AgB2*) gene, complete cds | Argentina | G1 | Human |
| | | 79.76 | LC780495.1 | *Echinococcus granulosus B2H5* gene for antigen B, partial cds | Turkey | ? | Human |

NCBI: National Center for Biotechnology Information

gene; however, they revealed potential pathogenicity in the experimentally infected murine model at the same degree as the isolates (1, 2, 3, 5, 7, 8, 9, 10, 11, and 14) with highly identical gene sequences did. BLAST revealed that those aberrant isolates have various degrees of analogy with different clones of *AgB* genes that were isolated from different hosts in different countries (Table II).

## IV. DISCUSSION

Recent studies have demonstrated the rising rate of human CE worldwide (Yan, et al., 2018). In 2013, the World Health Assembly classified this zoonotic infection as one of the eight neglected zoonotic diseases (Nusrath, et al., 2013). The results revealed that the sequencing profile of the PCR-amplified product (250 bp) of the *E. granulosus* clone EgB2G2v13 *AgB2* gene (Accession no.: AY569356.1), that was previously characterized as *E. granulosus* genotype G2 (Accession number, AY569356.1) (Kamenetzky, et al., 2005), has shown extreme polymorphic sequence profiles. PCR product sequences of five (1, 2, 3, 5, 11) of the studied isolates revealed 100% mimicry with the selected source of the *E. granulosus AgB2* gene. This finding indicates the possible occurrence of the *E. granulosus* G2 strain in the Erbil province. Previous records in this regard revealed similar figures while using other genes of *E. granulosus*, such as NAD1, NAD2, and COX1, in hydatid cyst samples extracted from humans and sheep in Baghdad, Iraq, and in Riyadh, Saudi Arabia (Khalf, et al., 2014) (Metwally, et al., 2018). However, common sheep strain G1 is the predominated genotype of *E. granulosus* in almost all countries with endemicity of this zoonotic parasite (Ergin, Saribas and Yuksel, 2010) (Khalf, et al., 2014) (Spotin, et al., 2015) (Hamoo, Mustafa and Abdulraheem, 2019). In the present study, four of the studied isolates (4, 6, 12, and 13) revealed 100% variability in comparison with the studied

source gene (Accession no.: AY569356.1). However, those isolates revealed polymorphic sequence profiles that matched with the parasite strains other than G2, including G1, G3, and G6, with analogy rates ranging between 80.33% and 89.36%. Similarly, high genetic diversity was reported worldwide (Khalf, et al., 2014) (Spotin, et al., 2015) (Kinkar, et al., 2017). Furthermore, various diagnostic sensitivity rates of immunological techniques using native hydatid antigens in the serodiagnosis of human CE have been reported by previously published data (Al-sakee, 2011) (Al-Olayan and Helmy, 2012) (Sadjjadi, et al., 2022) (Alsakee, 2023) could support the genetic diversity of the *E. granulosus* AgB that has been noticed in the present study.

A study carried out in Iran suggested that *E. granulosus* genotype G1 (the common sheep strain) is the most prevalent genotype in livestock and that genotype G2 (the Tasmanian sheep strain) is the second most common genotype in both cattle and sheep (Rostami Nejad, et al., 2012). The third most common genotype of *E. granulosus* is G3 (buffalo strain). This latter genotype of *E. granulosus* was mainly isolated from cattle, buffalo, and sheep, as well as from human cases with liver and lung involvement (Piccoli, et al., 2013) (Metwally, et al., 2018). In the present study, one of the isolates revealed 89.36% sequence analogy to *E. granulosus* genotype G6 (camel strain). Previous studies carried out in Iraq and Iran have demonstrated that G6, with proven infectivity to human beings, is predominant in those countries (Khalf, et al., 2014) (Ebrahimipour, et al., 2017).

The complex intraspecific genetic diversity within the cestode, *E. granulosus,* has obviously impacted the various characteristics of the parasite and parasite-host relationship, including parasite life cycle, host specificity, pathogenicity, host defense mechanisms, the disease process and prognosis, definitive diagnosis, and susceptibility to antihelminth drugs (Maglioco, et al., 2019) (Li, et al., 2019) (Babaei, et al., 2021) (Mardani, et al., 2021).

AgB, with significant immunological properties, constitutes approximately 10% of hydatid fluid (Siracusano, et al., 2008) and is synthesized and released by the hydatid cyst's germinal layer as well as by the germinated protoscoleces (Shirazi, et al., 2016). This hydatid antigenic fraction encoded by a gene family involves 10 unique genes of five subfamilies (EgAgB1-EgAgB5) expressed in different stages of the parasite (Sarkari Shahriari, et al., 2013). At present, similarly, a high level of AgB8 subunits 1 and 3 was observed in hydatid cyst samples isolated from humans and cattle (Esfedan, Sarkari, and Mikaeili, 2018). AgB is thought to be involved in crucial roles in the host-parasite relationship through disturbing both innate and adaptive immune responses, including disturbance of polymorphonuclear leukocyte chemotaxis and shifting the immune response toward a non-protective Th2 response that initiates a long-term chronic infection (Shirazi, et al., 2016).

In the present study, despite the studied isolates revealing various complexed polymorphic profiles in the amplified products of the *E. granulosus AgB2* gene (Accession no.: AY569356.1), the pathogenicity of the parasite did not significantly alter, and those findings might be explained by the significant levels of AgB that are expressed in all stages of *E. granulosus* throughout its life cycle, regardless of the parasite strain (Esfedan, Sarkari and Mikaeili, 2018) (Sadjjadi, et al., 2022). Furthermore, no significant alteration in the pathogenesis of the studied isolates might be attributed to both immunomodulation and lipid transport, contributing to the AgB in the host-parasite relationship (da Silva, et al., 2018).

## V. Conclusion

The polymorphic sequencing profile of the PCR-amplified product (250 bp) of the *E. granulosus* clone EgB2G2v13 *AgB2* gene (Accession no.: AY569356.1) has no significant impact on the pathogenicity of CE in a murine model. *E. granulosus* genotype G2 is the common cause of CE in Erbil province, northern Iraq. To upgrade the diagnostic sensitivity rates of the immunological techniques, and due to the highly polymorphic genes that encode for AgB2, a mixture of native hydatid antigens containing AgB is recommended to be used in the serodiagnosis procedures for this infection.

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