A Revisit to the Infamous Zoonotic Echinococcosis: A Molecular Review

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Abstract

Through the past five decades, significant phenotypic and genetic variabilities have been recognized and identified in various strains of Echinococcus granulosus isolated from different regions. Studies have revealed that the different strains of E. granulosus consist of heterogeneous groups of genetic variants, which may display variations in morphology, host specificity, development rate, pathogenicity and geographical distributions. Thus identification of strain types is very important in strategizing and implementing an Echinococcus control and management program.

Keywords: Zoonotic echinococcosis; Molecular

Molecular Characterization of Echinococcus granulosus

Taxonomic studies of E. granulosus strains have been carried out based on different analytical methods such as morphology, epidemiology, biochemistry and molecular genetics [1]. These methods have proven to be useful, especially when used collectively. Thus, when morphological and molecular methods are conducted in complement they could provide more accurate and reliable information regarding the type and range of variation of E. granulosus [2-4]. However in the case of diagnosis of E. granulosus in the final hosts, different methods are used based on DNA analysis using Polymerase Chain Reaction [5,6].

Based on molecular data, E. granulosus have been classified into several genotypes [7-11] namely:

a) G1, common sheep strain.
b) G2: Tasmania sheep strain.
c) G3: Buffalo strain.
d) G4: Horse strain.
e) G5: Cattle strain.
f) G6: Camel strain.
g) G7: Pig strain.
h) G8: Cervid strain.
i) G9: Human strain. (Poland)
j) G10: Fennoscandian cervid strain.

In addition, several researchers have suggested a review of this genus based on phylagenetic findings, specifically to re-classify several genotypes into species [9,12]. The identification of genotypes has at least two important applications. Firstly, it supports progressive DNA vaccination using recombinant DNA technology [13]. Secondly, it plays an extremely important role in studies on vaccination resistance [14]. In the past decade, molecular methods have been utilized globally to identify the most common strain of E. granulosus isolates including in studies in Algeria, Tunisia, and Mauritania [15-17].

However, only limited studies have been conducted in Libya [1,18]. In fact, on the whole, not much information is available regarding the molecular characterization of Echinococcus granulosus in North Africa. According to Eckert et al. [19], epidemiologic studies from different Middle Eastern regions indicate that camel is an important intermediate host that spreads the infection to humans.

Genetic Variation in Echinococcus granulosus

According to Thompson and McManus [12,20], a single important biological characteristic of E. granulosus is that it is composed of a number of intraspecific distinct features or strains that manifest in considerable variation at the genetic level. The term strain is used to describe variants that differ from other groups of the same species in gene frequencies or DNA sequences, and in one or more characters of factual significance to the epidemiology and control of hydatidosis [21,22].

Several researches have noted that the wide intraspecific variation in E. granulosus may be linked to life cycle patterns, development rate, host specificity, transmission methods and pathology [12,20,21]. Such a situation has a significant influence on the design and development of diagnostic reagents and vaccines regarding the epidemiology and control of echinococcosis.

Research in molecular methods based mainly on mitochondrial DNA sequences has identified 9 different genetic types of E. granulosus. In addition, genotype 10 was recently identified as a strain present in reindeer and moose in northeastern Finland [9]. Usually, the molecular approach is used when it is difficult to differentiate at the morphological characteristics.

Molecular Methods Utilized for Genetic Assessment of Echinococcus

The genetic variation of Echinococcus has been extensively explored based on sequences from mitochondrial and nuclear genomes. In this regard, Polymerase Chain Reaction (PCR)-based methods are highly sensitive and at present widely used for Echinococcus identification targets, including discrimination of eggs. The following is a description:

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of the various molecular methods used to study genetic variation in *Echinococcus* [23,24].

**PCR-amplified DNA sequences**

This method is based on a direct comparison of the nucleotide sequences between organisms and provides a highly reliable and sensitive diagnosis. The fragments of the mitochondrial genes cytochrome c oxidase subunit 1 (cox1), and the NADH dehydrogenase subunit 1 gene (nad1) have proven to be very useful in *E. granulosus* strain identification [23]. Dinkel et al. [25] developed rapid diagnostic approach by using the specific and sensitive semi-nested PCR system for *E. granulosus* genotypes G1 and G6/7 and *E. ortleppi* G5 genotype. The diagnosis of G1, G6 and G7 was accomplished by a simple PCR, whereas the differentiation between G5 and G6/7 included a subsequent semi-nested PCR step. In addition, the mitochondrial 12S ribosomal RNA gene was evaluated on isolates of 16 species of cestodes including E. equinus G4 and E. ortleppi G1, G5, G6 and G7 genotype. Saad and Magzoub [26] and Elmahdi et al. [27] revealed for the first time a camel RNA gene was evaluated on isolates of 16 species of cestodes including E. equinus G4 and E. ortleppi G1, G5, G6 and G7 genotype. Saad and Magzoub [26] and Elmahdi et al. [27] revealed for the first time a camel strain G6 infection in humans in eastern Africa and cattle strain G5 in livestock from Sudan and Kenya respectively based on the PCR system.

Daniel Mwambete, et al. [28] tested genotype isolates of *E. granulosus* from different intermediate hosts based on the RAPD- PCR analysis in Spanish strains. Three strains, namely sheep-dog, horse-dog, and pig-dog of *E. granulosus* had been previously identified in Spain [28]. Daniel Mwambete, et al. [28] confirmed that the sheep strain G1 corresponded with genotype 1 but also infected sheep, goats, pigs, cattle and human. In addition they also confirmed that the horse strain corresponded to genotype 4 and only infected horse while the pig strain corresponded with genotype 7 and infected pigs and goats.

**RFLP/RAPD analysis**

Early DNA studies of genetic variation in *Echinococcus* had been focused on Restriction Fragment Length Polymorphism (RFLP) analysis based on the Southern blotting method [29-31]. In addition, Bowles and McManus [32] observed that the then current (RFLP) analysis was a simple process through binding rDNA RFLP analysis with PCR with no loss of solution or precision.

Azab, et al. [33] stated that previous studies of genetic variation which focused on RFLP analysis based on conventional southern blotting were able to differentiate between several strains of *E. granulosus*, were stable during analysis of a particular strain. The utility of Random Amplified Polymorphic DNA (RAPD-PCR) analysis has also been highlighted [34], when conducted under carefully controlled conditions. The method successfully characterized the four recognized *Echinococcus* species and different strains of *E. granulosus*. Furthermore, this method has been used in Egypt to identify the camel-dog strain [33].

**Microsatellite markers**

Microsatellites are short segments of DNA which have a repeated sequence and tend to occur in non-coding DNA. In comparison to the other methods, microsatellite DNA analysis is still underutilized in studying diversity in *Echinococcus* and only a few microsatellite markers are available for *E. multilocularis*. Microsatellite DNA was used for the first time by Bretagne et al. [35] to successfully assign *E. multilocularis* isolates into three groups. In addition, Nakao et al. [36] identified two microsatellite loci to demonstrate population level polymorphisms in *E. multilocularis* adults from wild foxes. In another study, Bartholomé-Santos, et al. [37] used eight oligonucleotides, including specific repeats as probes to characterize for the first time microsatellites of *E. granulosus* from Brazil and Argentina.

**Classification of Echinococcus granulosus Strains**

**Sheep-dog (G1) and horse-dog (G4) strains**

In their studies, McManus and Simpson [29]; McManus et al. [30] and McManus and Rishi [31] demonstrated the presence of sheep-dog and horse-dog strains in United Kingdom. Their findings were corroborated by Wachira et al. [38] who also detected the host predilection of sheep strain from Kenya while in Australia; Hope, et al. [39] demonstrated the presence of a single sheep genotypic strain in livestock animals.

In addition, it has been found that there are different strains of sheep-dog and horse-dog forms of *E. granulosus* which vary greatly in terms of biological criteria. For instance, Le, et al. [40] found that the sheep strain infects humans, whereas it may not be infective to horses. On the other hand, the horse strain appeared to be poorly infective to sheep as well as humans. Presently, based on DNA data, the sheep strain and horse strain differ by 12.4% in nucleotides and 11.6% in amino acids. Bowles, et al. [22] demonstrated an alternative process to detect levels of divergence based on the phylogenetic tree by using phylogenetic analysis of the sequences of mitochondrial and nuclear data. In another study, Le, et al. [40] used phylogenetic analysis to detect the level of divergence based on the phylogenetic tree of concatenated nad 1 and ATP6 genes from *E. multilocularis*, *E. oligarthrus*, *E. vogeli* and five genotypes from *E. granulosus* as illustrated in Figure 1.

**Cattle-dog strain (G5)**

According to McManus [41], up to the early 1990s, all human samples of *E. granulosus* examined by isoenzyme and DNA analysis belonged to the common sheep strain G1. However, the calcified hydatid cyst which was removed from young Dutch men and analyzed by PCR/RFLP test and cox1 and nad1 sequences, belonged to cattle strain G5, a strain found in Argentina [42,43]. Normally, cattle are the common host of hydatid cysts worldwide even though the sheep strain is more prevalent than the cattle strain. Moreover, according to Bowles, et al. [22], when cattle are infected by the sheep strain, the cattle is considered as an accidental host and the resultant cyst is usually infertile.

Although the molecular data for the cattle strain is not as rich...
compared to the sheep strain, nevertheless the cattle strain has a widespread geographical distribution, including South Africa, India, Central Europe and South America [12]. Recently, Obwaller, et al. [10] revealed that the cattle strain G5 was shown by nad1 and cox1 sequences to infect Namibian zebra. However, only a limited number of molecular studies have been conducted on Echinococcus isolates from South African hosts to confirm the prevalence of this strain [41].

Cervid strain (G6)

The cervid strain, which has been identified by DNA analysis, infects camels, cattle, goats and pigs in East Africa. It is also found in other countries including Iran, Argentina and China [41]. DNA studies have revealed that the camel strain infects camels, cattle and humans. In addition most of the studies from Mauritania, Egypt, and Iran have revealed the presence of the camel strain G6 in humans [15]. A recent study also revealed for the first time its presence in Kenyan human populations [25]. It was suggested that the camel strain has a shorter maturation time in dogs compared to the common sheep strain, hence more infective in humans.

Pig-dog strain (G7)

According to Scott, et al. [8], analysis of Echinococcus isolates from Poland indicated that the infection was not caused by the common sheep strain, G1 but the pig strain G7 as revealed by DNA analysis which showed very clear differences. However, DNA studies of pig and human isolates from Poland and Slovakia have failed to confirm the presence of this genotype, but have provided evidence for the almost exclusive existence of G7 [44,45].

Cervid strains (G8-10)

In North America and North Eurasia, the life cycle of the E. granulosus includes an intermediate moose and reindeer hosts with wolves and sledge dogs as the definitive hosts [46]. In Alaska, based on a single nad1 sequence and ITS PCR-RFLP pattern, the cervid strain, which obtained the G8 genotype in the moose [7]. However, Thompson and Lymbery [46] demonstrated that E. granulosus of cervid origin G8 differs in a number of biological assessments from domestic strains of E. granulosus.

Lavikainen, et al. [9] came up with a molecular guide to identify the presence of a new, distinct cervid strain. They observed that isolates of E. granulosus from reindeer and moose in Finland analyzed for mitochondrial cox1 and rDNA ITS-1 genes were similar, but a high sequence variation was found in the ITS-1 region. However, the mitochondrial and nuclear sequences of the cervid strain from Finland and camel strain G6 closely resembled each other. The phylogenetic analysis (Figure 2) indicated that the Finnish isolates presumed to be G8 had a close relationship with G6 and G7.

The cervid strain initially assigned G8, appeared to represent a distinct, previously undescibed genotype of E. granulosus and was then reclassified by Lavikainen, et al. [9] as the Fennoscandian cervid strain G10. According to McManus [41], it is important to evaluate the geographical distribution of this new genotype in order to determine whether it is infective to humans as demonstrated in the case of G8 strain.

Based on mitochondrial genes (nad1, cox1), Le, et al. [40] in China showed that G1, G4, E. vogeli, and E. oligarthrus are almost equidistant from each other and the G1, G4 genotypes are nearly equidistant from G6, G8 genotypes (Figure 2). However G6, G7 and G8 are closely related and most likely belong to the same origin (livestock and human).

Molecular Phylogenetic Analysis

Studies conducted before the 1990s failed to show the close phylogenetic relationships among Echinococcus strains based on morphological and incomprehensive biochemical data. However, through molecular analysis, Bowles, et al. [22] recorded many characters, in particular, DNA sequence analyses which were very useful in the refining of the available morphological data based on phylogenies. In this regard, sequence data, including three nucleotide data sets, two mitochondrial (cox1; nad1) and one nuclear (ITS1) were used to elucidate the phylogenetic relationships of the Echinococcus strains. The combined mitochondrial data (Figure 3) and the nuclear data (Figure 4) revealed at least three evolutionarily discrete groups of E. granulosus. Furthermore, the molecular distances between them was comparable to or greater than the molecular evolutionary distances observed among recognized species suggesting that they were distinct taxa.
DNA Detection of Infection in Definitive and Intermediate Hosts Based on Enzyme-Linked Immunosorbent Assay (ELISA) and Copro-DNA by PCR

Two methods have been successfully identified for the diagnosis of adult worms of *Echinococcus* in small intestines of definitive hosts [41]. The first is the investigation of E. granulosus specific coproantigens echinocystic-like in a cDNA-based coproantigen assay (ELISA) and the second is copro-DNA by PCR [47-52]. In addition to this application, these methods provide the means to study the transmission biology of *E. granulosus* as they allow investigation of infection in faecal samples collected from the environment.

According to Deplazes, et al. [48] the coproantigens (copro-PCR) method provides a sensitive, fast and cheap diagnosis compared to the PCR method alone, which is time consuming and expensive. In addition, the copro-PCR (antigens) is a useful method to confirm the positive coporantigen results based on ELISA, as taeniid eggs are difficult to differentiate morphologically. Therefore, the PCR (coproantigens) is the best method to use for identification of *Echinococcus* eggs in faecal samples, Dinkel, et al. [52].

Molecular Methods in Epidemiological Studies of Hydatid Cysts

On the other hand, Dinkel, et al. [52] described the routine PCR used in epidemiological studies and surveys of prevalence of hydatid cysts, especially small, atypical and calcified ones in intermediate hosts from different infected organs. In addition, Xiao, et al. [53] and Heath, et al. [54] discussed how PCR sequencing of mtDNA sequences have been utilized to reveal that Chinese yaks are unlikely to be infected by *Echinococcus* spp. Although the availability of a fairly comprehensive genetic database have provided a solid molecular basis for studying the taxonomy of the genus *Echinococcus*.

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