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Research Article

Molecular and Clinical Insights into *Otodectes cynotis* Infestation in Cats: A Novel Study from Babylon Governorate, Iraq

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AHM conducted and supervised the study, analysed data and prepared the manuscript. ADM took and processed samples, and collected epidemiological information. HAHA helped with molecular analysis, tabulated data and reviewed the manuscript. All the authors agreed to the final version of the manuscript.

Keywords
Otodectes cynotis, Prevalence, Cats, Molecular, *COX1*

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Abstract | Otodectes cynotis is a highly contagious ectoparasite. While it is not considered highly zoonotic, transmission to humans is rare but possible. This research was carried out at the Veterinary Teaching Hospital and Clinic in Babylon, Iraq, over the period from September 2023 to February 2024, with the objective of evaluating the prevalence and related risk factors of *O. cynotis* in pet felines. A sample of 200 cats was analyzed during the study, with epidemiological data collected through structured interviews with pet owners. This study revealed a 69% prevalence of *Otodectes cynotis* in cats in Babylon, Iraq. Significant associations were found with breed, age, hair type, and body condition. Molecular sequencing of the *COX1* and *ITS1* genes identified unique local variants. These findings highlight the importance of routine veterinary care and molecular monitoring to control ear mite infestations. Key risk factors identified included poor ear hygiene practices, lack of deworming protocols. This research represents the first molecular characterization of *O. cynotis* in Iraq, highlighting the need for improved preventive measures against this prevalent parasitic infection in cats.

Novelty Statement | The present study represents the first molecular characterization of *Otodectes cynotis* in cats from Iraq which identified unique variants of the COX1 and ITS1 genes, as well as highlighting key epidemiological risk factors for infestations in the Babylon Governorate.

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Introduction

All over the world, feline health is threatened by otodectes cynotis which are commonly referred to as ear mites. Among external parasites, these are found in pets, mainly cats and account for about half to two thirds of cases of skin inflammation (otitis externa), according to estimates (Acar and Altinok-Yipel, 2016). It is concerning because *O. cynotis* is not highly infectious for humans which allows it to impact public health. The way *O. cynotis* spreads may be changed by the age, breed and home envi-

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ronment of the infected pets. The contact of wildlife with pets has been proven to play a major role in the transmission of these pests (Souza *et al*., 2008). In addition, how clean a person keeps their ears and the general health of their cats can shape how severe and common ear infections become. The difficulties in treating and diagnosing ear mite infestations are increased because many pet owners do not realize that their pet's ears require specific attention and care different from other areas (Mahagna, 2024). In other words, This study aimed to determine how widespread *O. cynotis* is in pet cats living in Babylon Province, Iraq and what factors may increase the risk of infestation. Depending on clinical examinations and molecular techniques, this research aims to discover important facts about the spread of this parasite. Research like this promotes awareness among owners that keeping up with preventive care and seeking treatment fast can stop the development of ear mites. Essentially, the goals consist of describing how often *O. cynotis* appears and discovering main risks that can help improve feline care in the region.

Materials and Methods

Study design

The investigation took place at the Babylon Veterinary Teaching Hospital and Veterinary Clinic in Iraq, from September 2023 to February 2024. The main aim of the research was to check how many cats have Otodectes cynotis and what factors increase these cases. Owners brought their cats to the clinic for annual health check-ups, shots, deworming and ill-treatment consultations.

Data collection process and tools

A questionnaire designed for this study was used to collect the epidemiological data from each case. People who own cats were interviewed to understand more about their pets. The questionnaire asked about the demographic details of the cats, for example, their origin, how many they owned, their age, body condition score (BCS), gender, neutering status, breed, kind of fur and their relationships with other animals. By looking at all the factors, there was a better understanding of why ear mite infestations occur.

Sample collection and microscope examination

Direct samples of earwax were obtained from the ear canal, with a cotton swab and were then transferred to a clear microscope slide. The process of analyzing samples was done according to the method suggested by Peregrine (2007). 1-2 drops of paraffin were applied to a glass slide to assist the inspection of mites by examination under the microscope. The identification of mites in this group was based on studying their small jointed front legs, solid unjointed legs in the middle (pedicels), fewer rear legs and the presence of long, whip-like setae at the end of the third and fourth leg pairs (Akter and Akter, 2022). The mite eggs had an oval form and a dark embryo inside them, as can be seen with a microscope (DVM *et al*., 2022).

Primers were prepared by lyophilization and then dissolved in deionized distilled water (ddH2O) to get a stock solution that measured 100 pmol/µl. After synthesis, the stock solution was stored refrigerated at -20°C. A priming solution of 10 pmol/µl was set up from 10 µl of the stock solution and 90 µl of ddH2O, for a total volume of 100 µl see Table 1.

Results

The study revealed a high prevalence of *Otodectes cynotis* infestation among the examined cats in Babylon Province. Out of the 200 cats assessed, 138 were found to be infested, resulting in an overall prevalence rate of 69%. This finding underscores the high incidence of ear mite infestations in the region Table 2.

Prevalence by sex

The infection rates were analyzed based on sex, showing that 75% of male cats (81 out of 108) and 61.95% of female cats (57 out of 92) were affected. However, statistical analysis indicated no significant differences between the sexes regarding susceptibility to *O. cynotis* Table 3.

Prevalence by source of purchase

The source of purchase was also evaluated, with infestation rates of 77.41% in adopted cats (48 out of 62) and 65.21% in those obtained from other sources (90 out of 138). Again, no significant differences were observed between these group Table 4.

Prevalence by age

Age-related data indicated that senior cats showed the highest infestation rate at 80% (24 out of 30), followed by adult cats at 70.83% (68 out of 96), juvenile cats had a lower prevalence rate of 62.16% (46 out of 74). suggesting that infestation may increase with age Table 5.

Table 1: The sequence of primers that used this study.

Primer name	Sequence (5'→3')		Product size (bp)
COX1	F	TGATTTTTTGGTCACCCAGAAG	400
	R	TACAGCTCCTATAGATAAAAC	
Internal transcribed spacer 1 and 5.8S ribosomal RNA	F	CTGCGGAAGGATCATATCGGT	711
	R	ACGTGAGTCAGCACTATCAAA	

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Table 2: Prevalence of *Otodectes cynotis* according to microscopic examination.

Type of samples	Number of examined cats	Positive cases (%)
Ear wax	200	138 (69%)

Table 3: Prevalence of ear mite infestation according to sex.

Sex	Number of examined cats	Number of positive cases	Prevalence (%)
Males	108	81	75
Females	92	57	61.95
Total	200	138	69
X^2	0.112987		
P value	0.736769		

Non-significant differences at p > 0.05.

Table 4: Prevalence of ear mite infestation according to source of purchase.

Source of purchase	Number of examined cats	Number of positive cases	Prevalence (%)
Adopt	62	48	77.41
Stay	138	90	65.21
Total	200	138	69
X^2	0.0194		
P value	0.04769		

Significant differences at p ≤ 0.05.

Table 5: Prevalence of ear mite infestation according to age groups.

Age	Number of examined cats	Number of positive cases	Prevalence (%)
juvenile	74	46	62.16
adult	96	68	70.83
senior	30	24	80
Total	200	138	69
X^2	0.0345		
P value	0.0345		

Significant differences at P ≤ 0.05.

Prevalence by breed

Regarding breed, local cats showed a higher infestation rate of 83.33% (40 out of 48) compared to crossbred cats at 64.47% (98 out of 152). The differences were statistically significant, indicating that breed may influence susceptibility to *O. cynotis* Table 6.

Prevalence by number of cats owned

The number of cats owned also appeared to affect infestation rates, with households owning more than three cats showing an infection rate of 87.5% (42 out of 48). In contrast, households with one to two cats had a rate of 66.66% (68 out of 102), while those with three cats had a lower rate of 56% (28 out of 50). Statistical analysis confirmed significant differences across these categories Table 7.

Table 6: Prevalence of ear mite infestation according to cat breeds.

Cat breed	Number of examined cats	Number of positive cases	Prevalence (%)
Local	48	40	83.33
Cross	152	98	64.47
Total	200	138	69
X^2	0.03456		
P value	0.0465		

Non-significant differences at p > 0.05.

Table 7: Prevalence of ear mite infestation according to number of cats owned.

No of cats	Number of examined cats	Number of positive cases	Prevalence (%)
1-2	102	68	66.66
3	50	28	56
> 3	48	42	87.5
Total	200	138	69
X^2	0.0076		
P value	0.03583		

Significant differences (P ≤ 0.05)

Prevalence by body condition score

Body condition was another critical factor, with poor body condition correlating with an infestation rate of 87.5% (28 out of 32), followed by obese cats at 75% (18 out of 24) and normally cats at 63.88% (92 out of 144). These findings highlight the association between health status and susceptibility to ear mite infestations Table 8.

Table 8: Prevalence of ear mite infestation according to body condition score.

Body condition	Number of examined cats	Number of positive cases	Prevalence (%)
Poor	32	28	87.5
Normal	144	92	63.88
Obese	24	18	75
Total	200	138	69
X^2	0.05674		
P value	0.04923		

Significant differences (P ≤ 0.05)

Prevalence by neutering status

In terms of neutering status, the infection rate among neutered cats was recorded at 86.36% (38 out of 44), while non-neutered cats showed a rate of 64.1% (100 out of 156). However, no significant differences were found between

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these groups Table 9.

Table 9: Prevalence of ear mite infestation according to neutered status.

Neutered	Number of examined cats	Number of positive cases	Prevalence (%)
Yes	44	38	86.36
No	156	100	64.1
Total	200	138	69
X^2	0.11276		
P value	0.765569		

Non-significant differences at p > 0.05.

Prevalence by interaction with other animals

The study also examined the impact of interaction with other animals on infestation rates, revealing an infection rate of 71.42% in cats that interacted with other animals compared to a rate of 65.9% in those without such interactions. No significant differences were noted Table 10.

Table 10: Prevalence of ear mite infestation according to interaction with other cats/ animals.

Other cats/ animals	Number of examined cats	Number of positive cases	Prevalence (%)
Yes	112	80	71.42
No	88	58	65.9
Total	200	138	69
X^2	0.13465		
P value	0.657769		

Non-significant differences at p > 0.05.

Table 11: Prevalence of ear mite infestation according to interaction with other type of hair.

Type of hair	Number of examined cats	Number of positive cases	Prevalence (%)
Smooth	152	102	67.1
Coarse	32	22	68.75
Irregular	16	14	87.5
Total	200	138	69
X^2	0.8765		
P value	0.0378568		

Significant differences (P ≤ 0.05).

Table 12: Prevalence of ear mite infestation according to interaction with other severity of infections.

Severity of infection	Number of examined cats	Number of positive cases	Prevalence (%)
Mild	138	56	40.57
Moderate		44	31.88
Severe		38	27.53
Total		138	69
X^2	0.09657		
P value	0.03546		

Significant differences (P ≤ 0.05).

Prevalence by hair type

Lastly, hair type was analyzed, showing that irregular-haired cats had the highest infestation rate at 87.5% (14 out of 16), while smooth-haired and coarse-haired cats exhibited lower rates at 67.1% (102 out of 152) and 68.75% (22 out of 32), respectively. The differences across hair types were statistically significant Table 11.

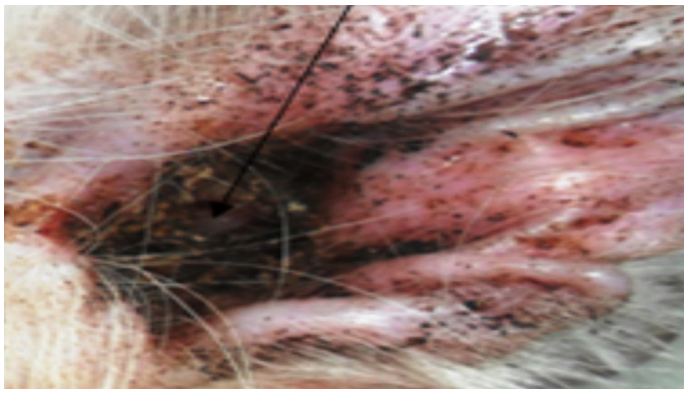

Figure 1: A Cat infected with *Otodectes cynotis* with clinical signs including redness, injures, inflammation and discharge.

Prevalence according to severity of infections

The study analyzed the infection rates of cats infested with *Otodectes cynotis* based on the severity of the infections including mild, moderate and severe depending on excessive scratching, fresh blood inside of ear canal, inflammation and excessive shaking of the head (Table 12, Figure 1). Among the 138 infested cats, the highest infection rate was observed in those with mild infections, accounting for 40.57% (56 out of 138). In contrast, moderate infections were recorded at a lower rate of 31.88% (44 out of 138), while severe infections exhibited the lowest rate at 27.53% (38 out of 138). These findings indicate a significant correlation between the severity of infection and the prevalence of *O. cynotis*, highlighting that mild infections are more common than moderate or severe cases. Additionally, statistical analysis confirmed significant differences in infection rates across these severity categories (P ≤ 0.05). Furthermore, it was noted that all hair type groups were infected by *Otodectes cynotis* at varying rates, with significant differences observed among them (P ≤ 0.05). This underscores the importance of understanding the relationship between infection severity and other demographic factors in managing and treating ear mite infestations in cats effective.

Phylogenetic analysis

Sequencing of the Partial COX1 *Gene in* Otodectes cynotis

The phylogenetic analysis involved sequencing the partial cytochrome c oxidase subunit I (*COX1*) gene in Otodectes cynotis, focusing on the most prevalent species. Multiple sequence alignment was conducted using the COX1 gene sequences from local isolates of *O. cynotis*

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Table 13: Relationship between partial *COX1* gene in *Otodectes cynotis* with most prevalent species.

Identities	Sequence ID	Predicted effect	Amino acid change	Nucleotide change	Nucleotide	Location	Type of substitution	No. of sample
99%	ID: AP023162.1	Sense	stop> Tyrosine acid	TAA>TAT	A>T	17766076	Tranversion	PP595771.1 and PP595772.1
		Sense	Stop> Tyrosine acid	TAG>TAT	G>T	17766249	Tranversion	
		Sense	stop > phenylalanine	TAA>AAA	T>A	17766274	Tranversion	
		Sense	stop > Tyrosine acid	TAG>TAT	G>T	17766279	Tranversion	
		Sense	stop > Tyrosine acid	TAA>TAT	A>T	17766282	Tranversion	

Table 14: Relationship between partial internal transcribed spacer 1 and 5.8S ribosomal *RNA* gene in *Otodectes cynotis* with most prevalent species.

Identities	Sequence ID	Predicted effect	Amino acid change	Nucleotide change	Nucleotide	Location	Type of substitution	No. of sample
98%	ID: HQ728005.1	Sense	stop> Cysteine acid	TGA>TGT	A>T	146	Tranversion	PP64646975.1 and pp646976.12
		Sense	Stop> Cysteine acid	TGA>TGT	A>T	218	Tranversion	
		Sense	Glutamine > leucine	CAA>TTA	C>T	243	Transition	
		Sense	Glutamine > leucine acid	CAA>TTA	A>T	244	Tranversion	
		Sense	tyrosine > Histidine acid	TAT>CAT	T>C	282	Transition	
		Sense	Stop > tyrosine acid	TAG>TAT	G>T	545	Tranversion	
		Sense	stop > tyrosine acid	TAA>TAC	A>C	554	Tranversion	
		Sense	Stop > cysteine acid	TGA>TGC	A>C	563	Tranversion	

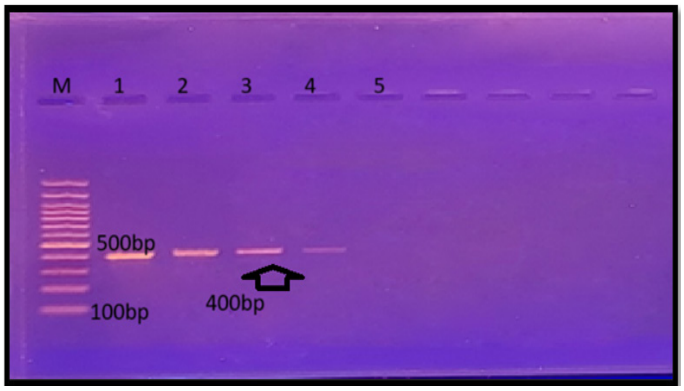

Figure 2: Agarose gel electrophoresis for *COX1* gene. Bands were fractionated by electrophoresis on a 1.5% agarose gel, and visualized under U.V. light after staining with red stain. Lane: M (M: 100bp ladder).

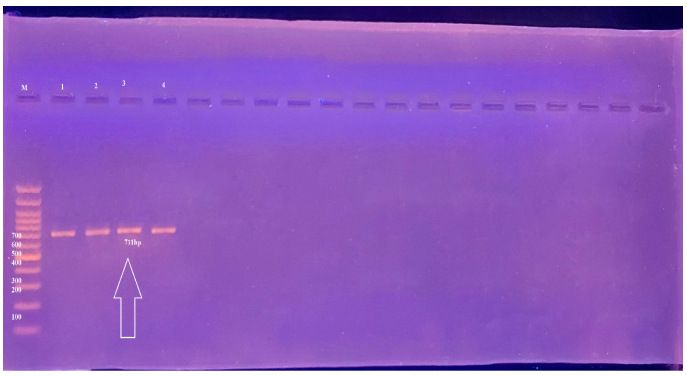

Figure 3: Agarose gel electrophoresis for Internal Transcribed Spacer and 5.8S Ribosomal RNA gene. Bands were fractionated by electrophoresis on a 1.5% agarose gel, and visualized under U.V. light after staining with red stain. Lane: M (M: 100bp ladder).

obtained from ear samples, alongside sequences from the NCBI GenBank database for various *Otodectes cynotis* species. The results of this analysis are summarized in Table 13 and Figure 2.

Multiple sequence alignment of internal transcribed spacer and 5.8S ribosomal RNA gene

In addition to the *COX1* gene, a multiple sequence alignment analysis was performed on the partial internal transcribed spacer 1 (ITS1) and 5.8S ribosomal RNA gene sequences from local isolates of *O. cynotis*. This analysis also incorporated sequences from the NCBI GenBank database for comparative purposes. The findings are presented in Table 14 and Figure 3.

Discussion

Nearly two-thirds of the cats from Babylon Governorate had *Otodectes cynotis* ear mites which must be recognized and addressed by veterinary staff. The findings match past studies on domestic cats in other areas of the Middle East, as they found infection rates ranging from 60% to 75% which is common in places where veterinary care is not regular (Al-Rubaye *et al*., 2021). Several things lead to this high rate such as limited knowledge about proper ear hygiene, fewer regular vaccinations or programs for controlling parasites and animals living together closely, both inside houses and outdoors (Kadhim and Mahmood, 2022). The study contributes to the first molecular and clinical understanding of this parasite in Iraq which will inform future strategies to prevent and treat it. As a result

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of these studies, strengthening awareness campaigns and recommending regular checkups for at-risk cats, since exposure to pets and bad health are sig0nificant problems. More males (75%) than females (61.95%) tested positive for the virus in this study, though the difference was not found to be significant. This is in line with studies before it that stated that gender impacts on being infected with parasites are often very small and may be caused by behaviors as well as biology (Lopez and Faraj, 2021). Adopted cats were found to have ear mites more often than cats from other sources, according to the study results (77.41% vs. 65.21%). Although the difference was not statistically significant, this difference suggests that where adopted cats lived before adoption may impact their risk of parasite infections, especially if they lived on the streets or at a shelter before they were adopted (Ahmed *et al.*, 2023). Often, there is not enough veterinary care in these places which puts them at higher risk for parasites. There is a strong connection between a cat's age and ear mites which suggests senior cats have the highest incidence of 80%, followed by adults with 70.83% and juvenile cats have an incidence rate of 62.16%. More infections in elderly cats are caused by weaker immunity as they get older, together with a longer history of contact with parasites (Hussein *et al.*, 2021). Elderly cats might have tougher times cleaning their ears which raises the risk of infection due to diseases they often have. The observation was that domestic cats had a much higher rate (83.33%) of developing FIP than crossbred cats (64.47%) and this difference was statistically significant. Because domestic cats wander the streets or outdoors more often and receive less veterinary care than hybrid cats, they are more likely to get parasites (Ali and Salman, 2022). Thus, this research underlines that both age and breed are significant factors in ensuring pets are protected from ear parasite infections, so care needs to be taken accordingly with a focus on at-risk animals. Research results indicated that the presence of ear mites was more common with a higher number of cats at home. In households with more than three cats, the rodent infestation rate was 87.5% which was higher than in households with one to two cats (66.66%) and three cats (56%). Such differences in infection rates happen since the increased number of cats within a confined space increases the likelihood of infecting each other by physical contact (Jassim and Al-Zubaidi, 2021). Those cats that were not in good health were most likely to catch an infection (87.5%), but obese cats were also at risk (75%) compared to normally cats (63.88%). It is clear from this data that having poor health increases people's chances of being infected by parasites which is in line with what is known from previous works about the harmful effects of malnutrition and chronic illnesses on the immune system (Saleh *et al.*, 2022). Clearly, handling the number of animals in the house and helping your cats physically is an important method for stopping the spread of Otodectes cynotis. Ear mite cases were found to be more frequent in cats that had been neutered (86.36%) compared to those that were not neutered (64.1%), though these were not differences that could be confirmed statistically. The reason for this could be the way cats are cared for and kept after surgery, since past research indicates that neutered cats are sometimes raised with more cats which increases the likelihood of parasites (Al-Khafaji *et al.*, 2023). Cats whose hair was untidy had a very high rate of this condition (87.5%) and a clear distinction from those with other types of fur was observed. This happens because it is hard to clean this type of ear hair and the environment is helpful for parasites to reproduce (Mahmood and Rasheed, 2022). Dirty or messy hair can hide some signs the doctor may notice during a clinical exam. Regarding animals other than cats, the results showed no difference, but cats who interacted were more likely to have the infection (71.42%). Direct contact is not usually enough by itself to pass on infection, except when someone has weak immunity or poor hygiene (Saleh *et al.*, 2021). The findings indicated that more sterilized cats carried ear mites (86.36%) than unsterilized cats (64.1%) even though this difference was not statistically significant. The reason for this infection difference might be related to the environment or behavior of sterilized cats, as old research indicates that those in crowded groups have a higher risk of getting infected with parasites (Al-Khafaji *et al.*, 2023). Cats whose hair was untidy had a very high rate of this condition (87.5%) and a clear distinction from those with other types of fur was observed. The reason for this is that cleaning ears with curly hair is not easy, allowing the parasite to populate (Mahmood and Rasheed, 2022). A lot of hair can block the view of small signs during a clinical examination. Seriousness and consequences for cats can be judged by the clinical signs caused by *Otodectes cynotis*. According to several studies, if a patient itches much, notices blood in the ear canal, experiences inflammation and suffers from many head shaking episodes, the infection is likely to be worse or more severe (Silva *et al.*, 2023). Mild cases make up most of the infections, showing that many cats are able to resist a mild disease or may recover thanks to prompt veterinary care (Costa *et al.*, 2021). Not treating the infection or managing it improperly can increase its severity, since the infection might spread and result in other bacterial infections in the cat which can make the cat experience chronic pain and a decline in health (Lopes and Almeida, 2022). This agrees with findings that suggest that more severe symptoms often mean the parasites spread through the ear canal more quickly (Martins *et al.*, 2020). This means that paying close attention to clinical symptoms helps select the best treatment plan and an early approach is important to keep the infection from advancing and to make the cat feel better (Ferreira *et al.*, 2024). Noticing variation in how severe symptoms are between various cat groups can help decide on suitable strategies for prevention by age or coat type (Silva *et al.*, 2023). Tool such as COX1 and ITS1 which include 5.8S ribosomal RNA, play a crucial role in exploring the genetic features and

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evolutionary paths of parasites including *Otodectes cynotis*. They make it possible to accurately tell apart species and distinguish local from global strains (Nguyen *et al*., 2021). Using the *COX1* gene from the mitochondria plays a role in finding genetic changes in the parasite that might lead to resistance to drugs (Zhang *et al*., 2023; Al-Khafaji *et al*., 2022). Phylogenetics is more accurate when ITS1 and 5.8S rRNA sequences are involved since their high variability within the same species helps spot small differences between strains and trace where parasites are circulating (Almeida *et al*., 2022). Combing these sequences with global data like NCBI GenBank can accurately determine where certain parasites exist and how things such as the environment or location might affect their genetic diversity (Martins *et al*., 2020). Multigene analysis is at the heart of recent studies that seek to improve how we control these infections using medication and helps keep Otodectes cynotis infections from getting spread in cats (Nguyen *et al*., 2021; Zhang *et al*., 2023).

Conclusion

A large number of *Otodectes cynotis* were found in the cats checked in Babylon, Iraq and each organism had certain unique traits. Not taking care of ears and not giving wormer were main contributors to ear problems. Researchers recommend regular veterinary check-ups and preventive care as a way to manage this widespread, but frequently neglected parasitic ailment.

Declarations

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IRB approval

The study was approved by the Institutional Committee for the Ethics of College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq (dated 19 April 2024, Ref. No. 3022).

Ethical statement

The study was approved by the Institutional Committee for the Ethics of College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq (dated 19 April 2024, Ref. No. 3022). Informed consent was obtained from all cat owners prior to sam-ple collection.

Generative AI and AI-assisted technology statement

No Generative AI and AI-assisted technologies were used in the writing process.

Conflict of interest

The authors have declared no conflict of interest.

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