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Research Article

Molecular and sequencing study of *Otodectes cynotis* in cats in Karbala province/Iraq

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Abstract

Background: *Otodectes cynotis* (Acari: Psoroptidae) is an obligate ectoparasite that primarily causes otodectic mange in both domestic and wild animals.

Aims: This study aimed to evaluate the prevalence and distribution of the obligate ectoparasite *O. cynotis* (Acari: Psoroptidae) mites and the molecular sequences of isolates in Karbala province/Iraq.

Methods: For this purpose, 187 cats visiting the Teaching Animal Hospital at the University of Kerbala's Faculty of Veterinary Medicine in Karbala, Iraq were monitored for mites. The diagnosis was based on otoscopic examination of both ears, followed by microscopic confirmation based on ear swab samples. The prevalence was 46%. No significant relationship was found between infestation status and season, sex, body weight, age, or breed of the cats. Polymerase chain reaction (PCR) analysis was performed to identify the species and evaluate the possible effects on domestic, isolated populations. PCR was performed for 10 isolates originating from three different breeds (Sherazi, Himalayan, and Scottish). The first internal transcribed spacer of rDNA was selected as the target sequence.

Results: Comparisons of sequences revealed two different newly sequenced Iraqi isolates, under accession numbers PV123832 and PV564189, which were closely related genotypes at the same node as the dog no. *O. cynotis* isolate. KP. 676677.1, although the closest genotyping was with one clade no. HK 728005.1.

Özet

Dayanak: *Otodectes cynotis* (Acari: Psoroptidae), hem evcil hem de yabani hayvanlarda birincil otodektik uyuz hastalığına neden olan zorunlu bir ektoparazittir.

Amaçlar: Bu çalışmanın amacı, Irak'ın Kerbela ilinde zorunlu ektoparazit *O. cynotis* (Acari: Psoroptidae) akarlarının yaygınlığını ve dağılımını ve izolatların moleküler dizilerini değerlendirmektir.

Yöntemler: Bu amaçla, Irak'ın Kerbela kentinde bulunan Kerbela Üniversitesi Veterinerlik Fakültesi'ne bağlı Eğitim Hayvan Hastanesi'ne gelen 187 kedi, akar açısından izlenmiştir. Tanı, her iki kulağın otoskopik muayenesine dayandırılmış, ardından kulak sürüntü örnekleri temelinde mikroskobik doğrulama yapılmıştır. Prevalans %46 olarak belirlenmiştir. Enfestasyon durumu ile mevsim, cinsiyet, vücut ağırlığı, yaş veya kedilerin cinsi arasında anlamlı bir ilişki bulunamamıştır. Türleri tanımlamak ve evcil, izole popülasyonlar üzerindeki olası etkileri değerlendirmek için polimeraz zincir reaksiyonu (PCR) analizi yapılmıştır. Üç farklı cinsten (Sherazi, Himalayan ve Scottish) kaynaklanan 10 izolat için PCR yapılmıştır. Hedef sekans olarak rDNA'nın birinci iç transkripsiyonlu ara bölgesi seçilmiştir.

Bulgular: Dizilerin karşılaştırılması, PV123832 ve PV564189 erişim numaraları altında, köpek kaynaklı *O. cynotis* izolatı KP. 676677.1 ile aynı düğümde yakın akraba genotipler olan iki farklı yeni dizilenmiş Irak izolatını ortaya çıkarmıştır. Bununla birlikte, en yakın genotipik benzerlik HK 728005.1 numaralı bir klad ile görülmüştür.

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Conclusion: Our findings clarified the genetic relationship, origins, and potential effects on endemic domestic cat populations, which do not distinguish between different host species or regions of origin. These findings should be considered in cat populations to avoid a potential epidemic of mite-borne zoonotic illnesses.

Sonuç: Bulgularımız, farklı konak türleri veya menşe bölgeleri arasında ayrım yapmayan endemik evcil kedi popülasyonları üzerindeki genetik ilişkiyi, kökenleri ve potansiyel etkileri açıklığa kavuşturmuştur. Akar kaynaklı zoonotik hastalıkların potansiyel bir salgını önlemek için bu bulgular kedi popülasyonlarında dikkate alınmalıdır.

Keywords: Cat, ear mite, ITS-1, *Otodectes cynotis*

Introduction

The ear mite *Otodectes cynotis* is one of the most common ectoparasites causing otitis externa and represents a clinically important disease in cats and dogs (Hassan & Barzinji, 2018; Lefkaditis et al., 2015). The parasite is highly contagious and is not restricted to cats; it can infect a wide range of animals, including dogs, nutria, mink, foxes, ferrets, raccoon dogs, wild carnivores, and occasionally humans (Antipov et al., 2017; Bisterfeld et al., 2024; Fanelli et al., 2020; Hu et al., 2019; Premaalatha et al., 2023; Silva et al., 2021). Severe infestations may lead to serious complications, and in rare cases, death has been reported in cats (Melezhyk et al., 2024). In addition, cats suffering from otodectosis are often coinfected with other pathogens, including viral (Zenad & Radhy, 2020), bacterial (Abbas & Rady, 2023), ectoparasitic (Karpovaitė et al., 2013), and fungal agents (Ivaškienė et al., 2009). Globally, *O. cynotis* is responsible for approximately 50%–80% of otitis externa cases in cats (Kumar et al., 2021). Cat owners usually recognize the clinical signs of severe irritation, including intense pruritus, erythema, dark brown ceruminous discharge, and a characteristic coffee-ground–like exudate (Sotiraki et al., 2001).

In Iraq, the first *O. cynotis* infestation in cats was recorded in Baghdad, where the parasite was detected in 3 out of 50 (6%) locally kept cats (Kallo, 2004). A study conducted in Fallujah reported an overall prevalence of 36.4% (51/140) among cats of different ages examined at veterinary clinics (Hussein et al., 2024). More recently, 150 cats were examined in Babylon City using both light and electron microscopy (Al-Khafaji & Al-Musawi, 2025). Several diagnostic methods, including direct ear swab examination, light microscopy, and otoscopic evaluation, have been used to confirm *O. cynotis* infestation in the external auditory canal (Coelho et al., 2024; Wilhelm et al., 2025).Recent studies have also applied advanced diagnostic techniques (Al-Khafaji & Al-Musawi, 2025). In Chile, *O. cynotis* has been detected in free-ranging southern pudus (Pudu puda) using both microscopic and molecular analyses (Hu et al., 2019).Similarly, ear mite infestations were identified in 26.6% (4/15) of the examined animals at Zoo Wuppertal through clinical in situ and ex situ investigations (Wilhelm et al., 2025).

Despite the global distribution of *O. cynotis*, only a limited number of molecular studies have been conducted, and few studies have explored the genetic differences between isolates from cats and dogs (Salib & Baraka, 2011). Only few *O. cynotis* gene sequences are currently available in public databases (Hu et al., 2019). Although mitochondrial DNA is useful for species identification, ribosomal DNA spacers—particularly internal transcribed spacer (ITS)-1 and ITS-2—are among the most widely used molecular markers for species confirmation due to their rapid evolutionary rate (Dabert, 2006). In Iraq, molecular investigations of ear mites remain scarce, and limited information is available regarding genetic characterization and association among sex, age, body weight, health status, breed, and clinical signs in domestic cats. Therefore, the present study aimed to molecularly identify *O. cynotis* isolates obtained from domestic cats and analyze and compare *ITS-1* gene sequences with reference sequences available in GenBank, using samples collected from different areas in Karbala province/Iraq.

Materials and Methods

Study Design

The current study was carried out at Kerbala University, College of Veterinary Medicine, Department of Veterinary Parasitology, from November 2023 to October 2024. Domesticated cats of different ages, breeds, and health conditions brought to the clinics of the department for different reasons (e.g., checkup, vaccination, etc.) were used as the study material.

Sample Collection

Cats with clinical symptoms of mite infection were included in the sample collection. The most common symptoms were itching and with wounds. With a prevalence of 78/86 (90.70%) and 8/86 (9.30%). Samples for mite monitoring were collected by ear swab from 52 male and 42 female cats with suspected cases of otodectic mange with the consent of the owners for continued screening for all animal infestations. Prevalence of the present study was 46% (86/187). A total of 187 ear swab specimens were collected during the study. The swab samples were placed in sterilized plastic/glass tubes for further use. Animal data on their identity, age, sex, vaccine status, deworming notification, and reason for clinic consultation were also recorded. The positive samples were then transferred to the laboratory for microscopy and other methods.

Recovery and Examination of the Ectoparasites

After soaking the samples in 10% potassium hydroxide for a whole day, skin mites were put on a glass slide. Cotton buds from the ear swab samples were scraped onto a sterile glass slide, followed by the application of 3–5 drops of glycerol and a coverslip. A compound microscope was used to view the prepared mounted slides ectoparasites at magnifications of 10x, 40x, and 100x. The identification of the specimens at the genus level was performed using specified morphological criteria and published keys (Ahn et al., 2013).

DNA Extraction

Whole genomic DNA was extracted from individual mites using a commercial DNA extraction kit (Bioneer, Korea) according to the manufacturer's instructions. Briefly, 1.4 mL of ASL buffer was added to 220 mg of ASL and incubated at 70 °C for 5 min. Then, the sample was homogenized for 6 min using a TissueLyser and centrifuged at 14,000 rpm for 1 min. One Inhibit EX tablet was added to the collected supernatant, vortexed thoroughly, incubated at room temperature for 1 min, and centrifuged at 14,000 rpm for 3 min. Subsequently, 200 µL of the clarified supernatant was mixed with 15 µL of proteinase K and 200 µL of lysis buffer and incubated at 70 °C for 10 min. After incubation, 200 µL of absolute ethanol was added, and the mixture was centrifuged at 14,000 rpm for 1 min. The column was washed according to the manufacturer's instructions. DNA was eluted using 100 µL of the kit's elution buffer. The concentration and purity of the extracted DNA were measured using a NanoDrop spectrophotometer, and the DNA samples were stored at −20 °C until further analysis.

Polymerase Chain Reaction (PCR) Amplification of ITS-1 rDNA

The sequence was downloaded from the National Center for Biotechnology Information (NCBI), and the primer was designed: The ITS-1 target sequence was retrieved from the NCBI GenBank database (KP676675.1). The primers were designed using the program (Primer3plus) Table 1.

The reaction mixture (Taq DNA polymerase, dNTPs, and reaction buffer), template DNA (4 µL), forward and reverse primers (10 pmol/µL; 2 µL of each), and nuclease-free water yielded a total volume of 20 µL. Reactions were performed in a thermal cycler (Model ND1000, Thermo Scientific, Willington, DE, USA) with an initial denaturation for 5 min at 95 °C denaturation at 95 °C for 30 s, and annealing at 55 °C for 30 s, followed by an extension for 55 s at 72 °C, and a final extension at 72 °C for 5 min (Bioneer Korea company manufacture) 35 cycle. The PCR products were separated on a 1.5% agarose gel using TBE buffer and a 100-bp DNA ladder.

Gel Electrophoresis, ITS-1 rDNA Sequencing, and Phylogenetic Analysis

PCR products were visualized by electrophoresis on a 1.5% agarose gel stained with Ethidium bromide. Bands of the expected size were purified using a commercial PCR Purification kit according to the manufacturer's instructions. ITS-1 region of *O. cynotis* the DNA recovered from cats was sequenced, and sequence alignment and editing were performed. Performed using BioEdit and ClustalW software. The ITS-1 sequences obtained were compared with previously published *O. cynotis* ITS-1 sequences in GenBank database. The newly generated sequences were deposited in GenBank under the accession numbers PV564189 and PV123832. Multiple sequence alignment was performed using ClustalW, and phylogenetic analysis was performed using the NJ method in MEGA software (version 5.2), with scale bars representing the number of nucleotides substitutions per site (Song et al., 2024).

Statistical Analysis

Data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS), version 24. Descriptive statistics were applied to summarize the data as frequencies and percentages. The chi-square test of independence was used to examine the association between health status and gender, age groups, weight categories, and seasons. A significance level of $p \leq 0.05$ was adopted, and results were considered statistically significant when the *p*-value was 0.05. The SPSS statistical software (version 24) was used.

Results

The total prevalence was 46% (86 mite-positive cats among 187 individuals) for the entire study period. The infestation is distributed among 13 breeds, with Sherazi, Himalayan, and Scottish being the most infested (Figure 1).

The lowest *O. cynotis* infection rates were recorded in Ragdoll, Panda, and Chinchilla, with one infestation confirmed. Anchor, British, and Calico are the two recorded cases. Three cases were detected in both Bersion Bekevís and Persian breeds. Shantela recorded four cases; additionally, five cases were reported in local breeds. The highest number of infections was found in Sherazi, Himalayan, and Scottish breeds (44, 11, and 7) (Figure 1). The type of infestation has single or mixed signs. The most common sign was itching (90.70%), and itching with wounds (9.30%).

Numerous factors were noted during the investigation of *O. cynotis* infestation, with seasons showing prevalence (winter, 8.10; spring and summer, 36.00%; autumn, 19.80%). The prevalence of males and females was 60.5% (52/86) and 39.5% (34/86), respectively. The prevalence rates were 25.60%, 20.90%, 22.10%, 15.10%, and 16.30% for animals weighing 1 kg, 1–2, 3, 4, and 1–5, respectively. Between the ages of <12, 12–24, 24–36, 36–48, and 48–60 months, the prevalence of infestation was 61.60, 22.10, 7.00, and 2.30%, respectively. All factors, such as sex, season, and weight, had no significant effects on infestation in cats ($p > 0.05$). The age was significant, with the most infestation occurring in the age of less than one year and decreasing with increasing age (Table 2).

Table 1. Primary information in this study.

Primer sequences	5-3 directions	Length	G-C content (%)	Accession number	Amplicon size	T	City/country
ITS-1-F	5′TAGGTGAACTGCGGAAGGATC-3′	21	52.4	KP676675.1	570 bp	64 oC	Macrogen/ Korea
ITS-1-R	5′-GTCGAGTGATCGGAGTGTCCT-3′	21	61.9			66 oC	

ITS-1 = internal transcribed spacer-1; T = temperature.

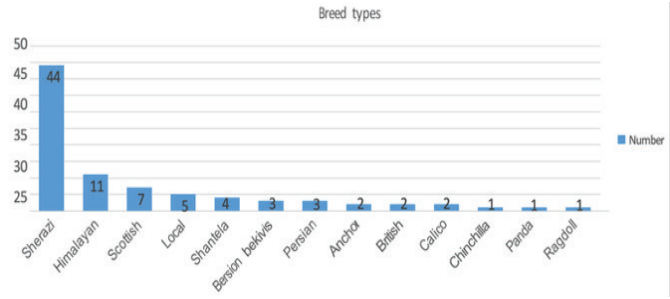

Figure 1. The presence of *Otodectes cynotis* depends on the breed.

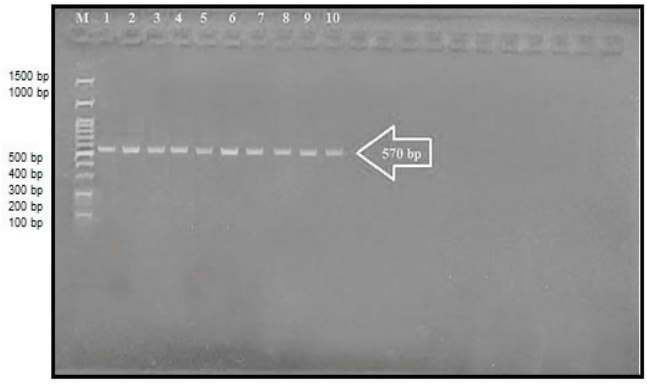

Figure 2. Agarose gel electrophoresis 1.5% of *Otodectes cynotis* isolate PCR product analysis Lane M: marker ladder (1500 bp); lanes 1–10: *O. cynotis* isolate positive for the rDNA gene (ITS-1) (570 bp).
ITS-1 = internal transcribed spacer-1; PCR = polymerase chain reaction.

Gel Electrophoresis and Phylogenetic Analysis

In the PCR of DNA samples extracted from *O. cynotis*, positive results for the rDNA gene (ITS-1) with a molecular size of 570 bp were obtained (Figure 2).

A phylogenetic tree was constructed to clarify the evolutionary relevance among the 10 *O. cynotis* isolates (Figure 3). The purpose of the tree's roots was to create an origin from which divergence events could be deduced. Several clades, each representing a group of taxa with a common evolutionary lineage, were observed from the root. Successful sequences were entered into GenBank using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The new Iraqi isolates, PV123832 and PV564189, have a separate clade. Several nodes representing a fictitious common ancestor from which descendant taxa have split off were created. While closely related genotypes at the same node as the dog no. KP. 676677.1, although the closest genotyping was with one clade no. HK 728005.1. All originated from *O. cynotis* clones that were collected in Iraq. Little genetic divergence was observed within this node and clade, as seen by the tight clustering of these taxa. Another isolate, KP676675.1 to KP676676.1, was grouped independently; they formed another unique clade, indicating genetic variability specific to the previously stated cluster. The different clades were composed of KP676675.1 in cat-1 to KP676676.1 in dog-1, which explains the cat- and dog-derived *O. cynotis* sequences, which were distinct from the KP676677.1 and HQ728005.1 sequences, which were clearly distinct from the

Table 2. *Otodectes cynotis* infestation with different factors.

p-value	X2	Percentage	Non-infected	Percentage	Infected	Numbers	Types	Variable factors
0.203	4.608	18.80%	19	8.10%	7	26	Winter	**Seasons**
		30.70%	31	36.00%	31	62	Springs	
		30.70%	31	36.00%	31	62	Summer	
		19.80%	20	19.80%	17	37	Autumn	
0.776	0.081	58.4%	59	60.5%	52	111	Male	**Sex**
		41.6%	42	39.5%	34	76	Female	
Weight category								
0.776	0.081	22.80%	23	25.60%	22	45	Less than 1 year	**Weight**
		20.80%	21	20.90%	18	39	1-2	
		23.80%	24	22.10%	19	43	1-3	
		16.80%	17	15.10%	13	30	1-4	
		15.80%	16	16.30%	14	30	1-5	
The age category								
1*	0.036	61.40%	62	61.60%	53	115	<12	**Age**
		22.80%	23	22.10%	19	42	12-24	
		6.90%	7	7.00%	6	13	24-36	
		6.90%	7	7.00%	6	13	36-48	
		2.00%	2	2.30%	2	4	48-60	

*Mean the factor was significantly at level $p < 0.05$ with infestation.

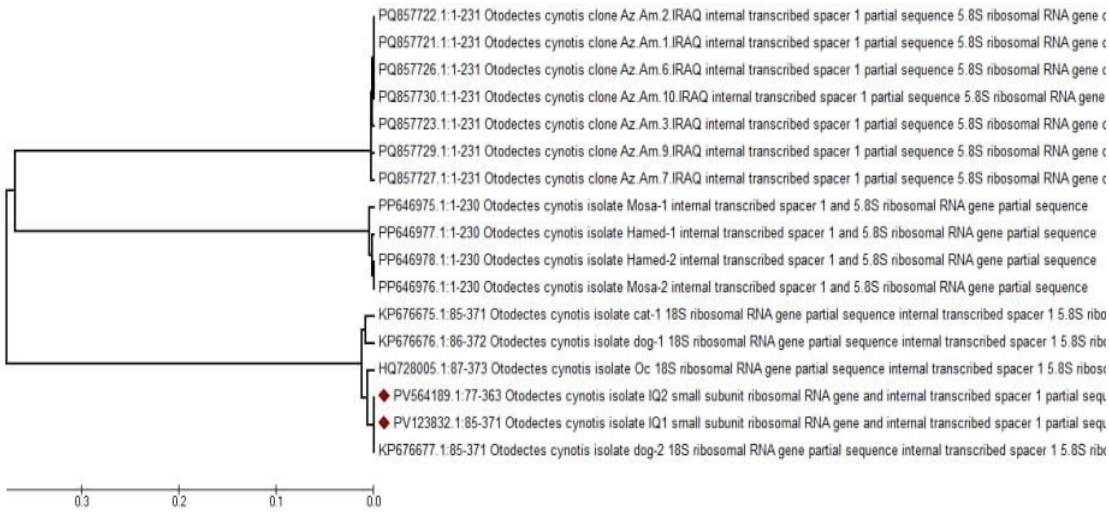

Figure 3. Phylogenetic tree analysis of ITS-1 homologs from different isolates of *Otodectes cynotis.*
internal transcribed spacer.
ITS-1 = internal transcribed spacer-1.

dog- and cat-associated strains, respectively. Both Iraqi isolates were localized in adjacent branches. These taxa formed a small, highly similar subclade once they were discovered to have a recent common ancestor. They were positioned to show that they were closely related genetically and that they differed significantly from other isolates, such as KP676675.1 and HQ728005.1. PV564189 and PV123832 split more recently from a common taxon than the other isolates in their clade since they shared a node. The degree of sequence variation was shown by the length of each branch; shorter branches suggested greater genetic similarity between isolates. Each terminal branch represented a different taxon and was linked to a distinct *O. cynotis* isolate.

Neighbor-joining reconstruction between the sequence of both isolates PV123832 and PV564189, obtained in this study and sequences were reported in GenBank and phylogenetic analysis from the NCBI BLAST database. The sequences were compared to independent analyses for each gene fragment using MEGA (5.2) software. The newly sequenced Iraqi isolates are identified by their red diamond markings and are demonstrated to group together under a certain subclade. The number of substitutions per site is indicated by a scale bar.

Discussion

Cats are recognized as primary hosts for various parasitic organisms and are among the most closely associated domestic companions of humans (Hassan & Gaeib, 2021). The ear mite *O. cynotis* is a leading cause of otitis externa and represents a major clinical concern in both cats and dogs (Ismail et al., 2017; Vidmantas et al., 2008). The prevalence recorded in this study is comparable to that in other regions.

Similar rates were reported in Indonesia, where 48.27% (14/29) of feral and domestic cats with otitis externa were positive for *O. cynotis* using otoscopy and direct smear examination (Siagian & Siregar, 2022). Similarly, a study in India reported a prevalence of 47.27% among clinically affected cats examined using similar diagnostic approaches (Radha et al., 2024). These consistent findings suggest that *O. cynotis* remains a highly prevalent ectoparasite in cats across diverse geographical regions. All examined breeds were susceptible to infestation, with higher infestation frequencies observed in Persian (Shirazi), Himalayan, and Scottish breeds. However, breed was not statistically associated with infestation, indicating that environmental and management factors influence susceptibility rather than genetic predisposition. Previous studies involving Angora, Himalayan, Persian, mixed-breed, and domestic cats have reported similar observations (Jannah & Siagian, 2021). Although a lower prevalence (3.2%) was documented in suspected cats of various breeds in Indonesia (Siagian et al., 2023), studies from India have also reported a higher infestation rate in Persian cats (65.62%) (Radha et al., 2024), which may be related to long hair coats facilitating mite survival and transmission. Clinically, pruritus was the most common symptom, either alone or accompanied by self-inflicted wounds.

In this study, itching was recorded in 90.7% of infected cats, whereas 9.3% showed itching with associated lesions. These findings are consistent with those of other studies (Radha et al., 2024; Silva et al., 2021). The intense irritation caused by mite activity and secondary inflammatory responses likely explain the high frequency of scratching and lesion development. Seasonal analysis revealed variation in prevalence, with higher infestation rates observed during summer (36.0%) and autumn (19.8%) than winter (8.1%). Although the seasonal differences were not statistically significant, this trend was similar to that of other studies from Ukraine and Egypt, where higher prevalence rates were reported during warmer months (El-Seify et al., 2016; Komisarova et al., 2025; Yousef et al., 2024). Increased cat contact, enhanced mite survival, and favorable environmental conditions during warm seasons may contribute to this pattern.

Sex-related prevalence showed slightly higher infestation rates in males (55.3%) than in females (44.7%), a trend also observed in studies from Egypt and Indonesia (El-Seify et al., 2016; Gunawan et al., 2024). Although not statistically significant, this difference may reflect behavioral factors, such as increased roaming and aggressive interactions among males, which facilitate parasite transmission. Body weight was also evaluated, and infestation was detected across all weight categories. Consistent with other studies, body weight alone is not a reliable predictor of infestation risk (Zakaria et al., 2022). Age-related analysis demonstrated a markedly higher prevalence in kittens aged 12 months (61.6%), with decreasing rates observed in older age groups. This pattern is similar to that of other studies from Indonesia and Iraq (Al-Khafaji & Al-Musawi, 2025; Gunawan et al., 2024). The increased susceptibility of young cats may be attributed to immature immune systems, close physical contact among littermates, and higher exposure risk in crowded environments (Komisarova et al., 2025). The traditional identification of ear mites relies primarily on morphological characteristics, which may be insufficient due to phenotypic variability and overlapping features among mite species. Therefore, molecular approaches, particularly those based on ribosomal DNA spacers such as ITS-1 and ITS-2, have become essential for accurate species identification and phylogenetic analysis (Dabert, 2006). Phylogenetic studies have consistently demonstrated that *O. cynotis* forms a monophyletic group, with ITS regions providing high resolution for species delimitation and population-level analyses (Pérez-Sayas et al., 2022).

These findings are similar to those of other studies showing limited host specificity and shared genotypes among *O. cynotis* isolates from cats, dogs, foxes, and other carnivores (Lohse et al., 2002; Salib & Baraka, 2011). However, the subtle genetic differentiation observed among geographic populations supports the hypothesis that regional variation and ecological factors may influence the genetic structure of *O. cynotis* populations. the same consistent, with other studies were drawn from studies in Chile, where distinct genotypes and novel alleles were identified in wild canids, highlighting the global distribution and genetic diversity of this species (Briceño et al., 2020).

Overall, the phylogenetic patterns observed in this study indicate that global *O. cynotis* populations exhibit strong genetic conservation and measurable divergence. The clustering of Iraqi isolates within well-supported clades emphasizes the value of ITS-1 sequencing for population-level studies and contributes to the development of novel genetic data for public databases. Continued molecular surveillance across different hosts and regions is essential to better understand the transmission dynamics, host adaptation, and evolutionary history of this clinically important ectoparasite.

Conclusion

In Karbala province/Iraq, *O. cynotis* is fairly common among the cats included in the study at a rate of approximately 46%, which is relatively high. Although most infestations in cats are less than one year old, in addition to being above one year old, which means highly contagious. Regular deworming of even stray cats is recommended to decrease the risk of transmission to humans. In addition, a vaccine must be developed in the future to stop *O. cynotis* infestations and their consequences. Further molecular studies, including different hosts and regions, are recommended to show the potential zoonotic implications and to explore genetic variability.

Ethics

Ethics Committee Approval: This study was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Kerbala (reference no. UOK.VET.MI.2025.113; dated 01.01.2025).

Data Sharing Statement: All data are available within the study.

Footnotes

Authorship Contributions: Conceptualization: F.A., M.I.J., S.M.A., M.J., and V.M.; Design/methodology: F.A., M.I.J., S.M.A., M.J., and V.M.; Execution/investigation: F.A., S.M.A., M.J., and V.M.; Resources/materials: F.A., M.I.J., S.M.A., and M.J.; Data analysis/interpretation: F.A., M.I.J., and M.J.; Writing – original draft: F.A.; Writing – review & editing/critical revision: F.A., M.J., and V.M.

Conflict of Interest: The author(s) have no conflicts of interest to declare.

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