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Morphorgical and microscopic diagnosis of the tapeworm *Raillietina tetragona* in domestic homing pigeons (Columba livia domestica) from various environments in Al-Najaf Governorate AL

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ABSTRACT: The present study aimed to identify the tapeworm Raillietina tetragona in domestic homing pigeons (Columba livia domestica) across various regions and environments within the Al-Najaf Governorate, Iraq, using morphological and microscopic examinations. The study involved the examination of 50 domestic homing pigeons collected from diverse locations, including areas near bodies of water as well as arid, terrestrial environments. The birds underwent necropsy, and their digestive tracts with a specific focus on the intestines were examined to detect and isolate the tapeworms. Examination results revealed the presence of tapeworms identified as R. tetragona in all 50 pigeons examined, with a total of 195 worms recovered. The number of worms per pigeon varied: 15 pigeons (30%) harbored three worms, 25 pigeons (50%) had four worms, and 10 pigeons (20%) contained five worms. The mean number of worms was 3.90 ± 0.71 per pigeon, and the prevalence of infection within the studied sample was 100%. Diagnosis was also performed using light and electron microscopy to examine the morphological characteristics, as well as the anatomical and surface details of the parasite, with the aim of enhancing the accuracy of species identification and documenting its diagnostic features. The study provides local data on the prevalence of this tapeworm infection in domestic homing pigeons within the Al-Najaf Governorate.

KEYWORDS: Domestic homing pigeons, tapeworms, Raillietina tetragona, light microscopy, scanning electron microscopy, Al-Najaf Governorate

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1. INTRODUCTION

Intestinal parasites represent a significant health issue for birds, as they exert varying effects on the host's health and digestive function. Furthermore, the prevalence of these parasites is linked to environmental factors, dietary conditions, and the circumstances under which the birds are kept (1). The domestic pigeon (Columba livia domestica) is a bird widely distributed across both urban and rural environments; it is raised for hobbies, racing, and ornamental purposes, and is notable for its ability to adapt to diverse environmental conditions. Tapeworms (Cestoda) are a group of internal parasites that inhabit the digestive tracts of birds. They are characterized by flattened, segmented bodies and specialized structures that enable them to attach to the intestinal wall and persist within the host. The genus Raillietina spp. belongs to the family Davaineidae and the order Cyclophyllidea; it comprises various species that infect birds, including Raillietina tetragona(2,3.)

Raillietina tetragona is a well-known tapeworm species that infects birds. It has been documented in domestic pigeons across various regions of the world, including Iraq, Iran, and Egypt. Its significance as a parasite lies in its colonization of the intestine and its capacity to induce intestinal alterations, the severity of which depends on the intensity of the infection and the health status of the host (4,5).

Worms of this type are characterized by a scolex (head) bearing four suckers and a rostellum armed with hooks, as well as a body composed of a series of segments (proglottids) that differentiate into immature, mature, and gravid (egg-bearing) segments. The features of the scolex such as the shape of the suckers, the arrangement of the hooks, the position of the genital pores, and the contents of the gravid segments are among the most important criteria used for the taxonomic identification of this type (6,7).

The importance of using a light microscope lies in the ability to study the general structure of the worms and observe their internal anatomical features. In contrast, a scanning electron microscope provides precise details regarding the parasite's external surface, including head structures, suckers, hooks, and the surface features of the proglottids (segments). Comparative morphological studies of species within Raillietina sp. have demonstrated the value of combining various examination techniques to distinguish between species that are morphologically similar (8,9)

From an ecological perspective, factors such as the conditions of pigeon-rearing sites, feeding habits, and exposure to insects or intermediate hosts can influence the likelihood of tapeworm transmission. The life cycle of many species within this genus requires an arthropod intermediate host such as an ant to complete their development into the infective stage (10,11).

Previous Iraqi studies have documented the presence of R. tetragona in pigeons, including research conducted in Al-Muthanna Governorate, as well as studies examining the intestinal parasites of pigeons in other regions of Iraq (1, 10). Furthermore, a study in Al-Najaf Governorate investigated the bioaccumulation of certain heavy metals within this tapeworm and the tissues of the pigeons, underscoring the local research significance of this parasite (12).

Despite these existing studies, the documentation of the morphological characteristics and microscopic details of tapeworms isolated from domestic homing pigeons across various environments in Al-Najaf Governorate represents a significant contribution to the body of knowledge regarding avian cestode parasites in Iraq.

MATERIALS AND METHODS

Study Area and Sample Collection

The study was conducted on domestic pigeons (Columba livia domestica) collected from various locations within Al- Najaf Governorate, Iraq. The collection sites encompassed diverse natural environments ranging from areas near bodies of water to terrestrial (dry-land) habitats, to represent the ecological variation of the locations where these pigeons are found and raised.

A total of 50 birds were included in the study. The birds were transported to the laboratory for anatomical and parasitological examinations. Bird Dissection and Examination of the Digestive System focused on the small intestine, as this is the typical intestinal site for R. tetragona in birds (13,14).

Collection and Isolation of Tapeworms

Following measurement of cestode length using a ruler, the worms were cut into suitable segments and stained with ready-made acid acetocarmine. Several drops of the stain were applied using a dropper in a watch glass, and staining was monitored until the specimens acquired an appropriate reddish color. Overstained specimens were destained by adding a few drops of 10% hydrochloric acid (HCl) to the stain, away from the specimens. The scolex was then separated, while the remaining stained segments were placed between two glass slides, secured with rubber bands, and immersed in 70% ethanol for 24 h at room temperature.(15,16)

The compressed specimens were subsequently released and dehydrated through an ascending ethanol series: 70% (10–15 min), 80% (5–10 min), 90% (5 min), and absolute ethanol (100%) for 1 min. They were then transferred to a 1:1 mixture of absolute ethanol and xylene for 1 min, followed by xylene for 1 min to clarify the internal structures. Finally, permanent microscopic slides were prepared by mounting the specimens in Canada balsam (17).

Diagnosis via Light Microscopy

A light microscope was used to examine the specimens and study the anatomical features essential for species identification. The study included the following structures (Scolex, Rostellum.,Suckers , Hooks., Neck and Strobila. (18).

Examination via Electron Microscopy

The specimens were dried and mounted on specimen stubs using double-sided adhesive tape. They were then placed in a gold sputter-coating chamber, where a nanometer-thin layer of gold was deposited onto their surfaces to prevent specimen damage caused by the high-energy electron beam. Finally, the prepared specimens were mounted in the designated positions for examination using a scanning electron microscope (SEM; Inspect S50, FEI Company, Netherlands) (19).

Taxonomic Diagnosis

Qualitative diagnosis was based on observed morphological and anatomical characteristics, compared against published taxonomic descriptions of tapeworms belonging to R. tetragona while accounting for distinguishing features that separate it from other closely related species, such as R. echinobothrida and R. cesticillus (20,21).

Statistical Analysis

Quantitative data regarding the tapeworms recovered from the pigeons were analyzed using descriptive statistics, including counts, frequencies, percentages, arithmetic means, and standard deviations.

RESULTS

Prevalence of R. tetragona Infection

The results of the parasitological examination of all the birds studied revealed the presence of tapeworms R. tetragona. A total of 50 out of the 50 examined pigeons were found to be infected, representing an infection rate of 100% within the studied sample. This figure refers specifically to the infection rate among the birds included in the study and does not necessarily represent the prevalence of the parasite among the entire pigeon population in Al-Najaf Governorate.

Number of R. tetragona Recovered from Pigeons

The results showed a variation in the number of R. tetragona recovered from the birds, with the count ranging from three to five tapeworms per bird. The total number of tapeworms recovered was 195.(Table 1)

Table (1): Numerical distribution of R. tetragona recovered from domestic homing pigeons.

Number of worms per pigeon	Number of pigeons	|Percentage
3	15	%30
4	25	%50
5	10	%20
Total	| 50	100%

Statistical Indicators of Infection Intensity

The total number of R. tetragona recovered from the 50 birds was 195. The arithmetic mean for the intensity of infection was 3.90 worms per pigeon, with a sample standard deviation of 0.71. Both the median and the mode were 4 worms (Table 2.)

Table (2): Statistical indicators for the number of R. tetragona recovered from the pigeons.

Statistical Indicator	Value
Number of pigeons examined	50
Number of infected pigeons	50
Infection rate	%100
Total number of worms	195
Arithmetic mean	3.90
Sample standard deviation	0.71
Mode	4
Minimum number of worms	3
Maximum number of worms|	5

Light Microscopic Description of Raillietina tetragona :

The Raillietina tetragona was isolated from the small intestine of domestic Columba livia domestica. The total body length was 12.6 cm. The scolex possessed four suckers, each measuring 19 µm in diameter and equipped with a single row of hooks measuring 8 µm in length (100× magnification). The scolex also contained a retractable rostellum measuring 4 µm in diameter (100× magnification), armed with a single row of hooks measuring 6 µm in length (100× magnification).

The mature proglottid measured 36 µm in length and 45 µm in width (100× magnification), whereas the gravid proglottid measured 47 µm in length and 50 µm in width (100× magnification) (Figure 1 A,B and C).

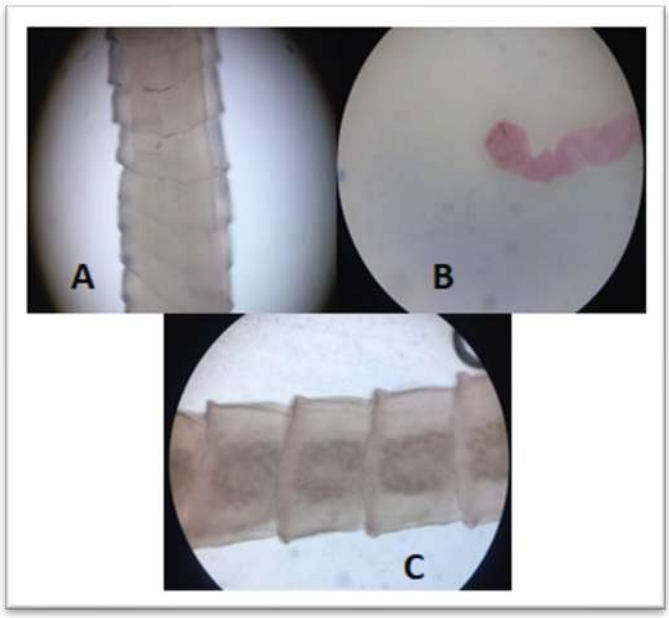


Figure (1): A: Imature proglottid of Raillietina tetragona B: Scolex of Raillietina tetragona, showing the rostellum, sucker, and hooks . Magnification: 40×. C:Mature proglottid of Raillietina tetragona, showing the genital pore (P). Magnification: 100×.

Scanning Electron Microscopic Description of Raillietina tetragona :

Scanning electron microscopy (SEM) revealed that the scolex of Raillietina tetragona was approximately oval in shape. It possessed a small, retractable rostellum measuring 25 µm in length, armed with a single row of hooks ranging from 8.09 to 10.73 µm in length. The scolex also exhibited four spherical suckers, each measuring 46.1 µm in diameter and armed with several rows of hooks.

The mature proglottid measured 0.44 mm in length and 0.25 mm in width, whereas the gravid proglottid was vase-shaped, measuring 0.35 mm in length and 0.32 mm in width (Figure 2- A,B,C and D).

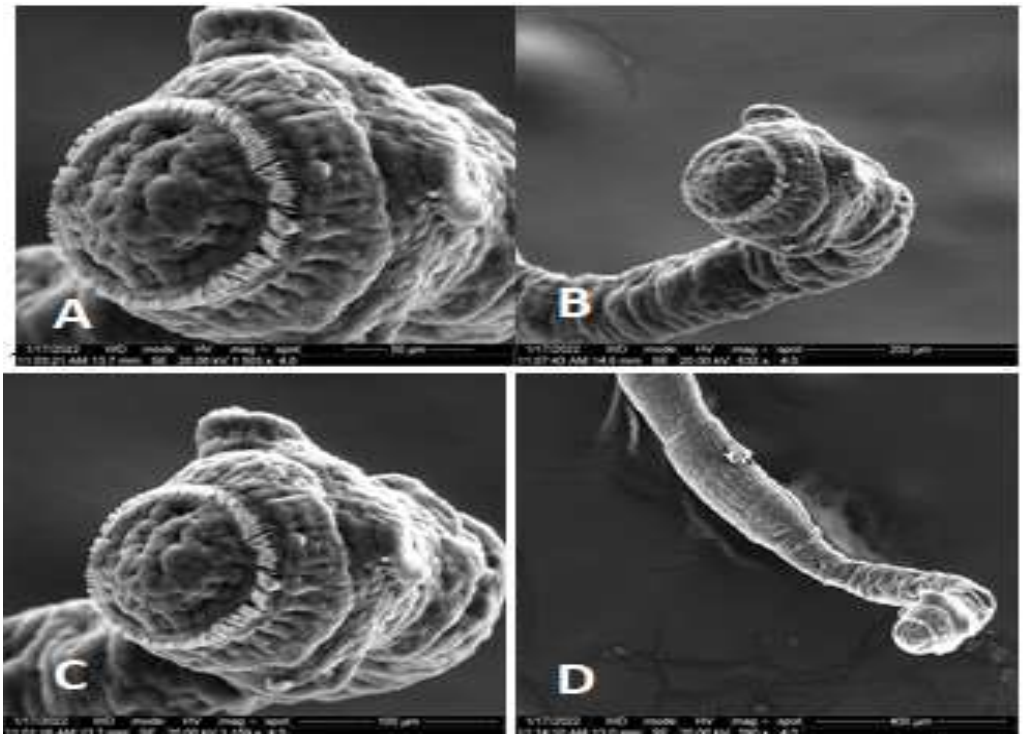


Figure (2): A and C: Anterior view of the scolex and adjacent body segments of Raillietina tetragona, showing the scolex, neck and beginning of the strobila , rostellum , hooks , and four suckers Scale bar A = 200 µm; (2) Scale bar C= 100 µm B and D: Anterior view of the scolex of Raillietina tetragona, showing the scolex, rostellum , hooks , and four suckers . Scale bar B= 50 µm; Scale bar D= 20 µm.

DISCUSSION

Tapeworms of the genus Raillietina sp. are significant intestinal parasites affecting birds; their presence has been documented in both domestic and wild pigeons across various geographical regions. R. tetragona is a well-recognized species within this group, characterized by specific morphological and anatomical features that facilitate its taxonomic identification (22,23,24).

The results of the current study revealed the presence of R. tetragona in all 50 pigeons examined, representing an infection rate of 100% within the studied sample. The total number of worms extracted was 195, with a mean of 3.90 ± 0.71 worms per pigeon. It should be noted that this percentage represents only the birds included in the study and should not be generalized to all pigeons in Al-Najaf Governorate without conducting a broader epidemiological survey using representative samples from all areas of the governorate).24,25)

The results showed that the group with four worms was the most frequent, recorded in 25 pigeons (50%), followed by the group with three worms in 15 pigeons (30%), and then the group with five worms in 10 pigeons (20%). This distribution may indicate variations in the number of worms found in infected birds within the studied group, but it is not sufficient on its own to prove the influence of environmental or dietary factors on the severity of the infestation. In the context of comparisons with Iraqi studies, a study by Alasadiy et al. (2022) documented the presence of R. tetragona in both domestic and wild pigeons in Al-Muthanna Governorate, the significance of this species among the cestode parasites recorded in Iraqi pigeons (26). Furthermore, a study by Al-Busaibar and Al-Awadi examined the occurrence of this species in pigeons within Najaf Governorate, investigating its relationship with the bioaccumulation of certain heavy metals in the host's tissues (27)

Additionally, a study by Radfar et al. (2012) in the Sistan region of Iran reported that domestic pigeons were infected with several types of parasites, including R. tetragona; this underscores the relevance of this species in parasitological research on pigeons in regions geographically adjacent to Iraq (28,29).

Regarding phenotypic diagnosis, a study by Butboonchoo et al. (2016) highlighted the importance of examining the morphology of the scolex, rostellum, suckers, hooks, and genital pores for distinguishing between species within the Raillietina sp. Furthermore, the study demonstrated the utility of scanning electron microscopy (SEM) for investigating the fine details of the parasite's surface structures (30,31,32).

The approach taken in the present study combining light microscopy with electron microscopy aligns with contemporary trends in the diagnosis of tapeworms. Accurate species-level identification cannot rely solely on general external morphology; rather, it requires the assessment of a range of anatomical and surface characteristics, which are then compared against established taxonomic descriptions. (33,34,35,36) From an ecological perspective, collecting pigeons from various locations—including both aquatic-proximate and terrestrial environments—provides a representation of the diverse environmental conditions present within the governorate. Variations in food sources and exposure to intermediate hosts are factors that warrant further investigation in this context. However, the current study cannot definitively establish a correlation between the type of environment and the prevalence or intensity of infection; such an analysis would require recording the number of pigeons and the worm burden for each specific site, followed by a formal statistical comparison. (37)

Furthermore, the presence of potential intermediate hosts such as certain ant species plays a critical role in the life cycle of these helminths; the infective stages are transmitted to the birds when they ingest an intermediate host carrying the larval stage (37,38). Thus, the present study contributes by providing diagnostic and quantitative data on the tapeworm R. tetragona in domestic homing pigeons in Najaf Governorate, and it underscores the importance of continuing morphological, microscopic, and epidemiological studies concerning intestinal parasites in Iraqi birds.

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