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Molecular Genotyping of *Echinococcus* species and *In-silico* analysis on NAD and 12SrRNA genes isolated from camels and cattle raised in Sudan

ELHag A. M [1], Abakar A. D [2], Elmahdi I. E [1], Abd Almalaik A. A[3], Altayb H. N[4], Babikir A. M, Salih M. A[5], Mohammed A. A[3], Peter Kern[6]

Abstract

Cystic echinococcosis (CE) is considered a re-emerging disease in various regions, like the Middle East, Central Asia, and northern and eastern Africa. In sub-Saharan Africa, CE is highly endemic. In Sudan, high prevalence estimates of CE in both livestock and definitive hosts were reported. Strain variation and genetic diversity still need to be elucidated in this area. The aim of this study is to genotype and to study the phylogenetic relations and taxonomic status of the *Echinococcus* species. A total of 418 hydatid cysts were collected from the abattoirs survey from camel and cattle isolates,12 isolates from camels in the Tamboul area, and 18 isolates from cattle collected from (10,6, and 2 from, Nyala, Addein, and Kass, respectively), isolates (of *Echinococcus* (*Camelus dromedaries*) were collected from (Tamboul, Nyala and Addein areas, Sudan. Molecular identification of *Echinococcus* species was determined by specific G5/6/7 genotype PCR and G6/7 genotype-specific PCR using 1073-1078 bp and 254 bp of mtDNA respectively. Nad1, 12srRNA gene 10 isolates (2,8 respectively) for DNA sequencing were conducted. Sequences alignments reported novel mutations of Nad1, cattle isolates of 12srRNA gene showed 98% Identity within database blasting between Gene bank *E. ortleppi* isolates, which have both and suggest that the Nad1 gene is continuing to evolve in the face of the current taxonomy profile. Very few bp exchanges differentiate G6 and G7, and 'intermediate' haplotypes have been observed, which merge them into a single genotype G6/7. A further molecular survey is needed to explore the situation of *Echinococcus* genotypes from human patients in Sudan.

Keywords: Cystic echinococcosis, *E. ortleppi*, Addein areas

* Correspondence author: ahmedmadani4@gmail.com
[1] University of Gezira, Faculty of Health and Environmental Sciences, Sudan.
[2] University of Gezira, Faculty of Medical Laboratory Sciences Department of Medical Parasitology, Wad Medani, Sudan.
[3] Faculty of Veterinary Science, University of Nyala, Sudan.
[4] University of Sudan, Faculty of Medical Laboratory Sciences.
[5] University of Gezira, National Cancer Institute (NCI).
[6] Head department of biotechnology, Biotechnology Park, Africa city of technology, Sudan.
[7]University Clinic of Ulm, Center for Internal Medicine, Albert-Einstein-Allee 23, 89081 Ulm, Germany.
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Introduction

Cystic echinococcosis (CE) is known as most neglected food-borne infection for human throughout the endemic areas in the tropics. Mammals of the family canidae play a major role in the transmission of the parasite. The life cycle of the parasite involves mainly dogs and wild carnivores as definitive hosts and a wide range of domestic and wild mammals as intermediate hosts, but also humans as aberrant intermediate hosts [1]; [2], 2006 [3] 2011 [4]; [5]. The genotype G6 was found to be responsible for 7.34% of infections worldwide. This strain is known from Africa and Asia, where it is transmitted mainly by camels (and goats), and South America, where it appears to be mainly transmitted by goats [6]. In the past, the G6 genotype had been identified as the etiological agent only in sporadic cases of CE and it was believed that this strain was less infective for humans or not infective at all [7]. Recently, many authors reported an increasing prevalence of G6 genotype in different countries [8]; [9]; [10]; [11]; [12]; [13]; Omer et al., 2007[14]; [15]; but also from areas where *E. granulosus*.

Echinococcus granulosus previously comprised up to 9 sub-specific genotypes (G1–G9) or strains, which develop in the larval (hydatid) stage as cystic echinococcosis (CE) in ungulates or other herbivores. The current view informed by biology, epidemiology and particularly molecular genotyping recommends the inclusion of at least 9 species in the genus. All those species of *Echinococcus* known to cause CE in the intermediate host may be referred to as E. granulosus sensu lato (s.l.), whereas strains G1–G3 (which are closely related) are now referred to as *E.granulosus sensu strictu* (s.s.). Among these, *Echinococcus canadensis* is genetically the most variable species, containing various 'strains' that are geographically and epidemiologically separated. Initially described as G6 (camel strain), G7 (pig strain), G8 ('American' cervid strain), and G10 ('Fennoscandian' cervid strain), they form a monophyletic cluster based on mitochondrial genomes and nuclear marker genes [16] [17]. Recently, the 'cattle strain' (G5) and the 'horse strain' (G4) have each been elevated to species level, as *E. ortleppi* and *E. equinus*, respectively [18]. However, CE is also reported in livestock, human and wildlife settings throughout Sudan than previously thought [19]. Up to now, the identification of the various Echinococcus taxa was done by PCR based methods such as RFLP-PCR, [20][21] species specific or multiplex PCRs [22] or DNA amplification with subsequent sequencing which requires even more sophisticated equipment. Recently, loop-mediated isothermal amplification (LAMP) assay has been shown to be highly accurate for the detection of echinococcosis in canine definitive hosts [23][24], where the DNA strand displacement and DNA synthesis occur under isothermal conditions. Therefore, instead of a thermal cycler a simple laboratory water bath

or heating block is sufficient to enable the amplification [25] [23]. particularly interest is the status in sub-Saharan Africa, where *E. canadensis* (G6) is known from a dog–camel lifecycle in vast regions in the northern and north-eastern parts of the continent, but also from further south, where camels are not present and other livestock species (particularly goats) act as intermediate hosts [26]. Despite high diversity, the regional populations of *E. Canadensis* G6/7 showed a lack of geographical genetic differentiation within Africa as well as between Africa and the Middle East (based on the Iranian isolates) as shown in the low values found here. Isolates from those regions originated mainly from camels with some specimens from human patients. Similarly, In a pairwise fixation measure of all *E. canadensis* G6/7 sub-populations and meta-populations included in the study, we observed a lack of genetic differentiation in Africa (Mauritania, Kenya and Sudan), the Middle East (Iran) and between the two regions when the African and Middle Eastern *E. canadensis* G6/7 cox1 sequences were compared with 26 available sequences from human isolates from Mongolia [27][28]. Additionally, collected data of haplotypes included, are reported and analyzed via different approaches such as haplotype networks phylogenetic trees.

The observation of lower nucleotide diversities of in *E. ortleppi* might be in support of the hypothesis that the younger clades of *Echinococcus* which also are the most widely distributed and 'domesticated' species in the genus have retained lower polymorphism due to small founder population introductions and disruption of ancestral wildlife transmission routes. In Sudan, high prevalence estimates of CE in both livestock and definitive hosts were reported, but train variation and genetic diversity is still in need to be elucidated. The aim of this study was to genotype and to study the phylogenetic relations and taxonomic status of the *Echinococcus* species using samples from *dromedarius* and Cattle species from various geographical areas of The Republic of Sudan.

Materials and Methods

Study area

Surveys were done in Tamboul town (Central Eastern, Sudan), and Addein, Rehed al Birdi and Kass areas (Western Sudan) (Fig 1). These two sites were reported to be endemic areas for CE.

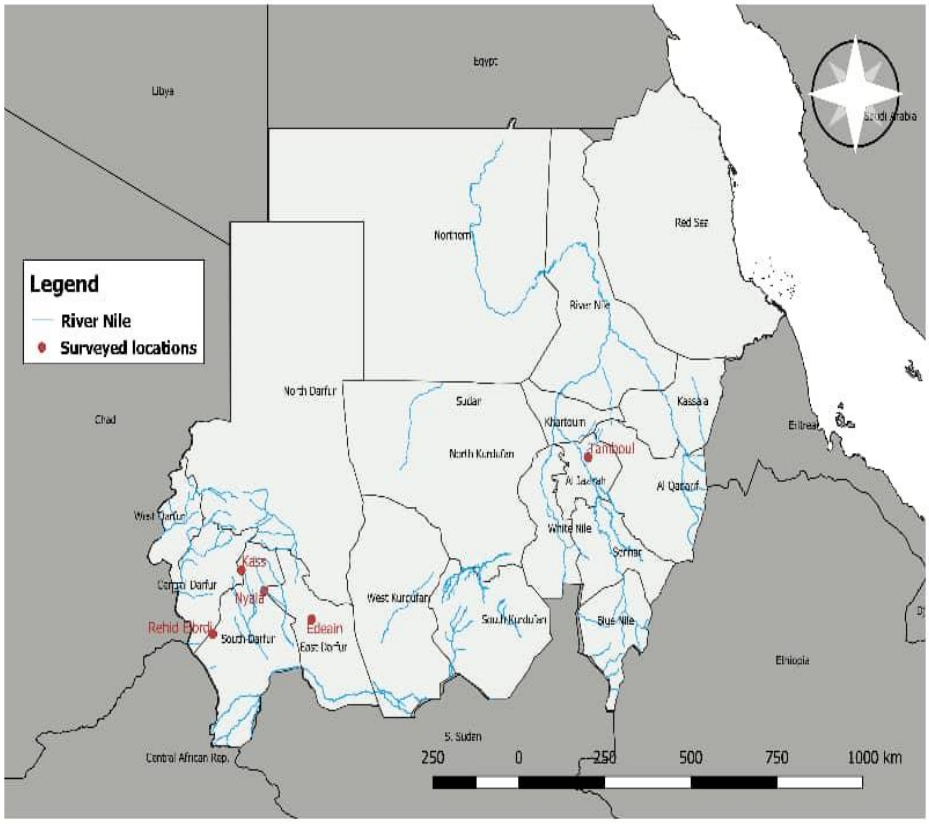


Figure 1.

Study area, Tamboul town (Central Eastern, Sudan) and Addein, Rehed al Birdi and Kass areas (Western Sudan).
Source: (GIS software 10.2)

Ethical approval

The field work of the study for animal investigation has been conducted at South Darfur State, Faculty of Veterinary Science, Nyala University and Gezira State, Faculty of Health and Environmental Sciences, University of Gezira. The authors received an ethical clearance from the Veterinary Ethics Committee (VEC). An approval for conducting this research has been obtained from Ministry of Animal Resource, Fisheries and Ranching, South Darfur and Gezira States.

Abattoir Survey

A total of 418 camel carcasses were examined in abattoirs (387, 228,16, 7, 5 and 20 from Tamboul, Wad Elnimer, Elgadarif, Addein, Rehed al Birdi and Kass, respectively), during routine meat inspection at May 2018 to September 2021, for presence of cystic

echinococcosis according to WHO (1981), where 63 isolates from camel (42 from Tamboul and 21 from Addein, Rehed al Birdi and Kass) were collected from abattoirs survey.

Phenotypic Detection

After removal of cysts from tissues of slaughtered animals protoscolices in cysts fluid were detect using stereo microscope.

PCR Technique

DNA extraction

Single protoscolices were separated using protocol described by (Nakao *et al*., 2003)[29]. DNA was also obtained, and the solution was used directly as a template DNA in the PCR.

PCR of the NAD1 gene

DNA extracted from protoscolices were used as template in PCR to amplify fragment with NADH dehydrogenase subunit 1 (NAD1) (mitochondrial gene). PCR was performed using PCR- buffer (conc. 10 mM Tris-Hcl, pH 8.3; 50 mM Kcl) (applied Biosystem, Germany), 2 mM of Mgcl2 (applied Biosystem, Germany), 200 µM of each deoxy nucleoside triphosphate (Genaxxon biosciences, Germany), 12.5 pmol of Taq DNA polymerase (applied Biosystem, Germany), two conserved primers, forward primer "nad B'' TATTAAAAATATTGAGTTTGGGTC reverse primer nad D" TCTTGAAGTTAACAGCATCACGAT and 2 µl from extract DNA in a 50 µl final volume of reaction mixture.

Positive and negative controls were included. The amplification reactions were carried out in primus 25 thermocycler (PeQlab, Germany) under the following condition; a hot start 94°C for 5 min followed by 35cycles of 30s denaturation at 94°C, 30s annealing at 55°C, 1min extension at 72°C followed by final extension of 5 min at 72°C [30][31]. Amplification product were separated by electrophoresis on 1.5% TBE agarose gel, stained with 2% ethidium bromide and visualized using U.V illumination (Kisker Biotechnology,Germany).

PCR of 12SrRNA gene by specific for *E. ortleppi* (G5) and *E. granulosus* G6/7

For the first PCR (G5/6/7) which amplifies 254 bp fragment of *E. ortleppi* (G5) and *E. granulosus* G6/7, the primer pair E.g.cs1for. (5'ATT TTT AAA ATG TTC GTC CTG 3') and E.g.cs1rev. (5'CTA AAT AAT ATC ATA TTA CAA C 3') was used. The100µl reaction mixture consisted of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM MgCl2, 200 µM of each dNTP, 25 pmol of each primer and 1.25 units Ampli-Taq Polymerase (Perkin Elmer Biosystems) for 40 cycles (denaturation for 30 sec at 94°C, annealing for 1min at 53°C and elongation for

40 sec at 72°C) (Dinkel *et al*., 2004) (Gene bank: AY462126–AY462129). The system for diagnosis of *E. granulosus* G6/7 and *E. ortleppi is shown* in Fig. 2*.* To discriminate between *E. ortleppi* and *E. granulosus* G6/7, semi-nested PCRs specific for G6/7(g6/7PCR; primer pair E.g. camel. for. 5' ATG GTC CAC CTA TTA TTT CA 3' and E.g.cs1rev.) and for *E. ortleppi* (g5 PCR; primer pair e.g. cattle. for. 5' ATG GTC CAC CTA TTA TTT TG 3' and E.g.cs1rev.) were used in a second step, each amplifying a different fragment of 171 bp. The reaction mixtures of 50 µl contained 1.5 µl of amplification product, 10 mM Tris– HCl (pH 8.3), 50 mM KCl, 2 mM MgCl2, 200 µM of each dNTP, 25 pmol of each primer and 1.25 units Ampli-Taq Polymerase (Perkin Elmer Biosystems) for 30 cycles (denaturation for 30 sec at 94°C, annealing for 1min at 60°C and elongation for 30 sec at 72°C). Amplification products were resolved on a 1.5% ethidium bromide-stained agarose gel.

Sequencing Analysis

DNA purification and standard sequencing was performed for both strands of NAD dehydrogenase subunit1,12SrRNA genes by Macrogen Company (Seoul, Korea, Netherlands).

Bioinformatics Analysis

The sequences chromatogram was viewed by Finch TV program, (http://www.geospiza.com/Products/finchtv.shtml).
The nucleotides sequences of the NAD1 dehydrogenase sub unit 1 gene were searched for sequences similarity using nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) Hailey similarity ratio for all two isolates sequences were retrieved from NCBI and subjected to multiple sequence alignment using Bio Edit software were applied ClustalW Multiple Alignment also to improve the sensitivity of progressive multiple sequencing alignment through sequence weighting, position specific gap penalties and weight matrix choice (Thompson et al 1994) [32].

Results

Phenotypic Detection

Out of 418 carcasses of camel examined in Tamboul, Kass, Addein and Rehed al Birdi abattoirs, 140 (33.5%) camel was found infected with C.E contains fertile protoscolices and fertility rate of 45.6% (Fig. 3,4). About 71% of camels slaughtered at Western Sudan abattoirs were females which 71.4% of infected animals up to 9 years ($P \leq 0.01$). In addition to that, only one animal 6 year age was found to be infected with CE and the three cysts

encountered were found to be fertile, whereas 25.4% of cysts up to 10 cm with mean volume of cysts (ml) (44.6±18.4) and mean number of protoscolices/ml 67772.0±41095.0 were observed. The lungs were found to be the main predilection site of camel cysts (Fig. 2).

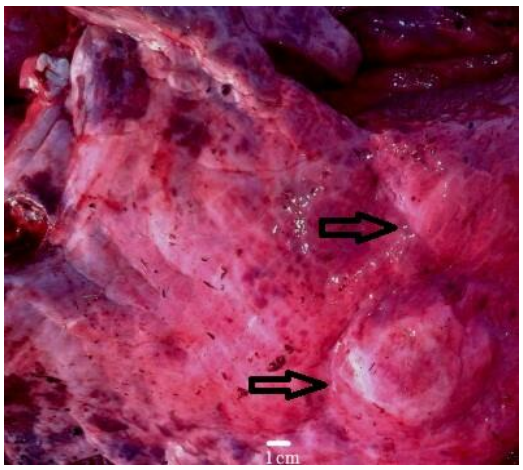


Figure 2.
Hydatid cyst on camel lung tissue.

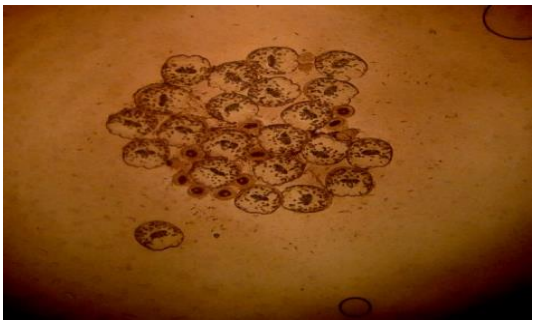


Figure 3.
Protoscolices under stereo microscope 40x.

Genotypic Detection of NAD I dehydrogenase

Amplified protoscolices isolates revealed similar band pattern of 1073-1078 bp long fragments (Figures 4 ,5 ,6 ,7).

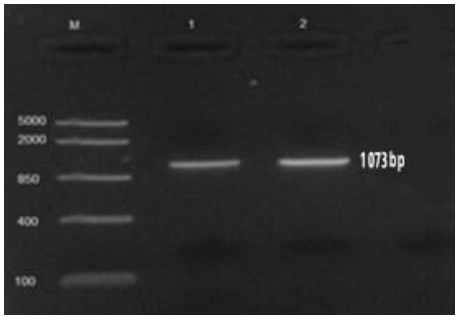


Figure 4.

PCR product of nad1 gene. Lane M, Marker; Lane 1, G6; Lane 2, positive control.

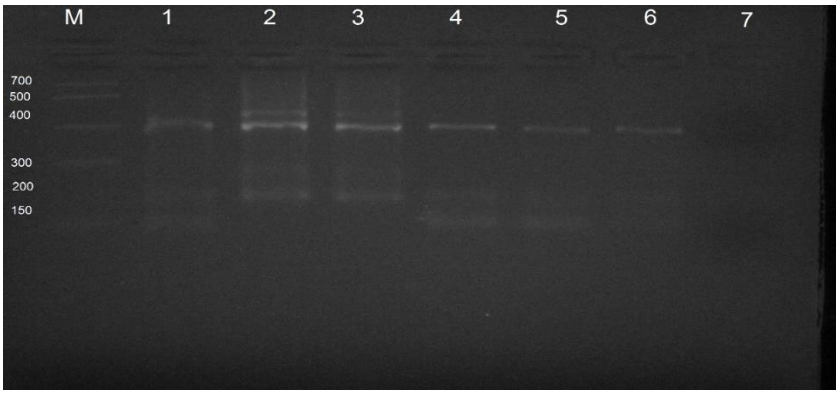


Figure 5.

PCR digest of restriction enzyme hph1 for nad1 gene. Lane M, Marker; Lane 1, G6, Lane 2, 3 Mutant isolates; Lane 4, 5 G6, Lane6 positive control, Lane 7 Negative control.

Genotypic Detection of 12S rRNA gene

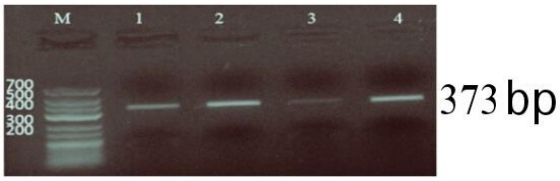


Figure 6.

Cestode specific PCR. M; marker, 1-3 cattle protoscolice, 4; positive control.

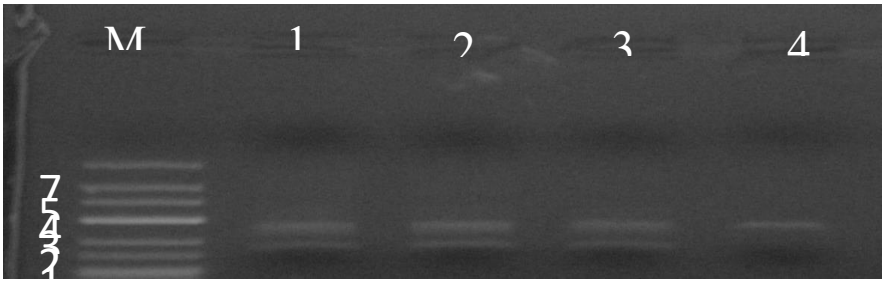


Figure 7.

G6/7 specific PCR. M; marker, 1-2 G6/7, 3; G6/7 positive control, 4; G5 isolate.

Bioinformatics Analysis

Multiple Sequence Alignment

The multiple sequence alignment of the mutant isolate with similar nucleotide sequences that obtained from BLASTn was carried out to find the homology and evolutionary relation between these sequences.

ClustalW Alignment

As shown by BioEdit software there was deleted A base in mutant Tamboul isolate (Figure 8). Insertion mutation on Abase in Tamboul NAD1 gene camel isolate was shown in (Figure 9). Insertion mutation on Abase in Mutant on addein cattle isolate was shown in (Figure 10). Two deletions in addein cattle isolate were shown in (Figure 11). Some information related on data base isolates showed highly heterogeneity with some strains isolated from the same camel species from Sudan and many counters (Table1). Multiple alignments sequencing between mutant isolate and other countries strains was shown in (Figure 12). Multiple Sequencing Alignment of Addein 12S rRNA gene cattle isolate was shown in **(**Figure 13**).** Further result on multiple sequencing alignment of the mutant isolate found highly similarity with Iranian strain (Iran2 (ID HM749615)) isolated in camel larval stage and shown novel haplotype with it (Figure 14).

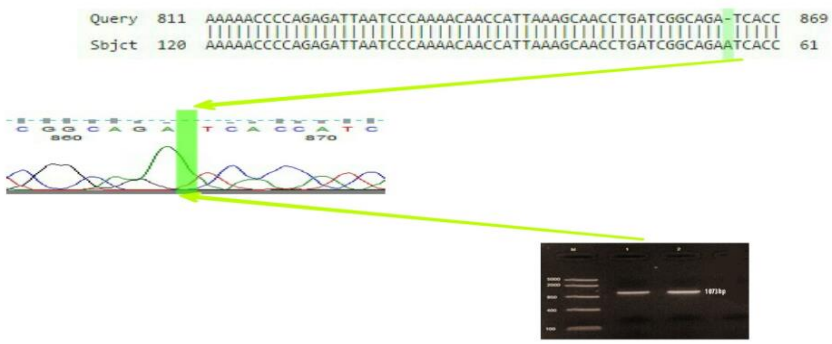


Figure 8.

Deletion mutation on A base on Tamboul camel isolate within site 864 in query

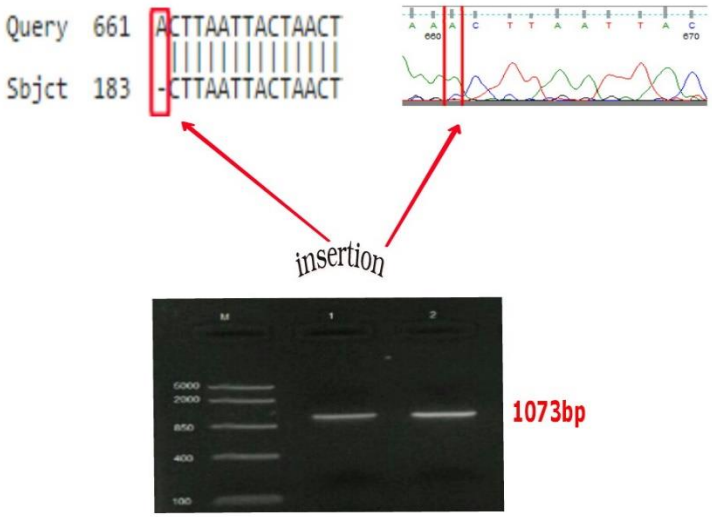


Figure 9.

Insertion mutation on Abase in Tamboul camel isolate.

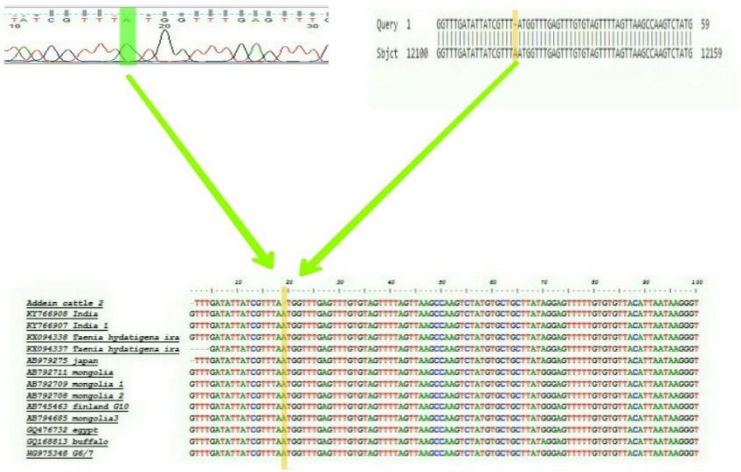


Figure 10.

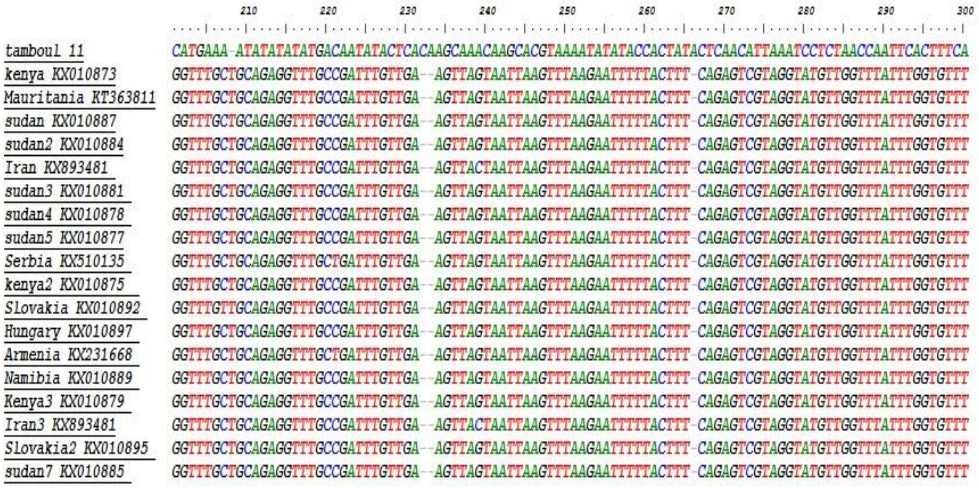
Deletion mutation on addein cattle isolate.

Figure 11.

Two deletion sites on T, A base on Addein 12SrRNA grne cattle isolate

Table 1.
Multiple Alignment Information

ID	Country	Host	Type	Genotype
KX 010873	*Kenya*	*dromedary*	*CE*	*Echinococcus canadensis*
KT363811	Mauritania	*dromedary*	*CE*	*Echinococcus canadensis*
KX010887	*Sudan*	*dromedary*	*CE*	*Echinococcus canadensis*
KX010884	*Sudan*	*dromedary*	*CE*	*Echinococcus canadensis*
KX893481	*Iran*	H.sapiens	*CE*	*Echinococcus canadensis*
KX010881	*Sudan*	*dromedary*	*CE*	*Echinococcus canadensis*
KX010878	*Kenya*	*goat*	*CE*	*Echinococcus canadensis*
KX010887	*Sudan*	*dromedary*	*CE*	*Echinococcus canadensis*
KX010877	*Sudan*	*dromedary*	*CE*	*Echinococcus canadensis*
HM749615	*Iran*	*dromedary*	*CE*	*Echinococcus granulosus*
KX510135	*Serbia*	*Pig*	*CE*	*Echinococcus canadensis*
KX010875	*Kenya*	*dromedary*	*CE*	*Echinococcus canadensis*
KX010892	Slovakia	*Pig*	*CE*	*Echinococcus canadensis*
KX010897	Hungary	*Pig*	*CE*	*Echinococcus canadensis*
KX231668	Armenia	*Pig*	*CE*	*Echinococcus canadensis*
KX010889	Namibia	oryx	*CE*	*Echinococcus canadensis*


210 220 230 240 250 260 270 280 290 300
tamboul 11 CATGAAA ATATATATATGACAATATACTCACAAGCAAACAAGCACGTAAAATATATACCACTATACTCAACATTAAATCCTCTAACCAATTCACTTTCA
kenya KX010873 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Mauritania KT363811 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
sudan KX010887 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
sudan2 KX010884 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Iran KX893481 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTACTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
sudan3 KX010881 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
sudan4 KX010878 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
sudan5 KX010877 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Serbia KX510135 GGTTTGCTGCAGAGGTTTGCTGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
kenya2 KX010875 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Slovakia KX010892 GGTTTGTTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Hungary KX010897 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Armenia KX231668 GGTTTGCTGCAGAGGTTTGCTGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Namibia KX010889 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Kenya3 KX010879 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Iran3 KX893481 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTACTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
Slovakia2 KX010895 GGTTTGCTGCAGAGGTTTGCCGATTTGTTGA AGTTAGTAATTAAGTTTAAGAATTTTTACTTT CAGAGTCGTAGGTATGTTGGTTTATTTGGTGTTT
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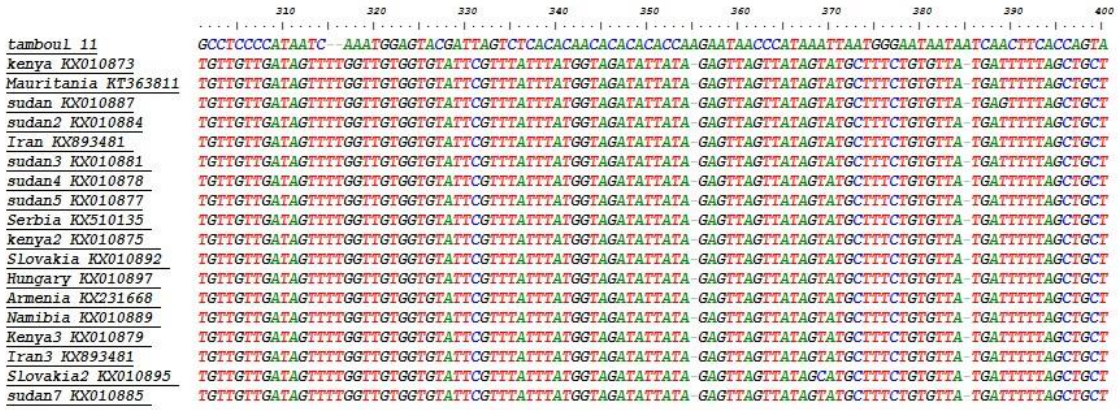
310 320 330 340 350 360 370 380 390 400
tamboul 11 GCCTCCCCATAATC AAATGGAGTACGATTAGTCTCACACAACACACACCAAGAATAACCCATAAATTAATGGGAATAATAATCAACTTCACCAGTA
kenya KX010873 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
Mauritania KT363811 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
sudan KX010887 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGAGTTTTAGCTGCT
sudan2 KX010884 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
Iran KX893481 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
sudan3 KX010881 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
sudan4 KX010878 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
sudan5 KX010877 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
Serbia KX510135 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
kenya2 KX010875 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
Slovakia KX010892 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
Hungary KX010897 TGTTGTTGATAGTTTTGGTTGTGGTGTATTCGTTTATTTATGGTAGATATTATA GAGTTAGTTATAGTATGCTTTCTGTGTTA TGATTTTTAGCTGCT
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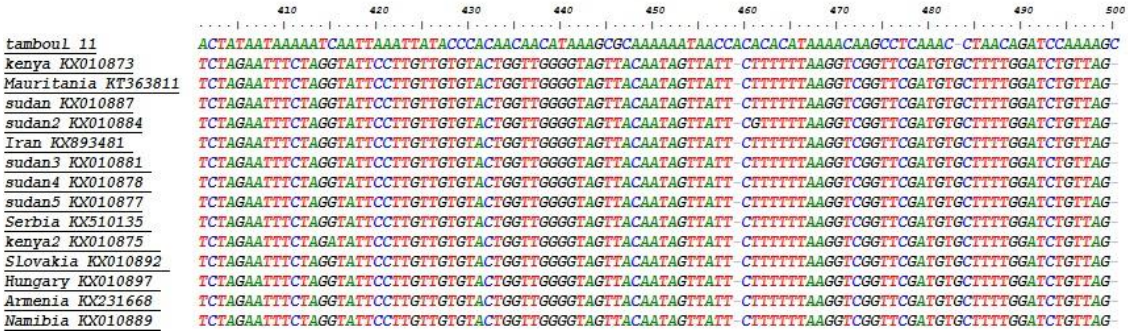
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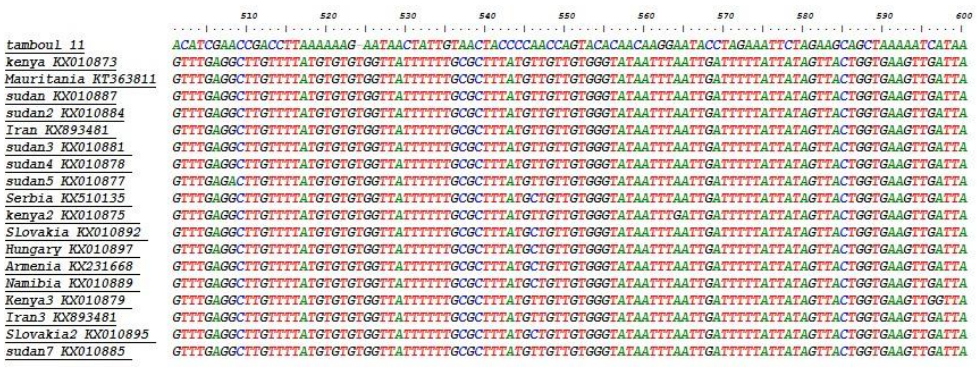
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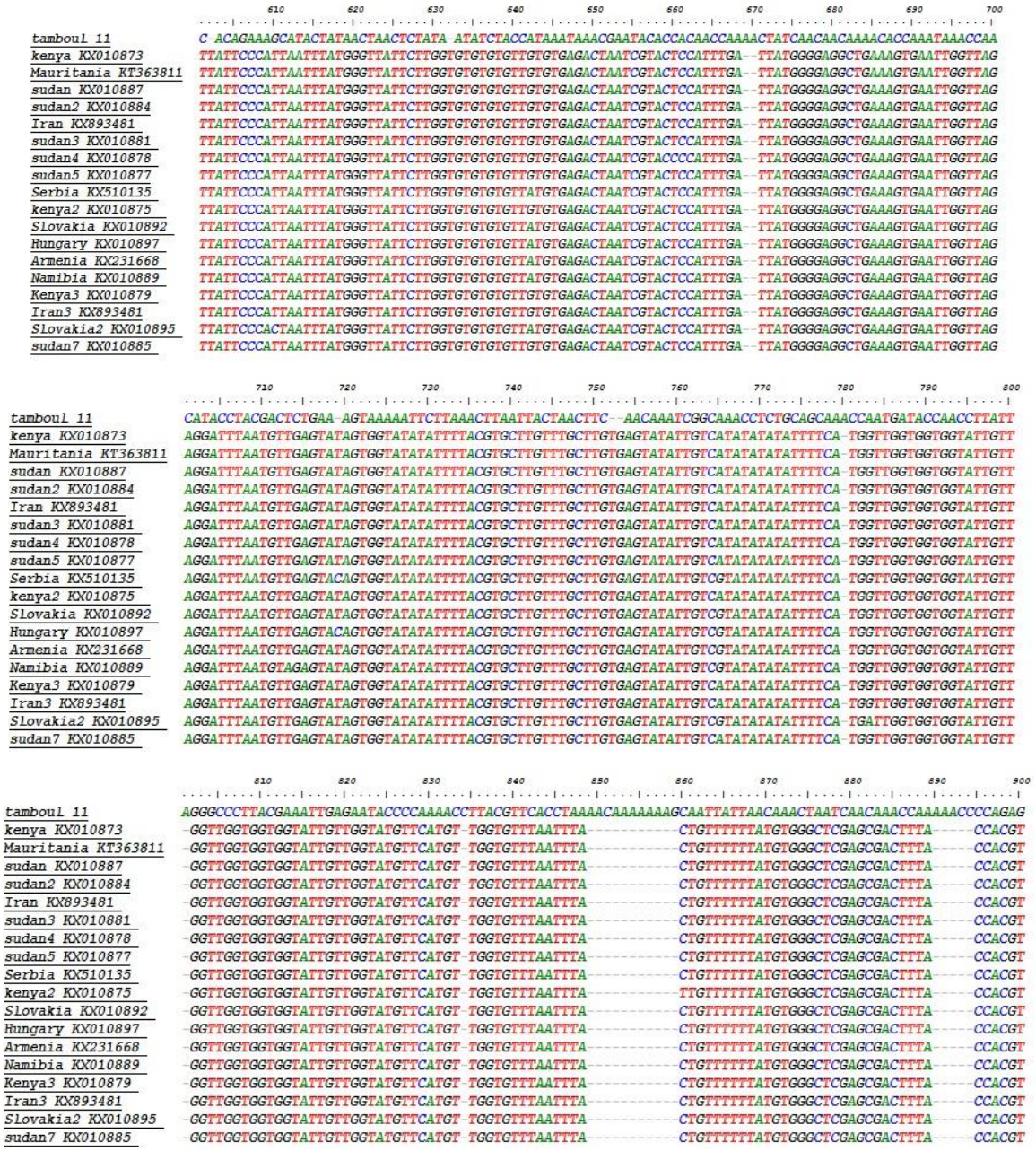

Figure 12.

Multiple Sequencing Alignment of Tamboul NAD1 gene camel isolate.

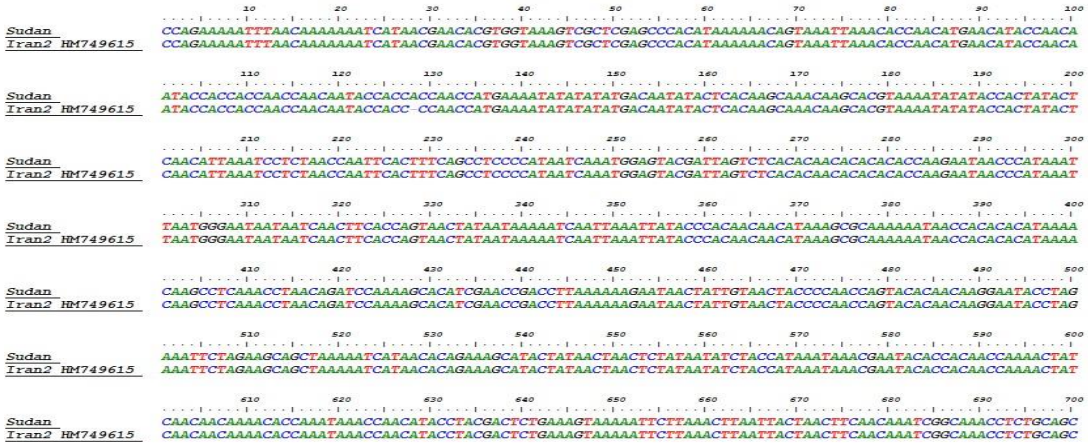

Figure 13.

Multiple Sequencing Alignment of Addein 12S rRNA gene cattle isolate

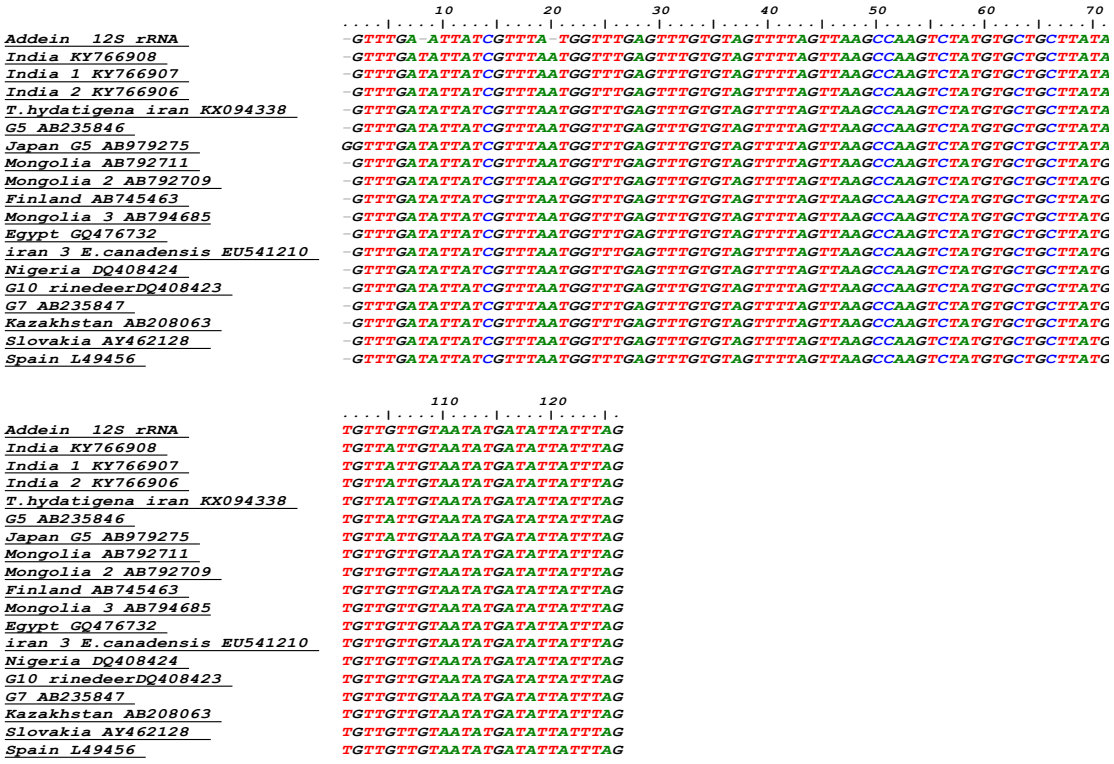

Figure 14.

Multiple Sequencing Alignment of Addein cattle isolate

Phylogenetic Tree

The drawn phylogenetic tree of Tamboul camel isolate revealed that our mutant Nad 1 gene was so closed to Iranian Nad1 gene (Figure 15**).** The drawn phylogenetic tree of addein cattle isolate 12SrRNA gene with some cattle's isolate was presented in (Figure 16).

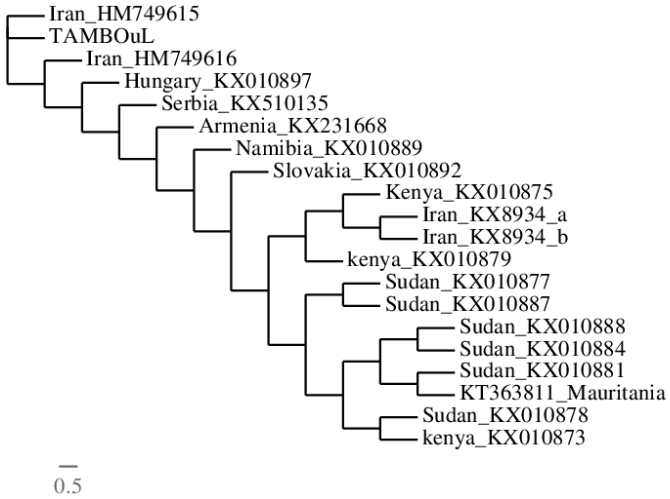

Figure 15.

Tamboul camel isolate phylogenetic tree

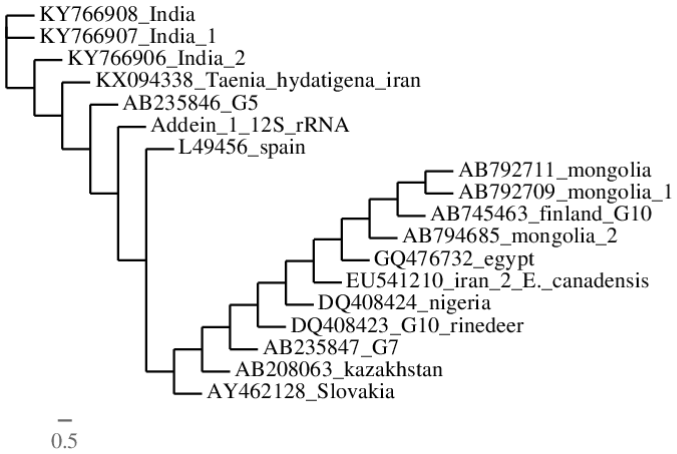

Figure 16.

Addein cattle isolate phylogenetic tree

Discussions

The reports of novel mutation of Nad 1 gene that have both suggest that the Nad1 gene are continuing to evolve in the face of current taxonomy profile. Highly similarity between Tamboul isolate and Camel Iranian strain, those mutations are modified the phylogenetic taxonomy. In addition, the extent and phylogenetic relevance of interbreeding among species is unclear, because most recent studies focused on mitochondrial sequences, which are not subject to recombination. Differences between the phylogenies of nuclear and mitochondrial genomes could explain biological differences between isolates of the same or closely related mitochondrial genotypes. In addition, the extent of intraspecific diversity is insufficiently known, particularly within *E. canadensis* [17]. Although DNA-based methods are useful for taxonomy at the level of genus, species and subspecies, use of such methods often requires careful attention to design of primers and preparation of pure DNA in adequate quantities (McManus & Thompson, 2003; Rahimi et al., 2007) [33] [34]. Mitochondrial and nuclear DNA analyses have previously been used successfully in a few molecular studies on *E. granulosus* in Iran. In the initial study by (Zhang et al. 1998) [35], the DNA sequence variation (assessed using PCR-RFLP) within regions of the mitochondrial cox1 and nad1 genes of 16 isolates of *E. granulosus* indicated the transmission of two strains, G1 and G6. In the study by (Fasihi Harandi et al. 2002)[36], Very few bp exchanges differentiate G6 and G7, and 'intermediate' haplotypes have been observed, which merge them into a single genotype G6/7(Addy et al., 2017) [28]. The Mitochondrial and nuclear DNA analyses have previously been used successfully in a few molecular studies on *E. granulosus* in Sudan, this close affinity between G6 and G7 was later confirmed by analysis of longer sequences including the entire mitochondrial genomes (Nakao et al., 2013) [16], G6 has been identified from goats and G7 from pigs (Soriano et al., 2010) [37] that is opening the suggest more infectivity with in deferent intermediate hosts , also the low level of nucleotide diversity within G6/7. This study showed a rather high haplotype diversity, suggesting that there is a higher degree of isolate variance than would be naturally observed under random mutational conditions (Addy et al., 2017) [38].

Conclusions

From the results obtained we summarize these findings on NAD1 Gene detected in Sudan *Echinococcus canadensis* strain, this is new mutant isolate is completely different from NAD1 gene in its genotypic characterization by using ClustalW Multiple Alignment, also the some mutations are done it change phylogenetic tree of *E. canadensis*, also with the present study, the described LAMP assay should facilitate rapid detection and genotyping

of hydatid cyst strains in a poorly molecular diagnosis tools in the tropics. In the present study, the potential of LAMP assay for rapid and accurate detection of CE was investigated, on a practical scale for the first time in Sudan. The LAMP assay provides high levels of diagnostic sensitivity and specificity when testing a variety of cysts sampled from human and domestic livestock.

Abbreviations

Not applicable

Declarations

Ethics approval and consent to participate

Funding

No funds from any institute

Authors' contributions

All authors are contributed in study design, analysis results, and match proof manuscript.

Competing Interests

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Acknowledgments

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References

1. J. Eckert, M. A. Gemmell, F.-X. Meslin, and Z. S. Pawlowski, “WHO-OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern.” World organisation for animal health Paris, 2001.
2. E. MOKS, I. J. GISALU, H. VALDMANN, and U. SAARMA, “First report of Echinococcus granulosus G8 in Eurasiaand a reappraisal of the phylogenetic relationshipsof ‘genotypes’ G5-G10,” *Parasitology*. 2008;135:647 654.
3. E. Moks, I. Jõgisalu, U. Saarma, H. Talvik, T. Järvis, and H. Valdmann, “Helminthologic survey of the wolf (Canis lupus) in Estonia, with an emphasis on Echinococcus granulosus,” *J. Wildl. Dis.*, vol. 42, no. 2, pp. 359–365, 2006.
4. P. Deplazes, F. van Knapen, A. Schweiger, and P. A. M. Overgaauw, “Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis,” *Vet. Parasitol.*, vol. 182, no. 1, pp. 41–53, 2011.
5. L. Laurimaa *et al.*, “First report of highly pathogenic Echinococcus granulosus genotype G1 in dogs in a European urban environment,” *Parasit. Vectors*, vol. 8, no. 1, p. 182, 2015.
6. C. A. A. Rojas, T. Romig, and M. W. Lightowlers, “Echinococcus granulosus sensu lato genotypes infecting humans--review of current knowledge,” *Int. J. Parasitol.*, vol. 44, no. 1, pp. 9–18, 2014.
7. A. Casulli *et al.*, “Molecular evidence of the camel strain (G6 genotype) of Echinococcus granulosus in humans from Turkana, Kenya,” *Trans. R. Soc. Trop. Med. Hyg.*, vol. 104, no. 1, pp. 29–32, 2010.
8. J. L. Badaraco, F. J. Ayala, J. M. Bart, B. Gottstein, and K. L. Haag, “Using mitochondrial and nuclear markers to evaluate the degree of genetic cohesion among Echinococcus populations,” *Exp. Parasitol.*, vol. 119, no. 4, pp. 453–459, 2008, doi: 10.1016/j.exppara.2008.02.004.
9. J. M. Bart *et al.*, “EmsB, a tandem repeated multi-loci microsatellite, new tool to investigate the genetic diversity of Echinococcus multilocularis,” *Infect. Genet.*

Evol., vol. 6, no. 5, pp. 390–400, 2006, doi: 10.1016/j.meegid.2006.01.006.

10. A. Dinkel *et al.*, “A PCR system for detection of species and genotypes of the Echinococcus granulosus-complex, with reference to the epidemiological situation in eastern Africa,” *Int. J. Parasitol.*, vol. 34, no. 5, pp. 645–653, 2004, doi: 10.1016/j.ijpara.2003.12.013.
11. E. A. Guarnera, A. Parra, L. Kamenetzky, G. García, and A. Gutiérrez, “Cystic echinococcosis in Argentina: Evolution of metacestode and clinical expression in various Echinococcus granulosus strains,” *Acta Trop.*, vol. 92, no. 2, pp. 153–159, 2004, doi: 10.1016/j.actatropica.2004.06.008.
12. L. Kamenetzky *et al.*, “Several strains of Echinococcus granulosus infect livestock and humans in Argentina,” *Infect. Genet. Evol.*, vol. 2, no. 2, pp. 129–136, 2002.
13. C. Manterola, F. Benavente, A. Melo, M. Vial, and J. C. Roa, “Description of Echinococcus granulosus genotypes in human hydatidosis in a region of southern Chile,” *Parasitol. Int.*, vol. 57, no. 3, pp. 342–346, 2008, doi: 10.1016/j.parint.2008.02.005.
14. R. A. Omer *et al.*, “Strain characterisation of human hydatidosis in Sudan,” in *AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE*, 2007, vol. 77, no. 5, p. 107.
15. S. J. Santivañez *et al.*, “Human hydatid disease in Peru is basically restricted to Echinococcus granulosus genotype G1,” *Am. J. Trop. Med. Hyg.*, vol. 79, no. 1, pp. 89–92, 2008.
16. M. Nakao *et al.*, “Mitochondrial phylogeny of the genus Echinococcus (Cestoda: Taeniidae) with emphasis on relationships among Echinococcus canadensis genotypes.,” *Parasitology*, vol. 140, no. 13, pp. 1625–36, 2013, doi: 10.1017/S0031182013000565.
17. T. Romig, D. Ebi, and M. Wassermann, “Taxonomy and molecular epidemiology of Echinococcus granulosus sensu lato,” *Vet. Parasitol.*, vol. 213, no. 3, pp. 76–84, 2015.
18. R. C. A. Thompson and D. P. McManus, “Towards a taxonomic revision of the genus Echinococcus,” *Trends Parasitol.*, vol. 18, no. 10, pp. 452–457, 2002.
19. I. E. Elmahdi, Q. M. Ali, M. M. a Magzoub, a M. Ibrahim, M. B. Saad, and T. Romig, “Cystic echinococcosis of livestock and humans in central Sudan.,” *Ann. Trop. Med. Parasitol.*, vol. 98, no. 5, pp. 473–479, 2004.
20. “Bowles McManus 1993 Molecular variation in echinococcus.pdf.”
21. M. Hüttner and T. Romig, “Echinococcus species in African wildlife.,” *Parasitology*, vol. 136, no. 10, pp. 1089–95, 2009.

22. S. Štefanić, B. S. Shaikenov, P. Deplazes, A. Dinkel, P. R. Torgerson, and A. Mathis, “Polymerase chain reaction for detection of patent infections of Echinococcus granulosus (‘sheep strain’) in naturally infected dogs,” *Parasitol. Res.*, vol. 92, no. 4, pp. 347–351, 2004.
23. M. Wassermann, U. Mackenstedt, and T. Romig, “A loop-mediated isothermal amplification (LAMP) method for the identification of species within the Echinococcus granulosus complex,” *Vet. Parasitol.*, 2014.
24. X.-W. Ni *et al.*, “A comparison of loop-mediated isothermal amplification (LAMP) with other surveillance tools for Echinococcus granulosus diagnosis in canine definitive hosts,” *PLoS One*, vol. 9, no. 7, p. e100877, 2014.
25. . Notomi *et al.*, “Loop-mediated isothermal amplification of DNA,” *Nucleic Acids Res*, vol. 28, no. 12, p. E63, 2000.
26. F. Addy *et al.*, “Prevalence and diversity of cystic echinococcosis in livestock in Maasailand, Kenya,” *Parasitol. Res.*, vol. 111, no. 6, pp. 2289–2294, 2012.
27. A. Ito *et al.*, “Echinococcus species from red foxes, corsac foxes, and wolves in Mongolia,” *Parasitology*, vol. 140, no. 13, pp. 1648–1654, 2013.
28. F. Addy *et al.*, “Genetic differentiation of the G6/7 cluster of Echinococcus canadensis based on mitochondrial marker genes,” *Int. J. Parasitol.*, no. August, pp. 1–9, 2017.
29. M. Nakao, Y. Sako, and A. Ito, “Isolation of polymorphic microsatellite loci from the tapeworm Echinococcus multilocularis,” *Infect. Genet. Evol.*, vol. 3, no. 3, pp. 159–163, 2003.
30. M. Hüttner *et al.*, “Genetic characterization and phylogenetic position of Echinococcus felidis Ortlepp, 1937 (Cestoda: Taeniidae) from the African lion,” *Int. J. Parasitol.*, vol. 38, no. 7, pp. 861–868, 2008.
31. M. Hüttner, L. Siefert, U. Mackenstedt, and T. Romig, “A survey of Echinococcus species in wild carnivores and livestock in East Africa,” *Int. J. Parasitol.*, vol. 39, no. 11, pp. 1269–1276, 2009.
32. J. D. Thompson, D. G. Higgins, and T. J. Gibson, “CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice,” *Nucleic Acids Res.*, vol. 22, no. 22, pp. 4673–4680, 1994.
33. D. P. McManus and R. C. a Thompson, “Molecular epidemiology of cystic echinococcosis.,” *Parasitology*, vol. 127 Suppl, no. Suppl 1, pp. S37–S51, 2003.
34. H. R. Rahimi *et al.*, “A new primer pair in ITS1 region for molecular studies on Echinococcus granulosus,” *Iran. J. Public Health*, vol. 36, no. 1, pp. 45–49, 2007.

35. L. Zhang, A. Eslami, S. H. Hosseini, and D. P. McManus, “Indication of the presence of two distinct strains of Echinococcus granulosus in Iran by mitochondrial DNA markers,” *Am. J. Trop. Med. Hyg.*, vol. 59, no. 1, pp. 171–174, 1998.
36. M. F. Harandi, R. P. Hobbs, P. J. Adams, I. Mobedi, U. M. Morgan-Ryan, and R. C. A. Thompson, “Molecular and morphological characterization of Echinococcus granulosus of human and animal origin in Iran,” *Parasitology*, vol. 125, no. 4, pp. 367–373, 2002.
37. S. V Soriano *et al.*, “Molecular characterization of Echinococcus isolates indicates goats as reservoir for Echinococcus canadensis G6 genotype in Neuquén, Patagonia Argentina,” *Parasitol. Int.*, vol. 59, no. 4, pp. 626–628, 2010.
38. F. Addy *et al.*, “Genetic differentiation of the G6/7 cluster of Echinococcus canadensis based on mitochondrial marker genes,” *Int. J. Parasitol.*, 2017.