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Original Article

Molecular Characterization and Phylogenetic Analysis of Raillietina Species Infecting Domestic Pigeons (*Columba livia domestica*): Insights from Genetic Sequencing

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Abstract:

Raillietina spp. are significant intestinal parasites affecting domestic pigeons (*Columba livia domestica*), causing considerable health challenges and economic losses in avian populations. So, the current study aimed to determine its prevalence in one-hundred pigeon intestine samples that were collected from different butcher in El-Menoufiya Governorate, Egypt. Results revealed its detection in 20.0% of the examined samples, reflecting the considerable burden of Raillietina infections in this region. The diagnosis of Raillietina spp. based solely on morphological features is often challenging due to intraspecific variation and morphological similarities among related species. Therefore, molecular diagnosis targeting the mitochondrial cytochrome c oxidase subunit I (*coi*) gene was utilized to provide precise and accurate identification of Raillietina isolates; where it gave positive band at 450 bP. Moreover, amplification and sequencing of the *coi* gene allowed for definitive species confirmation and discrimination from closely related cestodes. Phylogenetic analysis showed that the obtained *coi* gene sequence was significantly identical, with more than 99.0% similarity to recorded *Raillietina hymenolipidoides* genes from various sources deposited in GenBank. This high genetic similarity underscores the conserved nature of this gene region among Raillietina species infecting different avian hosts and highlights the utility of molecular tools for epidemiological and taxonomic studies. In conclusion, the integration of prevalence data with molecular diagnosis and phylogenetic analysis enhances understanding of Raillietina infections in domestic pigeons, supporting improved surveillance, control, and prevention strategies to mitigate their impact on poultry health and productivity.

Key words: *coi* gene, Domestic pigeon, Raillietina Species

Introduction

Cestodes, commonly known as tapeworms, are parasitic flatworms that infect a wide range of vertebrate hosts, including pigeons (*Columba livia*). These parasites typically reside in the intestines of their hosts, where they attach to the intestinal lining using specialized structures such as suckers and hooks. In pigeons, cestode infections are a significant concern due to their impact on the health and productivity of these birds (Mukaratirwa and Khumalo, 2010). The life cycle of cestodes in pigeons often involves intermediate hosts, such as insects or other invertebrates, which harbor the larval stages before transmission to the pigeon occurs (Lucas *et al.*, 2010)

The presence of cestodes in pigeons can lead to various health issues, including malnutrition, weight loss, and intestinal damage. Heavy infestations may cause mechanical irritation of the intestinal mucosa, leading to inflammation, diarrhea, and reduced nutrient absorption. In severe cases, cestode infections can compromise the immune system, making pigeons more vulnerable to secondary infections (Albogami *et al.*, 2023).

The commonest cestode parasites of pigeon include Raillietina, Hymenolepis and Davainea species. Raillietina group represents over 200 species, with infected ones may cause high rates of morbidity and mortality. However, the morphological criteria of Raillietina spp. in particular, showed a wide range of variations within and between species, it is difficult to identify by morphology (Caira *et al.*, 2014).

Morphological identification of cestodes found in pigeons traditionally relies on microscopic examination of scolex and proglottid features. However, this approach can be challenging due to the similarity between different cestode species and the variation in developmental stages. Molecular techniques, such as polymerase chain reaction (PCR) and DNA sequencing, have revolutionized the identification and classification of cestode species by providing more precise genetic information. These tools allow researchers to detect genetic variation and clarify phylogenetic relationships among cestode isolates from pigeons (Al-Quraishy *et al.*, 2019).

Molecular sequencing has also enhanced the understanding of the transmission dynamics and epidemiology of cestodes in pigeon populations. By analyzing specific genetic markers, researchers can track the spread of different cestode strains and assess the influence of environmental factors on infection rates (Ahmes *et al.*, 2025).

Research on cestodes in pigeons is not only important for avian health, but also has broader ecological and public health implications. Pigeons often live in close proximity to humans and other animals, raising concerns about the potential zoonotic transmission of cestode parasites (Abd El-Salam *et al.*, 2025). Although most pigeon cestodes are species-specific, some may pose a risk of infection to other birds or mammals, including humans, under certain conditions (Mohebati *et al.*, 2024).

Integrating morphological and molecular approaches in the study of cestodes from pigeons provides a comprehensive framework for parasite identification and zoonotic threats; therefore, the current study was planned to detect Raillietina spp. in domestic pigeon morphologically, accompanied by molecular sequencing and identification of the detected Raillietina spp.

Material and Methods:

Collection and preparation of samples

The study was performed on 100 slaughtered domestic pigeons (*Columba livia*) carcasses randomly collected from slaughter shops and home-rearing points in El-Menoufiya Governorate, Egypt.

Small intestine and caecum were dissected out and opened longitudinally, rinsed with physiological saline (0.9% normal saline) and mucosa was scraped to collect the worms embedded in the mucosal layer. Worms were carefully washed in saline in Petri dishes, examined under light microscope and the

specimen of the collected worms which examined microscopically were kept in bottles with 70% alcohol in refrigerator for molecular investigation (Thrusfield, 1995).

Molecular characterization of Raillietina samples

DNA extraction

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 25 mg of the sample was incubated with 20 µl of proteinase K and 180 µl of ATL buffer at 56^{O}C overnight. After incubation, 200 µl of AL buffer was added to the lysate, incubated for 10 min. at 72^{O}C, then 200 µl of 100% ethanol was added to the lysate. The lysate was then transferred to silica column and centrifuged. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide primers

Primers used were supplied from Metabion (Germany) are listed in Table (1).

Analysis of the PCR Products

After amplification of the obtained DNA extract, the products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

DNA sequencing and phylogenetic analysis

PCR products were purified using QIAquick PCR Product extraction kit. (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit(Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) (Altschul *et al.,* 1990) was initially performed to establish sequence identity to GenBank accessions. The sequence identites were determined by Lasergene DNAStar version 12.1 Thompson *et al.* (1994) and Phylogenetic analyses was done using maximum likelihood, neighbour joining and maximum parsimony in MEGA7 (Tamura *et al.*, 2013).

Results

Out of 100 examined samples, *Raillietina* spp. was detected in twenty samples (20%). Morphological characterization of the detected worms revealed medium-sized tapeworm, segmented into numerous proglottides characterized by rounded scolex with four muscular suckers with armed, weakly muscular rostellum.

Molecular identification of *coi* gene in the detected isolate (Fig. 1) revealed positive band at the base pair 450.

Moreover, Figs. (2 and 3) showed the phylogenetic analysis and the percent identity of *coi* gene of the present isolate's sequence that was recorded in GeneBank under the name of Sameh_1 with accession number of PV992729. Results revealed 99% identity with the recorded sequences with accession numbers of MN590289, MN590290, ON228190 and ON228191.

Discussion

Raillietina spp. is a significant cestode parasite commonly infecting domesticated pigeons (*Columba livia domestica*), posing important health challenges in avian populations. As an intestinal tapeworm, Raillietina spp. inhabit the ileum and jejunum, where they cause severe pathological effects including intestinal obstruction, hemorrhage, and tissue damage. These parasitic infections lead to clinical signs such as diarrhea, emaciation, weakness, and droopiness in pigeons, significantly impairing their growth and overall health. The high infestation rates of Raillietina spp. in pigeon populations exacerbate these effects, often resulting in substantial economic losses in poultry farming and pigeon breeding (Aljoburi

et al., 2019).

From a parasitological viewpoint, *Raillietina* spp. is prevalent and highly adaptive, with transmission involving intermediate hosts like beetles, small wasps, ants, and termites. Its life cycle facilitates rapid spread within pigeon flocks, often resulting in heavy worm burdens that can fully obstruct the intestines. Such heavy infestations trigger inflammation, thickening of the intestinal walls, mucosal hemorrhage, and disruption of normal digestive processes (Kamal *et al.*, 2020).

The rate of infestation by Raillietina species in pigeons has been reported to be notably high in various geographic regions, with prevalence rates reaching up to 77.8% or more in some studies (Al Quraishy *et al.*, 2019). In addition to their veterinary importance, Raillietina spp. carry zoonotic potential. Although human infections with Raillietina are rare, documented cases from diverse regions including Costa Rica, French Polynesia, and Indonesia reflect their capacity to infect humans under certain conditions. Pigeons, frequently cohabiting or sharing environments with humans, may act as reservoirs for zoonotic transmission, highlighting the need for awareness and preventive measures to reduce the risk of human infections originating from avian hosts (Siddiqui *et al.*, 2023).

Regarding the prevalence of *Raillietina* spp. as a cestodal infestation, it came in line with the recorded results of Safi-Eldin *et al.* (2019) who recorded that the total prevalence of Raillietina spp. was 19.5% regarding different seasons of collection; while, it came higher than Al-Barwari and Saeed (2012) and Eljadar *et al.* (2012) who found prevalence values of 6.25 and 5.0% in the collected samples from Iraq and Libya, respectively; but, relatively low with other studies worldwide; such as in Iran, prevalence was 84.78% (Radfar et al, 2012), in India was 91% (Parsani et al, 2014), in Libya was 56.0% (Alkharigy et al, 2018), in Nigeria was 60.0% (Buba *et al.*, 2018), in Egypt was 55.8% (El-Dakhly et al, 2018), and was 77.8% in the samples collected from Saudi Arabia by Al Quraishy *et al.* (2019).

Variations in the prevalence between different records may be attributed to difference in the season of collection, the area of study, and the management system of pigeon rearing.

The anatomy of cestode parasites in the genus Raillietina that infect domestic pigeons has not been well documented (Al Quraishy *et al.*, 2019; Ali *et al.*, 2020; Al Quraishy *et al.*, 2021); so, the reliability in the morphological traits that form the basis of cestode classification has not been extensively recorded. Few studies have tried to ascertain the frequencies at which these variants occurred in the species exhibiting them, despite the fact that numerous reports have detailed the variation in the morphological characters of cestode species (Franzese and Ivanov 2018 and Al Quraishy *et al.*, 2019).

Morphological characteristics of Raillietina spp. show considerable variation within and between species, making species identification based solely on morphology difficult and sometimes unreliable (Butboonchoo *et al.*, 2016). Due to overlapping features and intraspecific variability, relying on morphological traits alone can lead to misidentification or confusion among closely related cestode species. Therefore, molecular characterization has become a more accurate and reliable approach for species identification in the genus Raillietina (Alhayali *et al.*, 2025).

Molecular techniques, such as polymerase chain reaction (PCR) amplification and sequencing of specific genetic markers like the mitochondrial cytochrome c oxidase subunit I (*coi*) gene, and other genes such as nuclear internal transcribed spacer 2 (*its*-2), and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*nd*1), provide detailed genetic information that helps differentiate closely related species. These gene regions exhibit sufficient interspecific variation, allowing precise discrimination and confirmation of species identity even in morphologically similar individuals (Anslan and Tedersoo, 2015).

Regarding the molecular detection of *coi* gene of the detected Raillietina isolate, positive

band was detected at 450 bp (Fig. 1); followed by DNA sequencing and phylogenetic analysis for identity confirmation (Figs. 2 and 3) that came 99.0% identical with the recorded sequences, on the GeneBank, by Mariaux and Georgiev (2020) with accession number MN590289, Anwer *et al.* (2022) with accession number of ON228189, and Abdelmoneim *et al.* (2025) with accession number of PV628154; which was isolated from poultry, rats and wild birds, respectively that prove the interrelation between different sources of cestoda infestation that may reach domestic pigeon.

When the recovered Raillietina from this study was compared to other tapeworm species from around the world, it was shown to share similarities with other comparable species that inhabit the same host species (*Columba livia domestica*) and share genetic characteristic traits. In line with similar studies conducted around the world, which discovered that worms of the genus Raillietina are the most common internal parasites infecting domestic pigeons (Safi-Eldin *et al.*, 2019; Ali *et al.*, 2020; Hassan *et al.* 2024).

Conclusion

Raillietina spp. showed a high prevalence in the examined pigeon samples, highlighting its significance as a common intestinal parasite affecting pigeons. Molecular detection using the *coi* gene proved to be a reliable and accurate tool for identifying *Raillietina* spp., overcoming challenges posed by morphological variations. Phylogenetic analysis of the *coi* sequences confirmed the genetic relatedness of the isolates to known *Raillietina hymenolepidoides* strains, enhancing our understanding of the parasite's taxonomy and evolutionary relationships. This combined prevalence, molecular, and phylogenetic approach provides essential insights for effective diagnosis, control, and prevention of Raillietina infestation in domesticated pigeons.

Limitations of the study

There were no limitations in the current study.

Ethical approval

There was no need for ethical approval as the study has been conducted on dead pigeon

Conflict of interest: The authors have no competing interests to declare that are relevant to the content of this article.

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Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene		Primers sequences (5\-3\)	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
Cestode COI	F	TTT TTT GGG CAT CCT GAG GTT TAT	450	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Aboelhadid *et al.*, 2013**
	R	TAA AGA AAG AAC ATA ATG AAA ATG							

Fig (1). Molecular characterization of the *coi* virulence gene of *Raillietina* spp. worm using agarose gel electrophoresis.

L: 100 bps DNA StepLadder, P: positive control, and N: negative control

Lane S provide positive results for the *coi* gene, which amplified at 450 bps.

Fig. (2). Phylogenetic analysis of the detected sequence of *R. hymenolepidoides* isolate with the other recorded sequence

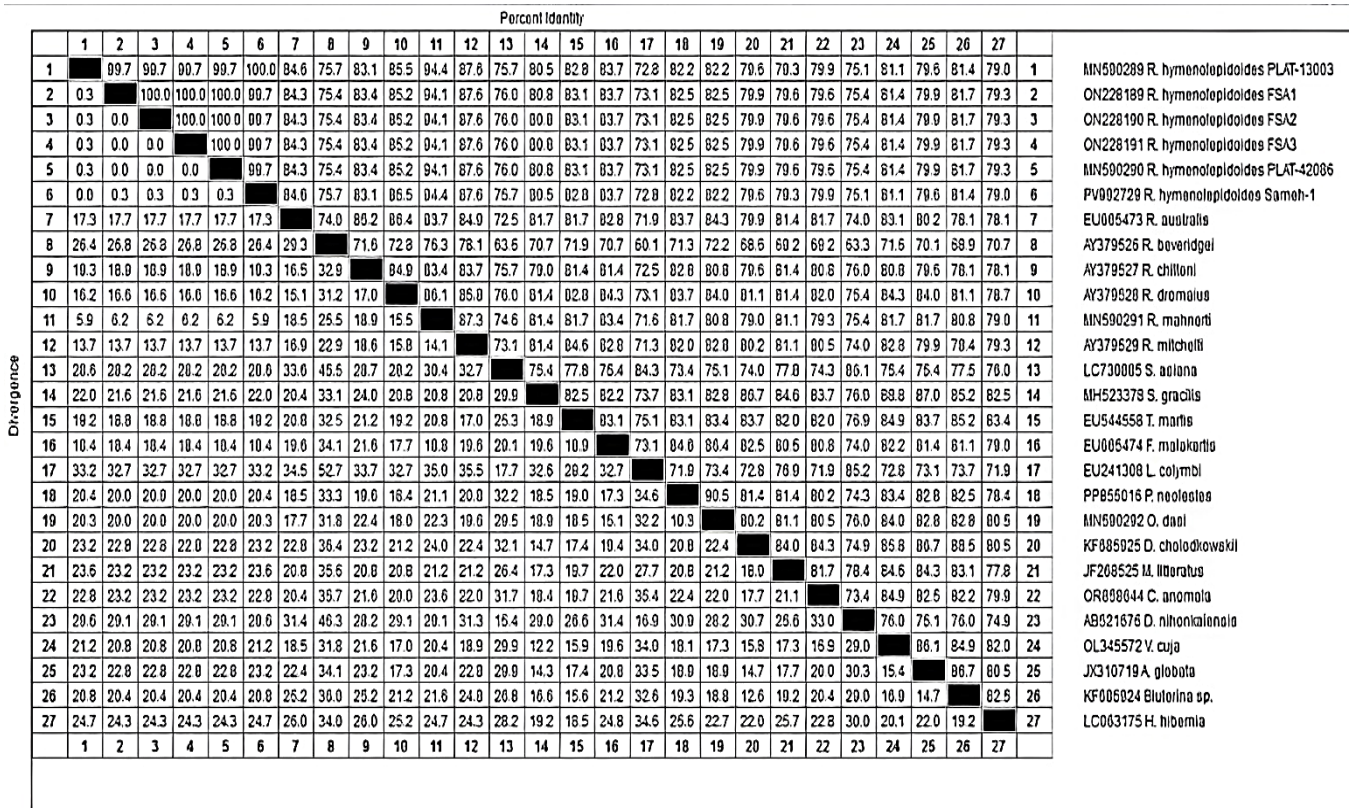

Fig. (3). % of identity of the recent *R. hymenolepidoides* isolate with the recorded sequences

ملخص عربى

التوصيف الجزيئي والتحليل الجيني لأنواع الرايليتينا التي تصيب الحمام المحلي: رؤى من التسلسل الجيني

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الملخص

تمثل سلالات الرايليتينا أحد أهم الديدان الشريطية المعوية التي تصيب الحمام، وتتسبب في مشاكل صحية كبيرة وخسائر اقتصادية في الطيور بشكل عام. لذا هدفت الدراسة الحالية إلى تحديد مدى انتشار هذه الديدان في مائة عينة من أمعاء الحمام والتي تم جمعها من المجازر ومحال ذبح الطيور في محافظة المنوفية، مصر. كشفت النتائج عن وجود الطفيليات في 20.0% من العينات المفحوصة، ما يعكس الأهمية الكبيرة لدراسة عدوى الرايليتينا في هذه المنطقة. ويعد التشخيص المعتمد فقط على الخصائص المورفولوجية لهذه الديدان تحديًا بسبب التباين بين السلالات والتشابهات الشكلية بين الأنواع القريبة. لذلك، تم استخدام التشخيص الجزيئي الذي يستهدف جين السيتوكروم سي أوكسيداز الفرعي الأول (*coi*) في الميتوكوندريا للمساعدة في تشخيص دقيق لأنواع الرايليتينا المعزولة. علاوة على ذلك، فقد تمكن الفحص الجزيئي لتسلسل جين *coi* بتأكيد تطابق السلالة المعزولة بنسبة تفوق 99.0% لجينات الرايليتينا هيمينولبيديد المسجلة في قاعدة بيانات بنك الجينات والمعزولة من مصادر مختلفة والتي تبرز أهمية الاختبارات الجزيئية للدراسات الوبائية والتصنيفية. في الختام، يعزز دمج بيانات الانتشار مع التشخيص الجزيئي وتحليل التتابع الجيني الفهم المتعمق لعدوى الرايليتينا في الحمام المحلي، مما يدعم تحسين المراقبة والاستراتيجيات الوقائية والسيطرة لتقليل تأثيرها على صحة وإنتاجية الطيور الداجنة.

الكلمات المفتاحية: رايليتينا، الحمام المحلي، التتابع الجيني