CHAPTER 13

MOLECULAR CHARACTERIZATION OF CYSTIC HYDATIDOSIS IN BASRAH GOVERNORATE- SOUTHERN IRAQ

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INTRODUCTION

Hydatid disease is considered as one of the most dangerous diseases that spread in all parts of the world, and Iraq is one of the countries where this disease is present in a large and widespread among ruminants, human and stray dogs (Lazim, 2019). Typically, hydatidosis can be classified according to the larval stage and divided into two types: Cystic Echinococcosis (CE) and Alveolar Echinococcosis (AE), Cystic Echinococcosis is a zoonotic disease and occurs by the larval stage of *Echinococcus granulosus* that has a cosmopolitan allocation and it is one of the most significant zoonotic diseases around the world (Thompson 2008). CE cause huge economic losses and has great public health significance worldwide (Romig et al. 2011). While, alveolar Echinococcosis is caused by *E. multilocularis* (Wen and New 1993), and this disease has a serious impact on human and animal health (Snabel et al. 2009). It also results in a significant economic and public health problem in many parts of the world especially in rural areas where dogs and livestock are raised together (Groeneveld et al. 2010; Sikó et al. 2011).

Types of Echinococcus

The genus *Echinococcus* is known to include nine recognized species according to (Thompson and McManus 2002):
1- *Echinococcus granulosus* has two types of hosts: definitive hosts (dogs and other canids) and intermediate hosts (sheep, goats, cattle, pigs and human).
2- *Echinococcus equines*: definitive hosts (dogs and other canids), intermediate hosts (horses and donkeys).
3-*Echinococcus canadensis*: definitive hosts (dogs and other canids),intermediate hosts (human as well as domestic and wild animal).
4-*Echinococcus felidis*: definitive hosts (dogs and other members of family canidae), intermediate hosts (lions).
5-*Echinococcus ortleppi*: definitive hosts (dogs and other canids),intermediate hosts (cattle and human) which cause cystic Echinococcosis (CE).
6-*Echinococcus multiocularis*: definitive hosts (dogs and other canids)intermediate hosts(human) which causes alveolar Echinococcosis.
7-*Echinococcus oligarthrus* and *Echinococcus vogeli*: definitive hosts (dogs and other canids), intermediate hosts are rodents and ungulates and accidentally, human, causes Polycystic Echinococcosis (PE).
8-*Echinococcus shiquicus*: definitive hosts (dogs and other canids), intermediate hosts (small mammals) with cysts similar to CE or PE but of unknown zoonotic status (Nakao et al. 2007; Badaraco et. al. 2008).

Clinical Diagnosis

The clinical diagnosis of (CE) in human and animals were difficult because the disease continues without symptoms and the morbid identification of the causative species was difficult in the cases of irregular forms (Eckert and Depalazes 2004). Also, Nakao et al. (2010) noticed that *Echinococcus* spp. Must be subjected to molecular diagnosis for species identification. Now a day, clinical samples taken at biopsy are subjected to PCR, and the amplified the fragments of mitochondrial and nuclear DNA are subsequently sequenced and strains are determined.

Clinical Sings of Hydatidosis in Animals

Clinical sings depend on location and size of hydatid cyst in their intermediate hosts (David and Petri 2006). Infection remains asymptomatic for many years before increasing the number and size of cysts, which are able to cause symptoms in the infected organs, However, this disease may be in progress and result in obstructive symptoms (Paniker 2013). Sometimes, hosts show clinical symptoms, such as slow growth, weakness and lameness (OIE 2008). The degree of symptoms varies depending on the severity of the disease and the location of the hydatid cyst. Clinical indicators in the affected animal include decreased milk production, poor wool, and organ damage in the affected area (Eckert and Deplazes 2004; Eddi et al. 2006).

Clinical Sings of Hydatidosis in Human

The symptoms in humans stay reliant on the involvement of precious organ and the liver is the most vulnerable organ, with a rate of infectivity roughly around 60-70 percent, followed by the lungs (20-22%), spleen, heart, muscles, eye, and thyroid gland (6%), kidneys, brain and bones (1%), Also,

How to cite this chapter: Abdulazizz SA, Mustafa JY, Al-Ataby FHA and Lazim AR, 2022. Molecular characterization of cystic hydatidosis in Basrah Governorate- Southern Iraq. In: Abbas RZ, Khan A, Liu P and Saleemi MK (eds), Animal Health Perspectives, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. I, pp: 95-102. https://doi.org/10.47278/book.ahp/2022.13

there is no organ in the body that is free of hydatid cysts to preserve teeth, nails and hair (6%) (Marquardt et al. 2000). Even if the cyst is tiny, symptoms appear, and the majority of instances of brain cyst illness are discovered in youngsters. This infection is dangerous enough to cause death in certain circumstances (Moro and Schantz 2009). Also, there is inflammation and sensitivity on palpation of liver abscesses, as well as discomfort in the stomach, vomiting, nausea, as well as increased hepatic blood pressure and in the lumen of the inferior vena cava, are indicators of the liver. The bile ducts have secondary fibrosis, and the hydatid cyst puts a lot of strain on the diaphragm (Brunetti 2015).

Diagnosis of Hydatid Cysts

Hydatidosis can go unnoticed for years until the quantity and size of cysts grow large enough to produce symptoms in the afflicted organs. The diagnosis of *Echinococcus granulosus* in the intermediate host is based on the detection of a hydatid cyst, which can occur in almost any organ but is most common in the liver and lungs. Ultrasound and computed tomography examinations for abdominal Echinococcosis, X-ray for lung Echinococcosis, and immunodiagnostic tests are used (Pawlowski et al. 2001). While the discovery of adult Echinococcus spp. in feces or the small intestine, or the detection of particular coproantigens or coproDNA, is required for the diagnosis of Echinococcosis in dogs or other carnivores (OIE2008).

Distribution of Echinococcosis

Echinococcosis is one of the serious public health problems in all the parts of Iraq in both human and animals. This is because of distribution of stray dogs in all parts of Iraq. Also, hydatid cysts have been frequently reported in livestock slaughtered at slaughterhouses in most of the Iraqi provinces and human. For example, a case of AE has been reported from the north of Iraq, in Zakho (Al-Attar et al. 1983) in human. Amr et al. (1994) recorded that CE was prevalent 60% female and 40% male. With major affect in liver 60% and lung 26.4% respectively. Another investigation found a high prevalence of hydatid cysts in sheep (14.75) prevalence of 1.5%, 5.9%, and 13.7% in North, Middle and South of Iraq Mohamad et al. (2008). In the north of Iraq, the prevalence of hydatid cysts was reported at 12.7% in sheep, 4.8% in goats and 4.3% in cattle (Mero et al. 2013), while, in Al-Najaf the prevalence of hydatidosis was 0.62% in sheep (Al-Shabbani 2014). In Arbil province, the rate of infection in sheep was 15.0% (Saeed et al. 2000), and in Mosul 3.16 % (Jarjees and Al-Bakri 2012), in Thi-Qar the rate was 15.15% (Al-abady et al. 2010) and hydatid cysts were found in 42% of sheep in Baghdad (Imari 1962). By the other hand, hydatid disease is one of the common zoonotic parasitic infection in human, in Sulaimaniya province for example the rates was 5%, 7.3%, 3.5% and 7.7% among veterinarians, assistant veterinarian, slaughterhouse workers and animal breeders (Abdulla et al. 2014). In Baghdad a total of sixty cases of human hydatidosis were collected, with (73%) were liver cysts, (20%) were lung cysts (Khalf et al. 2014). However, most of the scientific research conducted on hydatidosis in Iraq has not investigated risk factors for infection or evaluated the impact, including economic burden, of the disease on the community and only one study performed has evaluated economic losses from the condemnation of affected viscera of sheep, cattle and goats. In that study, conducted in Kirkuk, an overall annual economic lossof 10,430,000 Iraqi dinars (approximately US$ 8,800) was estimated (Kadir et al. 2012).

In Basrah province- southern Iraq, a study recorded by Al-Shalabbi (2007) showed the rate of infection in sheep 4.2%, also a study showed the effect on killing of the protoscolices of *E. granulosus* in vitro and in vivo in the laboratory mice. A case with *E. multilocularis* has been recovered from the liver of a woman in Basrah Southern Iraq from 55 years-old living in Al-Hartha region, southern that was in contact with sheep and dogs (Benyan et al. 2013).

The prevalence of hydatid cysts in slaughtered sheep found to be 14.75%. The female sheep 22.9% (123/536) was observed to be more infected with hydatid cysts than male's sheep7.5% (46/609) (Mutar et al. 2017), the prevalence in liver and lungs constituted 61.6% (104/169) and the lungs 38.4% (65/169) were recorded by Abdulhameed et al. (2018). Other study in Basrah province recorded the infection with hydatidosis in sheep, donkey and human with prevalence rate 43.15, 28.5 and 8.8% respectively (Lazim 2019). According to the Iraqi CDC (2012), the number of cases of hydatidosis in humans has increased dramatically since 2000 and from 2011 to 2015 4,769 human CE cases were recorded by the Communicable Diseases Control Center (Parasitology and Helminthology Units) in Iraq (Saheb and Noori 2019). The strategic implementation of a control programme to eliminate or reduce the number of free roaming dogs, as well as owned domesticated dogs, has not been implemented in Iraq (Al-Shabbani 2014).

Molecular Study

Molecular diagnosis of Echinococcus is important for understanding the genetic structure and status of genetic variation of the parasite which contains important suggestions for epidemiology and effective control of Echinococcosis in different regions and countries.

Ten genotypes of *E. granulosus* are known worldwide and categorized as G1-G10 (McManus et al.2003), but just five strains affected humans like sheep (G1), Tasmanian sheep (G2), cattle (G5), camel (G6), and pig (G7) strains (Bart et al.2006). Also, the buffalo strain(G3) and equine strain (G4) have been recorded from Spain, Italy, Lebanon and Syria (Harandi et al. 2002).

Molecular Diagnosis of *Echinococcus* spp.

Human

The results of molecular diagnosis of human samples collected in Basrah suspected to be infected with Echinococcosis are in agreement with those of Hama et al. (2012); Barak (2014) and AL-Nakeeb et al. (2015). The results of this study are consistent with studies in different parts of the world showing that the sheep breed represents the most prevalent form of Echinococcosis responsible for human infection and disease in a wide range of intermediate hosts (Busi et al. 2007; Andresiuk et al. 2009; Guoa et al.2011). Pezeshki et al. (2013) reported that the sheep breed is more prevalent in humans, sheep and goats of Iran. Utuk et al. (2008) concluded that the sheep breed is the dominant phenotype in humans, cattle, sheep, goats and camels. Though, the species status of the

four *E. granulosus* genotypes G6, G7, G8 and G10 remains unsure (Romig et al. 2017). The G6, G7, and G8 genotypes have been isolated from humans. The human cases with the G9 genotype that was described in 1997 are now considered to be the G7 genotype (Cucher et al.2016). Also, camels are significant on the topic of the epidemiology *E. intermedius* (G6), which can be transmitted to human (Thompson 2008).

Molecular studies of *E. granulosus* genotypes in special parts of the world can create a useful place of data about the parasites' epidemiology, ecology, transmission and the sources of human infection. The newest organization indicates that *E. granulosus* includes G1, G2 and G3 genotypes in sheep, human and also in cats (G1) (Cucher et al. 2016). Unusual molecular strategies and genetic targets have been useful for the identification of *E. granulosus* which includes*cox1* (M'Rad et al. 2005), *nad1* and *cox1* (Abushhewa et al.2010). In addition, NADH dehydrogenase 1 gene (*nad1*) is most preserved nucleotide sequence among different genotypes (Bowles and McManus 1993). So, the polymerase chain reaction (PCR)it is basically accurate and responsive scan Bowles et al. (1992) and is performed using gene for cytochrome oxidase subunit I (*cox1*) gene (Osman et al.2009). The pleural cyst showed a difference in epidemiology, developmental biology, morphology and genetics. They are subjected to PCR and amplification of mitochondrial and DNA fragments and then sequenced and strained (Nakao et al. 2010). Molecular study showed that the specific gene for *E. granulosus* G1 sheep and human strain NADH dehydrogenase subunit 1 as dominant (Sanchez et al. 2012). Also, more than a few molecular studies have identified the presence of two genotypes including the common sheep strains G1 and camel strain G6 in Iran (Harandi et al. 2002).

Human cyst occurs generally as two forms which differ in pathology, morphology and epidemiology. Cystic hydatid disease (CHD) is caused by the larval stage of *E. granulosus* and alveolar hydatid disease (AHD) is caused by *E. multilocularis*. More responsive molecular techniques are applied for discriminating species (Wen and New 1993).

Some Animals (Sheep, Camels, Horses and Donkeys)

Sheep breed-specific genes (*G6, sh4-1, cox1*) were identified in Basrah, Iraq (Lazim 2019) which are in agreement with Hosseinzadeh et al. (2012) who extracted DNA and used the *G7-6* and *sh-1* genes in order to detect *E. granulosus* in sheep. The results of amplification of these genes were (234 bp) and (294 bp) for the sheep strain, while the aligned sequence array (792 bp) for the partial *cox1* gene contained 124 variable loci (Junying et al. 2012). It was used to determine the species identification of Echinococcus (Pour et al. 2011). While, these genes (*nad1*, *cox1*) were used for the human strain, but in this study, three of the extracted DNA showed positive results for the *nad1* gene and G6 for the *cox1* gene.

It was found that there are many reasons for sheep to be infected with the human race. The first reason is related to stray dogs, which are considered one of the important reasons for the distribution of the human race in sheep, because stray dogs are infected with multiple strains of *E. granulosus* and *E. multilocularis*, at the same time, then these organisms transfer information between each other and are then eliminated by defecation and then infect the sheep with feces. The important source of spread of this disease is the occasion of offering sacrifices Basrah. The last reason is related to foxes, jackals or any other migratory animals because the borders are open with other countries and other governorates which has diverted this infection to Basrah.

The results of donkey samples detected through *cox1*genes agree with those of (Blutke et al. 2010). In this study the *cox1*gene is used to identify *E. equnis*. In the molecular diagnosis of Echinococcus spp. in Basrah, Iraq (Lazim 2019) representative of the sheep breed (*G6-7, sh4-1, cox1*) are in agreement with Hosseinzadeh et al. (2012) who extracted DNA and used *G7-6* and *sh4-1* genes in order to detect *E. granulosus* in sheep. The results of amplification of these genes were (234 pixels) and (294 pixels) for the sheep strain, while the aligned sequence array (792 bp) for the *cox1* partial gene contained 124 variable loci (Junying et al. 2012). This gene was used to determine the species identification of Echinococcus (Pour et al.2011).

The results of Iraq, Basrah are consistent with studies conducted in different parts of the world showing that the sheep breed represents the most important source for human infection and a wide range of intermediate hosts (Busi et al. 2007; Andresiuk et al. 2009; Guoa et al. 2011). Pezeshki et al. (2013) reported that the sheep breed is more prevalent in humans, sheep and goats in Iran. Utuk et al. (2008) reported that the sheep breed is the dominant phenotype in humans, cattle, sheep, goats and camels. *Cox1* is a partial gene of three of the DNA extracted from human show positive results for this gene due to multiple infections.

The sequencing results recorded in Basrah showed that the *G6-7* gene was an identification (96%) of an Estonia isolate that was recorded in GenBank at the entry number (KX039965.1), the partial gene registered by Laurimae et al. (2016). However, the sequencing result for the sh4-1 gene in the compartment with the database in GenBank shows that there was 99% similarity with a single isolate registered in accession number (HM563031.1) as recorded by (Harandi et al. 2011) in southern Iran. The sequencing result for the *cox1* gene compared to the database in GenBank shows that there was 99%similarity with the isolate recorded in the accession number (MF281540.1) as recorded by Yan et al. (2018), while in the *coxl* gene, the sequencing results show that there was 99% identification with Estonia isolates (Kinkar et al. 2018).

A study by Al- Ataby (2022) was done in Basrah province, isolated *nad1* gene from sheep samples, and found that there is 100% identification with the MG672293.1 strain submitted by Kinkar et al. (2018), while, the sequencing results for *cox1* from Donkey samples when compared with the database in GenBank found 100% matched with Estonia isolate strain number KY766905.1 registered by Kinkar et al. (2017).

The results of genetic analysis of the G6-7 gene in a study conducted in Iraq, Basra showed 100% identification compared with several other countries such as Iran, Turkey, Algeria and India, while the sh4-1 gene in the same study showed that the homology (99%) with isolate from Iran (Harandi et al. 2011) and the percentage was 100% with Iraqi isolation, and that the percentage was 100% identity with many countries such as Tunisia, Brazil, Turkey, India and Australia. One of the new studies (Al-Ataby 2022) in Iraq, Basrah recorded a molecular study using polymerase chain reaction (PCR) technology where four genes were used which are *nad1*gene (418 bp), *cox1*gene (370 bp), *nad1* gene subunit gene (674 bp) Basis) for *E. granulosus* and *nad2* (551bp) of Echinococcus in three hosts four strains, sheep scored five strains and camel scored two strains. And

studying the genetic sequences of those strains and comparing them with the strains registered in the gene bank by analyzing the phylogenetic tree. Isolates were scored in NCBI, under accession numbers (MW084709.1, MW077506.1, MW080539.1, MW093745.1, LC600749.1, LC600747.1, LC600748.1, LC600745.1, LC600746.1, LC600751. 1 and LC600750.1). The sequencing result showed that there is (99%) homology with the Estonian isolate in *cox1* while in the *nad1* gene, this sample identified 100% with a sample isolated from human, camel in Nigeria and from sheep in China. While in the gene *nad1* (674bp) some strains have 100% similarity in the genebank with the strain isolated from human and Iraq and in the identification of camels in an isolate sample of sheep in Nigeria and China. Finally, about (551bp) *nad2*of Echinococcus, sequencing result compared to five strains of *E. equines* recorded in the NCBI World database.

We can classify the genes detected in Basrah, Iraq from human, sheep and camel to three parts those are as below:

cox1 Gene in Human, Sheep and Camel

The *cox1* gene was responsible for encoding the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene. To 95% of the energy of living eukaryotic cells (Johnston, 2006), it directly affects metabolic performance. The *cox1* subunit I is the most conserved of the 3 genes coding for cytochrome oxidase, so it has been used in many genetic studies (Traversa et al. 2007).

When comparing the observed DNA sequences of these investigated samples with the retrieved DNA sequences from GenBank found matched with the isolate (GenBank acc. NC_044548.1). A phylogenetic tree was established in study of Al-Ataby 2022 (Fig. 1), which was based on the observed differences in DNA. This genetic tree contained samples (A1, A3, A4 and A5) along with other relative DNA sequences. The total number of DNA sequences aligned in this neighbor-binding method (Saitou and Nei 1987), was 10. Remarkably, the examined samples were grouped into two adjacent blocks within the *E. granulosus* sequences. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei 1987).

However, there was a genetic polymorphism that could be detected in sample (A1) identified in the samples examined in the study, this tree provided many informative data about all samples analyzed whether in terms of their actual location, phylogenetic tree or distances. The genetic proximity between these samples and their close sequences on one side and other sequences incorporated within the same tree forming the other side, resided all examined samples in the immediate vicinity of GenBank entry number MN787551.1, which belongs to the isolated human city of Kyrgyzstan. Interestingly, the examined samples were grouped into two phyla within the *E. granulosus* sequences. One of these branches was made of three samples (A3, A4 and A5).

While the other clade consists of other comparison samples; As well as sample (A1) in a separate branch. In fact, the positioning of (A1) is due to the presence of a mutation in this sample. In addition to the proximal position of this sample (A3, A4 and A5), it was also positioned near several reference sequences embedded in the same tree.

The three samples (A3, A4, and A5) were found in close proximity to the GenBank entry number MN787558.1, MN787556.1, MT537158.1, MT537159.1, MT537162.1 and MT537165.1, both belonging to *E. granulosus* sequences. In addition, another mode of this branch is also observed with four GenBank entry numbers MN787550.1, MN787552.1, MN787541.1 and MN787540.1, which belong to the same organism.

All examined samples settled in the immediate vicinity of GenBank entry number MN787551. Comparison with the database in GenBank showed that there was (99%) identification with the isolate recorded in the accession number (MF281540.1) as recorded by Yan et al. (2018), while in the *cox1* gene, the sequencing results showed that there was (99%) Identification with Estonia Isolate (Kinkar et al. 2018).

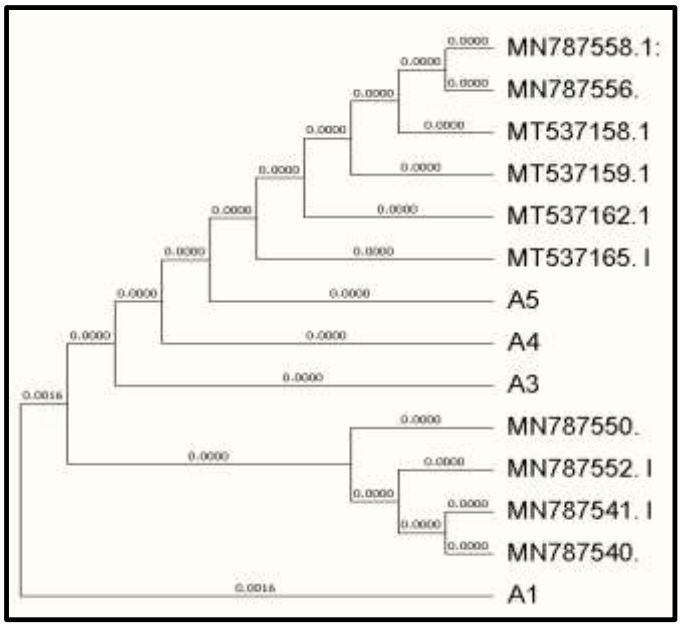

Fig. 1: Phylogenetic tree of genetic variants of the *Cox1* fragment of four *E. granulosus* local samples (Al-Ataby 2022): A1, A2 and A3(Samples showed genetic variation by sequences when used *Cox1*).

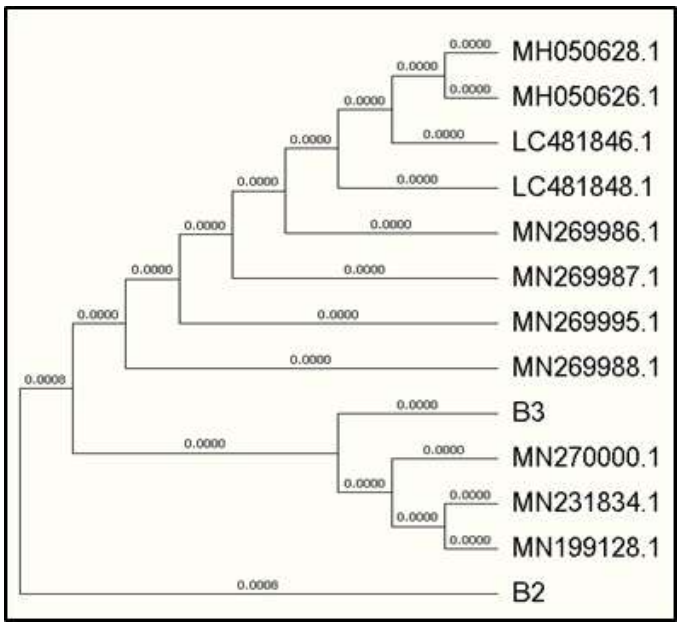

Fig. 2: Phylogenetic tree of genetic variants of the *Nad1* fragment of four *E. granulosus* local samples. (Al-Ataby 2022): B2 and B3 (Samples showed genetic variation by sequences when used *Nad1*).

Another study in the sequencing result of the *cox1* gene in human showed that there was (100%) with isolate recorded by Shafiei et al. (2018) in entry number MH010310.1. Whereas, the sequencing results compared with the database in GenBank showed that there was (99%) isolate identification recorded in strain number (FJ608748.1) as recorded by Calderini et al. (2018) in Italy, while other isolates from sheep showed that (100%) of Turkey isolate was recorded in the strain number (MF544127.1) recorded by Oguz et al. (2018).

Nad1 Subunit Dehydrogenase Gene in Human, Sheep and Camel

The study recorded by Al-Mohammad (2011), which showed three common genotypes found in Iraq based on *cox1* and *nad1* genetic sequence analysis including sheep (G1) breed, buffalo breed (G3) and camel breed (G6). (G6) did not appear in human and sheep hydatid isolates. Two strains in human isolates are sheep (G1) and buffalo (G3) with 92% matching for *cox1* and 99% matching for *nad1* in strain (G1) and 99-100% matching for *cox1* as well. At the same rate as *nad1* in strain (G3). These results did not agree with the study recorded by Lahmar et al. (2004) in Tunisia who discovered most infected camels of the sheep breed (G1) while Rahimi et al. (2011) detected (G1, G3, and G6) in camel isolates in Iran. The strain was the type dominant genetic.

Two samples were included in the Al-Ataby study (2022), these samples were screened for amplification of the *nad1* gene sequence in the genetic sequence of *E. granulosus*. The *nad1* gene is responsible for encoding the NADH dehydrogenase sub-gene1 (*nad1*). The ND1 protein is a subunit of NADH dehydrogenase, which is located in the inner mitochondrial membrane and is the largest of the five complexes of the electron transport chain (Voet et al. 2013). So far, ten remarkable genotypes (G1-G10 strains) of *E. granulosus* have been qualified in the world according to nucleotide sequence analysis of some genes such as NADH dehydrogenase 1 (*nad1*) gene and other genes such as (*cox1*) gene and transcribed spacer 1 (*ITS1*). These lineages are related to notable intermediate hosts including: sheep, goats, horses, swine, sardines, cattle and camels (Sánchez et al. 2010). Sequencing reactions indicated the exact identity after performing an NCBI blast of these PCR amplicon (Zhang et al. 2000). Regarding the 674bp amplicon, the NCBI blast engine showed about 100% sequence similarity of the B3 sample but 99% of the B2 sample between the sequenced samples and the intended reference target sequence. And when comparing the observed DNA sequences of these investigated samples with the retrieved DNA sequences from GenBank found matched with GenBank acc. MN_199128.1.

The alignment results of the 674 bp Basrah samples (Figure 2) revealed the presence of single mutations of interest in all samples analyzed compared to the reference DNA sequence, however, the majority of the mutations were localized in sample (B2) that matched 100% with MN231834.1 strain isolated from human, and MN199128.1 strain isolated from camel, while the strain isolated from sheep MN270000.1 which belongs to *E. granulosus*.

This similarity resulted from a mutant (B2) sample. Genotype (G1) was the most common infectious sheep strain of *E. granulosus* worldwide with a variety of hosts (Craig et al. 2003). In these areas, dogs were usually fed with the intestines of cattle which may have given rise to infection with *E. granulosus* (Sánchez et al. 2010). This efficiency may be sufficient for the reproduction of the current endemic state.

A phylogenetic tree was established in the Al-Ataby study, which was based on the observed differences in DNA. This genetic tree contained samples (B2 and B3) along with other relative DNA sequences. The total number of DNA sequences aligned in this neighbor-linking method was 11. Remarkably, the examined samples were grouped into contiguous groups within the *E. granulosus* sequences.

However, there was a genetic polymorphism that could be detected in the (B2) sample identified in the samples currently examined, this tree provided many informative data about all samples analyzed both in terms of their actual position in the phylogenetic tree or genetic distances between these samples and her samples. Sequences close to one side and other sequences combined in the same tree form the other side. Interestingly, the examined samples were grouped into two phyla within the *E. granulosus* sequences. One of these interfaces was made from sample (B3), while the other close layer was made from other reference spots; while (B2) is located in a separate branch.

In fact, the localization of (B2) in the branch is due to the presence of a mutation in this sample. A sample (B2) was found near the clade containing some strains with 100% similarity to (B2), and was erected near GenBank entry numbers MN231834.1 isolated from human, Iraq; MN199128.1 isolate from camel, Nigeria"; MN269987.1 isolate from sheep, China.

The reason may be attributed to the fact that sheep are clearly sensitive to the sheep strain (G1) of *E. granulosus* and the hydatid cysts in this intermediate host are mostly fertile, so sheep are a primary source of canine Echinococcosis. This sample was 100% identified with sample isolated from human, Iraq, camel, Nigeria and from sheep, China. (Rahimi et al.2011).

Nad2 of *Echinococcus equinus* Gene in Human, Sheep and Camels

The PCR product of the *nad2* gene in samples isolated from Basrah in Iraq showed moderation for four samples of 551 bp. This sample was isolated from humans, sheep and camels. Compare the current sequencing result with the identity of *E. equinus* isolated FSJ01 with five strains of *E. equinus* recorded in the NCBI Worldwide database found that completely different from five strains of *E. equinus* in the world.

Some studies of *E. equinus* in the world such as the study in Turkey is the first study of *E. equinus* isolated from the human host in Turkey and this study of 82 samples was identified as *E. granulosus*. The sequence obtained from *E. equinus* is submitted to the GenBank accession number (MT621047). These results are in agreement with the results recorded in Basrah, Iraq in isolated samples from humans, and the sequencing result is considered the first report on *E. equinus* and it is called FSJ01. To the present, *E. equinus* has been accepted as being specific to the subfamily Equidae, although there has been a study conducted on molecular characterization of *E. granulosus* by PCR-RFLP technology and it has been reported by Nakao et al. (2013) and Romig et al. (2017).

Another research found *E. equinus* in a horse and described the first case of a molecularly verified *E. equinus* infection. Restriction and sequencing analysis of gene 1 in subunit 1 of

the nicotinamide adenine dinucleotide gene verified the diagnosis of *E. equinus* in Germany (Andreas et al. 2010).

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