Kurdistan Journal of Applied Research (KJAR)
Print-ISSN: 2411-7684 | Electronic-ISSN: 2411-7706
Special Issue: 2nd International Conference on the Health & Medical Sciences | DOI: 10.24017/science.2018.2.30

Received: 18 May 2018 | Accepted: 25 June 2018

Prevalence and Molecular Characterization of *Cysticercus tenuicollis* Isolated from Some Intermediate Host in Kurdistan-Iraq

Abdullah Ahmed Hama
Medical Laboratory Department
Technical College of Health
Sulaimani Polytechnic university
Sulaimani, Iraq
Research Center/ Sulaimani Polytechnic university
abdullah.hama@spu.edu.iq

Rostam Hama Zorab
Directorate of Veterinary in Sulaimani
Sulaimani, Iraq
rostamhama@gmail.com

Fatima Mohammed Ali
Research Center | Nursing department
Technical Institute of Sulaimani
Sulaimani Polytechnic university
Sulaimani, Iraq
Fatimah.Ali@spu.edu.iq

Awaz M. Salih
Research Center, Sulaimani Polytechnic university
General Directorate of Health in Sulaimani
Sulaimani, Iraq
Awaz.salih@yahoo.com

Amer Abdullah Hassan
Medical Laboratory Department | Research Center
Technical college of Health
Sulaimani Polytechnic university
Sulaimani, Iraq
Amer..hassan@spu.edu.iq

Abstract: ***Cysticercus tenuicollis (C. tenuicollis) is the larval stage infection of Taenia hydatigena, a common tapeworm of dogs and other Canidae, which has a wide range of intermediate hosts including sheep, goat, cattle, deer, camel, horse, human and other wild ruminants, the disease spreads through a contaminated water, soil and food with feces of infected dogs or other carnivores, T. hydatigena lives in the intestinal of the definitive host (carnivores) and excretes a huge number of eggs with feces daily. The present study conducted to determine the prevalence rate and molecular characterization of C. tenuicollis among sheep and goats in Sulaimani province. A total of 14088 slaughtered animals were inspected postmortem from the new Sulaimani abattoir comprise, which involves 13395 sheep and 693 goats. The selected cysts were preserved in 70% Ethanol for DNA extraction and molecular study, The mt-CO1 gene was amplified with a conventional polymerase chain reaction (PCR), the PCR product purified and DNA sequencing for reverse and forward strands was determined by a genetic analyzer, the obtained sequences aligned with the DNA sequences of T. hydatigena in Iran, Turkey, and Palestine, which deposited in GenBank under the following accession number (JQ710588), (JN827307) and (KM032284) respectively. The prevalence rate of C. tenuicollis was 2.63% in sheep and 2.58% in goats. This result shows no significant differences of C. tenuicollis between sheep and goats (p>0.05). The nucleotide sequence alignment of cytochrome oxidase subunit 1 (CO 1) gene revealed that the amplified DNA fragment belongs to Taenia hydatigena and Echinococcus granulosus and the nucleotide sequences of T. hydatigena deposited in GenBank under accession number (MH638348). This finding concludes that the amplification of mt-CO1 gene cannot be depended on discriminate hydatid cyst and C. tenuicollis while the partial DNA sequences of the mt-CO1 gene are significantly valuable to differentiate C. tenuicollis from hydatid cyst, which is completely different in the pathology and control.***

Keywords: *Cysticercus tenuicollis*, *Taenia hydatigena*, *mt-CO1*, DNA, Molecular marker.

1. INTRODUCTION

Taenia hydatigena is a cestode of the family Taeniidae, the adult stage of the parasite lives in the intestine of dogs and other Canidae (definitive host), while the larval stage, which is called metacestode (*Cysticercus tenuicollis*) develops and residing in domestic and wild mammalians (intermediate hosts), the cyst is developed as fluid-filled larvae in tissues [1, 2]. The adult bladder worm is a large thin, measure about 75-500 cm and is mostly called a slender thin worm [3]. The life cycle of *T. hydatigena* is indirect and required both definitive and intermediate hosts to complete its life cycle, adult worm lives in the small intestine of dogs, wolves, jackals, and foxes, the gravid segments are detached and eggs are expelled out and spread in the environment, the eggs will contaminate soil, grass, water, and vegetables. Eggs transmit to the intermediate hosts when it is eaten with plants or grasses. The egg will be hatched after ingestion and the hexacanth embryo penetrates the intestinal wall and then enters the blood circulation to reach the liver and another organ. Most of the embryo leaves the liver and they enter the peritoneal cavity to grow and develop to *Cysticercus tenuicollis*.

The definitive host becomes infected when fed on infected animal's offals. The scolex evaginates in the small intestine and attaches to the mucosa, then grows to an adult in 51days post-infection [4].

Metacestode (*C. tenuicollis*) is a cyst loosely filled with transparent fluid, with size varies from one cm up to seven cm, and long necks which are usually found

attached to the omentum, mesentery, and sometimes on the liver surface, particularly among the sheep and goat; however, unusual locations of *C. tenuicollis* have been described as the lungs, kidneys, brain, ovaries, uterine tubes, uterus, cervix, and vagina [5, 6]. *Tenuicollosis* is responsible for a high degree of morbidity and mortality in livestock and particularly the domestic animal; sheep and goat [7], it is frequently associated with hemorrhagic tracts of the liver in acute cases [8]. In slaughter animals, it has an important economic loss due to the condemnation of offal's containing larvae of *T. hydatigena* [9]. The metacestode may serve as a predisposing cause to black disease or may lead to acute traumatic hepatitis as well as a contributory agent of peritonitis [10].

Diagnosis of taenia infection and cysticercosis is based on the morphology and molecular characterization of the parasites [11,12]. Number and length of the large and small hooks, number and layers of testes, number of uterine branches, and structure of cirrus sac are important characteristics for morphological identification [1]. Usually, the prevalence of infection by *C. tenuicollis* was different according to animal geographical distribution and it had taken a high level in poor countries and could not be controlled among wild animals [13]. Numerous surveys on the prevalence have been reported in different parts of the world and Iraqi neighbor countries for *C. tenuicollis*. In Iraq, for the first time, it was isolated from the peritoneal cavity of sheep by Leiper [14]. The parasite was also recorded from the peritoneal cavity of sheep in north of Iraq [15], from slaughtered sheep in Basra [16], also the *C. tenuicollis* were isolated from sheep, goat, and cow in Erbil city [17], in another epidemiological study in Mosul and Diwania, the high prevalence rate of *C. tenuicollis* was recorded among slaughtered sheep [18]. While the low prevalence rate (0.7%) of cysticercosis among sheep was reported in Dohuk [19]. The present work was conducted to determine the prevalence rate and molecular characterization (strain identification and genotyping) of *C. tenuicollis* among slaughtered sheep and goats in Sulaimani province for the first time.

2. METHODS AND MATERIALS

2.1 Study Area: This study was carried out in Sulaimani, Kurdistan, northeast of Iraq, which is located on the longitude (44.50- 46.16) east and latitude (35.04 - 36.30) north. The samples (cysts) of *Cysticercus tenuicullis* (Fig.1) were collected from November 2017 to April 2018 at the new abattoirs of Sulaimani (Qragol). The total inspected animal was 13395 sheep and 693 goats, the samples collected from different organs including liver, lung, mesentery, and momentum, the cysts were preserved in sterile containers contain one volume (v/v) of 70% ethanol at 4 °C [20].

2.2 DNA extraction: The cysts of (*T. hydatigena*) were washed with phosphate buffer saline /or normal saline three times to remove the ethanol [21], the genome (total DNA) was extracted from the cyst by the modified Genomic DNA Extraction Kits (Miniprep Tissue) provided by (Geneaid, Korea), the extracted DNA preserved in the TE buffer at -20°C until use [22].

2.2 PCR process: The amplification of a partial gene of the cytochrome oxidase one (CO1) gene was performed according to Nejad *et al*. [23]. Briefly, 50 µl of a master mix containing 10-100 ng of DNA and 50 pmol of each primer; forward (5' TTT TTT GGG CAT CCT GAG GTT TAT 3') and reverse (5' TAA AGA AAG AAC ATA ATG AAA ATG 3), the Thermocycler (AB Biomed) was used: an entail step (denaturation) at 95^0C for four minutes followed by 35 cycles at 94 ^{0}C for 45 seconds, 55 ^{0}C for the 30s, 72 ^{0}C for 45s and 72 ^{0}C for seven min, as a final extension step. The PCR product was detected on 1.5% ethidium bromide-stained agarose gel under UV illuminator (gel-documentation with the computerized system).

2.3 Nucleotide Sequence analysis: The PCR products purified from the residual of protein, RNA, primers, enzyme, salts, free nucleotides, to be ready for DNA sequencing. The PCR product was purified with a PCR product purification Kit (Amersham UK), The nucleotide sequencing was performed by the genetic analyzer after amplification of the partial gene (*CO1*) with forward and reverse primers separately to obtain forward and reverse sequences in CBAR Laboratory in Malaysia (Kualalampur Malaysia). The sequence analysis, multiple sequence alignment, sequence editing, and correction was done with genetic analyzing software Mega5 and Bioedit.

2.4 Statistical analysis: The epidemiological data analyzed with one way ANOVA and chi-square by statistical software SPSS version 17.

3. RESULTS

The overall prevalence rate in the current study of *C. tenuicollis* (Fig.1), among slaughtered animals (14088) including (13395) sheep and (693) goats was 352 (2.63%) and 18 (2.59%) respectively (Table 1). The statistically significant differences among sheep and goat were not observed ($P > 0.05$). Based on sex, also there were no significant differences ($p>0.05$) between male and female (Table 2).

The used primer in the current study was designed for identification of *Taenia spp*. [20], it has the ability to amplify a part of *CO1* gene of *E. granulosus* and *CO1* gene of *T. hydategena* according to the molecular result of this study, some cysts were belonged to *E. granulosus*, which has same DNA molecular weight and they excluded in this study. Generally, all the cysts were identified as *Taenia* spp. and the PCR product of mt-CO1 yielded 446 bp (Fig. 2).

The partial nucleotide sequence of (*mt-CO1*) gene was submitted to the GenBank and recorded under accession number (MH638348), the result was corresponding with *T. hydatigena*. The multiple sequences alignment of the nucleotide with published references (JQ710588, KM032284, JN827307, and JQ710627) for *T. hydatigena*, the result shows that our finding is 100% identical with *T. hydatigenia* strain in Iran (JQ710588) and *T. hydatigenia* strain in Palestine (KM032284),

while 99% was similar with *T. hydatigena* isolated from Turkey (JN827307) and Iran (JQ710627) the alignment and differences of nucleotide were represented in (Fig.3).

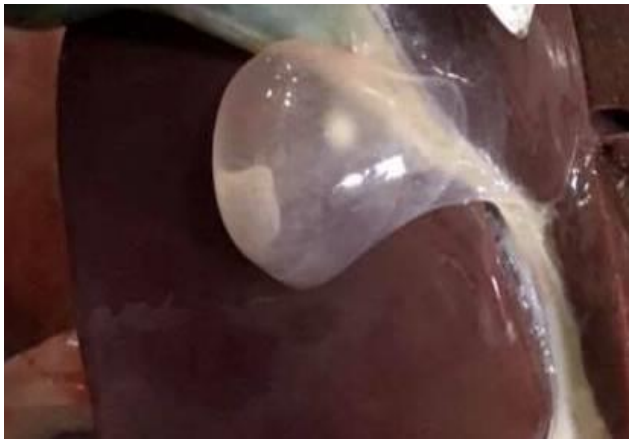

Figure (1): Full developed cyst (*Cysticercus tenuicollis*) on the liver surface of sheep.

Table (1): The prevalence rate of *Cysticercus tenuicollis* among slaughtered animals (sheep and goats) in Sulaimani province

Hosts	Total examined animals	Negative animal		Positive animal	
	N0.	N0.	%	N0.	%
Sheep	13395	13043	97.37	352	2.63
Goats	693	675	97.41	18	2.59
Total	14088	13719	97.38	369	2.62

X2=0.0789 p-value=.778818. (p>0.05)

Table (2): The prevalence rate of *Cysticercus tenuicollis* among slaughtered animals (sheep and goats) according to the sex in Sulaimani province.

Hosts	Total examined		Negative animal		Positive animal		P- value
	N0.		N0.	%	N0.	%	
Sheep	624	M	610	97.75	14	2.24	0.53
	12771	F	12433	97.35	338	2.65	
Goats	86	M	84	97.67	2	2.33	0.86
	607	F	591	97.36	16	2.63	

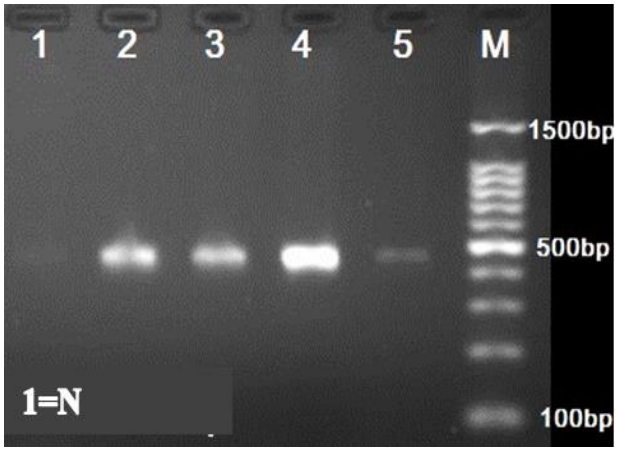

Figure (2): The Gel-electrophoresis of PCR product of mt-CO1 gene shows DNA bands (446 bp). M: Marker, 1=Negative control

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                                10        20        30
                     ....|....|....|....|....|....|
MH638348 Kurdistan   ATTATTAGTCATATATGTTTGAGAATAAGC
JQ710588.1Iran-go    ..............................
KM032284.1Palastin   ..............................
JN827307.1 Turkey    ..............................
JQ710627.1Iran       ..............................

                                40        50        60
                     ....|....|....|....|....|....|
MH638348 Kurdistan   ATGAGTCCTGATGCTTTTGGATTCTATGGA
JQ710588.1Iran-go    ..............................
KM032284.1Palastin   ..............................
JN827307.1 Turkey    ..............................
JQ710627.1Iran       ..............................

                                70        80        90
                     ....|....|....|....|....|....|
MH638348 Kurdistan   TTATTATTTGCTATGTTTTCAATAGTCTGT
JQ710588.1Iran-go    ..............................
KM032284.1Palastin   ..............................
JN827307.1 Turkey    ..............................
JQ710627.1Iran       ..............................

                               100       110       120
                     ....|....|....|....|....|....|
MH638348 Kurdistan   TTGGGTAGAAGTGTGTGGGGTCATCATATG
JQ710588.1Iran-go    ..............................
KM032284.1Palastin   ..............................
JN827307.1 Turkey    ..............................
JQ710627.1Iran       ..............................

                               130       140       150
                     ....|....|....|....|....|....|
MH638348 Kurdistan   TTTACTGTTGGATTAGATGTTAAGACTGCT
JQ710588.1Iran-go    ..............................
KM032284.1Palastin   ..............................
JN827307.1 Turkey    ..............................
JQ710627.1Iran       ..............................

                               160       170       180
                     ....|....|....|....|....|....|
MH638348 Kurdistan   GTTTTTTTTTAGTTCTGTCACTATGATTATA
JQ710588.1Iran-go    ..............................
KM032284.1Palastin   ..............................
JN827307.1 Turkey    ..............................
JQ710627.1Iran       ................T.............

                               190       200       210
                     ....|....|....|....|....|....|
MH638348 Kurdistan   GGTGTGCCTACTGGTATAAAGGTGTTTACT
JQ710588.1Iran-go    ..............................
KM032284.1Palastin   ..............................
JN827307.1 Turkey    ..............................
JQ710627.1Iran       ..............................
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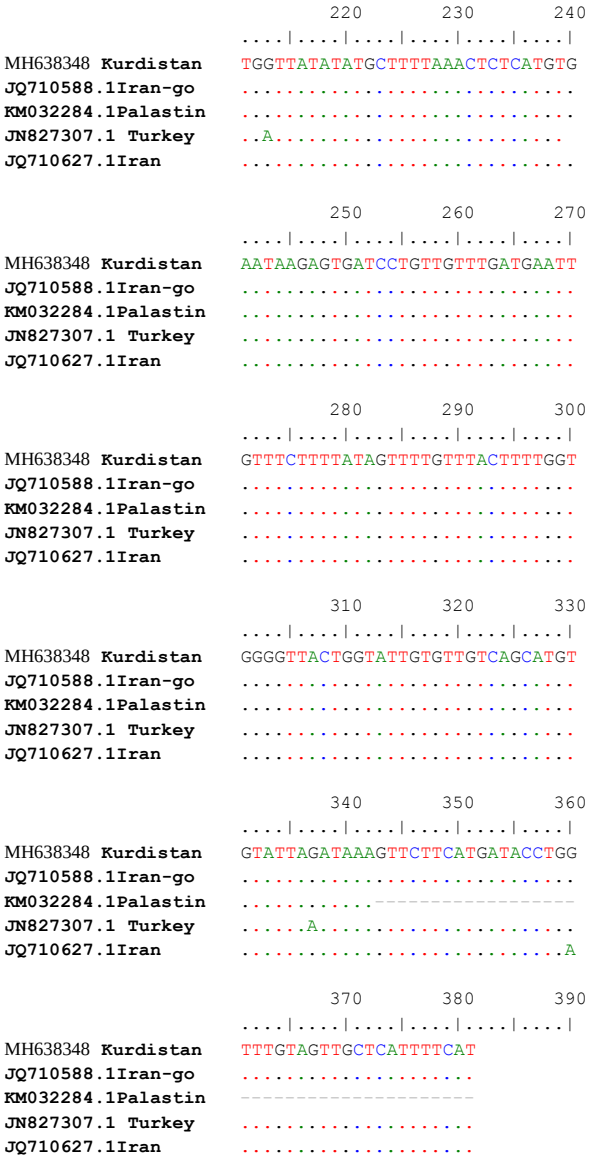

Figure (3): Nucleotide sequences of partial *mt-COX1* of *T. hydatigena* (MH638348) aligned with the published sequences of the *T. hydatigena* as a reference.

4. DISCUSSION

Cysticercus tenuicollis is a parasitic infection of herbivores caused by the larval stage (metacestode) of *T. hydatigena* with a global distribution, which has a veterinary, medical, and economic importance. The disease spreads through the contamination of water, soil, and vegetable with feces of infected dogs and other carnivores [24].

The present study recorded a low prevalence rate of *C. tenuicollis* among slaughtered animal sheep and goat (2.6%), this finding is considered a low prevalence rate in comparison with the most studies that carried out in Iraq; Esa and Al-Aziz [25] in Basra, they recorded 40.55% and 26.25% infection rate among sheep and goats respectively. Moreover, Khadair [26] in Baghdad recorded a higher prevalence rate among slaughtered animals; (14.22%) in sheep and (16%) in goats. In the recent study for Haddawee *et al.* [27] in Karbala, they recorded a high prevalence rate of *Cysticercus tenuicollis* among animals in the different months and session, the prevalence rate was (42.65%) in September, (45.57%) in August and (30.91%) and (28.40%) in October and November respectively, the authors explained the reason for the high prevalence rate of *Cysticercus tenuicollis* among sheep and goats may be due to inadequate knowledge and awareness about this disease among the Iraqi population and a lack of medical incinerator in the most slaughterhouses to collect and burn the infected organ and illegal slaughtering animals outside the abattoirs (on the street) makes difficult to control the disease, also the mostly infected organ (animal offal) will be accessed to the stray dogs due to improper management and a lack of medical incinerator, the national strategical plan to management and control of stray dog is required to reduce the infection with dog tapeworm *E. granulosus and T. hydatidena* [27].

While our result disagrees with Al-Saqur and Gorani [27] they recorded a lower prevalence rate of *Cysticercus tenuicollis* (1%) among sheep. Although Al-Bakri [28] in Naynava recorded a low prevalence rate (2%) of *C. tenuicollis* in sheep, which supports our finding. This variation may be due to the difference in management practice, environmental factors, also the knowledge about the disease and separating dogs from sheep and goats, also frequently cleaning of the domestic animal house has a direct effect on reducing the infection.

The significant differences in *C. tenuicollis* infection between sheep and goat were not observed, this finding disagrees with some studies [25, 29], they recorded a higher infection rate of *C. tenuicollis* among sheep than in goats, while Rdfar *et al.*[2] recorded 26.7% in sheep and, 27.9% in goats the infection rate among goats is slightly higher than in sheep, the author stated that grazing behavior and management can be considered as the major reasons, also Nejad *et al*. [23] stated that the high prevalence rate among sheep due to the immune system of sheep, the immunity of the sheep is developed quickly in comparison with goat's immune system and this immunity regulate and limited the parasite infection. The prevalence rate of *C. tenuicollis* among male and female relatively have the same prevalence rate of infection this may be due to both male and female have the same chance to get an infection after ingestion of the egg during feeding of contaminated food or water. In the present study, the significant differences of infection rates were not observed between a male and female of sheep and goat, while the females will be more susceptible this result agreed with [25] the slight differences of infection rate with *C. tenuicollis* between male and female may be due to physiological and hormonal effect. The inspection of meat for *C. tenuicollis* is more difficult if cysts are degenerated or low developed, the meat inspection procedure has a probability to detect about 20-50% of actual infected animals [30]. The molecular-based methods are not alternative of inspection methods for cyst detection, while the combination between direct inspection and DNA markers are important to understanding the nature and origin of cysts [31]

The morphological and biochemical markers are helpful for species and strain identification of *Taenia spp*. while the molecular and DNA markers are more accurate and dependable for molecular epidemiology study [32]. The

DNA marker and molecular characterization are valuable for species and strain identification of *Taenia species* [33, 34]. The alignment and blast of the present sequences which deposited in GenBank **(**MH638348**)** shows that 100% similar with (JQ710588) in Iran [35] and with (KM032284) in Palestine while 99% similar with (JN827307) in Turkey and (JQ710627)in Iran, this may be due to close contact of the Kurdistan Region of Iraq with Iran and Turkey and they relatively have the same climate, culture, and lifestyle also the majority of domestic animals were imported from Iran and Turkey to Kurdistan-Iraq [36, 37].

Different molecular markers have been developed for molecular characterization and species, strain identification of Taenia, the most common approaches are DNA sequencing, restriction enzyme fragment length (RFLP), PCR-linked RFLP, AFLP, and PCR-RFLPs of the rDNA internal transcribed spacers 1 and 2 (*ITS1 and ITS2*) [12]. In the current study the partial cytochrome oxidase gene (CO1) which is a mitochondrial DNA was studied, the mitochondrial DNA sequences have been used widely in world as a genetic and DNA marker to evaluate the genetic population structure, the DNA sequencing of met-CO1 is useful for evolutionary and relationship and also it can be used for species and strain identification of the Taenia and another worm.

4. CONCLUSION

Our result concludes that the prevalence rate of *C. tenuicollis* among sheep and goat in Kurdistan Region of Iraq is (2.6%), which is lower than those of most Iraqi neighbor countries.

The amplification of *CO1* gene with the current primer is not dependable to discriminate hydatid cyst (HC), which is caused by dog tapeworm *Echinococcus granulosus* and *C. tenuicollis*, DNA sequencing and accurate methods for species and strain identification of Taenia worm are required.

Molecular techniques are not an alternative to direct meat inspection, but the combination of both methods will be the most valuable for confirmation of *C. taenicollis* infection.

5. ACKNOWLEDGMENT

The authors would like to thank Sulaimani abattoir veterinarian and administration staff and special thanks for the Technical College of Health and Research center of Sulaimani Polytechnic University for their support and facilities.

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