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CLINICAL AND MOLECULAR DETECTION OF *OTODECTES CYNOTIS* IN DOGS IN BABYLON PROVINCE, IRAQ

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ABSTRACT

Otodectes cynotis in dogs is a common ear mite that causes *otodectic* mange, living in the ear canal and feeding on skin debris. Between October 2023 and April 2024, a comprehensive study was conducted to assess the prevalence of ear mite infestations in dogs in Babylon, Iraq. A total of 90 dogs exhibiting ear-related issues were selected from various sources, including the Veterinary Teaching Hospital in Babylon, veterinary clinics, and the stray dog population. A thorough clinical examination was conducted for each dog to document all signs of ear infestation and to facilitate diagnostic testing. Ear swab samples were collected and subjected to polymerase chain reaction (PCR) to detect the presence of ear mites. Out of the 90 dogs tested, 22 were found to be positive for ear mites, resulting in an overall infection rate of 24.44%. The infection rates among male and female dogs were closely matched, with 22.72% (10/44) of males and 26.08% (12/46) of females, indicating no significant gender-based differences. Regarding age susceptibility, dogs younger than one year showed a higher susceptibility with an infection rate of 40% (14/35). The infestation rate for dogs aged between one and two years was 20% (6/30). Dogs older than two years had a significantly lower infestation rate of 8% (2/25), highlighting a notable age-related difference in susceptibility. Dogs living in groups are more susceptible to infestations (25.71%) compared to solitary domestic pets or strays 20% (4/20). The clinical signs associated with these infestations include erythema 81.81% (18/22), head shaking 9.09% (2/22), ulceration 4.55% (1/22), ear discharge (waxy, crusty) 77.27% (17/22), pain 45.45% (10/22), swelling 50% (11/22), bad odor 68.18% (15/22), and ear pruritus 90.9% (20/22), respectively. The gene sequences were sequenced and submitted to the NCBI database, receiving the accession numbers PP646977.1 and PP646978.1. Phylogenetic analysis was conducted using the aforementioned database tools to determine the homology with other recorded strains.

Keywords: *Otodectes cynotis*, Dogs, Clinical, Molecular, Gene.

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INTRODUCTION

Otitis externa is a frequent issue that veterinarians encounter, whether they're working in general clinics or specialized referral practices. It's one of those conditions that pops up quite often, making it a staple in the world of veterinary care. Otitis externa is commonly caused by bacteria, primarily *Staphylococcus* species, *Streptococcus* species, *Proteus* species, *Pseudomonas* species, and *Escherichia coli*, as well as yeasts from the *Malassezia* genus and mites like *Otodectes cynotis*. According to Rosser (2004), mites are considered the primary cause and can affect up to 50% of cats and 5% to 10% of dogs. Bacteria and yeasts, on the other hand, are mostly perpetuating or predisposing factors rather than primary pathogens (Zur and Bdolah-Abram, 2011; Bugden, 2013). An awareness of the fundamental, predis-posing, and perpetuating variables that contribute to the pathophysiology of otitis externa is essential for effective management. These elements often play a role in mixed infections and are not easily separated from one another (Rosser, 2004). When ear mites are parasitized, opportunistic microflora and fungi belonging to the genera Candida and Malassezia frequently develop out of control. Otoacariasis is among the most prevalent parasitic illnesses in domestic animals (Chudnova and Vorontsova, 2017). *Otodectes cynotis* is a pathogen that damages the ceruminous glands and induces inflammation. The host may become sensitized to the salivary antigens produced by mites. It is well-recognized that secondary bacteria frequently exacerbate the illness. Therefore, the complex form of otodecosis should be regarded as an associated disease, with pathogenic and opportunistic bacteria and fungi as the secondary cause and mites *O. cynotis* as the primary cause. Clinical indicators include head shaking, ear scratching, auricular discharge, foul odor, erythema, ear swelling, and pain, which have to be used to make the diagnosis (Lyskova and Mazurova, 2007). *Otodectes cynotis*-induced otoacariasis is diagnosed by showing the parasite, either directly by otoscopy or indirectly through microscopic analysis of cerumen obtained using various methods (Rosanna Marsella, 2021). To treat ear mites, mineral oil, and cerumenolytic chemicals were used to thoroughly clean both of the puppies' ears. After that, Amitraz solution 2.5% was used topically once a week for a month. Additionally, a combination of topical antifungal and antibacterial drugs (otosporin) was applied every day for a week. After that, Amitraz solution 2.5% was used topically once a week for a month. To treat ear mites, mineral oil and cerumenolytic chemicals were used to thoroughly clean both puppies' ears (Dourmishev, 1998; Campbell, 2005).

MATERIALS AND METHODS

Samples collection

A total of 90 samples of wax, or debris, in the ear canal were collected from 90 dogs of various ages with ear problems, which was performed by using a cotton swab (He *et al.,* 2022). During the period from October 2023 to April 2024, the samples were placed in a sterile plastic container and stored in the freezer until they arrived at the laboratory. All information about the animals, such as clinical signs, case history, sex, age, lifestyle, ear pruritus, erythema, head shaking, ulceration, ear discharge, and pain was recorded.

DNA extraction

DNA extraction was done according to the PureLink™ Genomic DNA Mini Kit with Catalog Number K182002.

Primers

Table 1: Show the primers used in the study.

Primer		Primer sequence 5'-3'	Size of Product (bp)
internal transcribed spacer 1 and 5.8S ribosomal RNA	F	CTGCGGAAGGATCATATCGGT	711 bp
	R	ACGTGAGTCAGCACTATCAAA	

Table 2: Reaction components of all genes PCR.

Component	25 µL (Final volume)
Master mix.	12.5µl
Reverse primer.	10 picomols/µl (1 µl)
Forward primer.	10 picomols/µl (1 µl)
DNA.	1.5 µl
Distill water.	9 µl

Table 3: The optimum condition of detection.

phase	Temp. °C	Time	No. of cycle
Initial denaturation.	94 °C	3 min.	1 cycle.
Denaturation-2.	94 °C	30 sec.	35 cycle.
Annealing.	51 °C	30 sec.	
Extension-1.	72 °C	40 sec.	
Extension-2.	72 °C	7 min.	1 cycle.

Sequencing and phylogenetic analyses

One of the amplicons produced in Korea was sequenced using forward primers (Macrogen, Korea). The results were compared with the sequence data of the internal transcribed gene 1 (ITS1) and the 5.8S ribosomal RNA gene (5.8S rRNA) of *Otodectes cynotis,* which were available on the NCBI website using BLAST. Using MEGA 6.0, a number of phylogenetic trees were constructed, and the sequences were analyzed (Bezerra-Santos *et al*., 2023).

Statistical analysis

A significant level of P<0.005 was established. The data was compared between groups using the chi-square test. IBM SPSS Statistics software (version 26.0) was used to conduct statistical analysis.

RESULTS

The results of the current study were detected by PCR test, which diagnosed *Otodectes cynotis* in dogs with a prevalence rate of 22/90 (24.4%) (Table 4).

Table 4: Show the total number of samples, positive samples for the PCR test and Prevalence.

Ear Samples	PCR +ve	Prevalence	PCR -ve	Prevalence
90	22	24.44	68	75.55

Clinical examination

All dogs were examined clinically, and the observed frequencies and percentages of primary characteristic signs were variable (Table 5). Animals that infected with *otodectes cynotis* showed several clinical signs with highly significant difference (P<0.01) that were including erythema, head shaking, ulceration, discharge, Pain, swelling, bad odor and ear pruritus were 18 (81.81%), 2 (9.09%), 1 (4.55%), 17 (77.27%), 10 (45.45%), 11 (50%), 15 (68.18%), 2 (9.1%), respectively (Table 5).

Table 5: Clinical findings that were detected in dogs of the study.

Clinical signs		*Otodectes cynotis* infestation	
		Positive	Prevalence %
Erythema	Yes	18	81.81
	No	4	18.18
Head shaking	Yes	2	9.09
	No	20	90.9
Ulceration	Yes	1	4.55
	No	21	95.45
Discharge	Yes	17	77.27
	No	5	22.73
Pain	Yes	10	45.45
	No	12	54.55
Swelling	Yes	11	50
	No	11	50
Bad odor	Yes	15	68.18
	No	7	31.82
Ear pruritus	Yes	20	90.9
	No	2	9.09
X^2		63.564	
P value		0.00*	

S: significant difference (P<0.05).

Percentage of infection according to sex.
The infection rate in males was (22.72%) 10/44, while in females the infection rate was (26.08%) 12/46. These results showed that there is no significant difference between males and females (P>0.005).

Table 6: Sex-wise infection rate of *otodectes cynotis* in dogs as assessed by polymerase chain reaction.

Sex	tested animals	Positive (%)
Males	44	10 (22.72)
Female	46	12 (26.08)
Total	90	22 (24.44)
X^2		0.534(NS)
P value		0447

NS: non-significant difference (P>0.05).

Percentage of infection according to age groups.
As for age, the percentage of infection was distributed into three age groups: < 1 year, 1-2years, and >2 years. The results showed that the infection rate for those aged < 1 year was 14 (40%) out of 35, while those aged 1-2 years had an infection rate of 6 (20%) out of 30. As for the age >2 years, the infection rate reached 2 (23%) out of 25.

Table 7: Age-wise infection rate of *otodectes cynotis in dogs* as assessed by polymerase chain reaction

Age/ year	No. of animals	Positive (%)
<1years	35	14 (40)[a]
1-2years	30	6 (20)[b]
>2 years	25	2 (8)[c]
Total	90	22 (24.44)
X^2		1.78 (S)
P value		0.039

S: significant difference (P<0.05).

Percentage of infection according to lifestyle
The infection rate was concentrated in animals that live indoors 25.7% (18/7٠), which was divided into two parts: (alone) the ones that live on their own, 10% (2/20), while the (with other pets) ones that live with other animals were 32% (16/50). As for the animals that live free-roaming, the infection rate was 20% (4/20). This shows that animals that live indoors have a higher incidence of infection than animals that live free roaming.

Table 8: Sex-wise infection rate of *otodectes cynotis* in dogs as assessed by polymerase chain reaction.

Lifestyle	tested animals	Positive (%)
Indoor	70	18 (25.71)
Free roaming	20	4 (20)
Total	90	22 (20.44)
X^2		0.534(NS)
P value		0447

Phylogenetic Analysis
An evolutionary analysis was done through a distance tree according to the neighbor-joining method. The phylogenetic tree of the 1-5.8S ribosomal RNA gene of the dog *Otodectes cynotis* compared with other ribosomal RNA genes reported on NCBI. The results of the partial gene sequencing (PGS) showed that sequence samples (Seq1-2) PP646978.1and PP646977.1 were in close matches with isolates, HQ728005.1, KP676676.1, and KP676677.1 from China by 98.16%. %, 96.93% and 96.32%, respectively (Fig. 1).

DISCUSSIONS

Otodectes a major external parasite that frequently affects ear dogs and produces infestations is *Otodectes cynotis* (He *et al*., 2022; Thomson and Núñez, 2023). According to PCR, the prevalence of *Otodectes cynotis* infestation in this study was (22/90) 24.44%, which is consistent with earlier results. According to reports, *Otodectes cynotis* affects between 25.5% and 29% of dogs in Greece and London, and between 22.5% and 37%, 50%, and 69.23% of dogs in the US (Mircean *et al*., 2008; Thomson *et al*., 2023). Hasso (2007) was the first study to demonstrate the validity of ear mites in Iraq. Only a small number of *O. cynotis* genes have been added to public databases, indicating a lack of understanding of its molecular biology (He, 2022).

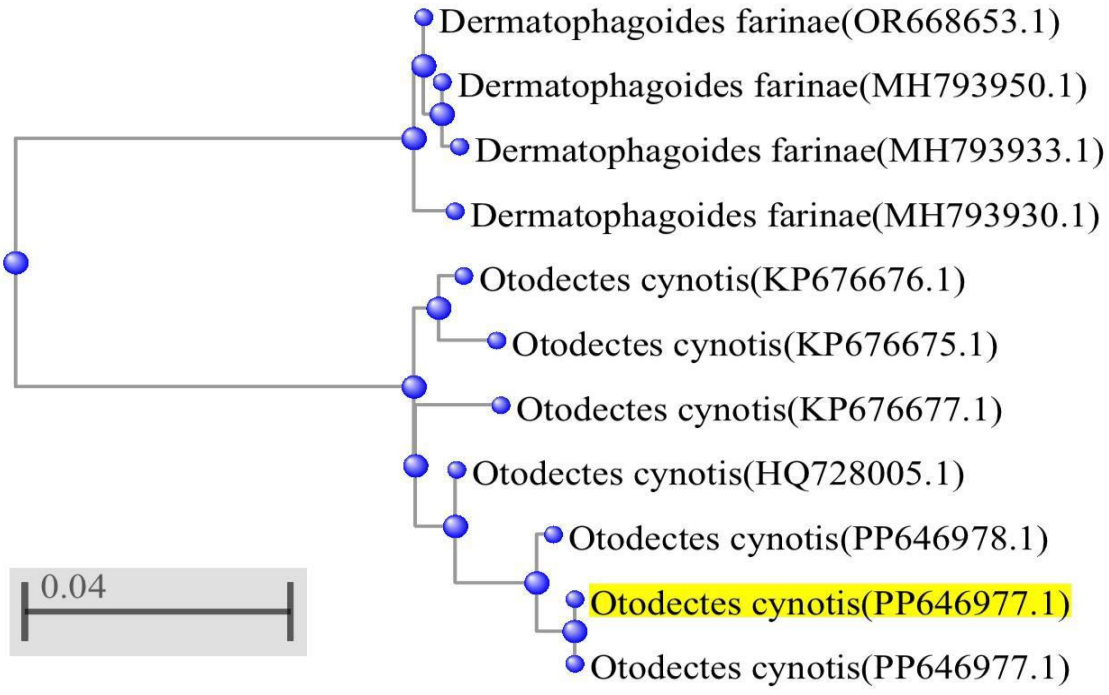

Figure 1: Distance tree according to the neighbor-joining joint to the local 5.8S ribosomal RNA gene of dogs *Otodectes cynotis* (NCBI-BLAST).

The parasite can infect dogs of any age, but because it is spread by infected mothers, it is more common in young dogs (less than a year old) (Salib and Baraka 2011; Hussein, 2024). Nonetheless, the current work discovered a correlation between age and mite infection, with statistical analysis indicating that the prevalence of infestation declined with age. According to certain research, dogs of any gender are vulnerable (Lefkaditis *et al*., 2009; Mosallanejad *et al*., 2011). Therefore, the prevalence of *Otodectes cynotis* in male dogs (22.72%) and female dogs (26.08%) did not differ significantly. There was a correlation between lifestyle and infestation, with door dogs being more common (25.71%). It has been suggested by several authors that infestations are more common among animals living in adverse conditions and among animals found on the streets (Mosallanejad *et al*., 2011). According to Degi *et al*., outdoor dogs are more impacted (84%) than indoor dogs (16%). These findings, however, contradict the assertion made by Sotiraki *et al*. (2001) that lifestyle has not been shown to significantly affect infection risk. Contact with infected animals, whether they belong to the same species or a different one, can easily spread the parasite. (Sotiraki *et al.,* 2001; Harwey and Mckeever 2009). The current work showed no statistically significant difference in the lifestyle of dogs. In any case, there was no significant difference in prevalence between indoor dogs living alone (10%) and indoor dogs living with other pets (32%). Furthermore, according to Degi *et al*. (2010), no permeated dogs have been observed contacting other animals. Up to half of 24.4% of instances worldwide may result in otitis externa due to *Otodectes cynotis*, and 77.3% of dogs with waxy or dry ear discharge which require veterinary consultation (Saridomichelakis, 1999; Sotiraki *et al*., 2001). Depending on the level of parasitism, contaminated creatures can cause clinical side effects such as pruritus, erythema, ulceration, ear discharge, and torment. They pierce the host's skin with their mouthparts and feed on its lymph, tissue fluid, and blood, causing dermatitis or hypersensitive reactions, as well as needless keratinization and epithelial cell proliferation (Taenzler, 2017; Roy *et al*., 2012). Dermatitis or

adverse sensitive reactions occur when germs attack a host. Although defensive, adversely susceptible reactions can also be harmful to the host, causing tissue damage and immunological sensitivity (Powell, 1980). This conclusion is consistent with the current review's findings. Ear pruritus in non-parasitized dogs has been seen by a few researchers (9.2%). The pervasion had the distinctive look of an external ear trench filled with various amounts of a reddish-earthy, dry substance (Montoya, 2018). It has been observed that approximately 85.4% of penetrated dogs exhibit unusual ear emission, and 41.5% suffer from the negative consequences of mechanical disruption caused by the parasite (Harwey and Mckeever, 2009; Sotiraki *et al*., 2001). Although only a small number of instances may be asymptomatic, we found a correlation between pervasion and side effects (ear discharge, pruritus) (Knaus *et al*., 2014; Montoya, 2018).

CONCLUSION

The study indicates a good level of statistical significance. The level of *Otodectes cynotis* infection in Babylon, Iraq, is lower than or similar to that in some neighboring and distant countries. Additionally, PCR techniques have successfully detected *Otodectes cynotis* infections in dogs, utilizing (ITS1) and (5.8S rRNA) genes that are important for enhancing the diagnosis of *Otodectes cynotis*.

ETHICAL APPROVAL

All study animal samples were handled following the required biosafety and security protocols. Before commencing this study, the Ethics and Scientific Committee in the Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, AL-Qasim Green University, Ministry of Higher Education and Scientific Research, Iraq, approved the research protocol (NO. 23 in 22/9/2023). This guideline for the care and use of dogs was fully adhered to throughout the research.

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الكشف السريري والجزيئي عن طفيلي *Otodectes cynotis* في الكلاب في محافظة بابل ، العراق

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Otodectes cynotis في الكلاب هو سوس الأذن الشائع الذي يسبب الجرب الأذني، ويعيش في قناة الأذن ويتغذى على حطام الجلد. أجريت دراسة شاملة بين أكتوبر ٢٠٢٣ وأبريل ٢٠٢٤، لتقييم انتشار الإصابة بسوس الأذن في الكلاب في محافظة بابل، العراق. تم اختيار ٩٠ كلبًا يعانون من مشاكل تتعلق بالأذن من اماكن مختلفة، بما في ذلك مستشفى التعليم البيطري في بابل والعيادات البيطرية وتجمعات الكلاب الضالة. تم إجراء فحص سريري شامل لكل كلب لتوثيق جميع علامات اختبار التشخيص الإصابة بالأذن. تم جمع عينات مسحة الأذن وإخضاعها لتفاعل البوليميراز المتسلسل (PCR) للكشف عن وجود سوس الأذن. من بين ٩٠ كلبًا تم اختبارهم، وجد أن ٢٢ كلبًا إيجابيًا لسوس الأذن، مما أدى إلى معدل إصابة إجمالي بنسبة ٢٤,٤٤٪. كانت معدلات الإصابة بين الكلاب الذكور والإناث متقاربة بشكل وثيق، حيث بلغت ٢٢,٧٢٪ (٤٤/١٠) من الذكور و٢٦,٠٨٪ (٤٦/١٢) من الإناث، مما يشير إلى عدم وجود فروق كبيرة بين الجنسين. و فيما يتعلق بالعمر أظهرت الكلاب التي يقل عمرها عن عام واحد قابلية أعلى للإصابة بمعدل إصابة بلغ ٤٠٪ (٣٥/١٤). وكان معدل الإصابة للكلاب التي تتراوح أعمارها بين عام وعامين ٢٠٪ (٣٠/٦). وكان معدل الإصابة لدى الكلاب التي يزيد عمرها عن عامين أقل بكثير حيث بلغ ٨٪ (٢٥/٢)، مما يسلط الضوء على اختلاف ملحوظ في قابلية الإصابة والعمر. و لقد لوحظ ان الكلاب التي تعيش في مجموعات أكثر عرضة للإصابة ٢٥,٧١٪ (٧٠/١٨) مقارنة بالحيوانات الأليفة المنزلية المنفردة أو الضالة ٢٠٪ (٢٠/٤). تشمل العلامات السريرية المرتبطة بهذه الإصابات احمرار الجلد بنسبة ٨١,٨١٪ (٢٢/١٨) ، واهتزاز الرأس بنسبة ٩,٠٩٪ (٢٢/٢) ، والتقرح بنسبة ٤,٥٥٪ (٢٢/١) ، وإفرازات الأذن (شمعية، متقشرة) بنسبة ٧٧,٢٧٪ (٢٢/١٧)، والألم بنسبة ٤٥,٤٥٪ (٢٢/١٠) ، والتورم بنسبة ٥٠٪ (٢٢/١١)، والرائحة الكريهة بنسبة ٦٨,١٨٪ (٢٢/١٥) ، وحكة الأذن بنسبة ٩٠,٩٪ (٢٢/٢٠) ، على التوالي. تم تسلسل تسلسلات الجينات وإرسالها إلى قاعدة بيانات NCBI ، تم تلقي أرقام الانضمام PP646977.1 و PP646978.1. و تم إجراء التحليل التطوري باستخدام أدوات قاعدة البيانات المذكورة أعلاه لتحديد التشابه مع سلالات مسجلة أخرى.