Comparative Molecular Detection of *Toxocara spp*. in Humans and Sheep Using *COX*١ Gene-Based PCR Assays

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الكشف الجزيئي المقارن عن أنواع Toxocara في البشر والأغنام باستخدام اختبارات تفاعل البوليميراز المتسلسل المعتمدة إلى جين COX١

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Abstract

Toxocariasis is a zoonotic parasitic infection caused by *Toxocara* spp., with varying clinical outcomes in different hosts. This study aimed to compare the molecular diagnostic efficiency between human and sheep samples using the COX١ gene as a molecular marker. DNA and RNA were extracted from infected human and ovine samples and subjected to PCR and real-time PCR analysis. Significant differences in gene expression levels, amplification efficiency, and melt curve profiles were observed. The COX١ gene expression was ٩٩-fold higher in human samples than in sheep, suggesting stronger mitochondrial activity or higher DNA quality in human hosts. These findings highlight the species-specific variation in molecular detection of *Toxocara* spp. and emphasize the importance of host-specific diagnostic strategies.

Keywords: Toxocariasis, human, sheep, PCR, COX١ gene

المستخلص

داء التوكسوكاريا هو عدوى طفيلية حيوانية المنشأ، تسببها فصيلة التوكسوكارا، وتتفاوت نتائجها السريرية لدى مختلف العوائل. هدفت هذه الدراسة إلى مقارنة كفاءة التشخيص الجزيئي بين عينات البشر والأغنام باستخدام جين COX١ كعلامة جزيئية. استخلص الحمض النووي الريبوزي (DNA) والحمض النووي الريبوزي (RNA) من عينات بشرية وأغنام مصابة، وأخضعت لتحليل تفاعل البوليميراز المتسلسل (PCR) وتحليل تفاعل البوليميراز المتسلسل الفوري (PCR) في الوقت الفعلي. لوحظت اختلافات كبيرة في مستويات التعبير الجيني، وكفاءة التضخيم، وأنماط منحنى الذوبان. كان التعبير الجيني لجين COX١ أعلى بـ ٩٩ ضعفا في العينات البشرية منه في الأغنام، مما يشير إلى نشاط ميتوكوندريا أقوى أو جودة أعلى للحمض النووي لدى العوائل البشرية. تبرز هذه النتائج التباين النوعي في الكشف الجزيئي عن طفيليات .Toxocara spp وتؤكد على أهمية استراتيجيات التشخيص الخاصة بكل عوائل.

١. Introduction

Toxocariasis is a neglected zoonotic illness predominantly caused by the larval stages of Toxocara canis and Toxocara cati, which infect many hosts, including humans and domestic animals. Transmission occurs through the intake of embryonated eggs found in contaminated soil, food, or through intimate contact with sick animals. In humans, who serve as incidental hosts, the larvae traverse tissues, resulting in several clinical syndromes including visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis, and hidden or asymptomatic infections [١, ٢]. Toxocariasis impacts millions worldwide, especially in underdeveloped or rural areas characterized by inadequate veterinary oversight and poor sanitation. While most infections are asymptomatic or subclinical, significant problems may occur, particularly in young or immunocompromised adults. Moreover, recent research has associated toxocariasis with allergic conditions like asthma and atopic dermatitis, underscoring the necessity for enhanced detection techniques to more accurately evaluate the disease's epidemiology and clinical implications [٣,٤].

Conventional diagnostic techniques, such as serological assays, offer indirect indications of exposure but fail to distinguish between active and

historical infections. Molecular diagnostics, especially polymerase chain reaction (PCR) and real-time PCR (qPCR), have emerged as essential instruments for identifying Toxocara DNA in clinical and environmental specimens, owing to their elevated sensitivity and specificity [٥،٦]. The mitochondrial cytochrome c oxidase subunit ١ (COX١) gene is often targeted due to its highly conserved and species-specific sequences among worms [٧،٨]. The involvement of domestic animals, particularly sheep, in the transmission cycle of toxocariasis is inadequately investigated. Sheep may consume infective eggs from polluted pastures and act as paratenic hosts, so potentially facilitating environmental contamination and human exposure via the food chain or the handling of animal products [٩].

This study aims to investigate and compare the molecular diagnostic performance of the COX١ gene in human and ovine samples. We assess amplification efficiency, gene expression levels, and melt curve profiles to identify host-specific differences in parasite detection. Understanding these variations is critical for improving diagnostic accuracy and tailoring control strategies for toxocariasis in both human and veterinary contexts.

٢. Materials and Methods

٢،١ Sample Collection

A total of ٢٠٠ DNA and ٥٠ RNA samples were extracted from suspected Toxocariasis hosts, including humans and sheep, collected from rural areas in Erbil and surrounding districts.

٢،٢ DNA/RNA Extraction

High-quality nucleic acid extraction is a critical prerequisite for successful molecular diagnostics. In this study, both DNA and RNA were extracted from clinical and tissue samples obtained from suspected human and ovine cases of toxocariasis. The extraction process was tailored to preserve nucleic acid integrity, maximize yield, and eliminate potential PCR inhibitors common in environmental and host tissues. DNA Extraction was

performed using silica-based spin column purification kits. Following extraction, DNA concentration and purity were assessed spectrophotometrically using a NanoDrop™ ٢٠٠٠ system (Thermo Scientific). Samples with A٢٦٠/٢٨٠ ratios between ١,٨ and ٢,٠ were considered of acceptable purity. Additional confirmation of DNA integrity was performed by ١٪ agarose gel electrophoresis stained with ethidium bromide, ensuring the presence of high-molecular-weight bands and absence of smearing[١٠,١١].

RNA Extraction was carried out using TRIzol® reagent (Invitrogen, USA), which provides effective disruption of cells and denaturation of protein complexes. This method is ideal for recovering RNA from tissue or whole-organism homogenates, where ribonucleases are abundant. Following extraction, RNA was treated with RNase-free DNase I to eliminate genomic DNA contamination. The integrity and purity of RNA were evaluated using both Nanodrop spectrophotometry and agarose gel visualization. Only samples with clear ٢٨S and ١٨S rRNA bands and A٢٦٠/٢٨٠ ratios above ١,٩ were used for downstream applications. To ensure consistency across samples and species, all extractions were carried out in sterile, RNase/DNase-free conditions, and all procedures were performed within ٢٤ hours of collection or after storage at -٨٠°C. For downstream quantitative PCR analysis, equal concentrations of DNA and RNA were used per reaction to minimize variability due to template load[١٢,١٣].

This rigorous and optimized extraction protocol was designed to overcome the variability in nucleic acid yield and quality that often arises in parasitological studies involving multiple host species [٨,١٤]. The adoption of dual-extraction strategies helped ensure that samples were of sufficient quality for both conventional PCR and quantitative real-time PCR (qPCR) assays.

PCR Amplification of COX١

PCR targeting the COX١ gene was performed using species-specific primers. Products were visualized on ١,٥٪ agarose gels to assess amplification quality and band intensity.

Real-Time PCR and Melt Curve Analysis

qPCR was conducted to quantify COX١ expression using SYBR Green chemistry. Melt curve analysis assessed the specificity of amplification and presence of secondary products.

ΔΔCt and Folding Calculation

Expression levels were calculated using the ٢^(-ΔΔCt) method, comparing sheep expression against human samples as the reference. β-actin was used as the endogenous control.

٣. Results

PCR Amplification Quality

PCR successfully amplified COX١ in both species; however, human DNA produced clearer, sharper bands compared to sheep samples. Agarose gel profiles indicated stronger amplification efficiency in human DNA.

As shown in Figure **٣,١:** PCR amplification of the COX١ gene from extracted DNA samples represent human samples showing strong and clear bands (~٤٥٢ bp); while figure **٣,٢** represent sheep samples with weaker amplification. M: ١٠٠ bp molecular weight marker. The band intensity confirms higher DNA quality or gene expression in human samples compared to sheep.

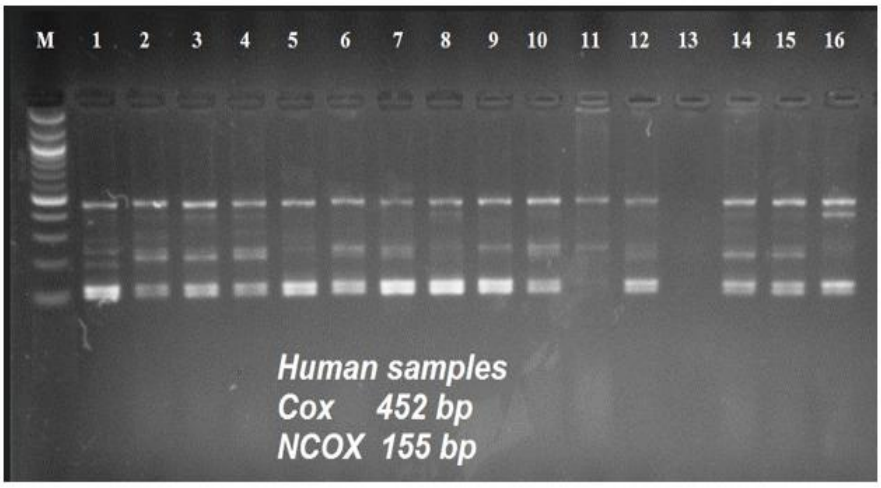

Figure (3.1): DNA samples extracted from humans

Figure (3.2): DNA samples extracted from sheep

Melt Curve and Amplification Profile

Melt curve analysis showed distinct thermal peaks for both species. Human samples exhibited a well-defined single peak at ~٨٢°C, indicating specific amplification. Sheep samples showed weaker peaks and slightly broader shoulders, suggesting lower template concentration or amplification inefficiency.

Gene Expression Analysis (ΔΔCt and Fold Change)

Sample	ΔΔCt Mean	Folding Mean	Interpretation
COX١ Human	-٥,٨٦	٩٩,٣١	High expression
COX١ Sheep	+١,٨٧	٠,٣٥	Low expression

Human samples demonstrated a ٩٩-fold higher expression of COX١ compared to sheep. The positive ΔΔCt and low fold-change in sheep indicated gene suppression or poor DNA quality.

As shown in figure (٣،١), humans have significantly higher COX١ expression than sheep, indicating more efficient amplification or greater gene activity in human samples

- The **blue and orange bars** represent the **fold change** in gene expression (2^-ΔΔCt).
- The **dashed black line** shows the corresponding **ΔΔCt values**.

An unpaired T-test comparing mean Ct values revealed a statistically significant difference ($p < ٠,٠٥$) between human and sheep groups:

- COX١ human: ٢٧,١٧ ± ١,٧٦
- COX١ sheep: ٣٢,٩٢ ± ٣,٠٤

٤. Discussion

The results illustrate substantial differences in molecular diagnostic efficiency between human and sheep samples. Higher COX١ expression in humans may be attributed to greater mitochondrial content or gene copy number, higher quality of DNA extraction and Intrinsic biological differences in host–parasite interaction. The suppressed COX١ expression in sheep could reflect lower parasitic load or host-specific immune

responses limiting parasite survival and replication. These results align with previous studies (٨, ١٥], which emphasize the reliability of COX١ for human diagnosis but suggest its limited efficiency in non-human hosts. Melt curve analysis validated the specificity of the qPCR assay and ruled out contamination or nonspecific products.

٥. Conclusion

This study highlights the importance of host-specific considerations in molecular diagnostics of Toxocariasis. The significant difference in COX١ gene expression between humans and sheep suggests that diagnostic sensitivity and accuracy can vary greatly depending on the host species. Molecular tools like qPCR and melt curve analysis provide reliable means for such comparisons, paving the way for improved diagnostic approaches in both human and veterinary medicine.

References

١. Smith, H. V., Holland, C. V., Taylor, M. R., Magnaval, J. F., Schantz, P. M., & Maizels, R. M. (٢٠٠٩). How common is human toxocariasis? Towards standardizing our knowledge. Trends in Parasitology, ٢٥(٤), ١٨٢–١٨٨.

٢. Zibaei, M., Sadjjadi, S. M., & Uga, S. (٢٠٢٢). Immunological aspects of toxocariasis: a review of current knowledge. Helminthologia, ٥٩(١), ١–١٠.

٣. Caldera, F., Pacheco, R., & Hernandez, L. (٢٠١٣). Immune modulation by helminths: potential application in allergic diseases. Current Allergy and Asthma Reports, ١٣(٤), ٣٥٧–٣٦٦.

٤. Martín-Cruz, L., González-Fuentes, M., & Rubio-Álvarez, V. (٢٠٢٤). Regulatory T-cells in allergic airway diseases: A focus on cytokine interplay. Immunology Letters, ٢٥٨, ٢٥–٣١.

٥. Yoshikawa, M., Hayashi, K., Mori, Y. (٢٠٠٨). Molecular diagnosis of toxocariasis by polymerase chain reaction using DNA extracted from blood samples. Journal of Helminthology, ٨٢(٣): ٢٣٩–٢٤٤.

٦. Nguyen, T. T., Le, Q. H., Tran, M. H., & Pham, D. V. (٢٠٢٣). Clinical and laboratory characteristics of Toxocara canis infection among children in Ho Chi Minh City, Vietnam. Journal of Clinical Microbiology and Immunology

٧. Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (١٩٩٤). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, ٣(٥), ٢٩٤–٢٩٩.

٨. Zibaei, M., Sadjjadi, S. M., & Uga, S. (٢٠٢٢). Comparative analysis of mitochondrial COX١ gene amplification in human and animal hosts infected with Toxocara. Parasitology International, ٨٥, ١٠٢٤٥٣.

٩. Overgaauw, P. A. M., van Knapen, F., & Boersema, J. H. (٢٠٢٣). Environmental persistence of Toxocara spp. eggs and risk of transmission. Veterinary Parasitology, ٣١٧, ١٠٩٨٨٩.

١٠. Nijsse, R., Ploeger, H. W., & Wagenaar, J. A. (٢٠١٤). Host species differences in immune response and cytokine expression following Toxocara infection. Veterinary Parasitology, ٢٠٥(٣–٤), ٢٨٥–٢٩٠.

١١. Waindok, P., Baska, P., & Sitko, J. (٢٠٢٢). Brain cytokine profiles in Toxocara canis and T. cati infections: species-specific immune deviation. Acta Parasitologica, ٦٧(٣), ٩٨٢–٩٩٠.

١٢. Leal-Silva, M., Cavalcante, L. F., & Mendes-de-Almeida, F. (٢٠٢٢). Host-specific gene expression responses in Toxocara-infected tissues. Experimental Parasitology, ٢٣٩, ١٠٨٢٢٦.

١٣. Sies, H., Belousov, V. V., Chandel, N. S., Davies, M. J., Jones, D. P., Mann, G. E., & Stocker, R. (٢٠٢٢). Defining roles of specific reactive oxygen species in cell biology and physiology. Nature Reviews Molecular Cell Biology, ٢٣(٧), ٤٩٩–٥١٥.

١٤. Zangana, A. J. M., & Al-Khafaji, Z. H. (٢٠٢٣). Genetic diversity and phylogenetic analysis of Toxocara spp. isolated from different definitive hosts in Iraq. Veterinary Parasitology: Regional Studies and Reports, ٣٥, ١٠٠٩٢٨.

١٥. Nguyen, Y. T., Pham, M. D., & Tran, H. T. (٢٠٢٣). Real-time PCR and melt curve analysis for molecular detection of Toxocara species in human hosts. Journal of Helminthology, ٩٧, e٧.