How to Cite:
Rhaman, Z. F., & Al-Amery, A. M. (2022). Morphological and molecular identification of Ascaridia galli isolated from local chicken (Gallus gallus domesticus) in Diayala Province, Iraq. *International Journal of Health Sciences*, *6*(S4), 5556–5568.
https://doi.org/10.53730/ijhs.v6nS4.9389

Morphological and molecular identification of Ascaridia galli isolated from local chicken (Gallus gallus domesticus) in Diayala Province, Iraq

Zainab Fadhil Rhaman
Department of Parasitology, College of Veterinary Medicine, University of Diayala, Iraq
Corresponding author email: zainab.khq90@gmail.com

Amer Murhum Al-Amery
Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Iraq

***Abstract*---**The current study was conducted to investigate the prevalence of *Ascaridia spp* in local chickens from the beginning of October 2020 till the end of the March 2021 included examination of 120 local chickens (*Gallus gallus domestica*) in Diayala province. The total prevalence rate of *Ascaridai spp*. was 41.66%. Also, this study objective to detect *A. spp*. by the molecular diagnosis and detection the presence of *18SrRNA gene* (724bp).The technique included genomic DNA extraction from adult worms isolated from small intestine of naturally infected local chickens with using tissue DNA extraction kits. This gene was amplified by using specific primers. Polymerase Chain Reaction (PCR) technique on the genomic DNA gave positive results at 724 bp . The randomly ten positive PCR products where sent for sequencing and phylogenetic analysis ,The sequences registered in NCBI gene bank under the accession numbers (MW732174.1), (MW732175.1), (MW732176.1), (MW732177.1), (MW732178.1), (MW732179.1), (MW732180.1), (MW732181.1), (MW732182.1), (MW732183.1) belong to *Ascardia galli*. Sequence analysis conducted by Blast- NCBI program showed (EF180058.1)USA isolate, with identity 99-100% to local isolate *A. galli* as well as closly related to local isolates with accession number(MK918847.1, MK918636.1, MK918635.1, MK919081.1) from Iraqi with 99% identity to our isolates. It is the first record of *Ascaridia galli* in Diayala province in local chickens by using conventional PCR.

Keywords---Ascaridia spp, (18S rRNA) gene, phylogenetic tree, local chicken, Iraq

Introduction

There are many helminthes parasites affecting and causing production losses to the poultry industrys, including cestodes, trematodes and nematodes(Sivajothi and Reddy ,2014). Among them, *Ascaridia galli* is a most common nematode of domestic fowl and causing ascaridiosis in the hens, turkeys, geese and some other birds (Radfar *et al.*,2012). Life cycle of the nematode is direct but earthworms can ingest eggs and act as a transport host. Birds become infected by ingestion of infective eggs directly with contaminated food and water or indirectly by consumption of transport host. After ingestion, the eggs are mechanically transported to the duodenum and hatch within 24hrs. After hatching larvae penetrates the intestine for histotrophic phase and return to the lumen and finally get matured (Tarbiat *et al.*,2015). Ingestion of such eggs can not cause any clinical disease in the human as nematode will be destroyed by peptic digestion (Bharat *et al.,* 2017). Although presence of parasite worm in the hen's egg is not considered as hazard for public health, it can cause potential consumer complaint. While this erratic migration, parasite may lead the mechanical transmission of bacterial, parasitic, or viral 24 enteric organisms like E. coli, Salmonella spp., Cryptosporidium spp., Giardia sp., Rotavirus, avian Influenza virus etc. into the egg (Roussan *et al.*, 2012; Zambrano *et al.*, 2014 Okorie-Kanu *et al.*, 2016). The objective of this study is to use the morphological and molecular identification of *Ascaridia galli* in local chickens and to study the phylogenetic analysis for this parasite in Iraq.

Materials and Methods

Sample collection and examination of parasite

A total of 120 local chickens of both sexes and different age were brought from many regions in study areas from the beginning of October 2020 until the of March 2021. The chicken were slaughtered and the abdomen region of each one was sectioned throughout mid ventral line using knife , the alimentary canal removed from abdominal cavity and preserved in a container inside ice- box that labeled with required information, like age and sex in addition to date of sample collection. , All collected samples were transported to the laboratory of Parasitology Department / collage of Veterinary Medicine of University of Baghdad (Baghdad, Iraq) . The small intestine were eviscerated a after legate both end and opened longitudinally with sharp scissor .the small intestine content scrapped in to petri dishes containing physiological saline .The recovered adult worms, washed by the physiological saline to remove the attached debris and transferred into plastic caps to be fixed in 70% ethyl alcohol for 24h. A hot mixture of 70% ethyl alcohol and glycerol (1:1) where used to straight the parasite. then cleared with lacto phenol under the light microscope the adult worms were examined and identified based on morphological keys according to (Soulsby 1982 ;Ramadan and Abo zanda .,1992), and male and female were measured for length using a ruler.

DNA extraction

Genomic DNA extracted from *Ascaridia galli* isolated from (50) chickens by using G- spin DNA extraction kit (IBT ,Korea) and the extraction was performed according to the company instructions.

Polymers chain reaction procedure

The primer was used to perform PCR technique supplied by IDT-DNA Company. Primer lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl(Table1).

Table(1):The specific primers sequence used for PCR

Primer	Sequence	Product size	Reference
Forward Reverse	5'- AGTGCTTAACGCGGGCTTAT - 3' 5'- AAAGCACGCTGATTCCTCCA - 3’	724 base pair	Faraj and AL-Amery,2020

PCR Component and program

PCR Component

Fifty random specimens of adult worms *Ascaridia galli* were used mix with PCR tubes components as in the Table(2).

Table (2): PCR Component

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

PCR Thermo cycler program

DNA amplification was performed by using Thermocycler apparatus sample were put after device was programing for each primer for optimal condition as in the table (3).

Table(3)PCR Program for DNA amplification

No.	Phase	Tm (^{o}C)	Time	No. of cycle
1-	Initial Denaturation	95^{o}C	5 min.	1 cycle
2-	Denaturation -2	95^{o}C	45sec	

3-	Annealing	57°C	45sec	
4-	Extension-1	72°C	45sec	35 cycle
5-	Extension -2	72°C	7 min.	1 cycle

Gel Electrophoresis

Electrophoresis has been done to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel, according to (Sambrook *et al* .,1989).

PCR product loading

PCR product analyzed by gel electrophoresis technique ,3µl of the processor loading buffer (Intron / Korea) has been mixed with 5 µl of the supposed DNA to be electrophoresis (loading dye), after the mixing process, the process of loading is now to the holes of the gel. An Electric current of 70 volt for 1-30 h till DNA moved from cathode to the Anode. The DNA bands tested by a source of the UV with 336 nm after put the gel in pool contain on 30µl Red safe Nucleic acid staining solution and 500 ml from distilled water.The red safe stained bands in gel were visualized by UV transilluminator and DNA bands size with the ladder as marker.

DNA Sequencing

Ten amplified PCR products sample were sent for species identification in Sanger sequencer (Korea) . Pro mega software program used in sequencing analysis. Sequencing of gene was performed by national instruction center for environmental management (nicem) company .NCBI –BLAST sub mission to find the relation .

Sequencing and Sequence Alignment

The PCR products were separated on a 1.5% agarose gel electrophoresis and visualized by exposure to ultra violate light (302 nm) after red Stain staining. Sequencing of gene was performed by national instrumentation center for environmental management (nicem) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), biotechnology lab, machine is DNA sequencer 3730XL, Applied Biosystem), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit program.

Results and Discussions

Results of macroscopic examination

Most of the worms isolated from infected intestine were adult *Ascaridia galli*. The adult worms were cylindrical in shape and semitransparent yellowish-white in color ,body length measured by ruler in which female longer than male (40 -

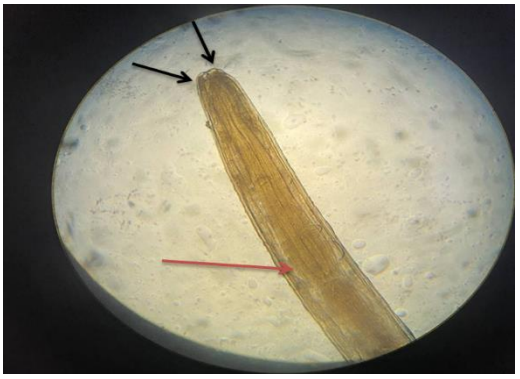
82)mm at average 69 and (36-55)mm at average 43.4 respectively. Sexual dimorphism characteristic in ascarids. Figure (1,2) .These features were identical key with (Bowman, 2009).

Figure(1)Adult female (red arrow), adult male (black arrow) *Ascaridia galli* isolated from small intestine local chicken.

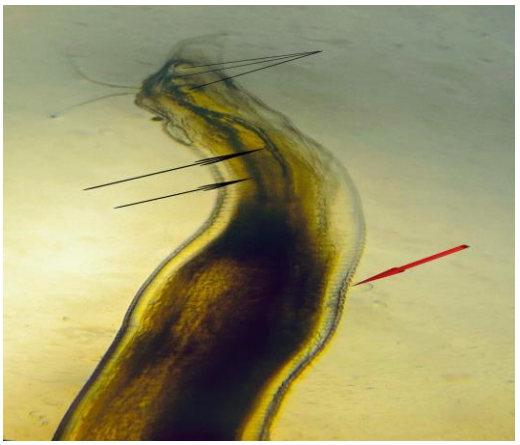
Figure(2) *Ascaridia galli* female

Results of microscopic examination

Results showed microscopic examination some feature characteristics of adult worm, the mouth in anterior end of *Ascaridia galli* surrounded by three lips, esophagus club shaped without distal bulb (figure 3). The enter body surrounded with transversally striated cuticle, in male posterior end pointed and curved with presence of two equal spicules protruded out at the anal opening with presence three pairs of caudal papillae (figure 4),also with circular pre- anal sucker ventrally located (Figure, 5) .while in female posterior end blunt and straight with presence anal opening before the posterior extremity(Figure 6).The vulvar is situated a short distance anterior to middle of the body(figure 7). These features were identical key with (Kassai, 1999; Bowman, 2009).



Figure(3): Anterior end of *Ascaridia galli* showing three lips(black arrow), the esophagus club in shape (red arrow)(10x)



Figure(4) : Posterior end of *A. galli* shows well developed spicules(black arrows) and caudal papillae (lines),with striated cuticle (red arrow) (10x)

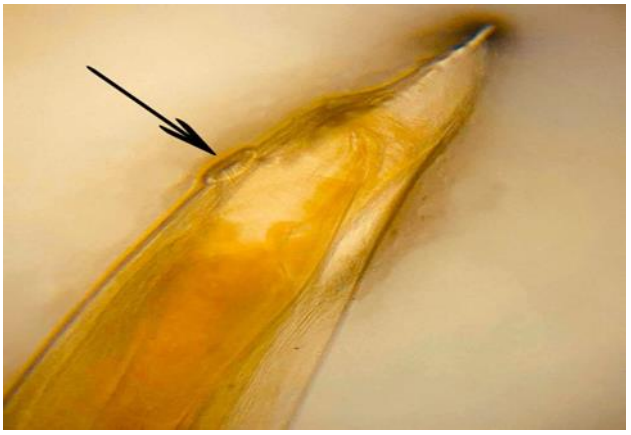
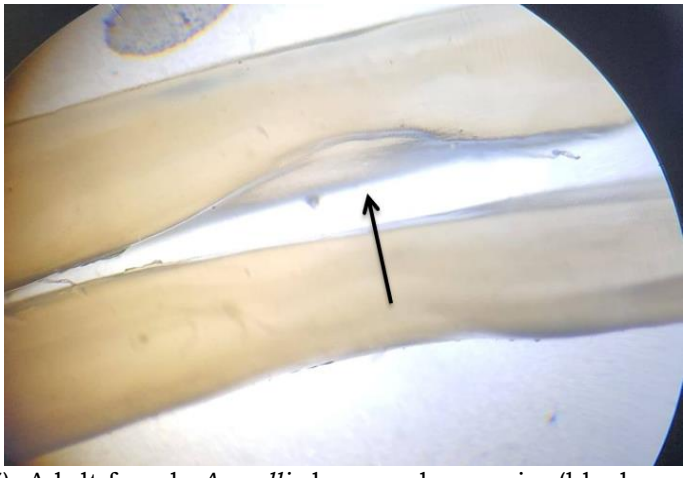


Figure (5) : Posterior end of adult male *A. galli* show pre -anal sucker or pre-clocal sucker(arrows(10x)

Figure(6): Posterior end of adult female *Ascaridia galli* showing the anus (black arrow)(10x)



Figure(7): Adult female *A. galli* shows vulvar region(black arrow)(10x)

Prevalence rate of *Ascaridia galli* in local chicken

The results that has been resulting from 50 infected birds out of 120 show that the overall prevalence percentage of 41.66 %, The studies another recorded different results included 36.9% in Baghdad, Iraq by Shthar (2010), 52.9% in Salah Al-Deen ,Iraq by AL-Jaumeili and Aljoburi (2015), 25.63% in Kenya by Kaingu *et al.* (2010), 25.7% in Pakistan by Sayyed *et al*,(2000) and 21.44 % in Mardan by Zada (2015). This difference may reflect that the management factors play a role in the spread of nematode infections within chicken fields. Skallerup *et al.* (2005) established that the environmental surroundings must be considered to be among the most important determining factors for transmitting infective eggs in natural helminthes infections. Therefore, factors other than wild bird for example farm to farm contamination via vehicle, machine, equipment or people

might also have contributed as the source of initial infections, especially for *Ascaridia galli*. The discrepancies among the result of the present and earlier works in other countries could be belongs to different reasons such as geographical location of the research area, method of detection and sample size and age and sex of the birds.

Table (4) The total infection rate of *Ascaridia galli* in local chicken

Total No.	No. of examined sample	No of positive sample	Percentage%
	120	50	41.66

PCR results

A total DNA was extracted from all positive samples (50). revealed that the PCR amplification was successful on all isolates for the *18S rRNA* gene. The amplified fragment size was approximately 724 bp. As seen in (figure 8) .

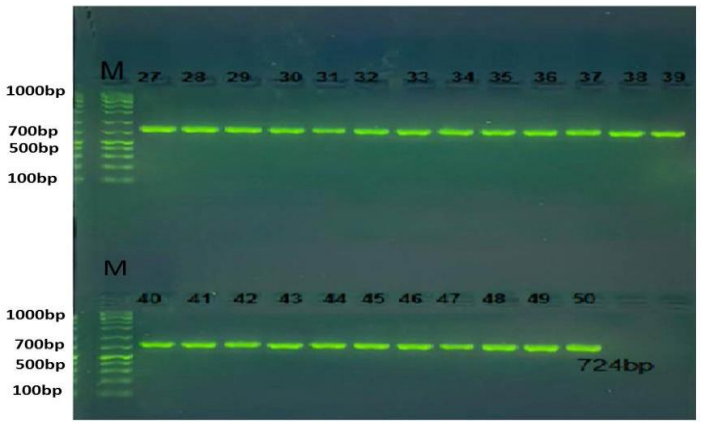


Figure (8): PCR product the band size 724 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100). PCR results of the mitochondrial DNA of single *Ascaridia galli* lanes 27 to 50 a positive results

Submission of local Iraq isolate in NCBI

Ten samples of PCR products were taken from fifty PCR samples chosen randomly positive sequencing by forward and reverse primers. The sequences were employed in the NCBI gene bank database No.1 (MW732174.1), No.2(MW732175.1), No.3 (MW732176.1), No.4 (MW732177.1), No.5 (MW732178.1), No.6 (MW732179.1), No.7 (MW732180.1),No.8 (MW732181.1),No.9 (MW732182.1),No.10 (MW732183.1) . These sequences were analyzed by BLAST- NCBI program to determine the converging sequences recorded in the gene bank. The sequences(No.1, No 2, No 3, No 5, No 7, No 8, No 10) were related to *Ascaridia galli* (EF180058.1)USA isolate, with identity 99-100% Table (5).

Table (5): The type of mutation of 18S ribosomal RNA gene from *Ascaridia galli* isolates

Gene: 18S ribosomal RNA gene						
No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Transvertion	772	T\G	ID: EF180058.1	Ascaridia galli	99%
	Transition	1255	T\C			
	Transvertion	1304	T\A			
2	Transvertion	1307	T\G	ID: EF180058.1	Ascaridia galli	99%
3	Transvertion	1307	T\G	ID: EF180058.1	Ascaridia galli	99%
4	Transvertion	1307	T\G	ID: EF180058.1	Ascaridia galli	99%
5	Transvertion	1144	G\C	ID: EF180058.1	Ascaridia galli	99%
	Transvertion	1255	T\G			
	Transvertion	1331	G\C			
	Transvertion	1377	T\A			
6	Transvertion	891	A\T	ID: EF180058.1	Ascaridia galli	99%
	Transition	1386	T\C			
7	-------	-------	------	ID: EF180058.1	Ascaridia galli	100%
8	Transvertion	848	G\C	ID: EF180058.1	Ascaridia galli	99%
	Transvertion	891	A\T			
	Transvertion	982	A\C			
	Transvertion	1331	G\C			
	Transition	1386	T\C			
9	Transvertion	1307	T\G	ID: EF180058.1	Ascaridia galli	99%
10	Transvertion	1307	T\G	ID: EF180058.1	Ascaridia galli	99%

Sequencing

Sequence alignment analysis

The 18S rRNA gene of *Ascaridia galli* were amplify by PCR method, and sent for sequencing to Macrogen company Korea. Sequence alignment analyses for 18S rRNA of *Ascaridia* isolate of Iraqi chickens were arranged by MEGA6 and NCBI. The nucleotide base alignment shows substitution modification as transversion and transition in the 18S rRNA gene as changes in the nucleotides sequence with proven isolate in data base NCBI GeneBank (Appendix 2).

The multiple sequences analyses of 1-10 sequence with other *A. galli* credited in GenBank NCBI (Figure 9 – 10- 11).

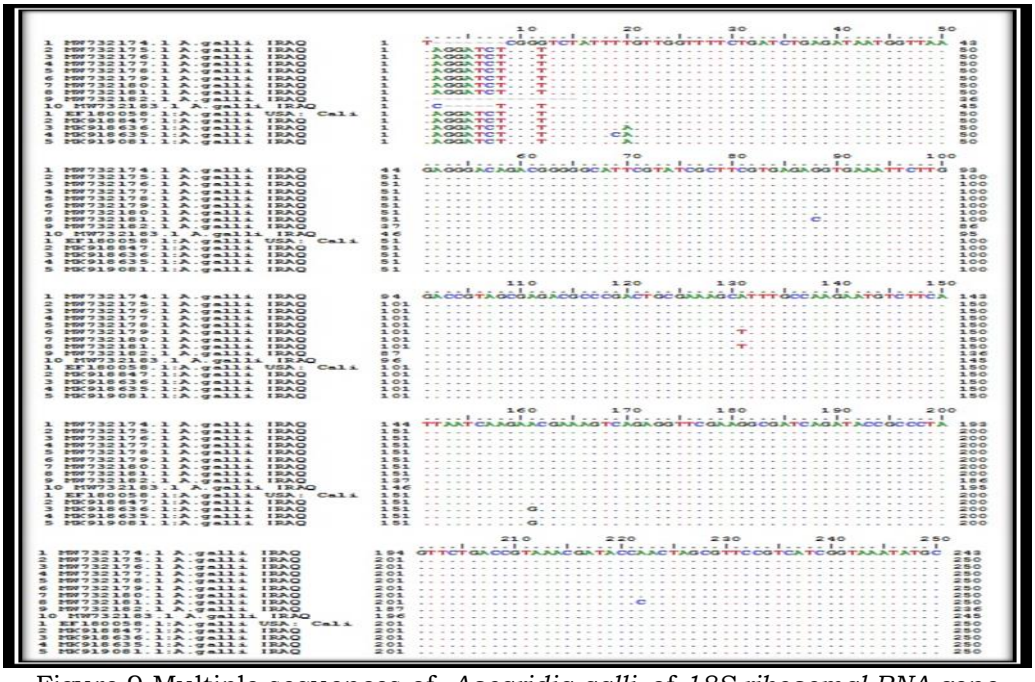


Figure 9.Multiple sequences of *Ascaridia galli* of *18S ribosomal RNA* gene

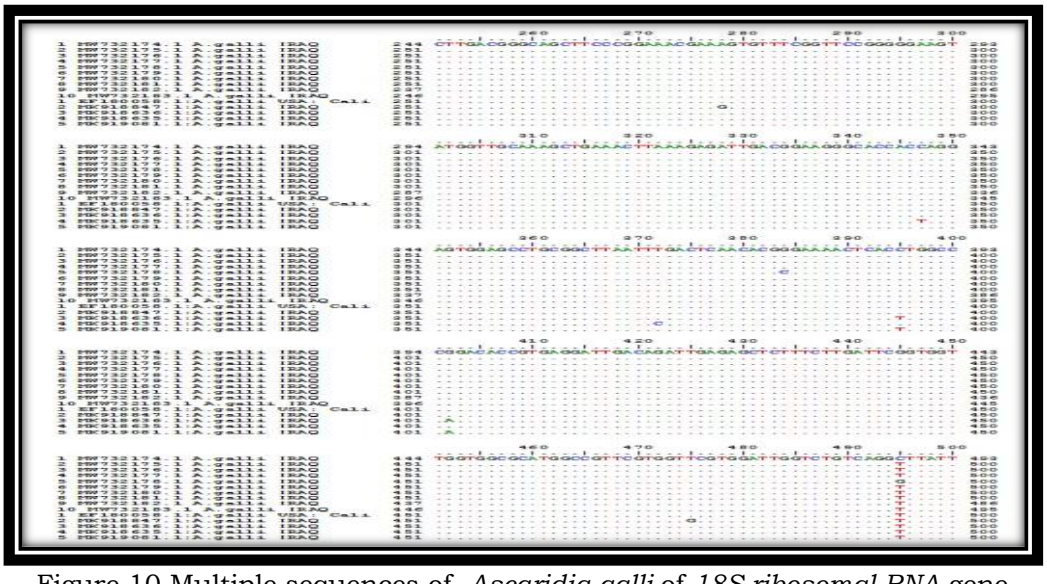


Figure 10.Multiple sequences of *Ascaridia galli* of *18S ribosomal RNA* gene

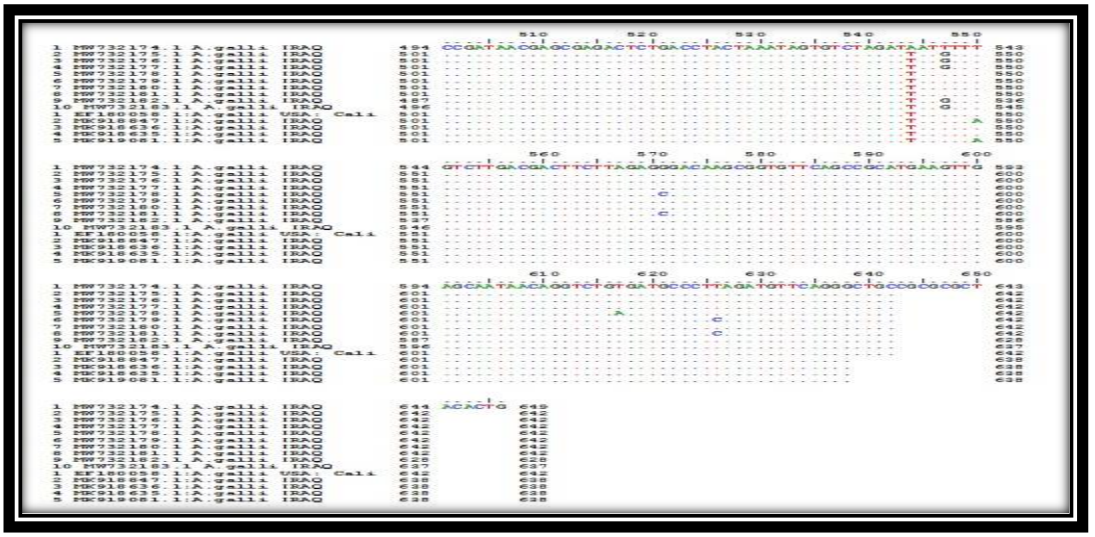


Figure 11.Multiple sequences of *Ascaridia galli* of *18S ribosomal RNA* gene

Phylogenetic Analysis

In the present study, sequences have been registered in NCBI under the following accession numbers: No. 1 (MW732174.1), No. 2 (MW732175.1), No. 3 (MW732176.1), No. 4 (MW732177.1), No. 5 (MW732178.1), No. 6 (MW732179.1), No. 7 (MW732180.1), No. 8 (MW732181.1), No. 9 (MW732182.1) and No. 10 (MW732183.1) and compared with the NCBI- GenBank *Ascaridia galli* isolates (Table 6, Figure 12). (EF180058.1) USA isolate closely related to local isolates with high identity 99%-100% and *A. galli* (MK918847.1, MK918636.1, MK918635.1, MK919081.1) Iraqi isolates showed 99% identity to our isolates

Table 6: The NCBI-BLAST Homology Sequence identity (%) between local

	Accession	Country	Host	Source	Compatibility
1	ID: EF180058.1	USA: California, UC Riverside	Gallus gallus (Zuk lab strain)	*Ascaridia galli*	99%
2	ID: MK918847.1	Iraq	Columba livia	*Ascaridia galli*	99%
3	ID: MK918636.1	Iraq	Columba livia	*Ascaridia galli*	99%
4	ID: MK918635.1	Iraq	Columba livia	*Ascaridia galli*	99%
5	ID: MK919081.1	Iraq	Columba livia	*Ascaridia galli*	99%

Ascaridia galli chicken isolates and NCBI-BLAST submitted *Ascaridia galli*Strain.

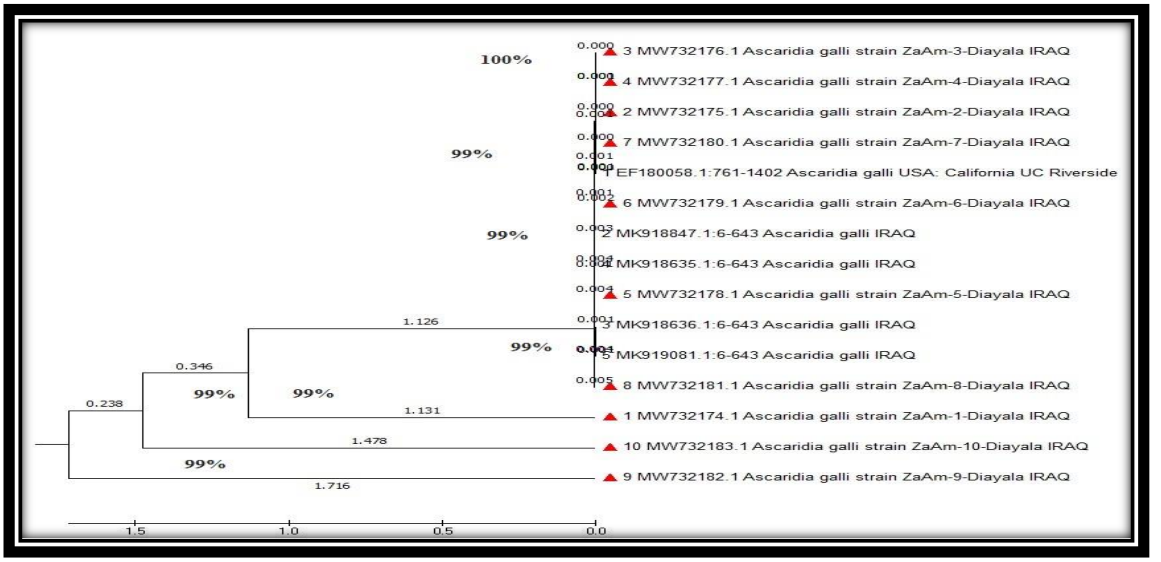


Figure (12):Neighbor-joining tree *Ascaridia galli* of *18S ribosomal RNA* gene. With genetic variation 1.5

Reference

AL-jaumeili, A. H., & Aljoburi, A. U. J. M. (2015). The Parasites That Infect the Females for Domestic Fowl (Gallus Gallus Domesticus) in The Villages of The City of Sharqat, Salah Al-Deen Province. Tikrit Journal for Agricultural Sciences, 15(4):144-148.

Bharat, G. A., Kumar, N. P., Subhasish, B., & Ria, B. (2017). A report of Ascaridia galli in commercial poultry egg from India. J. World Poult. Res, 7: 23-26.

Bowman DD. 2009.Georgis' Parasitology for Veterinarians.9th Ed. Westline industrial drive St. Louis, Missouri, p 142.

Eigaard, N. M., Schou, T. W., Permin, A., Christensen, J. P., Ekstrøm, C. T., Ambrosini, F., ... & Bisgaard, M. (2006). Infection and excretion of Salmonella enteritidis in two different chicken lines with concurrent Ascaridia galli infection. Avian Pathology, 35(6): 487-493.

Faraj, A. A., & Al-Amery, A. M. (2020). Microscopic and molecular diagnosis of Ascaridia spp. in domestic pigeons (*Columba livia domestica*) in baghdad city, iraq. The Iraqi Journal of Agricultural Science, 51(4): 734-743.

Kaingu F; Kibor B; Shivairo A.C(2010). Prevalence of gastrointestinal helminthes and coccidia in indigenous chicken from different agroclimatic zones in Kenya. African J Agri Res.5(6):458–462.

Kassai T. 1999. Veterinary Helminthology, Butterworth-Heineman Publishing, Oxford, UK., p 222**.**

Okorie-Kanu, O. J., Ezenduka, E. V., Okorie-Kanu, C. O., Ugwu, L. C., & Nnamani, U. J. (2016). Occurrence and antimicrobial resistance of pathogenic Escherichia coli and Salmonella spp. in retail raw table eggs sold for human consumption in Enugu state, Nigeria. Veterinary world, 9(11): 1312- 1319.

Radfar, M. H., Asl, E. N., Seghinsara, H. R., Dehaghi, M. M., & Fathi, S. (2012). Biodiversity and prevalence of parasites of domestic pigeons (Columba livia

domestica) in a selected semiarid zone of South Khorasan, Iran. Tropical animal health and production, 44(2), 225-229.
Ramadan, H. H., & abouznada, N. Y. (1992). Morphology and life history of Ascaridia galli in the domestic fowl that are raised in Jeddah. Science, 4(1): 87-99.
Roussan, D. A., Shaheen, I. A., Khawaldeh, G. Y., Totanji, W. S., & Al-Rifai, R. H. (2012). Simultaneous detection of astrovirus, rotavirus, reovirus and adenovirus type I in broiler chicken flocks. Polish journal of veterinary sciences, 15(2) : 337-344.
Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular cloning: a laboratory manual (No. Ed. 2). Cold spring harbor laboratory press.
Sayyed R; Phulan M; Bhatti W(2000). Incidence of nematode parasites in commercial layers in swat. Pak Vet J. 20(2):107–108.
Shthar, M. M. (2010). A comparative study of gastrointestinal helminthes infection between local and farm breeding houses in Baghdad province. Al-Anbar Journal of Veterinary Sciences, 3(1): 18-23.
Sivajothi, S., & Reddy, B. S. (2014). Lousicidal effect of deltamethrin in domestic pigeons. Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology, 40(3): 838-839.
Skallerup, P., Luna, L. A., Johansen, M. V., & Kyvsgaard, N. C. (2005). The impact of natural helminth infections and supplementary protein on growth performance of free-range chickens on smallholder farms in El Sauce, Nicaragua. Preventive veterinary medicine, 69(3-4): 229-244.
Soulsby, E. J. L. (1982). Helminths, arthropods and protozoa of domesticated animals (No. Ed. 7). Bailliere Tindall.
Tarbiat, B., Jansson, D. S., & Höglund, J. (2015). Environmental tolerance of free-living stages of the poultry roundworm Ascaridia galli. Veterinary parasitology, 209(1-2): 101-107.
Zada L; Rehman T, Niaz S(2015). Prevalence of *Ascaridia galli* in some poultry farms of district Mardan. J Adv Parasitol. 2(4):75–79.
Zambrano, L. D., Levy, K., Menezes, N. P., & Freeman, M. C. (2014). Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 108(6): 313-325.
Aryani, L. N. A., & Lesmana, C. B. J. (2019). Neuropsychiatric factor and polymorphism gene in internet addiction. *International Journal of Health & Medical Sciences*, *2*(1), 39-44. https://doi.org/10.31295/ijhms.v2n1.90
Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864