

<https://doi.org/10.48047/AFJBS.5.4.2023.66-74>



African Journal of Biological Sciences



Research Paper

Open Access

Molecular diagnosis of isolated nematodes from chickens

Rasha Shamil Hussein

Tikrit University / College of Education for Pure Sciences

Prof. Dr. Omaima Ibrahim Mahmoud

Tikrit University / Faculty of Veterinary Medicine

Email: rasha.sh.huseen@tu.edu.iq

Article Info

Volume 5, Issue 4, October 2023

Received: 13 Aug 2023

Accepted: 18 Sept 2023

Published: 09 Oct 2023

doi:10.48047/AFJBS.5.4.2023.66-74

Abstract

360 samples of local chickens were collected from different areas in Salah Al-Din and Hawija Governorate during the period from May 2022 to February 2023, and when dissecting the chickens, nematodes were isolated and examined using a light microscope. The following types were diagnosed: *Ascaridia galli*, *Ascaridia columbae*, *Heterakis gallinarum*, *Trichostrongylus tenius* and *Subulure brumpti*. The results of the genetic diagnosis showed positive genes studied COX1, GCOX for nematode isolates in chickens infected in the genus *Ascaridia* spp. and the genus *Heterakis* spp. Worm and *Subulure brumpti* while negative in the genus *Trichostrongylus tenius*, while the ITS gene adopted in the diagnosis of intestinal worm types proved positive in both *Ascaridia columbae*, *Trichostrongylus tenius* and *Subulure brumpti*.

Keywords: Nematodes, *Ascaridia galli*, *Ascaridia columbae*, *Heterakis gallinarum*, parasites

© 2023 Rasha Shamil Hussein, This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made

Introduction

Chickens occupy an important economic position in Iraq, and they are raised for the purposes of producing human food (meat and eggs), and domestic birds are usually housed in limited areas with many types of domestic animals with them, and they are therefore exposed to a wide variety of digestive parasites (Attree *et al.*, 2021), as they are exposed to a variety of intestinal parasites such as primary parasites (protozoa) and worms, which are still responsible for severe health and economic concerns in poultry farms and other animals worldwide (Carrisoa *et al.*, 2021).

Common internal parasitic infections occur in oral poultry and include nematodes, cestodes, protozoans and primary parasites that may cause significant damage and economic losses to poultry due to malnutrition, weight loss, low egg production and the death of young chicks (Rizwana *et al.*, 2019). Nematodes and shine Rofa also in the name of roundworms is a large group of worms with different shapes, hosts and life cycles and may exist in tissues and intestines, and is characterized by its cylindrical body covered with a complete acellular layer called cuticle, and has a sophisticated digestive channel, and be separate races, and most of them have a direct life cycle, but some of them need a middle host (Muna and Enas, Nematode helminths are widespread in poultry such as *Ascaridia galli* and *Heterakis*, for example *Heterakis gallinarum* and species belonging to *Capillaria* spp. These species are the most prevalent nematodes and cause diseases in poultry, peacocks, turkeys and pigeons (Christopher *et al.*, 2021).).

Thematerials and methods ofwork

Collection and inspection of chickens

60 samples of local chickens were collected from different regions in Salah Al-Din Governorate during the period from May 2022 to February 2023. The information for each table was recorded according to a questionnaire prepared to show many effects that may be significant in increasing or decreasing infection or injury, including the months of the year in which the study was conducted, the age group of the chicken samples and the geographical location from which the samples were brought.

Bringing the chicken to the studies laboratory, Department of Life Sciences, College of Education for Pure Sciences, slaughtered the chicken, and then it was dissected by opening the chest and abdomen areas after removing the feathers from them, then the internal viscera was isolated and placed in Petri dishes containing warm water in order to rest the worms, and the small intestine was isolated and placed in special dishes.

3.9 Nematode isolation

The small intestine was isolated from the rest of the members of the gut of chickens and the small intestine was opened longitudinally and its contents were emptied in a Petri dish and was examined with a light microscope for worms and parasites in its cavity and then the nematodes were isolated and the worms were washed and placed in warm water to relax their bodies, then they were kept in Petri dishes containing phosphate buffer saline It has a

pH of 7-7.3 and was placed in an incubator with a temperature of 36-39 °C to ensure its survival for the purpose of diagnosis and laboratory experiments.

3.10 Diagnosis of worms

The worms were washed three times with the physiological solution to remove all the materials and impurities attached to them, then placed on a clean glass slide and the lengths of the worms were measured using the ruler. Image (1), then a drop of lactophenol solution was added to tame the worms and then examined using the usual microscope under the force of X 4 and 10X.



Image (1) Isolated nematodes

PCR Molecular Diagnostic Study

3.15.1 Genomic DNA Extraction

The principle of DNA extraction is that the nuclear material is present in the nucleus surrounded by proteins and then the cell wall, and to obtain pure DNA, DNA was extracted through the use of the extraction kit prepared by Geneaid as follows:

Table of Contents of the DNA Extraction Kit

Material	Volume
Gram + Buffer	30 ml
GT Buffer	30 ml
GB Buffer	40 ml
W1 Buffer	45 ml
W2 Buffer	25 ml
Lysozyme	110 mg
Proteinase K	11 mg
GD Columns	100
2m1 Collection tube	200
Elution Buffer	30ml

The lacarose gel was prepared at a concentration of 1% in order to relay DNA in order to detect its quality and safety, while the gel for the PCR reaction was prepared at a

concentration of 1.5-2%. To obtain a concentration of 1%, 1.5 g of acarose was dissolved in 150 ml of SB relay solution with a strength of 1 X and the microwave was used as a heat source for minutes with continuous stirring to homogenize the solution and ensure the dissolution of the acarose.

Prefixes for a PCR reaction

Specific primers are designed to detect the genetic influence on genes in polymerase chain reaction (PCR) based on information mentioned in (Hector *et al.*2022.)

Table of GCOXgene prefixes

الحجم	Sequence 5-3	Primer
540	F ATTATTACTGCTCATGCTATTTTGATG R CAAAACAAATGTTGAAAATCAAAGG	GCOX
285	F TTTCATACAGAATAAATATCAGGA R AGTTCTAATCATAAGGATATTGGGA	COX1
600	F TTTCCGTAGGTGAACCT R TCCTCCGCTTAGTGATA	ITS

Results and discussion

Genetic diagnosis

DNA extraction and measurement of concentration and purity

The DNA of five types of nematodes isolated from the cecum and small intestine of local fertilized chickens was extracted, and their DNA concentrations ranged between (22-121) ng / Microliters with purity estimated (1.6-2.00) and the percentage is acceptable for the study.

DNA genomic relay

The genomic relay of the extracted DNA samples showed a positive result for all samples when the electroplating was carried out on the prepared lacrose medium at a concentration of 1% as shown in Figure (4-1), which confirms the validity of the samples extracted for the following molecular study, the isolates were coded with letters as follows:

First isolation (A) of *the species Ascaridia galli* .

Second isolation (B) of *the type Ascaridia columbae*.

Third isolation (C) of *the type gallinarium Heterakis* .

Fourth isolation (D) of *Trichostrongylus tenuis* .

Fifth isolation (E) of *Subulura brumpti*.

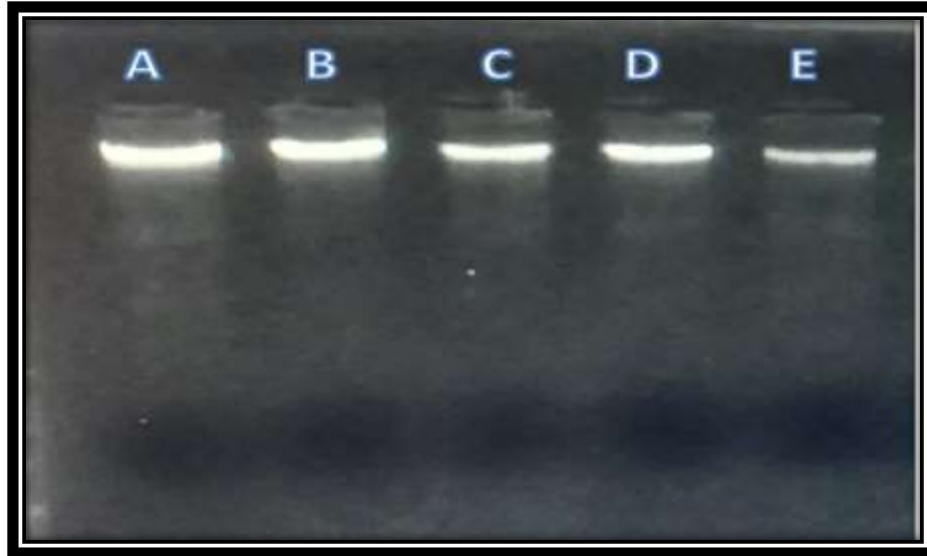


Image (2) Genogenetic relay of DNA extracted from cecum and intestinal tissue Micro for chickens for five types of nematodes.

Diagnosis of the *COX1* gene for intestinal nematodes

The results of amplification of the *cox1* gene using PCR technique and the electro-migration of DNA extracted for five types of nematodes under study isolated from the cecum and small intestine area of local fertilized chickens shown in Figure 4-2 showed a positive result for all isolates with a molecular weight of 285 bp. That is, the rate of genetic influence is 100%. The results of the sequence

Multiplex is an essential step for genetic analysis of species of organisms, which explains the changes that occurred during the evolutionary system of different sequences (Hector *et al*, 2022).).

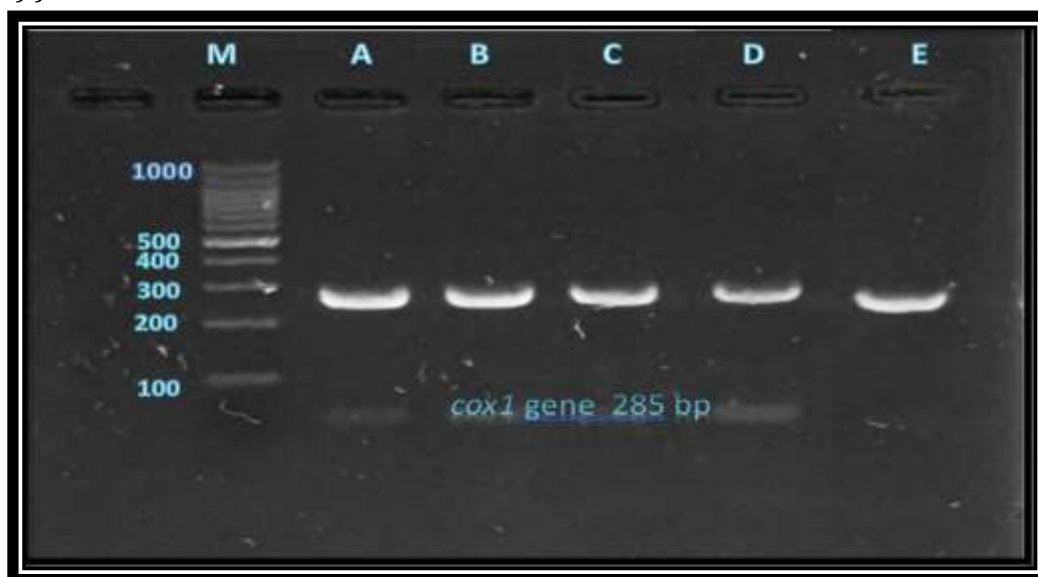


Image (3) Electroplating of *COX1* gene amplification product for intestinal nematode

Isolated from cecum and small intestine samples of local velvet chickens on agarose gel at a concentration of 1.5% Using volumetric index 1000 bp.

Diagnosis of *the GCOX* gene for intestinal nematodes

The results of the polymerase chain reaction (PCR) shown in the image (2) for amplification of the gene primers *gcox* showed the appearance of a genetic packet weighing 540 bp in all five isolates of nematodes isolated from the cecum and small intestine of the local ferocious chickens under study except isolation (D). *Trichostrongylus tenuis* is a genetic susceptibility of 80%.

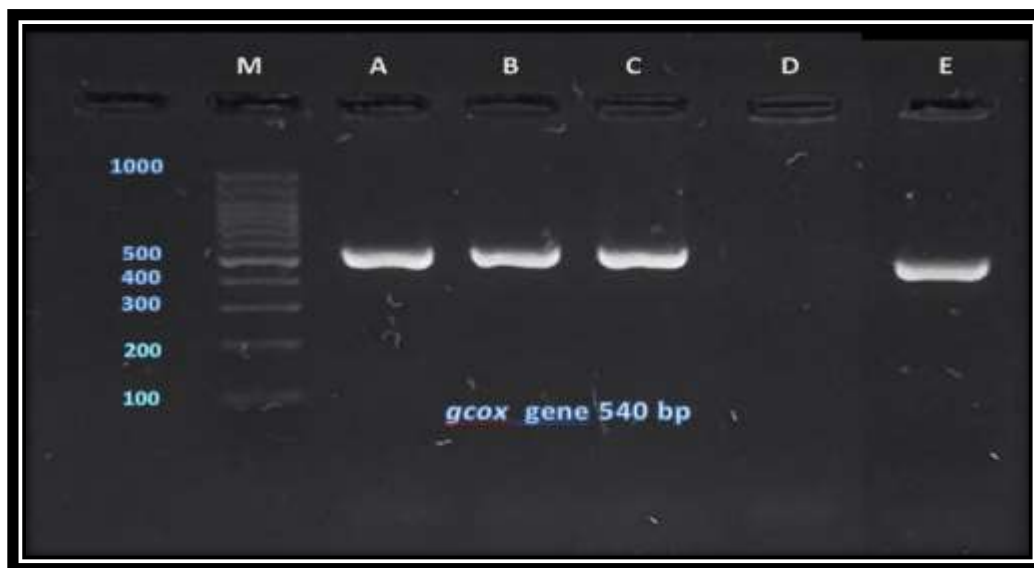
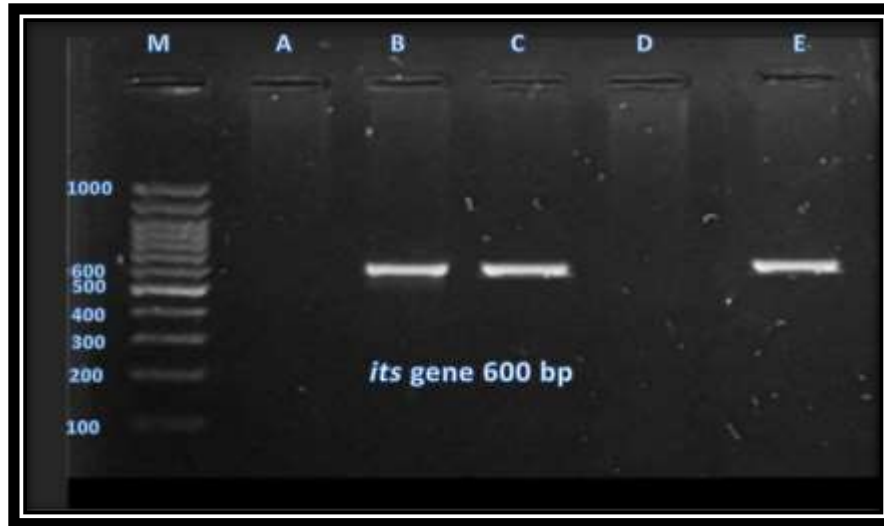


Image (4) Electroplating of *the GCOX* gene amplification product for the isolation of intestinal nematodes Isolated from cecum and small intestine samples of local velvet chickens on agarose gel at a concentration of 1.5% Using volumetric index 1000 bp .

Diagnosis of *the ITS* gene for intestinal nematodes

The results of amplification of the IS gene using thermal polymerase PCR sequencing technique of DNA extracted for five types of nematodes isolated from the cecum and small intestine of domestic fertilized chickens under study revealed the appearance of a genetic packet weighing 600 bp in each of the isolates (B, C and D). While the isolation A and E, represented by *the types Ascaridia galli* and *Trichostrongylus tenuis*, did not show. Respectively, from the results obtained above, the genetic influence can be estimated at 50%.



Image(5) Electrical relay of the product of amplification of the *gene s* for the isolation of isolated intestinal nematodes From one-eyed and small intestine samples of local chickens on acarose gel at a concentration of 1.5% Using a 1000 bp volumetric guide .

Table (1) Positive and Negative Nematode Results for *Cox1,Gcox,ITS* Genes

Sample Code	The name of the worm	Genes		
		<i>cox1</i>	<i>gcox</i>	<i>S</i>
A	<i>Ascaridia galli</i>	+	+	-
B	<i>Ascaridia columbae</i>	+	+	+
C	<i>gallinarium Heterakis</i>	+	+	+
D	<i>Trichostrongylus tenuis</i>	+	-	-
E	<i>Subulura brumpti</i>	+	+	+

References

- **Hector Lorent-Martinez , Ainhoa Agorreta, Diego San Mauro , (2022).** Genomic Chicken and Data Processing for Molecular Evolution Research, Journals , MPs, Volum 5 , Issu2,10,3390/mps 5020026.
- **Carrisosa Lozon , Cristina Almeida , Ana Claudia , Victorio , PedroMelo,(2021).** Implementation of mini-floTAC in Routine Diagnosis of Coccidia and Helminth infections in Domestic and Exotic Birds , Vet Sci , Aug ; 8(8): 160 . published online.

- **Christopher Andrew Lamb , Nicholas. A. Kennedy , Tim Rain , Philip Anthony Hendy,(2021).** Birtish Society of Gastroenterology Consensus guidelines on the management of in flammatory bowel disease in adults ,Volume 68, issue suppl 3.
- **Maha .M.A. Basyoni , and Enas M.A. Rizk, (2016).** Nematodes ultrastructure :Complex systems and Processes , J Parasite Dis, 40(4): 1130-1140.
- **Rizwana, A.,** Saroj , Kumar , Rajat Garg, Tira Ram , P.S. Maury and P.S. Banderjce , (2019). Gastrointestinal parasitic infections in chickens of upply gangetic plains of India with special reference to poultry Coccidiosis , J parasite 39(1): 22-26, published.
- **Attia, M. M., N. Yehia, M. M. Soliman, M. Shukry, M. T. El-Saadony, and H. M. Salem. (2021).** Evaluation of the antiparasitic activity of the chitosan-silver nanocomposites in the treatment of experimentally infested pigeons with *Pseudolynchia canariensis*. Saudi J. Biol. Sci, doi:10.1016/j.sjbs.2021.10.067 in press.
- Karupusamy, S., Mustafa, M. A., Jos, B. M., Dahiya, P., Bhardwaj, R., Kanani, P., & Kumar, A. (2023). Torque control-based induction motor speed control using Anticipating Power Impulse Technique. The International Journal of Advanced Manufacturing Technology, 1-9.
- Govindarajan, S., Mustafa, M. A., Kiyosov, S., Duong, N. D., Raju, M. N., & Gola, K. K. (2023). An optimization based feature extraction and machine learning techniques for named entity identification. Optik, 272, 170348.
- Sudha, I., Mustafa, M. A., Suguna, R., Karupusamy, S., Ammisetty, V., Shavkatovich, S. N., ... & Kanani, P. (2023). Pulse jamming attack detection using swarm intelligence in wireless sensor networks. Optik, 272, 170251.
- Hassan, J. A., & Rasheed, M. K. (2022, November). Synthesis and characterization of some benzimidazole derivatives from 4-methyl ortho-phenylene diamine and evaluating their effectiveness against bacteria and fungi. In AIP Conference Proceedings (Vol. 2394, No. 1). AIP Publishing.
- Nijris, O. N., Khaleel, Z. I., Hamady, S. Y., & Mustafa, M. A. (2020). The effectiveness of Aqueous Extract of Grape Seeds *Vitis vinifera* as an antibiotic for some microorganisms and its Protective Role Histology for Liver, Kidney in Mice. Indian Journal of Forensic Medicine & Toxicology, 14(2), 1838-1845.
- Mustafa, H. A., Majid, H. H., Abdulqader, A. T., Mustafa, M. A., & Salih, A. A. (2019). Study On Some Physiological, Biochemical And Hormonal Parameters Of Seminal Fluid Of Infertile Men. Biochem. Cell. Arch, 19(Supplement 1), 1943-1947.
- Fadhil, K. B., Majeed, M. A. A., & Mustafa, M. A. (2019). Electronic study of fresh enzyme complexes of antifungal drugs-P450 and *Aspergillus kojic acid* biosynthesis. W: w saccharose flavus: fructose as a substratum. Annals of Tropical Medicine and Health, 22, 65-72.

- Abdulazeez, M., Hussein, A. A., Hamdi, A. Q., & Mustafa, M. A. (2020). Estimate the Complications That Resulting from Delayed Management of Dental Trauma in Tikrit City. *Journal of Cardiovascular Disease Research*, 11(2), 80-82.
- Hama Hasan, T. A., Erzaiq, Z. S., Khalaf, T. M., & Mustafa, M. A. (2020). Effect of Equisetum Arvense Phenolic Extract in Treatment of Entamoeba Histolytica Infection. *Systematic Reviews in Pharmacy*, 11(11).
- Hama Hasan, T. A., Erzaiq, Z. S., Khalaf, T. M., & Mustafa, M. A. (2020). Effect of Equisetum Arvense Phenolic Extract in Treatment of Entamoeba Histolytica Infection. *Systematic Reviews in Pharmacy*, 11(11).
- Nijris, O. N., Khaleel, Z. I., Hamady, S. Y., & Mustafa, M. A. (2020). The effectiveness of Aqueous Extract of Grape Seeds *Vitis vinifera* as an antibiotic for some microorganisms and its Protective Role Histology for Liver, Kidney in Mice. *Indian Journal of Forensic Medicine & Toxicology*, 14(2), 1838-1845.
- Ali, A., Jassim, A.F., Muhsin, S.N., & Mustafa, M.A. (2020). Study of Lycium Shawii Phenolic Compounds in Treatment of Hyperlipidemia. *Journal of cardiovascular disease research*, 11, 196-199.
- Ibrahim, H. M., Jumaah, L. F., Khalaf, S. A., & Mustafa, M. A. (2021). KNOWLEDGE AND PRACTICE OF BREASTFEEDING AND WEANING IN MOTHERS LIVES SAMARRA CITY, IRAQ. *Biochemical & Cellular Archives*, 21.

Cite this article as: Rasha Shamil Hussein (2023).

Molecular diagnosis of isolated nematodes from chickens. *African Journal of Biological Sciences*. 5(4), 66-74.
doi: 10.33472/AFJBS.5.4.2023.66-74