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ORIGINAL ARTICLE

Molecular Identification and Phylogeny of Ec-4 Antigen B-Like Gene of Hydatid Disease Caused by *Echinococcus granulosus* Isolated from Cattle in Basrah South Iraq

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ABSTRACT

Background: *Cystic hydatid disease is an important infection that affects a wide range of animal species and humans in many world countries with low-quality control procedures of Echinococcus granulosus in canines, especially dogs.* ***Objective:*** *The current study was conducted to identify and explore the genetic evolution in E. granulosus based on the utilization of Ec-4 antigen B-like (EgAgB) gene as molecular target.* ***Methodology:*** *The study included 67 expected liver samples cysts. These samples were subjected to PCR and partial gene sequencing of the EgAgB gene.* ***Results:*** *The results revealed the presence of these cysts in 16 (23.9%) using morphological identification. The PCR revealed that only 4/16 (25%) of the samples were positive for the gene. The genetic evolution-based phylogenetic tree showed that the current study isolates were nucleotide-similar to isolates from the neighbor country; Turkey.* ***Conclusion:*** *The data of this work showed continued existence of the hydatid disease in cattle in the examined city, still involved in a bovine-dog cycle and so represents a reservoir of the parasite which can apply dangerous health impacts on consumers in the city.*

INTRODUCTION

Hydatidosis is an infectious parasitic disease caused through animal-to-human transmission due to the larval stages of some species of tapeworms belonging to the genera *Echinococcus* and the family Taeniidae. The hydatid cyst of the *Echinococcus* parasite is fluid filled a fluid-filled cyst, which grows in different organs and tissues of the patient due to the development of *Echinococcus* parasite embryos or oncospheres in these organs. The larval stage takes its course of developing in a different array of intermediate hosts which includes humans. However, the adult stage is generally found in carnivorous animals[1-3].

Echinococcosis is endemic to regions where sheep and cattle are raised all over the world. Great prevalence of human infection with echinococosis in regions of Africa and South America, E. multilocularis occurs in different regions of Eurasia[4,5].

Echinococcus granulosus is the most common strain affecting humans worldwide, although *E. multilocularis*, *E. oligarthrus* and *E. vogeli* were also recorded. Both species (*Echinococcus granulosus* and *E. multilocularis*) cause great human suffering and are of major public health concern. Signs from all over the world indicate that echinococcosis is increasing in its impact on human health. Besides the consequences for animal and human health[6,7], Hydatidosis also causes economic damage, since organs and carcasses are condemned, and illnesses in animals and humans must be treated at considerable costs. According to the World Health Organization, epidemiology of the disease, more than 1 million people are affected by echinococcosis at any given time, in livestock, the prevalence of cystic echinococcosis found in slaughterhouses in hyperendemic areas, the disease is typically categorized as a veterinary problem due to its importance in management parasitic infection in animals[4]. Nonetheless, effective management of hydatidosis requires cooperation between veterinary sectors and human health systems. Because the infection is generally asymptomatic (subclinical) in both the terminal and intermediate hosts, its diagnosis is problematic and its transmission is risky[8-10].

EgAgB is the protein encoded by the larval stage of *E. granulosus*, and it is expressed and secreted by both the cyst germinal layer and the protoscoleces. It is highly immunogenic antigen that causes an offensive response and therefore it is detected in more than 80% of patients' serum, though its exact physiological role still has not been defined, other than speculative evidence regarding putative lipid binding characteristics[11-14]. The hypothesis is that it plays an important role in the interface with the host defends on several recent mechanistic investigations showing that *EgAgB* acts as a serine protease inhibitor that modulates chemotaxis of neutrophils to the site of infection. It also functions as an immune regulator that skews the Th1/Th2 balance toward a Th2 response (which favors

the survival of the parasitic organism within a mammalian host). In addition, *EgAgB* has been shown to be a product of a family of genes, which itself, displays high variability between different isolates and strains of *E. granulosus*[15-18].

Cystic hydatid disease is an important infection that affects a wide-range of animal species and human in many world countries with low-quality control procedures of *Echinococcus granulosus* in canine, especially dogs. The current study was conducted to identify and explore the genetic evolution in *E. granulosus* based on the utilization of Ec-4 antigen B-like gene as molecular target.

METHODOLOGY

Collection of Samples

A total of 67 hydatid cysts were collected from liver (35) and lung (37) samples of cattle from different areas in Basra Governorate during the period from May to December 2023. Hydatid cysts were identified in the liver samples by clearly observing them as a white or yellowish-white layer on the blister. All samples were placed in refrigerated plastic containers and then transported to the laboratory for study.

Morphological identification

After being preserved in polyvinyl lactophenol solution, protoscoleces are put on a glass slide and flattened with the proper pressure using a slide cover. The length and blade length (BL), as well as the size of the tiny and large hooks (SH and LH) for protoscoleces of two hooks from each rostellum, are then measured in order to determine the hooks' components[19]. To illustrate the variations in strains, at least ten Protoscoleces were examined under a microscope with a 100x oillens.

DNA extraction

The samples were used in the Promega DNA Extraction (Promega, USA). The protocol of the kit was employed for the extraction procedure. A NanoDrop was recruited to evaluate the extracted DNA.

Polymerase chain reaction (PCR)

The PCR employed the utilization of F: CCGTTCAAGCGTGAGTCTCA and R: TGTCCCGACGCATGACTTAC as a primer set that target the *EgAgB* gene at a piece of 308 bp. The Promega PCR master mix was used to prepare the master mix reaction (total: 25 µl). The PCR thermal cycler conditions were 95 °C-30s (94 °C-30s, 54 °C-30s, and 72 °C-30s), and 72 °C-30s for the initial denaturation (one cycle), (denaturation, annealing, and extension) for 35 cycles, and final extension (one cycle). The PCR products were 1.5%-agarose-gel-run using an electrophoresis process. A UV-visualizing imager was recruited to explore the amplification findings.

Gene sequencing

The PCR products purified from the gel were sent out for sequencing (Bioneer, Korea). The phylogenetic tree was built using information from NCBI websites and MEGA X software.

RESULTS

The results revealed the presence of these cysts in 16 (23.9%) (9 in the liver (56.2%) and 7(43.8%) in the lung) by using morphological identification. The Polymerase chain reaction (PCR) revealed that only 4/16 (25%) of the samples were positive for the gene.

The genetic evolution-based phylogenetic tree showed that the current study isolates were nucleotide-similar to isolates from the neighbor country; Turkey (Figure 2).

Fig. 1: Image of agarose gel electrophoresis of positive PCR products of the *EgAgB* gene that belong to *E. granulosus*

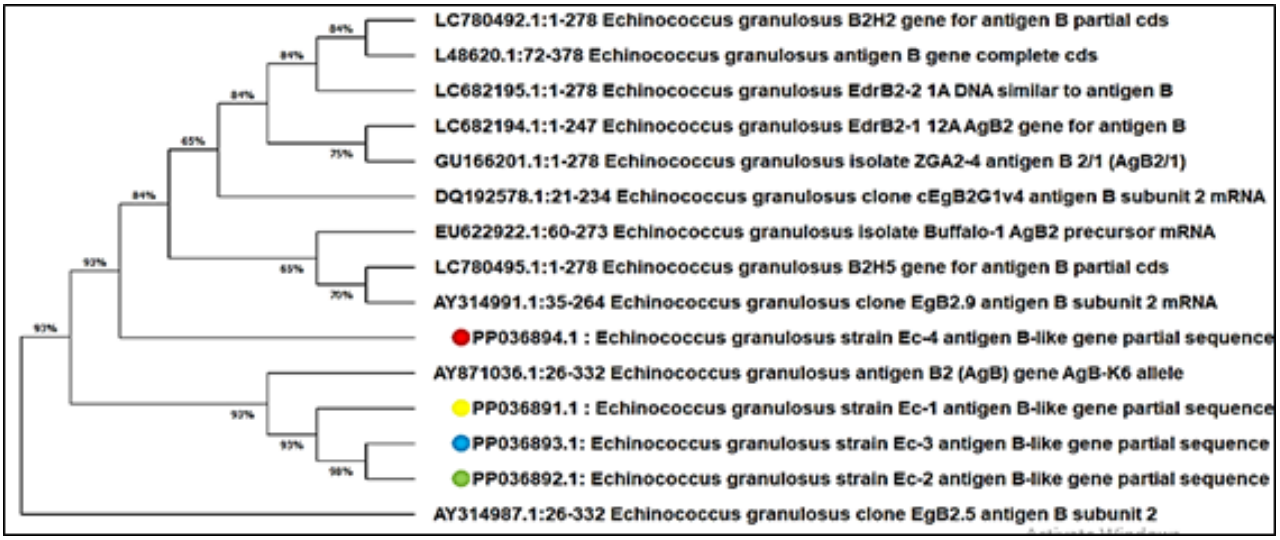

Fig. 2: Phylogenetic tree based on the partial *EgAgB* gene of *Echinococcus granulosus* isolated from cattle. Color filled-circles: Current study isolates

Table 1: Maximum likelihood estimate of substitution matrix

	A	T/U	C	G
A	-	4.35	3.18	13.49
T/U	5.00	-	15.28	3.86
C	5.00	20.93	-	3.86
G	17.51	4.35	3.18	-

DISCUSSION

By using morphological identification, detected hydatid cysts in 16 samples (23.9%) (9 in the liver (56.2%) and 7(43.8%) in the lung), and Polymerase chain reaction (PCR) revealed that only 4 of the 16 samples (25%) were positive for the gene, various studies have shown the presence of varying proportions of *E. granulosus*. Azami *et al.*[20] showed the most prevalence in sheep lungs (27%), and the least one in liver at (12%). In another study carried by Ehsan *et al.*[21], they described the cyst in cattle with an average of 9% and 5% in the liver and lungs, respectively. In another investigation, Haleem *et al.* [22]found 64% and 24%, in the same organs respectively, they described 69 livers from cow, 9 lungs from cows, 12 livers from lambs, 14 lungs from lambs, 3 livers, and one lung[22]. In a study dedicated to the analysis of the hydatid cyst in the liver and the lungs in Pakistan showed the following: 68% and 32% rates in sheep liver and lung, 66% and 33% rates in goat livers and lungs, 85% and 16% rates in cattle livers and lungs, 83% and 17% rates in camel livers and lungs [23]. In the other research that was conducted also in Pakistan found 47% and 17% in sheep livers and lungs, respectively, and 23% and 14% in goat livers and lungs, respectively[24]. Singh *et al*. concluded that the infection was 50% in liver and 36% in lung[25].

The phylogenetic analysis based on nucleotide sequences revealed that the isolates obtained in the current study cluster closely with isolates previously reported from Turkey, indicating a high degree of genetic similarity, the current isolates group with known *E. granulosus* strains such as B2H2, EdBr2.1A, and other antigen B-related sequences from Turkish sources, with strong bootstrap support values ranging from 65% to 99%. This close genetic relationship suggests a potential regional pattern of parasite circulation and may reflect a shared evolutionary origin across neighboring geographic regions.

The sequences at the top (starting with LC780492.1 and ending with EU622922.1) form a well-supported cluster (high bootstrap values at the connecting nodes). These represent various "antigen B" or "antigen B-like" genes and related DNA/mRNA sequences from different *Echinococcus granulosus* isolates. They are inferred to be relatively closely related to each other. The sequences highlighted with colored circles (PP036894.1 - red, AY871036.1 - yellow, PP036891.1 - yellow, PP036893.1 - blue, PP036892.1 - green) form another distinct cluster. These are also described as "antigen B-like" genes or alleles from different *E. granulosus* strains. The high bootstrap values (93%) at the nodes connecting them indicate strong support for their close relationship. The bootstrap value (62%) at the deeper node connecting the top cluster and the central colored cluster suggests moderate support for their relationship, meaning they are inferred to be related but less strongly than the sequences within each cluster. The sequence AY314987.1 is placed as a sister group to the central colored cluster with strong support (93%). This "Egb2.5 antigen B subunit 2" gene is inferred to be closely related to the strains in the colored cluster. The tree suggests at least two genotype

groupings: group 1: Likely G1-G3 (sheep strain group) genotypes (classical antigen B), and group 2: Likely G6-G10 (camel, pig, and other host-adapted strains) genotypes (antigen B-like variants).

Many studies suggested that the *EgAgB* gene family is variable between isolates and genotypic strains of *E. granulosus*, Zhang et al.,[26] cloned and sequenced ten unique genes of this gene family, each was identical in both larval and adult *E. granulosus* isolates.

Sharbatkhori *et al*[27] reported that %78.3 of all hydatidosis cysts, collected from cattle, small ruminants, and buffaloes, were G1. Pezeshki *et al*[28] documented %92 prevalence of G1 genotype among animals in Iran. Nematdoost *et al*[29] documented the prevalence of %7.2 G3 genotype among hydatidosis cysts collected from livestock. There are several studies showing the detection of the G3 genotype in Iran and in other countries[30]. *E. granulosus* includes *E. canadensis* (G6/G7) and *E. ortleppi* (G5). These genotypes have been detected in slaughtered livestock at (12% and 6%) of 19 isolates of *E. canadensis* (G6/G7), 53%, 21%, %11, and %16 in camels, sheep, goats, and cattle, respectively[31]. In addition to the fact that goats[32] camel[33] and goats[34] in different parts of the world are reported. The camel is the main intermediate host of the G6 genotype[35]. In Pakistan and Scotland[36], G6 was reported in humans. Although it was claimed that G6 genotype, has lesser ability to infect humans than the G1 genotype, but according to Alvarez Rojas *et al*, it is the second main cause of the disease after *E. granulosus*[37]. Simsek and Kaplan[38], reported two human cases with G6 in Turkey.

CONCLUSION

The data of this work showed continued existence of the hydatid disease in cattle in the examined city, still involved in a bovine-dog cycle and so represents a reservoir of the parasite which can apply dangerous health impacts on consumers in the city.

Conflict of interest

There is no conflict of interest.

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Ethics statement

The Ethical Committee of the University of Basrah - College of Pharmacy approved this study under Protocol No. EC 60 dated 14-10-2024.

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