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CASE REPORT

Detection of *Diplotriaena* spp. in the Abdominal Cavity of the Common Myna (*Acridotheres tristis*) in Central Iran (Isfahan)

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Abstract

In February 2023, eight common Mynas (*Acridotheres tristis*) were found dead in the Bird Garden of Isfahan. The mynas had a diet composed of bird pellets, fruits, vegetables, and dates. Postmortem examination revealed the presence of nematodes in their peritoneal and abdominal cavities. Ascites, necrotic spots in the liver, cardiomegaly, and pericardial effusion were observed during postmortem examination. Based on their morphological features observed through light microscopy, the nematodes were identified as belonging to the genus *Diplotriaena*. They had a milky white color, slender, elongated bodies, simple mouths with no lips, and a pair of chitinous tridents (0.12 mm × 0.02 mm) at their anterior ends. The worms had a body length of 130 mm and a diameter of 0.65 mm. The cephalic region measured 0.3 mm in width, while the caudal region measured 0.5 mm in width. Although *Diplotriaena* spp. infections have previously been reported in other regions of Iran, this represents the first record of the nematode in central Iran, specifically in Isfahan, within an aviary housing diverse bird species from around the world.

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Introduction

Diplotriaena spp. infections have previously been reported in the north (1), northeast (2), and southeast (3) regions of Iran. However, no report exists from central Iran. The genus *Diplotriaena* belongs to Diplotriaenoidea, a superfamily of spirurid nematodes distinguished by its large body size, oviparous reproduction, and the tropism of its adult worms for the air sacs of Sauropsids. *Diplotriaena* species infect the air sacs, lungs, and body cavity of birds. A single bird can be infected by multiple species concurrently. To date, species of *Anseriformes*, *Apodiformes*, *Galliformes*, *Charadriiformes*, *Piciformes*, and *Passeriformes* have been recorded as definitive hosts of *Diplotriaena* (4, 5).

Grasshoppers (Orthoptera) serve as the primary intermediate hosts (6), becoming infected during their nymphal stage by ingesting first-stage larvae (L1). Inside the grasshopper, *Diplotriaena* larvae develop from L1 to the third stage (L3). Birds become infected by consuming adult grasshoppers containing L3 larvae. After ingestion, the parasite develops into a fourth-stage larva (L4) within the liver of the definitive host, then migrates to the lungs as a sub-adult. Adult worms reside in the abdominal air sacs, producing eggs that are expectorated, swallowed, and eventually passed through the digestive system to be excreted in the feces (7) (Figure 1).

The bird in question, the common Myna (*Acridotheres tristis*), is a member of the order Passeriformes and family Sturnidae, and is native to Iran. Being an omnivorous species, this bird feeds on a variety of food sources, including arthropods such as insects. *Diplotriaena* infection can cause severe symptoms, including pneumonia, pulmonary consolidation, central nervous system disruption, diarrhea, marked weight loss, loss of appetite, and sudden death resulting from larval and adult migration within the host's body. Additionally, subcutaneous emphysema, pneumonia, and air sacculitis are frequently observed, some resulting in fatality (6, 8, 9). Despite these reports, our knowledge about the effects of this parasite on avian health remains incompletely understood. Therefore, this case report presents *Diplotriaena* infection in eight common Mynas.

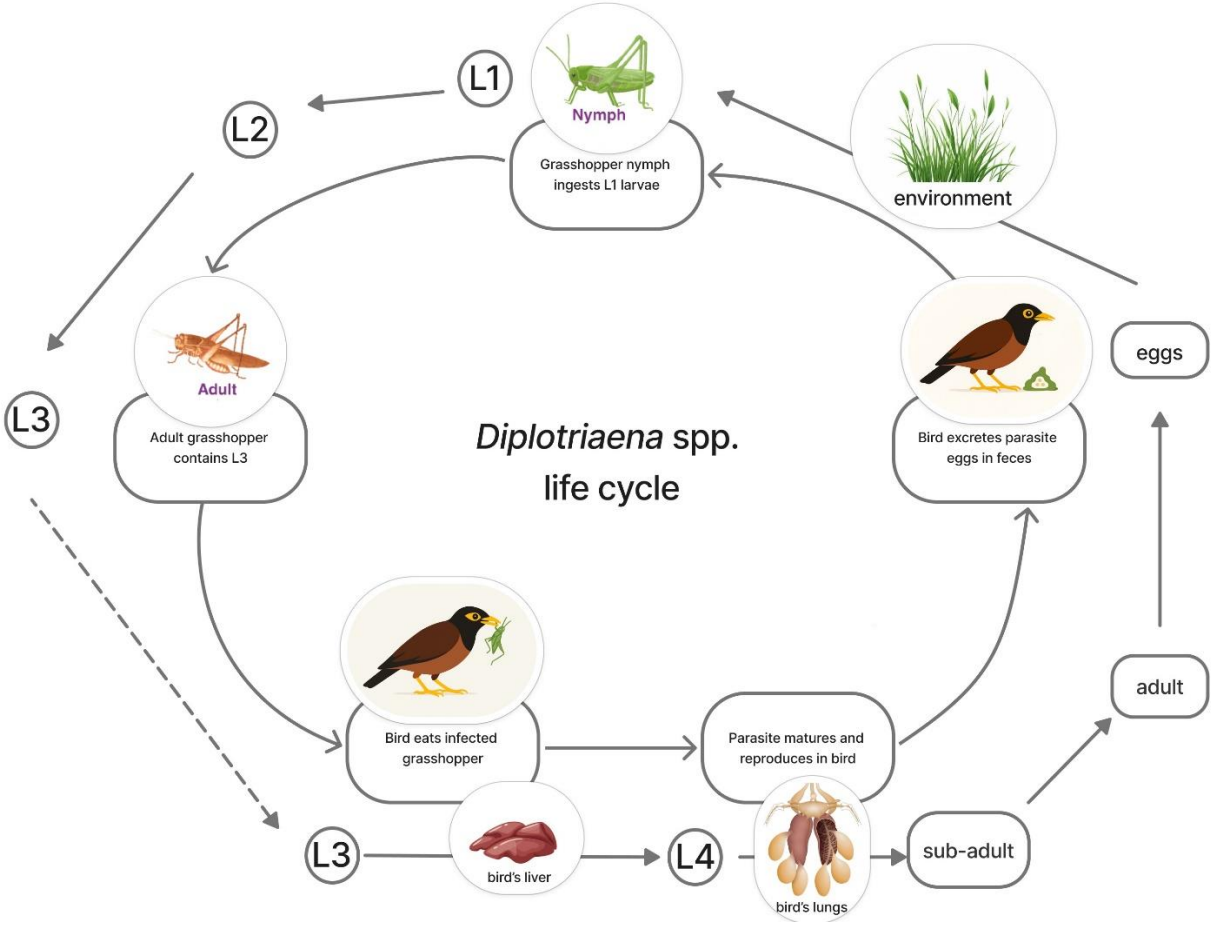

Figure 1. Schematic illustration of the *Diplotriaena* life cycle, showing transmission through insect intermediate hosts and infection of avian definitive hosts (created by M. Tavangar).

Clinical History

In February 2023, eight common Mynas (*Acridotheres tristis*) were discovered dead in the Bird Garden of Isfahan. The garden is enclosed by a chain-link fence and houses more than 5,000 birds representing 130 species. The mynas were fed a diet consisting of bird pellets, apples, bananas, vegetables, and dates; insects were not intentionally included but may have been incidentally ingested within the enclosures. A sudden drop in ambient temperature during February 2023 was considered a potential contributing factor (10), as meteorological data indicated average daytime and nighttime temperatures of 18 °C and 3 °C, respectively.

No antemortem clinical or hematological examinations were conducted, as there had been no prior suspicion of endoparasitic infection and the mortalities occurred suddenly. The nematodes recovered during postmortem examination were therefore considered unexpected findings.

The nematodes were collected from the carcasses in February 2023 and preserved in 10% formalin at room temperature for eight months. In October 2023, the specimens were transferred to the Parasitology Laboratory, Faculty of Veterinary Medicine, Shahrekord University, for detailed examination. Worms were initially examined under a stereomicroscope (Figure 2), then cleared in lactophenol for 24 h and mounted on slides for microscopic observation. Morphological features were studied under a light microscope at ×10 and ×40 magnifications to identify the nematodes to the genus level (Figure 3).

Morphometric analyses were performed using a light microscope equipped with an ocular micrometer calibrated against a 1 mm stage micrometer (100 divisions = 10 µm per unit). Actual measurements (µm) were obtained by multiplying the number of ocular divisions by the corresponding calibration factor for each objective lens.

Results

All eight carcasses underwent postmortem examination; nematodes were detected in the abdominal and peritoneal cavities of four birds, while no parasites were found in the remaining four. During their lifetime, five of the eight birds displayed respiratory signs, including tachypnea. All of the birds had a normal body condition score (Table 1). Postmortem findings included ascites, necrotic spots in the liver, cardiomegaly, and pericardial effusion.

Figure 2. *Diplotriaena* spp. obtained from the abdominal and peritoneal cavities of a common myna (*Acridotheres tristis*) observed under a stereomicroscope (×4).

Using a light microscope, the nematodes were identified as *Diplotriaena* spp., by examining key features, such as the tridents and other characteristic traits (Figure 3). The helminths appeared milky white in color, with slender, elongated bodies, and simple lipless mouths (Figure 2). Morphometric analysis revealed an average body length of approximately 130 mm and a width of 0.65 mm. The cephalic region measured about 0.3 mm in diameter, and the posterior end was approximately 0.5 mm wide. The trident branches had a mean length of 0.12 mm and a width of 0.02 mm (Table 2).

Discussion

Several species of nematodes possess cephalic armature, characterized by chitinous projections, spines, or hooks near the anterior end, which may function in sensory perception, locomotion, or host tissue penetration. The anterior end of *Diplotriaena* spp. typically bears paired chitinous tridents, a defining morphological feature shared among most species (2). Both males and females possess chitinous projections, though the number and length of prongs can vary slightly between species and regions (3). Similar cephalic armature was described in *D. manipoli* from the Republic of Korea, where trident pairs measured approximately 0.11 × 0.02 mm and were clearly visible under the light microscope (11). Comparable trident morphology has also been reported from *D. obtusa* in Germany and *D. saheefi* in Pakistan (4, 5, 12).

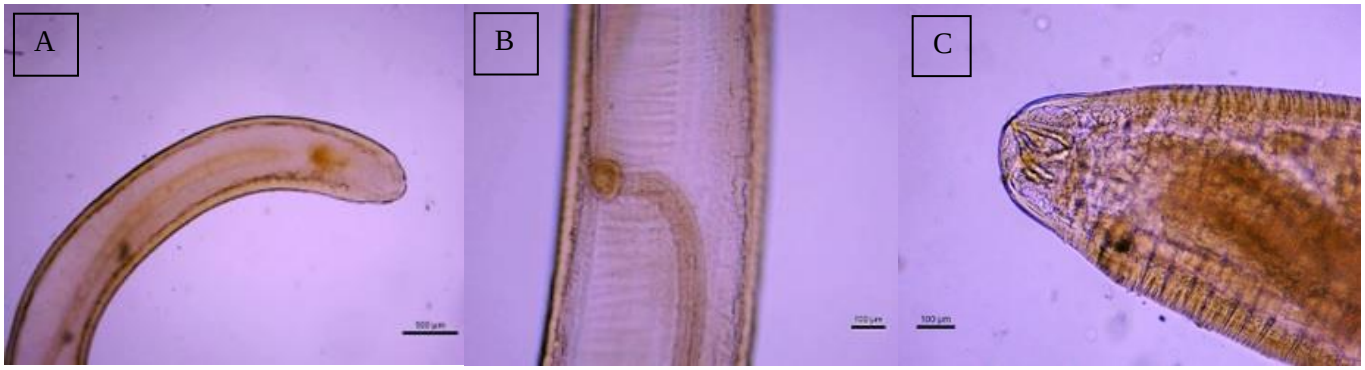

Figure 3. Microscopic images of *Diplotriaena* spp. (A) Posterior end of the nematode (×10). (B) Excretory duct indicated by an arrow (×40). (C) Anterior end showing paired chitinous tridents (arrows) (×40).

Table 1. Postmortem findings for eight common Mynas (*Acridotheres tristis*) from Isfahan (February 2023).

Total examined birds	Number infected (nematodes)	Number uninfected	Number with clinical signs	Body condition score
8	4 (50.0%)	4 (50.0%)	5 (62.5%)	All normal (100%)

Table 2. Morphometric measurements of *Diplotriaena* spp. (in millimeters).

Morphometric feature	Measurements (mm)
Trident branch height	0.12
Trident branch width	0.02
Body length	130
Body width	0.65
Cephalic width	0.3
Caudal width	0.5

Although *Diplotriaena* species generally inhabit the air sacs and lungs of their avian hosts, our finding of adult worms in the peritoneal and abdominal cavities resembles earlier observations from Pakistan and Southeast Asia. In Pakistan, *D. saheefi* n. spp. was recovered from the abdominal cavity of the Jungle Myna (*Acridotheres fuscus*), with up to 30 adult females in a single bird (5). Similarly, multiple *Diplotriaena* species have been documented in *Acridotheres* spp. and other passerines in India and Nepal, recovered from either the air sacs or body cavity (13, 14). In Poland, *Diplotriaena obtusa* was molecularly confirmed in a passerine host (*Sylvia atricapilla*), with adult worms localized in the air sacs (15). Dewi and Zhang (2010) described a new *Diplotriaena* species from a passerine in Indonesia, further confirming that body-cavity localizations are not geographically limited and may occur sporadically across avian hosts (16). Anderson (2000) suggested that the presence of these nematodes in the abdominal cavity may sometimes result from postmortem migration (17). These reports collectively indicate that while air sacs are the preferred niche, migration to the abdominal cavity can occur naturally in several host species across Asia.

In our study, *Diplotriaena* infection was characterized by respiratory signs and systemic lesions, including ascites and cardiac and hepatic changes, aligning with previously reported pulmonary and visceral involvement. Hamerton (as cited in Petrak, 1982) associated *Diplotriaena* infections with pneumonia and pulmonary consolidation (18). Similar lesions were described in infected passerines in Korea (11) and in Iran, where *Diplotriaena* spp. caused respiratory distress, reduced activity, and occasional sudden death (2, 3). Infections in Europe and North America (*D. obtusa* in tits and swallows) were also linked with respiratory compromise, weight loss, and neurological signs due to microfilarial congestion (4, 8). Collectively, these data indicate that the pathogenic potential of *Diplotriaena* infections is consistent across regions, though severity may vary with parasite load and environmental conditions.

Environmental factors likely contributed to the observed mortality in our study. Sudden declines in ambient temperature—particularly sharp nocturnal drops—can trigger cold stress, elevating stress hormone levels, increasing metabolic demands, and disrupting oxidative balance. These physiological responses collectively impair immune function and heighten susceptibility to infectious agents. Lv et al. (2023) and Zhao et al. (2014) demonstrated that abrupt temperature fluctuations compromise immune defenses through hormonal and oxidative stress pathways,

predisposing birds to infection (10, 15). Comparable effects have been reported in passerine populations during severe winters and rapid temperature shifts in temperate regions of Europe and East Asia (19-21). Such cold-induced physiological stress likely predisposed the mynas to heavy parasitic infection and diminished their ability to cope with concurrent disease challenges. Therefore, acute cold snaps and nocturnal temperature declines may be considered contributing factors to the observed mortality of common mynas.

Diagnosis of *Diplotriaena* infection in live birds remains challenging because microfilariae exhibit periodicity in circulation, often resulting in false negatives in blood smears. At postmortem examination, however, the identification of worms in air sacs or coelomic cavities remains a reliable diagnostic criterion (22). Molecular tools are increasingly being applied to confirm species identity and elucidate phylogenetic relationships (4, 11). Considering the wide geographic distribution of *Diplotriaena* and the occurrence of closely related species throughout Asia, comparative molecular analyses are recommended for future Iranian isolates.

The presence of diverse bird species from various continents in the Bird Garden of Isfahan underscores the potential for parasite introduction and cross-transmission. Previous reports from Iran (Mashhad, Zabol, Mazandaran) and other regions (Pakistan, India, Korea, Indonesia, and Europe) collectively highlight the cosmopolitan nature of *Diplotriaena* infections. This first record from central Iran extends the known range of the genus and emphasizes the need for continued parasitological surveillance and integrated control measures to protect both native and exotic avian populations.

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Authors' Contributions

Mahsa Tavangar: Investigation, Resources, Writing – original draft, Visualization. **Seyyed Sattar Tohidifar:** Conceptualization, Supervision, Project administration, Validation, Writing – review & editing. **Nader Ahmadi-Baberi:** Investigation, Methodology, Formal analysis, Data curation. **Mohammad-Javad Azizi-Dasteneai:** Resources, Investigation, Data curation. **Hamidreza Azizi:** Investigation, Methodology, Formal analysis, Writing – review & editing.

Data Availability

The data presented in this study are available on request from the corresponding author.

Ethical Approval

Not applicable.

Conflict of Interest

The authors declare no conflicts of interest.

Consent for Publication

Not applicable.

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