

Molecular identification of *Ascaridia columbae* in the Local Healthy Pigeon (*Columba livia domestica*, Gmelin, 1780) in Karbala Province

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Abstract

The current study was conducted to investigate dissemination of gastrointestinal Ascariasis in local pigeons (*Columba livia domestica*) living in Kerbala province. Forty domestic pigeons with different ages were purchased from different markets. Each bird was examined for intestinal parasites. Out of 40 birds examined, the prevalence of gastrointestinal Ascariasis in examined birds were (12.5%), *Ascaris* sp. was detected in 100% of intestinal contents using macroscopic and microscopic examination assays, molecular diagnosis from bird belly, with the emphasis on primer designed specificity it used in our study.

Key words: *Ascaridia columbae*, pigeon, primer design

Introduction

The rock pigeon (*Columba livia domestica*) is subspecies of the rock pigeon (*Columba livia*) which is a free-living in environment and live side by side with human and other animal species in the environment (2,3). The rock pigeon (*Columba livia*) belong to the order Columbiformes and spread widely in all the world 17. Columbiform pigeons were infect with *Ascaridia columbae* and causes significant infection 14. Untile now only ~25,000 species have been detected although bilateral animals with an estimate of 1–10 million species 9. The gastrointestinal helminth (*ascaridia spp*) that infect chickens, pigeons and parrots. Pigeons may be consider reservoirs for many of helminth infections of veterinary importance especially *ascaridia spp* to other avian 3. *A. scaridia spp* infestations have a major role and effect direct or indirect on health status of birds lead to economic losses in the production of poultry meat and eggs 16. *A. scaridia spp* are one of the most common gastrointestinal helminth problems of pigeons 3. They harbor *Ascaridia columbae* and *Ascaridia galli*. The infected pigeons suffer from various clinical signs which differ from loss of weight, growth, egg production, elementary canal disturbance and gastrointestinal blockage with increase mortality rates 4. The aim of the

present study to investigate prevalence of gastrointestinal helminth in healthy local pigeons in Kerbala province.

Materials and Method

Ethics statement

This experiment was approved by Veterinary medicine Animal Care and Animal Ethics Committees, Faculty of veterinary Medicine, University of kerbala, Iraq.

Study areas and Parasitological analysis

Forty local domestic pigeons (*Columba livia domestica*) with dubious clinical emaciated signs which include (inability to fly properly, Sitting too still, Drooping wings or slouched and Head listing to one side) were admitted to the Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Kerbala University. In this current study, several suspected *Ascaridia* eggs were examined in the sample stool of each emaciated bird, then was directed to the veterinary parasitological Laboratory, Faculty of veterinary Medicine, University of kerbala Iraq, from February till the end of April 2019. After the stool was mingled with 0.85 % physiological salt solution in order to refine it from several separate

particles and all pigeon were stool examined according to the ⁵. The infected birds were isolated and killed by neck dislocation in order to isolate the infected intestines from the belly of the birds, later on, the intestine was opened longitudinally from esophagus to cloaca. The intestinal contents were examined macroscopically for the presence of adult nematodes or other parasites. Microscopical examinations were included direct smear, sedimentation and floatation methods ¹⁴ and the ultra structures for both male and female ⁴. *Ascaridia spp.* that were presence in various places of intestine were collected and put it into Petri dishes then transferred in labeled bottles contain tap water ⁷, Amplification of specific DNA sequences by PCR and restriction endonuclease analysis of PCR products have been used for identification and characterization of ascariasis (6,11). The aim of this study was to determine the prevalence of pigeon ascariasis and to design a specific primer

selection of identified *Ascaridia columbae* in kerbala province.

DNA extraction:

Adult *Ascaridia columbae* were collected from luminal content in the pigeons were harvested , single worm was harvested using a procedure carried out according to ¹⁵ .the DNA of worm obtaining was extracted by genesig Easy DNA/RNA Extraction Kit (Southampton, UK) according to the manufacture company.

Primer designing:

Dual nucleotides primers were designed according to sequencing that obtained from NCBI website(<https://www.ncbi.nlm.nih.gov>) , PerlPrimer was used in the design of primers and calculates primer melting temperature (<http://perlprimer.sourceforge.net>) . Table 1.

Table 1. PerlPrimer was used in the design of primers and calculates primer melting temperature.

Primer	Sequence (5' →3' direction) Cytochrome oxidase 1 Cox1	G-C content	Melting temperature °C
Forword	TGGTGGTTTAAGTGTGACTG	40%	54
Reverse	CCAACAACAAAGGCAACATT	41%	55

The primers were manufactured by (Alpha DNA, Montreal, QC, Canada). PCR amplification was carried out with a thermal cycler (Analytik Jena’s Biometra thermal cycler, Germany.) and by using a premix PCR kit (AccuPower® PCR PreMix, Korea) and by following the instructions of the manufacturer instruments, PCR reaction assay were formed with a dual primers designed on the basis of the published nucleotide sequence of the *Ascaridia columbae* complete Cytochrome oxidase 1 Cox1 gene (length, 1,563 bp; GenBank database accession JX624729 and NC_021643.1). Briefly, A product of mixing PCR consisted of 5 µl of a sample containing DNA was added to each premix well, 7 µM each primer, and double-distilled water to a final volume of 25 µl. PCR optimizations were denatured at 94°C for 5 min. thirty five cycles were run under the following processes: DNA denaturation at 92°C for 3 minutes, annealing temperature at 52°C for 1 min, and extension process at 72°C for 1 min. After the final cycle, the reactions were terminated by an final extension at 72°C

for 7 min. The PCR products were analyzed by Safe-Red™ -agarose gel electrophoresis.

Results and Discussion

Ascariasis can probably affect any mammal. Its occurrence is sporadic and worldwide. Birds, particularly pigeons, often carry the agent in their intestinal contents and contribute to its reservoir (figure 1). They are rarely affected clinically, and then mostly on mucous surfaces, there were no clear signs of the birds unless they were weak, shivering emaciated and ruffled feathers, The internal organs are not clear due to the multiplication of worms forming almost like a cover enriched with roots of tree that cause intestinal blockage (figure 1) ¹⁸, Ascariasis most frequently affects the mucous surfaces on which the worm is abnormally found, possibly the digestive tract of pigeon; it typically remains confined to areas of villus epithelium due to blunting of the villi ¹³.

Pigeon is often associated with ascariasis (through eggs) or intensive exposure. The infestation of pigeon

has an important role in causing significantly reduce due to the economic losses in the eggs production. Also, they may have essentially harmful or lethal effects on birds infections, especially small birds (small squares), causing growth delay, intervention in healthy development, and making older birds susceptible to secondary infection ¹.

Microscopically and macroscopically methods were used to determine the difference between males and females, the male’s posterior end is curved ventrally and has a bluntly pointed tail with copulatory spicules which help the worm to dialate the vulva and can also work as a canal for the spermatozoa (figure 2). There was no apparent difference in parasite anterior end, so, this ends are more slender than the posterior ends . ¹⁰. Males have pineal, spices or spine like extensions nears its posterior opening whereas females lack these structure.

A female sediment from intestinal contents is mixed with an equal amount of ddH₂O on a slide and a cover slip is added. Microscopically, the internal organisms appear as bright circular lacunae in a dark field, containing the eggs in their centers.

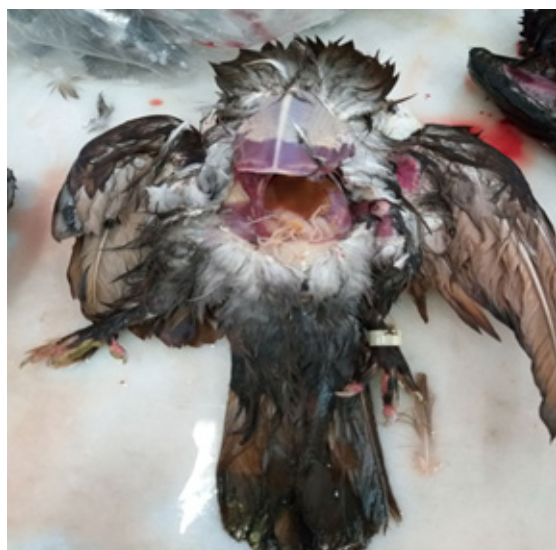


Figure 1 : Ascaris-induced intestinal obstruction

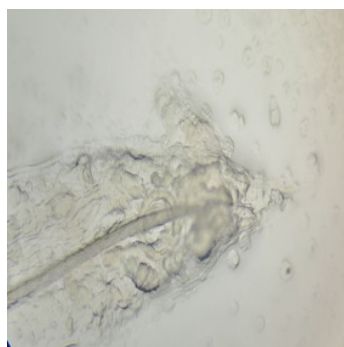


Figure 2 : Adult male worm with copulatory spicules in the posterior end 40X

Avian Ascariasis affects chickens, turkeys, pigeons, and other birds. It resembles ascariasis of humans involving the anterior digestive tract. In the young, it can be a blocking digestive disease and cause considerable mortality ⁸.

We searched the Cytochrome oxidase 1 Cox1 site, on the *Ascaridia columbae* genome Acession number (JX624729)using NCBI Nucleotide BLAST for complete sequences (<http://www.ncbi.nlm.nih.gov/BLAST/>) limited by the Entrez query ‘*Ascaridia columbae*’. among data obtaining, the study was selected the target gene containing the Cytochrome oxidase 1 in the *Ascaridia columbae*, and also tested the specificity of the 1563 bp sequence of the target, the selected 263 bp Cytochrome oxidase 1 and the 22 and 20 bp for forward and reverse primer regions, respectively, at both ends among the target region using NCBI Nucleotide BLAST figure 3.

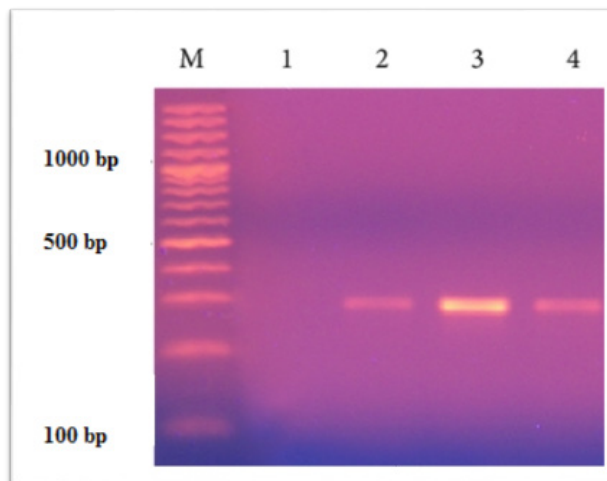


Figure (3): Molecular detection of *Ascaridia columbae*. Here, the product of PCR that had amplified 263 bp gene (Cytochrome oxidase 1) of *Ascaridia columbae* is showing on 1.5% of agarose staining dye concentration.

In PCR-based parasite identification, PCR-product amplified was also be shortly and appropriately detected. As a general, the amplify DNA sequences can be determined by agarose gel electrophoresis after Polymerase chain Reaction assay amplification. Numerous identification systems for specified of parasite investigation such as *Ascaridia columbae* based on the combination of PCR and gel electrophoresis have been already progressing and commercialized.

The primer design and aligned method was based on a number of available sequencing that upload on the the NIH genetic sequence database, unfortunately, in our current study, we found only two available

GenBank sequence database for *Ascaridia columbae* mitochondrion, complete genome which included two accession number (JX624729 and NC_021643.1) They belong to researchers¹² who record that accession number (NC_021643.1) has not yet been subject to final NCBI review and confirmed this reference sequence is identical to JX624729.

Our results was used gel electrophoresis is an easy method of PCR products, according to our knowledge, this primers can be used to differentiate between specific PCR amplification products and non-specific ones (figure 3), In our current study, we have not observed any convergence and alignment of the nucleotides sequences used between the *Ascaridia columbae* with other ascariasis except for some animals or the parasite far from a pigeon infestation, Therefore, we can say that this primer is considered as a specific diagnosis of the this strain.

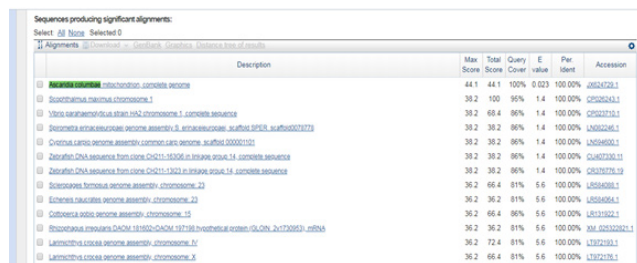


Figure 3: Align of *Ascaridia columbae* with more sequences with other prokaryotic and eukaryotic cells using BLAST

Conclusion

In conclusion our study was demonstrated the rapid and specific detection of PCR DNA amplified from an *Ascaridia columbae* genome using a specific primer sequences.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of pathology and poultry diseases, College of veterinary medicine, University of Al-Qadisiyah, Iraq and all experiments were carried out in accordance with approved guidelines.

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