INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 4, p. 71-77, 2018

RESEARCH PAPER OPEN ACCESS

Molecular identification of *Taenia hydatigena* isolated from domestic dogs in Baghdad city

Azhar Ali Faraj[1], Amer Murhum Al-Amery[2]

[1,2]Department of Parasitology, College of Veterinary Medicine, University of Baghdad , Iraq

Key words: *Taenia hydatigena*, (18S rRNA)gene, phylogenetic tree, dogs, Iraq.

http://dx.doi.org/10.12692/ijb/13.4.71-77 Article published on October 14, 2018

Abstract

The aim of this present study is to detect the 18S rRNA gene sequences of *T. hydatigena* and to compare their genetic relatedness utilizing phylogenetic analysis. Twenty of *T. hydatigena* adult worms were obtained from ten naturally infected domestic dogs after treatment with albendazol , at a dose of 0.3% g/kg body weight . We obtained samples from dogs that treated in a small animal private clinics in Baghdad, Iraq, during the period from January to May2018. The results of phylogenetic analysis showed that proximity and the genetic dimension among the *Taenia hydatigena* isolate isolated from of dogs among themselves and the world are with more than 99% compatibility values. The molecular tools, including the sequence data of (18S rRNA) gene. It was the first study on *Taenia hydatigena* genotypes in domestic dogs in Iraq.

Introduction

The family Taeniidae includes various species of *Echinococcus* and *Taenia*, distributed worldwide helminthes parasite of canids (Dalimi *et al.,* 2006).species of the genus *Taenia* causing economic losses in animals and medical problems in humans (Lavikainen *et al.,* 2008). *Taenia hydatigena* can infect a wide range of ruminants with its larval stage, *Cysticercus tenuicollis*, the adult worm for thisparasite is found in dogs and anthercinds, as the final hosts(Murat, 2005). The dogs released proglottids in the feces, which are ingested by ruminant intermediate hosts. Eggs hatches in the stomach to hexacanth emberyo (oncospheres) (Deplazes and Eckert, 1988). The freed oncospheres migrate to the liver via the portal system and leading to hemorrhagic, fibrotic hepatitis cysticercosa and the death of young animals due to the intense infections and traumatic hepatitis (Radfar *et al.,* 2005). Diagnosis of taeniosis depend on the morphologic and molecular characteristics of the parasites (Gasser *et al.,* 1999; González *et al.,* 2006; McManus, 2006). Molecular assay include direct comparison of PCR-amplified DNA sequences (González *et al.,* 2006; Lavikainen *et al.,* 2008). The objective of this study is to use the molecular identification of *T. hydatigena* in domestic dogs and to study the phylogenetic analysis for this parasite in Iraq.

Materials and methods

Samples collection

Twenty of *T. hydatigena* adult worms were obtained from ten naturally infected dogs(mixed breed and aged less than 2 year) after treatment with albendazol, at a dose of 0.3% g/kg body weight . We obtained the samples from dogs that treated in a small animal private clinics in Baghdad, Iraq, during the period from January to May2018. Place worms collected in a petri dish and rinsed several times with physiological normal saline and kept in 70% ethyl alcohol at –20°C for DNA extraction. Morphological features the adult worms were described according to Kassai, (1999).

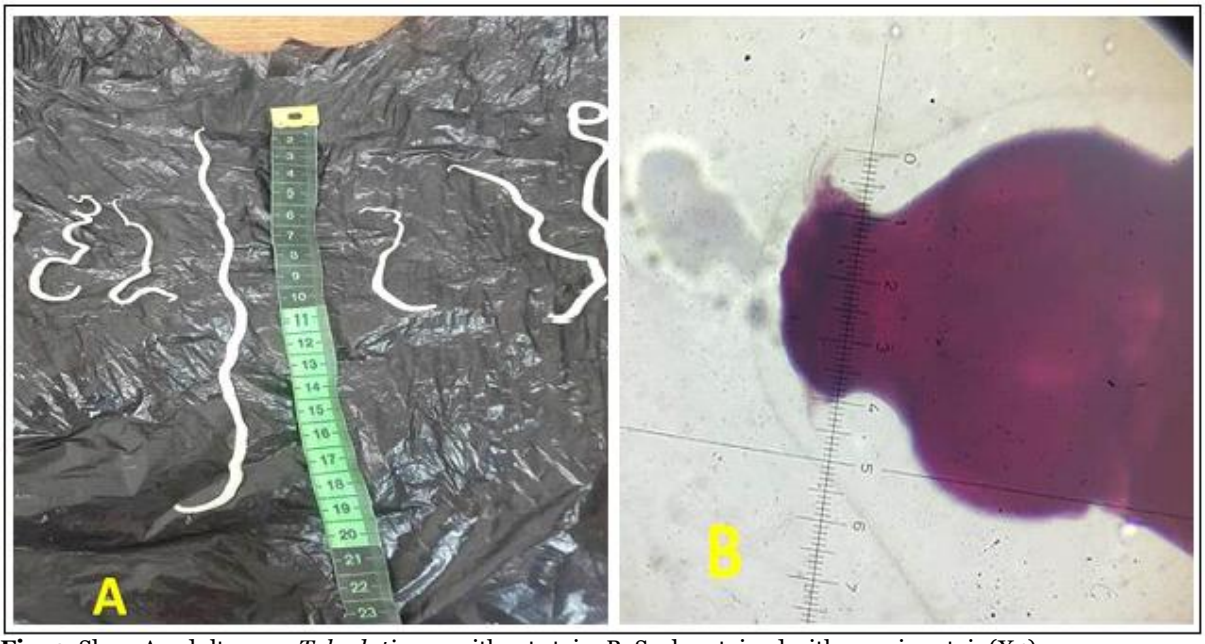

Fig. 1. Show A. adult worm *T. hydatigena* without stain, B. Scolex stained with carmine stain(X4).

DNA extraction

Extraction of DNA from 20adult worms collected, Genomic DNA of *T. hydatigena* isolate was extracted by using DNA mini kit that was supplied byG- spin DNA extraction kit, Korea according to manufacturer's instructions, primer were used in this study were obtained from IDT company (18S rRNA) Forward strand primers 5'-TCCGTAGGTGAACCTGCGG-3' and Reverse strand primers 5'- TCCTCCGCTTATTGATATGC -3'.

Sequencing

The sequencing of 18S rRNA gene was performed at Macrogen Inc., using their ABI 3730xl genetic analyzer (Applied Biosystems, US).

Results and discussion

Results showed microscopic examination some feature characteristics of adult worm, long adult worm with length average (35 cm).

They have a small scolex with armed rostellum which has double rows of hooks.

Table 1. Represent type of polymorphism of 18S rRNAgene from *Taenia hydatigena* isolate.

No. of sample	Tape of substitution	Location	Nucleotide	Sequence ID
Isolate 1	Transversion	122	T>A	ID: LC004202.1
	Transition	166	T>C	
	Transversion	209	C>G	
	Transversion	210	C>G	
	Transversion	217	T>A	
	Transversion	534	A>T	
	Transversion	535	C>G	

Table 2. Represent type of polymorphism of 18S rRNAgene from *Taenia hydatigena* isolate.

No. of sample	Tape of substitution	Location	Nucleotide	Range of nucleotide	Sequence ID
Isolate2	Transversion	122	T>A	78 to 548	ID: LC004202.1
	Transition	166	T>C		
	Transversion	203	G>C		
	Transversion	528	T>A		

The scolex connected to a relatively long neck , immature and gravid segments. These features were identical key with (Kassai, 1999; Bowman, 2009) (Figure 1).

Our study revealed that the PCR amplification was successful on all isolates for the 18S rRNA gene. The amplified fragment size was approximately 500 bp. As seen in (figure 2).This is the first study to detection *T. hydatigena* genotyping in dogs in Iraq using molecular technique.

Molecular assay is a highly sensitive diagnostic method for differentiation of dog taeniids. Allows identification of genetic links between different isolates and the valuation of parasite control by identifying the source from infection (Jia *et al.,* 2010; Rostami *et al.,* 2015; Omar *et al.,* 2016).

Table 3. Represent type of polymorphism of 18S rRNAgene from *Taenia hydatigena* isolate.

No. of sample	Type of substitution	Location	Nucleotide	Range of nucleotide	Sequence ID
Isolate3,4	Transversion	122	T>A	78 to 548	ID: LC004202.1
	Transition	166	T>C		
	Transversion	330	G>C		

Sequence alignment of 18S rRNAgene

The results obtained showed that 7 variations six Transversion T>A, C>G, and A>T and one Transition T>C have shown 99% compatibility as showed in table (1).Under sequence ID: LC004202.1(Polymorphism of *Taenia* species co-existing in the southwestern China)(Chen *et al.*, 2010). The results showed that 4 variations, three Trans version T>A , G>C, and one Transition T>C have shown 99% compatibility as showed in table (2).

Under sequence ID: LC004202.1 (Polymorphism of *Taenia* species co-existing in the South Western China) (Chen *et al*., 2010).

Table (3) shows3 variations, two Trans version T>A, G>C and one Transition T>C have shown 99% compatibility. Under sequence ID: LC004202.1(Polymorphism of *Taenia* species co-existing in the South Western China)(Chen *et al*., 2010).

Phylogenetic tree structuring

The phylogenetic tree diagrammatic by (MEGA) software version 6.0 is shown in Figure (6). The 4 isolated sequences have shown 99% compatibility. Neighbor-joining tree was constructed for phylogenetic analysis. These alignments appeared the genetic distance and other global strains by partial sequence similarity in 18S ribosomal RNA gene for translating specific region.

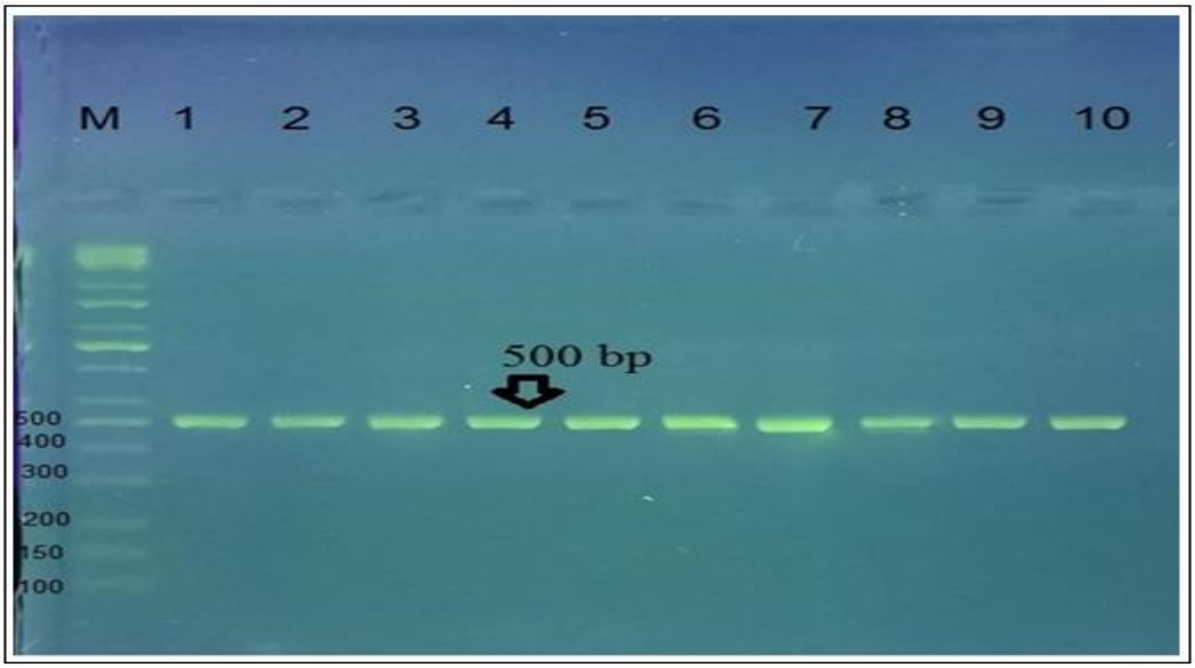

Fig. 2. Gel electrophoresis of PCR product of 18S rRNA (500bp), for *Taenia hydatigena* using 2% agarose gel at 5volt /cm for 2 hour. Lane 1- 10: PCR product positive for 18S rRNA genes.

Taenia hydatigena genes for 18S rRNA, ITS1, 5.8S rRNA, partial and complete sequence, clone: TE
Sequence ID: LC004202.1

Score	Expect	Identities	Gaps	Strand
818 bits(906)	0.0	464/471(99%)	0/471(0%)	Plus/Plus

```
Query  1    TGTTGGCAATGGTGGCGGTGGGgctgctgctgctgctgttgctgATGGACTATGAATCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  78   TGTTGGCAATGGTGGCGGTGGGGCTGCTGCTGCTGCTGTTGCTGTTGGACTATGAATCTA  137

Query  61   GACCTTGATATTGTCTTGCTGTTACTGCCGCGATGGGGTGCCTAGTCTGCCTCTAGCCTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  138  GACCTTGATATTGTCTTGCTGTTACTGCTGCGATGGGGTGCCTAGTCTGCCTCTAGCCTT  197

Query  121  TGCAGGTATGCGGGTCTTGACGCTCGCTTGCTCGCTAACTGGCTGGCTCATTGGCTCAGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  198  TGCAGGTATGCCCGTCTTGTCGCTCGCTTGCTCGCTAACTGGCTGGCTCATTGGCTCAGT  257
```

Fig. 3. Sequencing of *Taenia hydatigena* obtained from Gene Bank.

The genetic dimension between Iraq and the isolates of the world is detailed according to the Phylogenetic tree and the comparison table. The genetic dimension from *T. hydatigena* Iraq was by 2.1 it is close to china isolated from *T. hydatigena* by ID: FJ886761.1.The sequences that showed the highest identity (99%).

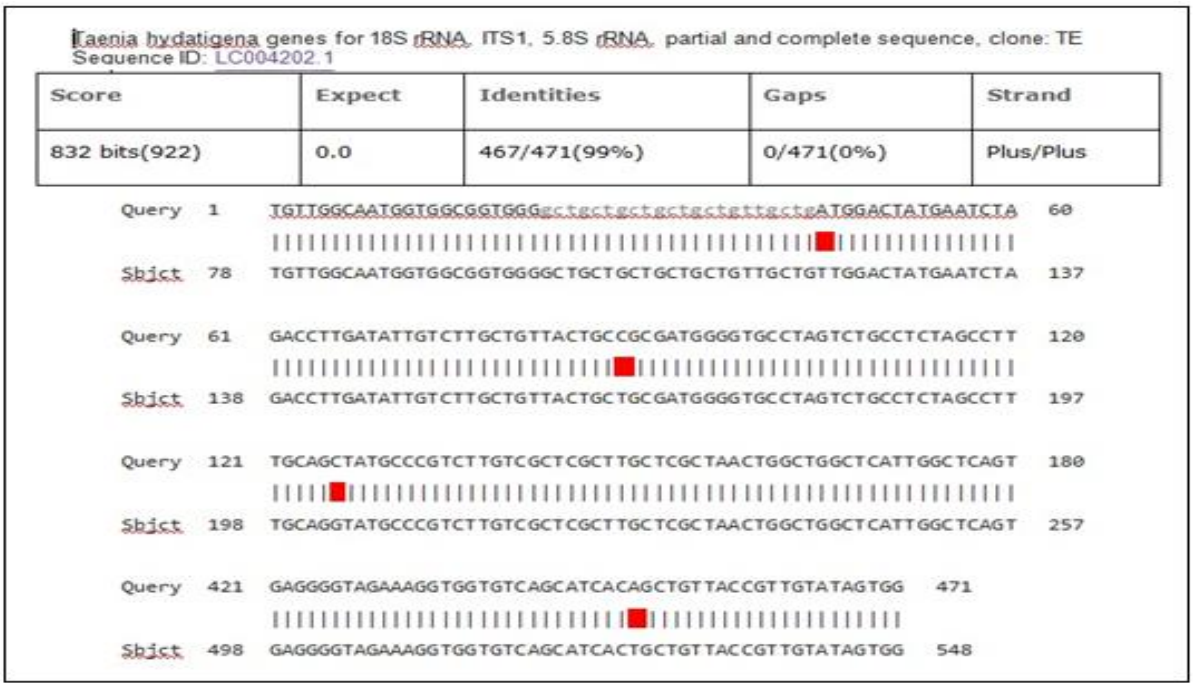

Fig. 4. Sequencing of *Taenia hydatigena* obtained from Gene Bank.

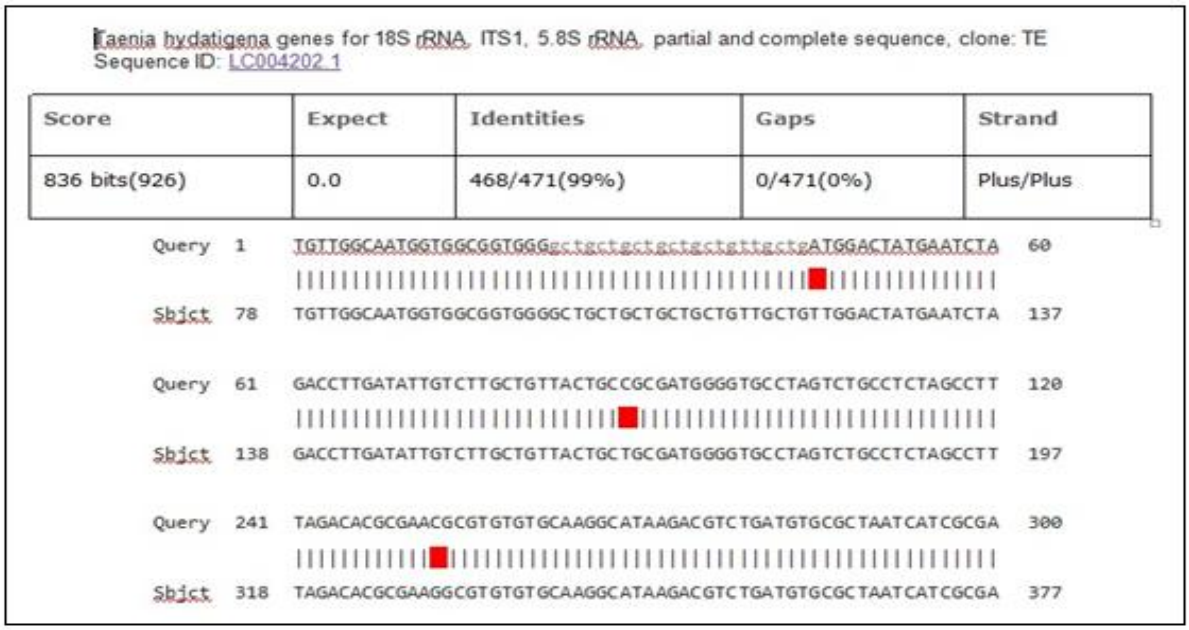

Fig. 5. Sequencing of *Taenia hydatigena* obtained from Gene Bank.

When comparison between *Taenia hydatigena* isolated recorded in the National Center Biotechnology Information (NCBI) and have under sequence (ID: FJ886757.1 , ID: LC004202.1 , ID: FJ886760.1 , ID: FJ886759.1 , ID: FJ886756.1, ID: FJ886761.1 , ID: FJ886758.1)(16), respectively with source of isolation and showed compatibility 99% and expect 0.0 with gene bank. Also, figure (6) revealed only sequences that showed the highest identity (>98%) and maximum coverage (>99%).

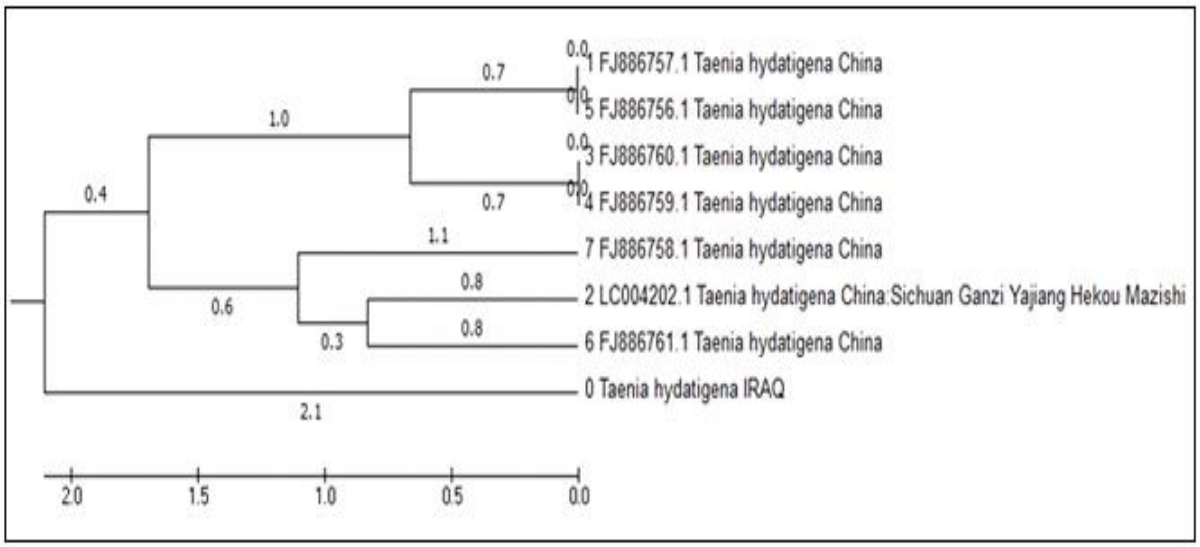

Fig. 6. Neighbor-joining tree *Taenia hydatigena* of 18S rRNA gene.

Conclusions

The current study was the first study on *Taenia hydatigena* genotypes in dogs in Iraq. Phylogenetic analysis of 18S rRNA gene sequence of *Taenia hydatigena* isolate of Iraqian origin revealed a close relationship to global isolate from china.

Submission of local Iraq isolate in NCBI

The18S rRNA gene was registered after the correspondence of (NCBI), and obtained accession number and became a reference to Iraq and the Middle East and the world. Ongoing work will add to this set as more type strains are publishedand it is available for download at NCBI: 587167.1. https://www.ncbi.nlm.nih.gov/nuccore/MH703534.1 , MH

Refreneces

Dalimi A, Sattari A, Motamedi GH. 2006. A studyon intestinal helminthes of dogs, foxes and jackals inthe western part of Iran. Veterinary Parasitology **142,** 129 – 133.

Lavikainen A, Haukisalmi V, Lehtinen MJ, Henttonen H, Oksanen A, Meri S. 2008. Aphylogeny of members of the family Taeniidae based on the mitochondrial cox1 and nad1 gene data. Parasitology **135,** 1457 – 1467.

Murat K. 2005. Investigation of antigenic specificity Against Cysticercus tenuicollis cyst fluid antigen in dogs experimentally infected with Taenia hydatigena. T urkishJournal of Veterinary Animal Sciences **29,** 835 – 840.

Deplazes P, Eckert J. 1988.Investigation of Taenia hydatigena infections in dogs Schweiz Arch Tierheilkd **130(6)**, 289-306.

Radfar MH, Tajalli S, Jalalzadeh M. 2005. Prevalence and morphological characterization of Cysticercus tenuicollis (Taenia hydatigena cysticerci) from sheep and goats in Iran. Veterinarski arhiv **75,** 469–476.

Gasser RB, Zhu X, McManus DP. 1999. NADH dehydrogenase subunit 1 and cytochrome c oxidase subunit I sequences compared for members of the genus Taenia (Cestoda). International Journal for Parasitology **29,** 1965–1970.

González LM1, Villalobos N, Montero E, Morales J, Sanz RA, Muro A, Harrison LJ, Parkhouse RM, Gárate T. 2006. Differential molecular identification of Taeniid spp. and Sarcocystis spp. cysts isolated from infected pigs and cattle. Veterinary Parasitology **142(1-2),** 95–101.

McManus DP. 2006. Molecular discrimination of taeniid cestodes. Parasitology International **55,** S31–37.

Lavikainen A, Haukisalmi V, Lehtinen MJ, Henttonen H, Oksanen A, Meri S. 2008. Aphylogeny of members of the family Taeniidae based on the mitochondrial cox1 and nad1 gene data. Parasitology **135,** 1457 – 1467.

Kassai T. 1999. Veterinary Helminthology, Butterworth-Heineman Publishing, Oxford, UK., p 222.

Bowman DD. 2009.Georgis' Parasitology for Veterinarians.9th Ed. Westline industrial drive St. Louis, Missouri, p **142**.

Rostami S, Salavati R, Beech RN, Babaei Z, Sharbatkhori M, Baneshi MR, Hajialilo E, Shad H, Harandi MF. 2015. Molecular and morphological characterization of the tapeworm Taenia hydatigena (Pallas, 1766) in sheep from Iran. Journal of Helminthology **89,** 150–157.

Omar MA, Elmajdoub LO, Al-Aboody MS, Elsify AM, Elkhtam AO, Hussien AA. 2016. Molecular characterization of Cysticercus tenuicollis of slaughtered livestock in Upper Egypt governorates. Asian Pacific Journal Tropical Biomedicine **6(8),** 706–708, 2016.

Jia WZ, Yan HB, Guo AJ, Zhu XQ, Wang YC, Shi WG. 2010. Complete mitochondrial genomes of Taenia multiceps, T. hydatigena and T. pisiformis additional molecular markers for a tapeworm genus of human and animal health significance. BMC Genomics **11(1),** 447.

Chen ZH, Bao HE, Wu XJ, Yang TX, Hu XZ. 2010. Identification of Taeniasolium with abnormal number of scolex booklets. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi **28(2),** 108-12

Dai RS, Liu GH, Song HQ, Lin RQ, Yuan ZG, Li MW, Huang SY, Liu W, Zhu XQ. 2012. Sequence variability in two mitochondrial DNA regions and internal transcribed spacer among three cestodes infecting animals and humans from China. Journal of Helminthology **86(2),** 245-51.