Research Article

Sequence and Morphology of *Cysticercus pisiformis* in Local Breed Rabbits

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Abstract | The current study was aimed to identified *Cysticercus pisiformis* of local breed rabbits (2 to 24 months old) based on morphologically and molecular detection by using *NAD1* and *COX1* genes and conventional PCR during the period from 1/ March / until 31/ May/ 2022 in Baghdad city, Iraq. Rabbits were humanely euthanized and abdomen opened longitudinally to looking for any cyst may be founded grossly in the peritoneal cavity or on the visceral organs, all cysts were examined for their structures scolex, membrane and the size measurements. The fluid was collected from each cyst for measurement the volume, and some cysts were stained with a cetoacetic acid carmine stain to seen the structure of cysts. To confirm the diagnosis of *C. pisiformis* (scolex and part of membrane) from these cysts were put in a 70% ethanol for further used in conventional molecular diagnosis. The results revealed that an overall infection rate was 23.33% (14/60). The females infection rate was 27.90% (12/43) and in the males 11.76% (2/17) without significant differences (P≥0.05). Fifty-five cysts were isolated from abdominal cavity of 14 positive samples as balloon filled with clear fluid, white floating scolex (evagenated or invagenated) or pus-filled in the older cysts , distribution of cysts was 47.27% (26/55) in mesentery, 21.82% (12/55) in liver, 21.82% (12/55) free within abdominal cavity and 9.09% (5/55) in omentum with Significant differences (P<0.05). The cyst diameters range between 7-21mm. in length and 4-10mm. in width. The PCR amplification results of 14 positive samples for *NAD1* (500bp.) and *COX1* (446bp.) genes and 3 isolates were squincing and submitted to GenBank under the accession numbers (LC731847.1, LC731848.1 and LC731849.1) for *NAD1* gene are 100% identical to the China isolate (JN870149.1), while less identically (97.15 - 99.47%) to Australia and Poland isolates, and OP274120.1, OP277616.1 and OP277618.1 for the *COX1* gene the matching between 99.56% -100% for China, Portugal and Poland. Finally, this is the first genetic investigation for the identification of local *C. pisiformis*, which has revealed a high infection rate of the parasite in local breed rabbits in Baghdad, Iraq.

Keywords | *Cysticercus pisiformis*, local breed rabbits, *NAD1* gene, *COX1* gene, Iraq

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INTRODUCTION

Since antiquity, tapeworm has caused considerable damage to wild and domestic animals, as well as human health, resulting in economic losses due to food safety, livestock output, and serious public health implications (Zhu *et al*., 2019). Rabbits are affected by many different species of parasites (El-Ashram *et al*., 2020). *Taenia pisiformis* which is one of these cestodes belonged to family Taeniidae and to the genus *Taenia* (Yang *et al*., 2013). The

final hosts of the parasite are canines and rarely felines, while their intermediate hosts are some rodents (Karamon *et al*., 2019).This parasite distributed in different areas of the world, Lahmar *et al*. (2008) found the prevalence with *T. pisiformis* of stray dogs in Tunisia was 6.36%, 3.33% in East-Azerbaijan Province, north-west Iran (Yagoob and Hossein, 2011), 26% in New Zealand (Hajipour and Zavarshani, 2020) and 9.4% in Ukraine (Bogach *et al*., 2022) and in Iraq, 8. 6% in Mosul (Al-Moula, 2005), 38. 75% in Baghdad Province (Athraa, 2014) and 54.55% in Al-Diwaniyah Province (Marhoon *et al*., 2018).

The adult worm colonizes the small intestine of dogs (Hadi and Faraj, 2016), and the larval stage causes cysticercosis in rabbits (Overgaauw, 2020). It mostly produces blood poisoning, depression, lethargic, loss of appetite and emaciation, slate themselves from normal activities of the flock, a syndrome that has been defined as sickness behaviors, but it can also impair resistance to other illnesses and may even result in mortality (Hart, 1991; Kakai, 1994; Zhou *et al*., 2008).

Because there is no phylogenetic analysis of *C. pisiformis* in local bred rabbits in Iraq, this study was achieved for confirmation and identification of this parasite from rabbits.

MATERIALS AND METHODS

Animals of the study

Sixty local breed rabbits were purchased from Al-Ghazel market in the center of Baghdad city, visited weekly during a period 1/March/ until 31/May/2022, the animals were placed under the optimal conditions (room temperature regulated at 24˚C, dry food and clean tap water) of rearing the laboratory animals in the Animal House, College of Veterinary Medicine/ University of Baghdad.

Necropsy finding

Five rabbits humanely euthanized weekly. The skin and muscles of abdomen of necropsied rabbits were opened longitudinally and the looking for any cyst may be founded grossly on the abdominal cavity or on the visceral organs, recorded the location of cysts and numbers (Abdulkareem *et al*., 2022). The isolated cysts were stored in clean containers before being sent to the Parasitology Department, College of Veterinary Medicine, University of Baghdad Laboratory for further analysis.

Identification of cysts

All cysts were examined for their structures scolex, membrane and the size measurements. The fluid was collected from each cyst for measurement the volume (Abbas, 2017; Abbas and Abbas, 2017). The cysts were identified as C. *pisiformis* according to the Loos-Frank (2000) description, and some cysts were stained with a cetoacetic acid carmine stain to seen the structure of cysts (Abbas, 1998). To confirm the diagnosis of *C. pisiformis* (scolex and part of membrane) from these cysts were put in a 70% ethanol for further used in conventional molecular diagnosis.

Molecular identification

Molecular confirmation was done on fourteen *C. pisiformis* isolated from naturally infected rabbits, they were achieved by DNA extraction from cyst tissue (scolex and part of membrane) by using commercially available kit (Add Prep Genomic DNA Extraction Kit/ Add Bio/ Korea) for tissue DNA extraction and according to instruction of manufacture's (Al-Azawi and Al-Biatee, 2019; Mraidi and Lafta, 2021).

Conventional polymerase chain reaction (PCR) technique were performed for (14 DNA samples) by using specific primers for *T. pisiformis,* first amplification of gene NADH dehydrogenase subunit1 (*NAD1*) using the primers JB11F (5'-AGATTCGTAAGGGGCCTAATA-3') forward and JB12R (5'-ACCACTAACTAATTCACTTTC-3') reveres, that amplify a fragment of about 500bp., the second conventional PCR was carried out for amplification of cytochrome oxidase subunit1 (*COX1)* using the primers CO1F (5'-TTTTTTGGCCATCCTGAGGTTTAT-3') forward andCO1R(5'-TAACGACATAACATAATGAAAATG-3') reveres, which amplifies a fragment of 446bp. according to (Samorek-Pier *et al*., 2021). Reaction of PCR technique Master Mix were performed by added 10µl of Master Mix to 3µl DNA template, 1.5µl primer forward, 1.5µl reverse, and completed by 4µl distilled water to make total volume of 20µl (Table 1).

Table 1: Contents for reaction of PCR technique.

Mixture PCR	Volume
Master-Mix	10 µL
DNA Template	3 µL
Primer Forward	1.5 µL
Primer Reverse	1.5 µL
Free nuclease water	4 µL
Total volume	20 µL

Thermocycler conditions were carried out as the following steps for both genes and for each sample (Table 2).

DNA sequencing and phylogenetic tree analysis

DNA sequencing was used to validate the presence of local *C. pisiformis* and to create a phylogenetic connection tree between local isolates and NCBI-Blast submissions,

as well as to submit local isolates to NCBI-Gen Bank. Three PCR positive isolate results for each gene were delivered in an ice bag to Bioneer Company in Korea for DNA sequencing using Applied Biosystem (AB) of DNA sequencing system. The NCBI-Gen Bank submission was completed using the Ban kit submission tool. Six PCR products' sequences were submitted to the National Center for Biotechnology Information (NCBI) under specific accession numbers for each strain. The sequences data were arranged into a FASTA format by saving the consensus as a single file (Chilton *et al*., 1997). The DNA sequencing analysis was conducted by using molecular evolutionary genetics analysis version 6.0. (Mega 6.0) and multiple sequence alignment analysis of the partial nicotinamide adenine dinucleotide dehydrogenase subunit1 (*NAD1*) and cytochrome oxidase subunit1 (*COX1)* genes, internal transcribed spacer each gene based Clustal W alignment analysis, the evolutionary distances were computed using the maximum composite likelihood method by phylogenetic tree UPGMA (unweighted pair group method with arithmetic mean) method (Tamura *et al*., 2011). Using molecular evolutionally genetics analysis version 6.0 (Mega 6.0) multiple sequencing alignment analysis of the partial *NAD1* gene and *COX1* gene. Sequence analysis of the *NAD1* and *COX1* genes has been used to show the similarity and differences between local and international genes sequence.

Table 2: Thermocycler conditions of PCR.

PCR steps	Temperature	Time	Repeated
Initial denaturation	94˚C	5 minutes	1
Denaturation	94˚C	30 Seco.	35 cyles
Annealing	52˚C	30 Seco.	
Extension	72˚C	60 Seco.	
Final extension	72˚C	5minutes	1
Hold	12˚C	∞	

Statistical analysis

To determine the significance of different components in research parameters, the Statistical Analysis System- SAS 9.3 (2012) application was utilized. In this study, the Chi-square test was utilized to compare percentages (0.05 and 0.01 likelihood).

RESULTS AND DISCUSSION

Fourteen of 60 (23.33%) necropsied local bred rabbits was found infected naturally with *C. pisiformis* (Table 3). The result of current study was less than result of Athraa (2014) who recorded 38.75% (31/80) infection rate of in local rabbits in Baghdad city-Iraq, also Jori (2016) who founded 40% (4/10) infection rate from domestic rabbits in Basrah city-Iraq, and less than Marhoon *et al*. (2018) recorded 54.55% (30/55) of *C. pisiformis* in a wild rabbits collected from the local animals market in Al-Diwaniyah Province-Iraq and less than Roldan (2019) in Spain 67.7% (21/31) infection rate with metacestodes *C. pisiformis* from the capturing wild rabbits, while the results enclose with study of Hajipour and Zavarshani (2020) in Iran which found the prevalence rate 26% (13/50) in new Zealand white rabbits. The results are higher than Al-Moula (2005) which recorded 8.6% (2/30) infection rate when examined domestic rabbits in Mosul city-Iraq. Also, more than Foronda *et al*. (2005) in Canary Islands which found the prevalence rate 16% in total 244 wild rabbits. Also higher than Yagoob and Hossein (2011) which isolated 3.33% (2/60) from abdominal cavity of wild rabbits in East Azerbaijan province of Iran.

According to the sex a total rate of infection in 27.90% (12/43) females was which was more than males' infection 11.76% (2/17) without significant differences (P≥0.05) (Table 3). This result agree with Roldan (2019) in Spain which recorded high infection rate in females 80% (17/21) than males 40% (4/10) with significant difference (P≤0.05). In contrast to Hajipour and Zavarshani (2020) in Iran who found males affected more than females and recorded infection rate 23.80% (5/21) in females and 27.58% (8/29) males without significant difference (P≥0.05). The disparities between the current study and prior studies might be attributed to variances in the quantity of samples obtained between investigations, difference in type of animals (domestic or wild), as well as differences in the distribution and proliferation of stray dogs.

Table 3: Total Infection rate with *C. pisiformis* in naturally infected rabbits according to the sex.

Sex	Number of rabbits	Number of infected	Ratio
Females	43	12	27.90%
Males	17	2	11.76%
Total	60	14	23.33%
Statistical analysis	χ^2= 1.17 DF=1 P value =0.27 Non-Significant (P≥0.05)		

Fifty-five cysts were isolated from peritoneal cavity of 14 positive samples (single or group) as balloon filled with clear fluid with white floating scolex (evagenated or invagenated) or pus-filled in others. The range of diameters of cyst between 7 to 21 in length and 4 to 10 mm in width. (Table 4) and (Figure 1), this cysts were confirmed to the Loos-Frank (2000) description as C. *pisiformis*.

According to the location of cyst into internal organs of 55 cysts, large number of larval cysts attached to the mesentery 47.27% (26/55), liver 21.82% (12/55), other cysts found

free within the abdominal cavity 21.82% (12/55) and on omentum only 9.09% (5/55) with Significant deffrences (P<0.05) (Table 5).

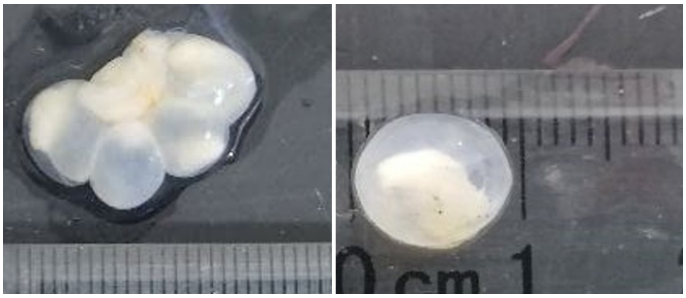

Figure 1: Shape and measurements of *Cysticercus pisiformis* cysts, grossly.

Table 4: Measurements of cysts size and volume.

Number of cysts	Range of size		Volume of fluid (µl)	Percentage (%)
	Range length (mm)	Range width (mm)		
6	18-21	9-10	12-17	10.90
17	12-15	4-10	8-11	30.90
27	7-10	4-9	4-7	49.09
5	5-6	3-4	2-3	9.09

Table 5: The site and number of cysts in fourteen naturally infected rabbits.

Site No.	Mesentery	Liver	Free in abdominal cavity	Omentum
1		7		
7	4			
13	2			
14	1			
18	4			
29	3			
30	2			
31	3		6	
36	1		2	2
38	2			
39	2			
41	2			3
56		2	2	
59		3	2	
Total cysts	26	12	12	5
Ratio	47.27%	21.82%	21.82%	9.09%
Statistical analysis	X2= 41.13 DF=4 P value =0.001 Significant (P<0.05)			

Cysts staining with acetoacetic carmine stain appeared scolex with armed rostellum with tow raw of hooks and four suckers (Figure 2) like the method of (Abbas, 1998).

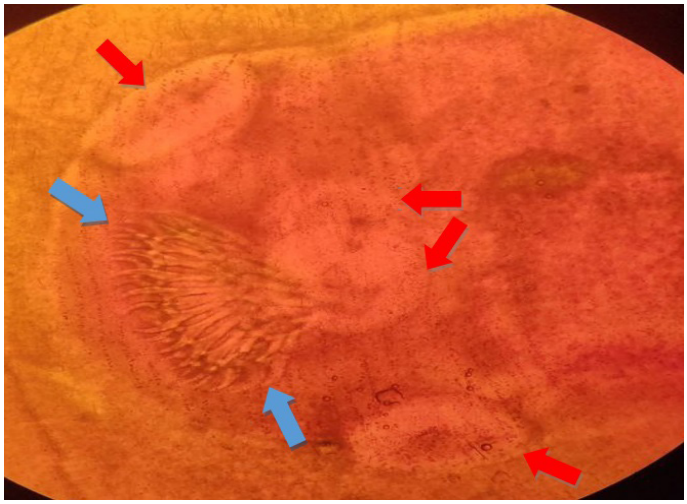

Figure 2: Structure of *Cysticercus pisiformis* Scolex staining by acetoacetic carmine stain, Suckers (red arrow) and Hooks (blue arrow), X40.

Result of moleculer study

The result of convential PCR technique to which used for confirm *C. pisiformis* based on using (*NAD1*) genes and primer 500bp the result was 100(14/14) (Figure 3) , also 14 DNA sample were subgected to PCR to amplification (*COX1*) genes with primer 446 bp the result was was 100(14/14) (Figure 4).

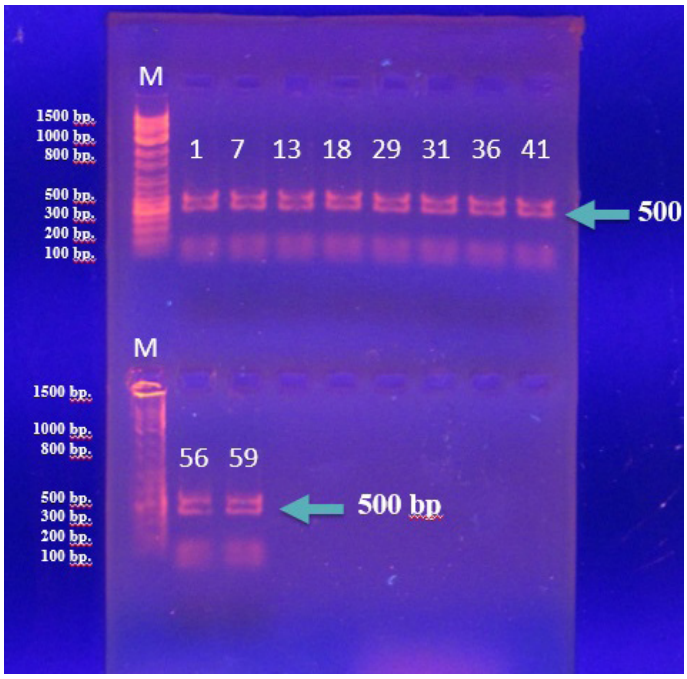

Figure 3: Agarose (1%) gel electrophoresis of PCR products for (NAD1) gene of C. pisiformis of rabbits and DNA Marker Ladder (M) (100- 1500bp), PCR products amplicons band lances (1, 7, 13, 18, 29, 31, 36, 41, 56 and 59) sized 500bp. (1h, 7 volt/cm2 TBE).

Results of DNA sequencing and phylogenetic tree construction

The nucleotide sequences of 3 local *C. pisiformis* isolates

for each genes were submitted to GenBank under the accession numbers (LC731847.1, LC731848.1 and LC731849.1) for *NAD1* gene (Table 6) and (OP274120.1, OP277616.1 and OP277618.1) for *COX1* gene (Table 7).

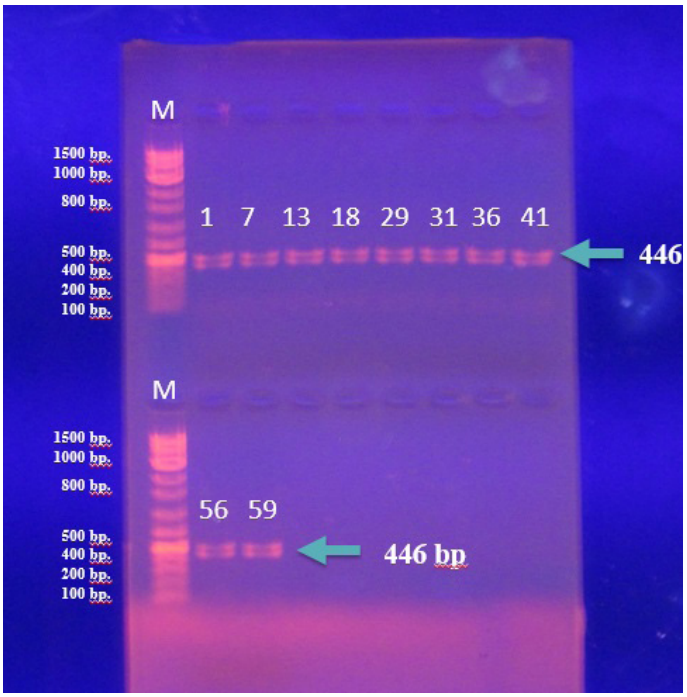

Figure 4: Agarose(1%) gel electrophoresis of PCR products for (COX1) gene of C. pisiformis of rabbits and DNA Marker ladder (M) (100- 1500bp), PCR products amplicons band lances (1, 7, 13, 18, 29, 31, 36, 41, 56 and 59) sized 446bp. (1h, 7 volt/cm2 TBE).

Three sequences of *NAD1* gene (LC731847.1, LC731848.1 and LC731849.1) was 100% identical to the China sequence JN870149.1 while less identically (97.15-99.47%) in Australia and Poland (Table 6 and Figure 5), and sequences of *COX1* gene (OP274120.1, OP277616.1 and OP277618.1) were matching variance between 100% to 99.56% in the China, Portugal and Poland (Table 7 and Figure 6).

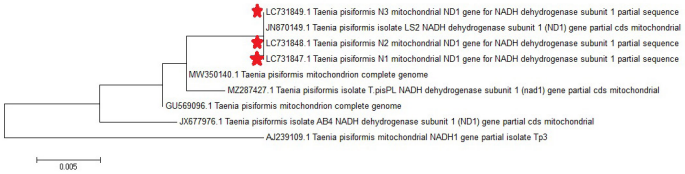

Figure 5: Phylogenetic tree study based on Taenia pisiformis NAD1 gene partial sequence.

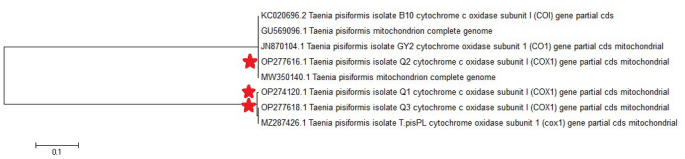

Figure 6: Phylogenetic tree study based on Taenia pisiformis COX1 gene partial sequence.

Table 6: Isolates of *T. pisiformis* from natural infected by *NAD1* gene sequencing ID in gene bank and nucleotide sequence identity from NCBI.

Local isolates	Accession numbers for local isolates	NCBI-BLAST homology sequence Identity(%)		
		Accession numbers	Country	Identity (%)
T. pisiformis No.1	LC731847.1	JN870149.1	China	100
		MW350140.1	China	99.44
		GU569096.1	China	99.44
		MZ287427.1	Poland	99.16
		JX677976.1	China	98.88
		AJ239109.1	Australia	97.21
T. pisiformis No.2	LC731848.1	JN870149.1	China	100
		MW350140.1	China	99.47
		GU569096.1	China	99.47
		MZ287427.1	Poland	99.20
		JX677976.1	China	98.93
		AJ239109.1	Australia	97.34
T. pisiformis No.3	LC731849.1	JN870149.1	China	100
		MW350140.1	China	99.43
		GU569096.1	China	99.43
		MZ287427.1	Poland	99.14
		JX677976.1	China	98.86
		AJ239109.1	Australia	97.15

Table 7: Isolates of *T. pisiformis* from natural infected by *COX1* gene sequencing ID in gene bank and nucleotide sequence identity from NCBI.

Local isolates	Accession numbers for local isolates	NCBI-BLAST Homology sequence		
		Accession numbers	Country	Identity (%)
T. pisiformis No.1	OP274120.1	MW350140.1	China	99.56
		JN870104.1	China	99.56
		GU569096.1	China	99.56
		KC020696.2	Portugal	99.56
		MZ287426.1	Poland	99.56
T. pisiformis No.2	OP277616.1	MW350140.1	China	100
		JN870104.1	China	100
		GU569096.1	China	100
		KC020696.2	Portugal	100
T. pisiformis No.3	OP277618.1	MZ287426.1	Poland	100
		MW350140.1	China	99.67
		JN870104.1	China	99.67
		GU569096.1	China	99.67
		KC020696.2	Portugal	99.64

This study is the first one by using conventional PCR and sequence identification of *Cysticercus. pisiformis* in Baghdad, Iraq.

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NOVELTY STATEMENT

This study is the first one by using conventional PCR and sequence identification of Cysticercus pisiformis in Baghdad, Iraq and study the highly distribution of the infection of local bred rabbits in Souq al-Ghazal in the Baghdad city.

AUTHOR'S CONTRIBUTION

Both authors conceived and designed the study, reviewed and approved the final manuscript. Kadhim Kh. K. Al-Khayat performed the experiments. Athmar K. A. Al-Azawi analyzed the data. Kadhim Kh. K. Al-Khayat contributed materials and wrote the manuscript.

Ethics statement

All animals in this study were handled and cared for according to the appropriate biosecurity procedures. The study was performed in accordance with the rules of the 'Guide for the Care and Use of Laboratory Animals that were approved by the Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Iraq (Number 472 P.G at 28/2/2023) before starting this study.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abbas AK (1998). Study in the epidemiology of cysticercus tenuicollis of sheep and goats. College of Veterinary Medicine, University of Baghdad.

Abbas AK (2017). Detection some chemical and biochemical constituents of Cysticercus tenuicollis cyst fluid of Iraqi goat. J. Entomol. Zool. Stud., 5(2): 814–818.

Abbas HB, and Abbas AK (2017). Analysis of chemical and biochemical composition of fertile and sterile hydatid cyst fluids of human origin. Iraqi J. Vet. Med., 41(1): 66–70. https://doi.org/10.30539/iraqijvm.v41i1.82

Abdulkareem MH, Razook BRF, Ameer AHA, Alrubaye B (2022). Histopathological changes of rabbits' vital organs associated with corynebacterium pseudotuberculosis infection. Int. J. Special Educ., 37(3). https://doi.org/10.51470/bca.2022.22.2.3743

Al-Azawi AKA, Al-Biatee ST (2019). Prevalence and phylogeny of *Cysticercus tenuicollis* sp. determined by PCR from 6909 slaughtered sheep over 12 months. Online J. Vet. Res., 23(6): 594–605.

Al-Moula IDH (2005). Study of some endo and ecto parasites in domestic rabbits in Mosul/Iraq. Iraqi J. Vet. Sci., 19(2): 143–153. https://doi.org/10.33899/ijvs.2005.46707

Athraa TW (2014). Study of Prevalence of gastrointestinal and blood parasites with some histopathological changes of local rabbits in Baghdad province. M. Sc. thesis, Coll. Vet. Med. Univ. Baghdad.

Bagrade G, Kirjušina M, Vismanis K, Ozoliņš J (2009). Helminth parasites of the wolf Canis lupus from Latvia. J. Helminthol., 83(1): 63–68. https://doi.org/10.1017/S0022149X08123860

Bajalan MMM (2010). Prevalence of intestinal helminths in stray dogs of Kalar city, Sulaimani province. Iraqi J. Vet. Med., 34(1): 151–157. https://doi.org/10.30539/iraqijvm.v34i1.673

Bogach MV, Paliy AP, Horobei OO, Perotska LV, Kushnir VY, Bohach DM (2022). Endoparasites of rabbits (*Oryctolagus cuniculus domesticus*) in Southern Ukraine. Biosyst. Diver., 30(2): 173–178. https://doi.org/10.15421/012218

Borji H, Azizzadeh M, Kamelli M (2012). A retrospective study of abattoir condemnation due to parasitic infections: economic importance in Ahwaz, southwestern Iran. J. Parasitol., 98(5): 954–957. https://doi.org/10.1645/GE-2988.1

Chilton NB, Gasser RB, Beveridge I (1997). Phylogenetic relationships of Australian strongyloid nematodes inferred from ribosomal DNA sequence data. Int. J. Parasitol., 27(12): 1481–1494. https://doi.org/10.1016/S0020-7519(97)00134-3

El-Ashram S, Aboelhadid SM, Abdel-Kafy ESM, Hashem SA, Mahrous LN, Farghly EM, Kamel AA (2020). Investigation of pre-and post-weaning mortalities in rabbits bred in Egypt, with reference to parasitic and bacterial causes. Animals, 10(3): 537. https://doi.org/10.3390/ani10030537

Ezenwa VO (2016). Helminth–microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits. Parasit. Immunol., 38(9): 527–534. https://doi.org/10.1111/pim.12348

Foronda PR, Figueruelo EO, Ortega AR, Abreu NA, Casanova JC (2005). Parasites (viruses, coccidia and helminths) of the wild rabbit (*Oryctolagus cuniculus*) introduced to Canary Islands from Iberian Peninsula. Acta Parasitol., 50(1): 80–84.

Garcia LS, Ash LR (1979). Diagnostic parasitology: Clinical laboratory manual CV Mosby Co. St. Louis, Missouri, USA, pp. 145–146.

Hadi AM, Faraj AA (2016). Prevalence of gastrointestinal Helminthes and protozoa among stray dogs in Baghdad. Iraqi J. Vet. Med., 40(1): 1–4. https://doi.org/10.30539/iraqijvm.v40i1.129

Hajipour N, Zavarshani M (2020). Ectoparasites and endoparasites of New Zealand white rabbits from North West of Iran. Iran. J. Parasitol., 15(2): 266. https://doi.org/10.18502/ijpa.v15i2.3310

Hart BL (1991). The behavior of sick animals. The Veterinary Clinics of North America. Small Anim. Pract., 21(2): 225–237. https://doi.org/10.1016/S0195-5616(91)50028-0

Hoberg EP (2002). Taenia tapeworms: Their biology, evolution and socioeconomic significance. Microb. Infect., 4(8): 859–866. https://doi.org/10.1016/S1286-4579(02)01606-4

Jori MM (2016). The effect of Cysticercus pisiformis on the haematological and biochemical parameters of rabbits in Basrah Province. Life Sci. Arch., 2(January): 458–463.

Kakai R (1994). A study of factors associated with intestinal

immunoglobulin A in children with diarrhoea only and those with diarrhoea and malnutrition. University of Nairobi.

Karamon J, Sroka J, Dąbrowska J, Bilska-Zając E, Zdybel J, Kochanowski M, Różycki M, Cencek T (2019). First report of *Echinococcus multilocularis* in cats in Poland: A monitoring study in cats and dogs from a rural area and animal shelter in a highly endemic region. Parasit. Vectors, 12: 1–8. https://doi.org/10.1186/s13071-019-3573-x

Khademvatan S, Majidiani H, Foroutan M, Tappeh KH, Aryamand S, Khalkhali HR (2019). Echinococcus granulosus genotypes in Iran: A systematic review. J. Helminthol., 93(2): 131–138. https://doi.org/10.1017/S0022149X18000275

Kornyushin VV, Malyshko EI, Malega AM (2011). The helminths of wild predatory mammals of Ukraine. Cestodes. Vestnik Zool., 45(6): 483–490. https://doi.org/10.2478/v10058-011-0031-4

Lahmar S, Sarciron M, Rouiss M, Mensi M (2008). Echinococcus granulosus and other intestinal helminths in semi-stray dogs in Tunisia: infection and re-infection rates. La Tunisie Méd., 86(3): 279–286.

Loos-Frank B (2000). An up-date of Verster's (1969) Taxonomic revision of the genus Taenia Linnaeus'(Cestoda) in table format. Syst. Parasitol., 45(3): 155–184. https://doi.org/10.1023/A:1006219625792

Marhoon IA, Mattar KT, Mohammad FI (2018). Parasitic infection in wild rabbits *Oryctolagus cuniculus*. Eur. J. Anal. Chem., 13(5): 55. https://doi.org/10.29333/ejac/95252

Mraidi DA, Lafta IJ (2021). RAPD-PCR and phylogenetic analysis of E. coli isolated from human and cattle urinary tract infections. Iraqi J. Vet. Sci., 35: 59–66. https://doi.org/10.33899/ijvs.2021.131100.1918

Nakao M, Yanagida T, Okamoto M, Knapp J, Nkouawa A, Sako Y, Ito A (2010). State-of-the-art Echinococcus and Taenia: Phylogenetic taxonomy of human-pathogenic tapeworms and its application to molecular diagnosis. Infect. Genet. Evol., 10(4): 444–452. https://doi.org/10.1016/j.meegid.2010.01.011

Overgaauw PAM (2020). Parasite risks from raw meat-based diets for companion animals. Compan. Anim., 25(11): 261–267. https://doi.org/10.12968/coan.2020.0065

Roldan RD (2019). Aspectos de dimorfismo sexual, reproductivos e histológicos asociados a la teniosis (taenia pisiformis) cisticercosis en dos especies de lagomorfos y en el hámster dorado.

Samorek-Pieróg M, Karamon J, Brzana A, Bilska-Zajacą E, Zdybel J, Cencek T (2021). Molecular confirmation of massive taenia pisiformis cysticercosis in one rabbit in Poland. Pathogens, 10(8).

Steele JH (1973). A bookshelf on veterinary public health. Am. J. Publ. Health, 63(4): 291–311. https://doi.org/10.2105/AJPH.63.4.366

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol., 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121

Yagoob G, Hossein H (2011). Prevalence rate of endoparasites in wild rabbits of East-Azerbaijan Province, Iran. Ann. Biol. Res., 2: 31–35.

Yang D, Ren Y, Fu Y, Xie Y, Nie H, Nong X, Gu X, Wang S, Peng X, Yang G (2013). Genetic variation of Taenia pisiformis collected from Sichuan, China, based on the mitochondrial cytochrome B gene. Korean J. Parasitol., 51(4): 449. https://doi.org/10.3347/kjp.2013.51.4.449

Zhou YX, Du AF, Zhang XJ, Wu YM, Tong FY, Wu GY (2008). Research of harmfulness of *Cysticercus pisiformis* in rabbit. J. Zhejiang Agric. Sci., 3: 372–373.

Zhu GQ, Li L, Ohiolei JA, Wu YT, Li WH, Zhang NZ, Fu BQ, Yan HB, Jia WZ (2019). A multiplex PCR assay for the simultaneous detection of *Taenia hydatigena, T. multiceps, T. pisiformis*, and *Dipylidium caninum* infections. BMC Infect. Dis., 19: 1–7. https://doi.org/10.1186/s12879-019-4512-3