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## **First documentation of canine dipylidiasis by molecular detection in Baghdad City, Iraq**

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**Abstract**--Canine dipylidiasis is a zoonotic intestinal cestode infects cands, fields and rarely human. The goal of this study was to determine morphological characteristics by traditional parasitological methods and molecular techniques among stray dogs in Baghdad, Iraq. Totally, thirty-two adults of *D.caninum* were isolated from 15 small intestines that were naturally infected obtained from 100 necropsied stray dogs (15%) and used for morphological description after using Semichon-acetocarmine stain, on the basis of morphological description of the scolex, immature, mature, and gravid segments of *D.caninum* The results revealed a significant difference ( $p > 0.01$ ) in the dipylidiasis infection based on the dogs' sex, females had a higher infection rate (28%) than males (10.7%). In between aged groups, infection rates showed a relatively significant difference ( $P \leq 0.01$ ) with a higher rate in puppies (20%) than in adults (13.8%). The records of *D.caninum* increased in March compared to the other months, with nil infection in August. By using the DC28SrRNA region at size (653bp) se, Polymerase Chain Reaction (PCR), DNA sequencing, and phylogeny were used for identification and genetic variation between thirty-two local *D.caninum* and NCBI. Ten positive PCR products (DC28SrRNA region-653bp) from DNA templates gave profiles characteristic of *D.caninum* and indicated that all Iraqi *D.caninum* were identified with accession no. (MZ677330, OL460637, OL460638, OL410552, OL413443, OL376660, OL413444, OL466918, OL413445, and OL413446). To our knowledge, this is the first morphological and molecular study of *D.caninum* in stray dogs in Baghdad, Iraq. The results pointed to the risk of zoonotic dipylidiasis transmission to humans.

**Keywords**---Baghdad, Iraq, dipylidium caninum, morphology, molecular study, prevalence, stray dogs.

## Introduction

Dogs are popular companion animals, and they are reservoirs for some pathogenic zoonotic parasites. Such as *Dipylidium caninum*, belonging to the family *Dipylidiidae* (Eucestoda), described for the first time by Linnaeus (1758). The tapeworm was discovered in the small intestines of canids and felines, which are the most common definitive hosts and are only rarely found in humans (1). Fleas and lice (Order: *Siphonaptera*, Family: *Pulicidae* and *Phthiraptera*) are intermediate hosts, typical of *Ctenocephalides felis*, *Ctenocephalides canis* (2), *Xenopsylla cheopis* (3), *Trichodectes canis* (4) and *Felicola subrostratus* (5). According to Pugh (3). This cestodes' occurrence in the environment depends on the definitive and arthropod intermediate hosts' presence at the optimum temperature, when the definitive host accidentally ingests the infected intermediate host with cysticercoid (larval stage), then the cysticercoid is released into the stomach and enters the small intestine to develop into an adult cestode and attach to the intestinal wall of the definitive host (6). In contrast, the adult cestode is less pathogenic and may cause anal irritation in some instances, causing concern for dog owners when emerging from the anus (7). Prevalence studies of dipylidiasis have been reported in several countries in stray dogs (6), in Egypt (8), Africa (9, 10), Nigeria (11), and more recently, throughout Palestine (12).

Currently, *D.caninum* infection is diagnosed by morphological examination of proglottids as rice-grain-like and/or egg capsules passed with faeces obtained by microscopic examination methods such as flotation and sedimentation techniques (13). A polymerase chain reaction (PCR) based molecular approach included detection of genetic variation and phylogenetic relationships of *D.caninum* based on nuclear and mitochondrial gene sequences (14). Despite these advances, there is currently no data on genomes and genetic diversity of *Dipylidiidae* cestodes. So this study was aimed at adding to the description of *D.caninum* morphological features as well as providing a new source for the first molecular identification in stray dogs, from Baghdad City, Iraq.

## Materials and Methods

### Sample collection and morphological study

one hundred stray dogs were necropsied from different areas in Baghdad City, via exterminations, from January to October 2021. Each small intestine was transferred to the parasitology laboratory- College of Veterinary Medicine, University of Baghdad, and then opened languidly to collect and wash the adult cestode with physiological saline. According to Kim and Shin, (15), adult *D.caninum* was stained with Semichonurd-acetocarmine stain, then matched with the descriptive data and figures given by Soulsby (16). Ethical approval was granted through the local committee of animal care and use at the College of Veterinary Medicine within the University of Baghdad (Number 962 at 2022/04/27).

### Statistical analysis

Was based on SAS (17) with the data of age (puppies and adults), sex (adults and puppies), and 9-months. Adult specimens were fixed in ethanol (70%) and stored (-80°C) until DNA extraction.

### Molecular study – PCR

DNA was extracted directly from all individual adult tissue by using (G-Spin DNA Extraction Kit-Intron Biotechnology, Korea). A final DNA elution was done in 300µl and stored (-20°C) until needed. As described by Beugnet *et al.*, (1), an amplification a volume of 25µl consisting of DNA template (1.5µl) at the 28S rRNA gene of DNA with 1 µl of each primer (20 mM) (a forward (DC28S-1F:5'-GCATGCAAGTCAAAGGGTCCTACG-3'); and reverse (DC28S-1R: 5'-CACATTCAACGCCCGACTCCTGTAG-3')) were amplifying (653bp) fragments 5µl of the Maxime PCR PreMix kit (i-Taq) according to the manufacture of the company (the components are: 5U/ µl of Color Taq DNA Polymerase, 2.5mM of dNTPs, reaction buffer (10X) and gel loading buffer (1X)) and mixed with 16.5 µl of distill water. PCR cycling conditions included many step: pre-heating at 95°C for 5 min., denaturing (35 cycles of 95°C for 45s), annealing (60°C for 45s), extension (70°C for 45s), and final extension (72°C for 7 min.), and a holding temperature of 14°C. An Applied Biosystems 2720 Thermo-cycler condition was used to do a conventional PCR thermo-cycler (T100 thermal cycler BioRad USA). A 1.5% agarose gel was prepared according to (18) using to visualize target amplicons and identify of positive samples on the gel from a specific band (~653bp). All chromatograms were viewed using a UV transilluminator. Ten positive products were sent to Bioneer Company in to Korea for sequencing by AB-Applied Biosystem DNA sequencing system in an ice bag. Submission-NCBI-Gen-Bank using the Bankit tool for comparing sequences and get accession number for any genetic variation between Iraqi *D.caninum* isolates of GenBank for similarity. Sequencing of DNA study was directed using utilizing Mega 6.0. The development distances were computed utilizing; Phylogenetic analyses were carried out employing (Phylogny.fr/advanced method).

### Results and Discussion

#### Morphological examination

Stained Scolex showed by light microscope a very tiny sensory organ, with four suckers and having an armed, retractable rostellum with many small rows of crowns and a “rose-thorn” shaped hook (Fig.1). Mature proglottid contains two sets of reproductive organs: female and male sexual organs, with double genital pores located laterally at the proglottid edges of each margin just below the midline that classifies as a hermaphrodite cestode. Testes are numerous and occupy most of the space. Ovaries are paired, the proglottid appears longer than wide (Fig. 2). Stained gravid proglottid were oval and longer than wide, the uterus was replaced by capsules, containing eggs, measuring about 20-25 µm. *D.cninum* eggs are spherical to oval in shape, colorless with a thin shell, and contain a visible embryo within the egg. Each terminal proglottid is less broad while the

middles are broadest with the subtlest indentation on the lateral side of the proglottid (Fig.3).



Figure 1. Stained *Dipylidium caninum* scolex, under microscopic (40X)

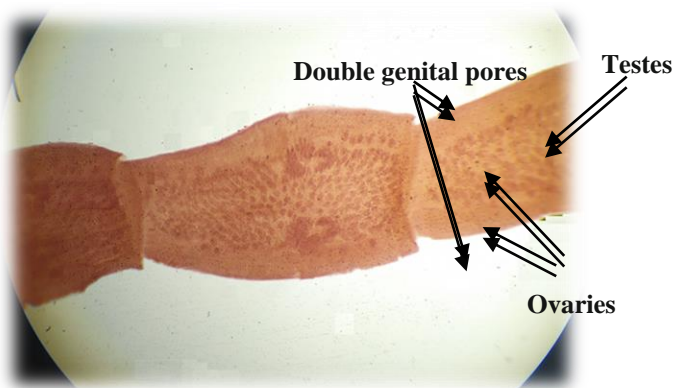


Figure 2. Stained *Dipylidium caninum* mature proglottid, under microscopic (40X)

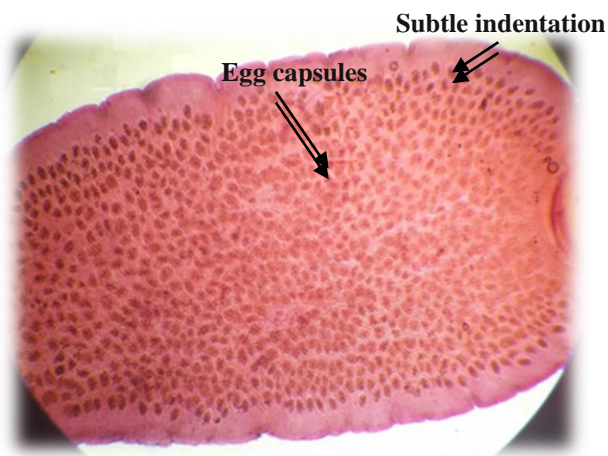


Figure 3. Stained *Dipylidium caninum* gravid proglottid under microscopic 10X.

***Dipylidium caninum* infection rate from stray dogs**

Fifteen (15%) out of one hundred necropsied stray dogs were found infected with dipylidiasis in different regions of Baghdad city. The results also revealed differences in the parasites' distribution between sexes. Out of 25 female examined 28% (7/25) were found infected; out of 75 males examined 10.7% (8/75) were infected with *D.caninum* (Table.1). According to age, the infection rate with *D.caninum* in the puppies was 20% (4/20) compared with adults' 13.8% (11/80) with a significant ( $P<0.01$ ) difference (Table.2). The results revealed a higher infection was 36.36% (4/11) in March than the other months, with significant differences ( $P\leq 0.01$ ), and no infection was recorded in August (0%) (Table3).

Table 1  
*Dipylidium caninum* infection rate according to the sex

Sex	No. examined	No. infected (+)	Percentage%
Females	25	7	28
Males	75	8	10.7
Total	100	15	15%
Chi-Square ( $\chi^2$ )	--	--	3.0549
P value	--	--	0.08
** ( $P\leq 0.01$ )			

Table 2  
*Dipylidium caninum* infection rate according to the age

Aged	No. of examined	No. infected(+)	Percentage %
Puppies	20	4	20
Adult	80	11	13.8
Total	100	15	15
Chi-Square ( $\chi^2$ )	--	--	13.603 **
P value	--	--	0.0001
** ( $P\leq 0.01$ )			

Table 3  
*Dipylidium caninum* infection rate, according to the months

Months	No. examined	No. infected	Percentage %
January	11	1	9.09
February	11	2	18.18
March	11	4	36.36
April	11	2	18.18

May	11	2	18.18
Jun	11	2	18.18
July	11	1	9.09
August	11	0	0
September	12	1	8.33
Total	100	15	15
Chi-Square (x2)	--	--	5.021
P value	--	--	0.755
** (P≤0.01)			

### Molecular analysis

From thirty-two DNA samples, using conventional-PCR for amplification of 28SrRNA-Large Subunit ribosomal-RNA gene (653bp) was used to determine *D.caninum* genes and identify specific species. The PCR product of *D.aninum*'s DC28SrRNA is shown in an agarose gel electrophoresis image (Fig.4).The DNA marker ladder (M: 1000-100bp) showed all 32 positive PCR *D.caninum* at 653bp. Analysis by sequence alignment showed the nucleotide alignment similarity as (\*) in the DC28SrRNA gene between local isolated and NCBI-Genbank (Fig. 5). Phylogenetic tree analysis of ten products in stray dog's *D.caninum* isolates revealed that the isolates (MZ677330, OL460637, OL460638, OL410552, OL413443, OL376660, OL413444, OL466918, OL413445, and OL413446) infected stray dogs in Baghdad, Iraq. The local isolates OL376660 and OL413444 had a close relationship with NCBI-Blast *D.caninum* of USA isolates (MH182478), South Africa (MH045471), with 100%, and less with NCBI-Blast *D.caninum* of Iran (MG774549), and Malaysia (KY751956) with 99.39%, 95.50%, respectively, in total genetic changes (0.0-0.000%). The local *D.caninum* dog isolates OL460637 and OL460638 were less related to identity with NCBI-Blast of USA isolates (MH182478), and South Africa (MH045471), with 80.73% and 89.32%, respectively. Isolates OL376660 and OL413444 had a 99.39% relationship with NCBI-Blast *D.caninum* of Iran (MG4549), while isolates MZ677330, OL413443, OL466918, and OL413446 had a 99.02% relationship with NCBI-Blast *D.caninum* of South Africa (MH045471) and USA (MH182478) (Table, 3-4 and Figure, 8). Identify and isolate OL466918 and OL413446, which had a 99.83% identity with NCBI-Blast *D.caninum* of USA (MH182478) and South Africa (MH045471) (Table 3-4 and figure, 6).

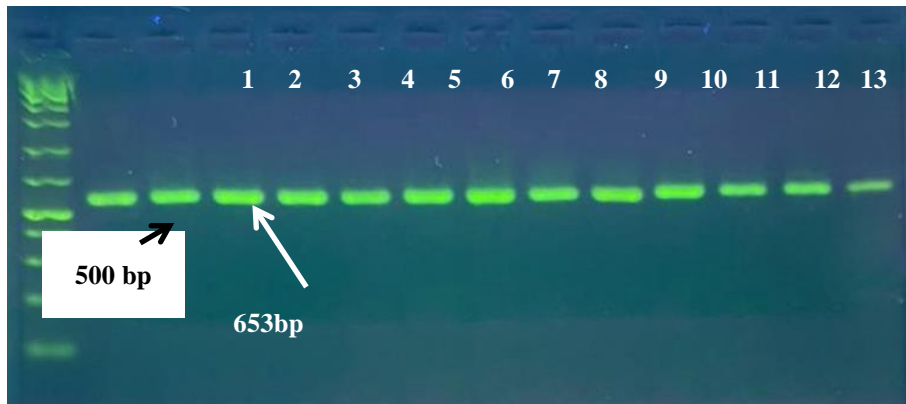


Figure 4. Agarose gel electrophoresis image showed PCR products analysis of 28SrRNA gen (653bp) in *D. caninum* adult samples, lane: (M) Molecular marker (1000-100bp)

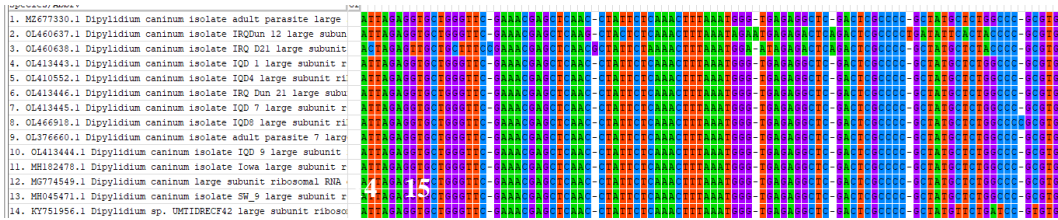


Figure 5. Sequence alignment analysis of DCSrRNA in local *Dipylidium caninum* isolates and NCBI-Genbank

Table 8

*D. caninum* genotypes in stray dogs used partial sequence (28SrRNA gene) based on analysis of phylogenetic tree and NCBI- BLAST alignment tool

Local <i>D. caninum</i> isolate No.	Genbank Accession No.	NCBI-BLAST Homology Sequence identification			
		NCBI Blast <i>D. caninum</i> USA (MH182478)(%) )	NCBI Blast <i>D. caninum</i> Iran (MG774549)(%) )	NCBI Blast <i>D. caninum</i> S. Africa (MH045471 ) (%) )	NCBI Blast <i>D. caninum</i> Malaysia (KY751956 ) (%) )
No.1	MZ677330	99.67	99.02	99.83	95.50
No.2	OL460637	80.73	80.40	80.73	87.04
No.3	OL460638	89.32	88.81	89.32	85.23
No.4	OL410552	99.67	99.02	99.66	95.50
No.5	OL413443	99.83	99.14	99.83	95.17
No.6	OL376660	100	99.39	100	95.50

No.7	OL413444 4	100	99.39	100	95.50
No.8	OL466918	99.83	99.16	99.83	95.29
No.9	OL413444 5	99.67	99.02	99.66	95.50
No.10	OL413444 6	99.67	99.02	99.83	95.50

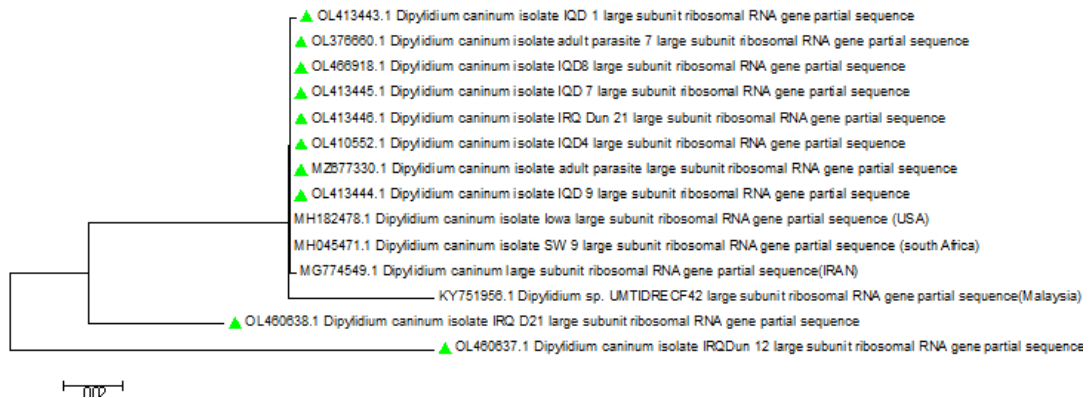


Figure 6. Phylogenetic tree analysis based on Large Subunit ribosomal RNA gene partial sequences in *D. caninum* local isolates is used for genetic relationships analysis. Local *D. caninum* isolates (MZ677330, OL460637, OL460638, OL410552, OL413443, OL376660, OL413444, OL466918, OL413445 and OL413446) were shown to be closely similar to NCBI-BLAST *D. caninum* USA isolates with total genetic variation (0.0-0.000%)

Some researchers described the same morphology of *D. caninum*, like Patricia *et al.*, (19) recorded the length of the body reaching 50 cm. The difference in measurements of length may be related to many factors, such as: type of animal feeding, environmental effects, and the parasite accumulation effect (20). Patricia *et al.*, (19) means the body was having a scolex, the neck, and a proglottids section (called the strobilus), the scolex is small, rhomboid in shape, with four suckers and an apical armed rostellum, which has many crowns of hooks. Saari *et al.*, (21) described the mature proglottid that has genitals paired. The mature proglottid has both female and male sexual (reproductive) organs, situated laterally at midline of the proglottid. Many testes are scattered, ovaries are paired with vitelline glands are posterior location to theirs. Gravid proglottids are longer than wide and filled with egg capsules contains more 30 eggs as is typical *D. caninum* characteristic. Isolates of *D. caninum* infecting stray dogs are morphologically identical to cucumber seed tape worms. Thus it's got typical name: cucumber tapeworm (22). A main reason to increasing tendency of *Dipylidium* cases is a lack of information about the deworming schedule and choice of anthelmintic, presence of fleas in the surrounding areas is related to the occurrence of tapeworm infection in dogs, as these fleas act as intermediate hosts for *D. caninum* (23). Despite disagreement with Ash *et al.*, (24) and Taylor and

Zitzmann (25), they recorded that the proglottids range between 7mm by 3mm (length and width) and egg capsules containing 8-10 eggs surrounded by a thinner membrane.

Our study of infection rats was close to Das *et al.*, (26) from Bangladesh, recorded of dipylidiasis infection rats at 15%. While our results are disagree with other studies by Tarish *et al.*, (27) recorded that the infection rate (50%) from Baghdad/Iraq, El Malaik *et al.*, (9) recorded the infection rates (1.7%) in Sudan. Bajalan, (28) recorded an overall infection rate of 26% from autopsied stray dogs in Sulaimani province, Iraq. Infected rats revealed the adult cestode were attached to the intestinal wall parasitized stray dogs. The causes of this variation was associated with variation of the presence of suitable intermediate hosts and the geographical study regions, affecting intermediate hosts distribution that relatively present at a higher density in stray dogs. Although dipylidiasis infections are rare, it is a zoonotic disease for humans, especially children can acquire the infection accidentally by ingesting infected adults fleas due to their playing habits and proximity to pet dogs and cats (6).

The results of the present study revealed there was a significant difference ( $P \leq 0.01$ ) associated with infection rate of positive samples according to sex. This result is in agreement with those observed by Das *et al.*, (26) who noted that the infection rate in female were higher 17.85% than male 12.5%. On the other hand, this result is in disagreement with Emamapourm *et al.*, (29) who recorded the infection rate of male were higher (44.2%) than female (30.7%). In the present study, stray dogs of younger age apparently showed higher susceptibility to *D.caninum* infections compared to adults. This result was in agreement with the findings of Matthew *et al.*, (11). During the month's study, the present study of killed stray dogs showed the infection rate was 15%. Infection rate has been reported to vary from a minimum of 2.0% in Iran (30) to a maximum of 100% from Egypt (8). The present finding corresponds with reports of Chiad, (31) in Baghdad, Iraq (12.30%), but shows similarity with the reports of Das *et al.*, (26) in Bangladesh (15%); Mahmoud, (32) in Salahdin province/ Iraq (19.68%) and Trasviña-Muñoz *et al.*, (33) in Mexicali Valley (16.50%). In an identical study carried out in China a high infection rate of *D. caninum* was encountered 72.5%, (34). It was also reported by Emamampourm *et al.*, (29) in Iran (39%).

In present study, thirty-two adult cestode tissues were extracted for genomic DNA from different geographical locations in Baghdad/Iraq, using primers for 28SrRNA region and positive PCR amplification, this technique is considered one of the most specific diagnosis methods, and can be useful in genetic and biological studies in comparison with previous local or global studies (1). By the phylogenetic tree analysis, agreement bases for *D.caninum* with those samples globally recorded have been identified: *D.caninum* samples in the current study having Serial no. OL376660 and OL413444 registered in USA and Malaysia by (35), and Serial no. MZ677330 registered in South Africa by (4). Besides, it has been noticed that the samples of *D.caninum* in the current study are approaching those having the serial no. MZ677330 and OL413443 registered in Iran (nonpolisher).

Conclusion: from monitoring stray dogs' populations and probable intermediate hosts to detect dipylidiasis sources in poor areas and limit *D.caninum* transmission to humans. The morphological and phylogenetic analyses allowed us to correctly identify this cestode. The reason behind this difference refers to diversity in environment, geographical location of being near or far from the seaside and wet, abundance of animals and providence of predisposing factors, especially contacts with the intermediate host. Further studies are needed on the impact on the population of Baghdad City. The rural areas of Baghdad city are the regions at higher risk for dipylidiasis, mainly because of the large number of stray dogs living in close proximity to domestic animal management systems.

### **Conclusion**

28S rRNA gene in size 653bp was an efficient molecular marker for their *Dipylidium* species detection, *Dipylidium caninum* sequencing with phylogeny confirms phylogenetic relationship of all *Dipylidium* species in NCBI database and determines the percentage of genetic similarity among them.

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### **Conflict of interest**

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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37. دنيا عبد الملك محمد صالح أثمار خضير عباس
38. الخلاصة
39. داء ذات المنفذين من الامراض المعوية الشريطيات تصيب الكلاب والقطط ونادرًا الإنسان. الهدف من الدراسة هو تحدي الخصائص الشكلية بالطرق التقليدية والجزيئية بين الكلاب السائبة في بغداد ، العراق. إجمالاً ، تم عزل اثنين وثلاثين دودة بالغه من 15 أمعاء دقيقة مصابة طبيعيًا تم الحصول عليها من 100 كلب سائبة مشرح 15%) (واستخدمت للوصف *D.caninum*.. الشكلي بعد استخدام صبغة -أسيتوكارمينز، على أساس القطع الراس ، غير الناضجة ، والحاملة من في الإصابة بداء ثنائي البويضات بناءً على جنس الكلاب ، حيث كانت ( $p > 0.01$ ) و أظهرت النتائج وجود فرق معنوي أعلى معدل إصابة في الإناث 28%) (من الذكور) 10.7%. (وبين الفئات العمرية ، أظهرت معدلات الإصابة فرقًا كبيرًا مع معدل أعلى في الجراء 20%) (منه لدى البالغين) 13.8%. (زادت سجلات داء الكينوم في اذار ( $P \leq 0.01$ ) نسبيًا مقارنة بالأشهر الأخرى ، مع عدم وجود إصابة في اب. تم استخدام تفاعل البلمرة المتسلسل وباستخدام منطقة المحلي و *D. caninum* والتطور الوراثي لتحديد الاختلاف الوراثي بين اثنين وثلاثين من ،DC28SrRNA من قوالب الحمض النووي وأشارت DC28SrRNA region-653bp أرسلت عشرة نتا نج موجبة لـ NCBI. و OL460637 و MZ677330) العزلة العراقية قد سجلت بالارقام *D.caninum* إلى أن جميع و OL460638 و OL410552 و OL413443 و OL376660 و OL413444 و OL466918 و

على حد علمنا ، هذه هي أول دراسة شكلية وجزئية لدودة ذات المنفذين الكلبية في (OL413445 و OL413446). العراق ، بغداد ، أشارت النتائج إلى خطر انتقال هذا الداء إلى البشر  
40. كلاب السائبة. *Dipylidium caninum*. الكلمات المفتاحية: انتشار؛ دراسة شكلية ؛ دراسة جزئية.