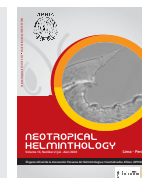




Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

OCCURRENCE OF *HETERAKIS GALLINARUM* (SCHRANK, 1788) (NEMATODA: HETERAKIDAE) IN *GALLUS GALLUS DOMESTICUS* LINNAEUS, 1758 IN VITORIA, ESPIRITO SANTO, BRAZIL

OCURRENCIA DE *HETERAKIS GALLINARUM* (SCHRANK, 1788) (NEMATODA: HETERAKIDAE) EN *GALLUS GALLUS DOMESTICUS* LINNAEUS, 1758 EN VITORIA, ESPIRITO SANTO, BRASIL

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ABSTRACT

Heterakis Dujardin, 1845 is a nematode parasite of birds with cosmopolitan distribution. In Brazil, there are reports of 14 species occurring in different species of domestic and wild birds. Among the reported species, *Heterakis gallinarum* (Schrank, 1788) presents a potential threat to commercial breeding sites as it is a vector of *Histomonas meleagridis* Smith, 1895. The Trichomonadida protozoans causing histomoniasis in birds represent critical infection that can be lethal and cause catastrophic economic losses. The present study reports the occurrence of *H. gallinarum* in the caecum of free-range chicken (*Gallus gallus domesticus* Linnaeus, 1758) sold at a street market in Vitoria, Espírito Santo, Brazil. The three male and three female specimens found were studied and illustrated.

Keywords: Nematodes – *Histomonas meleagridis* – histomoniasis – morphology – taxonomy – life cycle

RESUMO

Heterakis Dujardin, 1845 é um nematódeo parasito de aves e de distribuição cosmopolita. No Brasil são reportadas 14 espécies decorrentes de diferentes espécies domesticadas e selvagens. Entre as espécies já publicadas, *Heterakis gallinarum* (Schrank, 1788) apresenta ameaça para os criadores comerciais de aves pois é um vetor potencial para *Histomonas meleagridis* Smith, 1895, um protozoário Trichomonadida causador da histomoníase em ave, uma severa infecção que pode ser letal e causar perdas econômicas catastróficas. O presente estudo apresenta a ocorrência de *Heterakis gallinarum* em ceco intestinal de uma galinha (*Gallus gallus domesticus* Linnaeus, 1758) comercializada num grande centro comercial de Vitória, Espírito Santo, Brasil. Três machos e três fêmeas são descritos e ilustrados neste estudo.

Palavras-chave: Nematódeos – *Histomonas meleagridis* – histomoníase – morfologia – taxonomia – ciclo de vida

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RESUMEN

Heterakis Dujardin, 1845 es un nemátodo que parasita las aves y presenta distribución cosmopolita. En Brasil, se registran 14 especies que ocurren en diferentes especies domesticadas y silvestres. Entre las especies reportadas, *Heterakis gallinarum* (Schrank, 1788) representa una amenaza potencial para los criaderos comerciales ya que es un vector de *Histomonas meleagridis* Smith, 1895, protozoario Trichomonadida que causa histomoniasis en las aves, una infección grave que puede ser letal y causar pérdidas económicas catastróficas. El presente estudio registra la ocurrencia de *H. gallinarum* en el ciego de pollos de corral (*Gallus gallus domesticus* Linnaeus, 1758) vendidos en un gran centro comercial de Vitoria, Espírito Santo, Brasil. En este estudio se describen e ilustran los tres ejemplares machos y tres hembras encontrados.

Palabras clave: Nematodos – *Histomonas meleagridis* – histomoniasis – morfología – taxonomía – ciclo de vida

INTRODUCTION

Nematodes of the genus *Heterakis* Dujardin, 1845 are common and generalist parasites (Mendoza, 1953; Inglis, 1967; Cupo & Beckstead, 2019). According to Vicente *et al.* (1993, 1995), 14 species of *Heterakis* were reported in different Brazilian birds [*Heterakis alata* Schneider, 1866; *Heterakis arquata* (Schneider, 1866); *Heterakis brasiliensis* Linstow, 1899; *Heterakis brevispiculum* Gendre 1911; *Heterakis dispar* (Schrank, 1790), *Heterakis gallinarum* (Schrank, 1788), *Heterakis ibanezi* (Freitas, Vicente & Santos, 1969); *Heterakis mackerruensis* von Linstow, 1906; *Heterakis macabuensis* Vicente & Santos, 1967; *Heterakos nattereri* Travassos 1923; *Heterakis psophiae* Travassos, 1913; *Heterakis valdemucronata* (Molin, 1860); *Heterakis spiculatus* (Cobbold, 1861); and *Heterakis inglisi* Vicente, Pinto & Noronha, 1993].

Heterakis gallinarum was registered in *Gallus gallus domesticus* Linnaeus, 1758 in all regions of Brazil, in addition to registers in other bird's species in different locations in the country (Table 1).

There are also records of *H. gallinarum* infections in other countries, mainly in poultry production systems. The parasite was registered in Denmark, Germany, and the United States of America (Valadão, 2018). It is commonly found within the caecum of captive birds (Madsen, 1950) occasionally provoking bleeding in the intestinal mucosa thus causing serious inconvenience for breeders of slaughter birds, considering that severe

infections are often deadly (Cupo & Beckstead, 2019).

This parasite thrives in hosts such as *Numida meleagris* (Linnaeus, 1758) (helmeted guineafowl), *Phasianus colchicus* Linnaeus, 1758 (common pheasant), and *G. gallus domesticus* Linnaeus, 1758 (chicken), while in other host species there seems to be no adaptation efficiency (Lund & Chute, 1974). The prophylaxis to prevent this helminthiasis is also critical in avoiding the transmission of Trichomonadida protozoan *Histomonas meleagridis* Smith, 1895, which causes histomoniasis in birds, another critical infection that can be lethal and cause significant economic losses, because *H. gallinarum* is a vector species for this pathogen. Birds reared under improper space conditions are at higher risk of infection by these parasites (Cupo & Beckstead, 2019).

Regarding the nematode lifecycle, non-embryonic fertilized eggs are eliminated by the females in the host's caecum and are passed with the feces. Depending on environmental conditions (temperature and soil), the larvae can develop inside the egg (Saunders *et al.*, 2000). These eggs can remain infectious for several years until they are ingested by a new definitive (birds), paratenic or transport (earthworms - mainly *Lumbricus* Linnaeus, 1758 and *Eisenia* Michaelsen, 1900 and flies) hosts that may be consumed by the birds. The oviposition by the fertilized adult females starts between 24 up to 35 days.

Therefore, a better understanding and disclosure the presence of the parasites in different birds from

other locations can promote prevention and improve the morphological and evolutionary study of the nematode.

The present survey reports the occurrence of *H. gallinarum* in a bird commercialized at the metropolitan region of Vitoria, Espirito Santo, Brazil.

Table 1. Species of birds reported with *Heterakis gallinarum* in different localities of Brazil.

Bird species	Common name	Regions
<i>Cairina moschata domesticus</i> (Donkin, 1989)	Muscovy ducks	Southeast: MG Mid West: DF
<i>Chrysolophus pictus</i> (Linnaeus, 1758)	Golden pheasant	Southeast: SP
<i>Crypturellus noctivagus</i> (Wied, 1820)	Yellow-legged tinamou	Southeast: ES
<i>Crypturellus undulates</i> (Temminck, 1815)	Undulated tinamou	Southeast: MG Mid West: MT
<i>Crypturellus variegatus</i> (Gmelin, 1789)	Variegated tinamou	Southeast: ES
<i>Meleagris gallopavo</i> Linnaeus, 1758	Wild turkey	Southeast: MG Mid West: DF South: PR, RS
<i>Nothura maculosa</i> (Temminck, 1815)	Spotted nothura	Southeast: SP
<i>Numida meleagris</i> (Linnaeus, 1758)	Helmeted guineafowl	North: RO South: RS
<i>Pavo cristatus</i> Linnaeus, 1758	Indian peafowl	Southeast: SP Mid West: GO
<i>Phasianus colchicus</i> Linnaeus, 1758	Common pheasant	Southeast: SP
<i>Rhynchotus rufescens</i> (Temminck, 1815)	Red-winged tinamou	Southeast: SP
<i>Tinamus solitaries</i> (Vieillot, 1819)	Solitary tinamou	Southeast: ES, RJ North: PA

Source: Boroviec *et al.* (2020), Ramos *et al.* (2018), Vicente *et al.* (1993) and Vicente *et al.* (1995).

MATERIAL AND METHODS

A female of *G. gallus domesticus* acquired at the metropolitan region of Vitoria, Espirito Santo, in December 2019, was used for the study. After cervical dislocation euthanasia followed by exsanguination, as stated by the CFMV (2012), digestive tract (which was separated into parts), lungs, heart, kidneys, and liver were removed and placed separately in Petri dishes containing 0.85% saline.

Each organ was inspected using a stereomicroscope. The existing nematodes were collected, fixed in 10% hot formalin, and later transferred to 70% ethanol.

After clarification with Aman's lactophenol, the nematodes were mounted on a microscope slide, examined by a light microscope and their morphological characters measured using TSView 6.1 (Tucsen Imaging Technology Co. Limited). Drawings were made with CorelDraw® Home & Student Suite X8. The identification of the parasites was performed by using the taxonomic keys proposed by Vicente *et al.* (1995) and Anderson *et al.* (2009). The measurement units for the structures are given in micrometers unless otherwise specified. CHIOC 38924 a-b: *Heterakis gallinarum* (male = a; female = b).

Ethics aspects: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation

of data; in the writing of the manuscript, or in the decision to publish the results. The experimental procedures were conducted in accordance to institutional ethical guidelines.

RESULTS

Six specimens of nematodes (3 females and 3 males) found in the caecum of *G. gallus domesticus* from Vitoria, Espírito Santo, Brazil were identified as *H. gallinarum*.

Heterakis gallinarum (Schrank, 1788) Madsen, 1949

Diagnosis general. Robust body, whitish color, transversal ridges. Cephalic end with 3 round lips not connected by lateral lobes. Interlabia and labial tooth absent. One cephalic papilla on each lip. Esophagus relatively long, with a posterior bulb with a valve. Males shorter than females. Precloacal chitinous-rimmed sucker, caudal alae can be found together ventrally. The tail has short pedunculated and sessile papillae in pairs. Spicules unequal in size, similar in shape. Vulva near to half body. Uterus anfidelphic and didelphic.

Synonymies

Ascaris gallinarus Schrank, 1788

Ascaris gallinae Gmelin, 1790

Heterakis gallinae Gmelin, 1970

Ascaris vesicularis Froelich, 1791

Heterakis vesicularis Dujardin, 1845

Heterakis papilosa Railliet, 1885.

Male: (N=3) Length 6.64 mm (5.48–7.28) by 308.08 (403.90–251.65) wide. Pharynx 54.75 (51.87–57.62) long. Nerve ring 210.10 (160.35–293.64) and excretory pore 365.64 (319.36–411.93) from cephalic apex. Esophagus 681.84 (585.07–739.72) long and bulb 186.06 (178.40–190.29) long by 182.76 (159.52–197.48) wide. Present caudal alae, 368.70 (191.10–472.44) long by 161.84 (87.66–200.32) wide. Precloacal chitinous-rimmed sucker (Fig. 1, 2) 56.42 (34.11–70.99) long by 36.08 (31.42–38.95) wide, located 185.71 (111.98–231.81) from the cloaca and 689.27 (650.84–712.93) from the posterior end. Unequal spicules (Fig. 1), larger 1.41 mm (0.975–2.080), and smaller 625.39 (472.35–784.45) long. Eleven pairs of caudal papillae present and 2 unpaired: 2 pairs aside of the sucker; 1 sessile and 1 pedunculated pairs of precloacal papillae; 3 pedunculated and 1 sessile pairs of adcloacal papillae; 2 pedunculated and 1 sessile pairs plus 2 unpaired postcloacal papillae (Fig. 2). Slender tail with a pointed tip 227.40 (169.08–262.34) long (Fig. 1, 2).

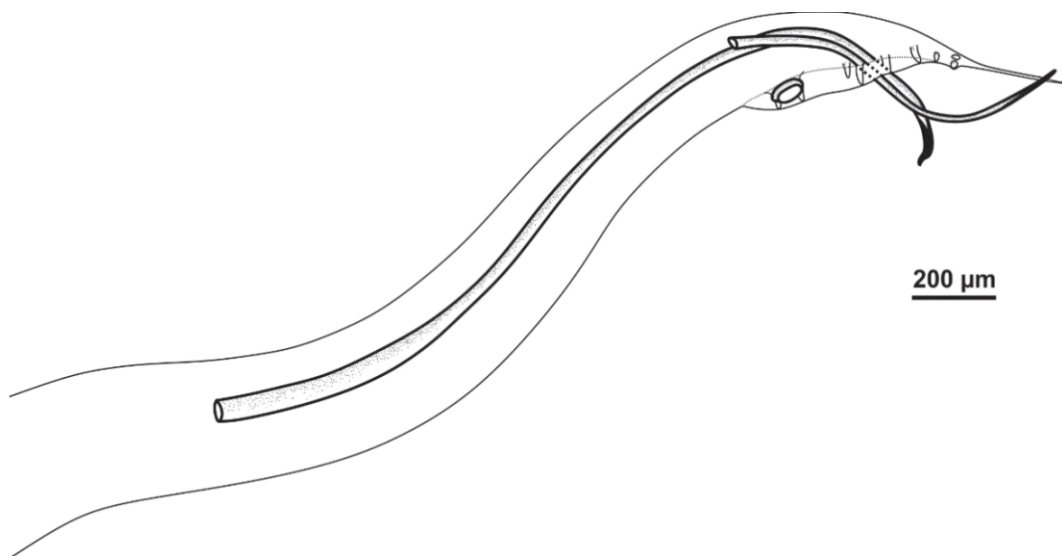


Figure 1. *Heterakis gallinarum*, male. Posterior end: spicules.

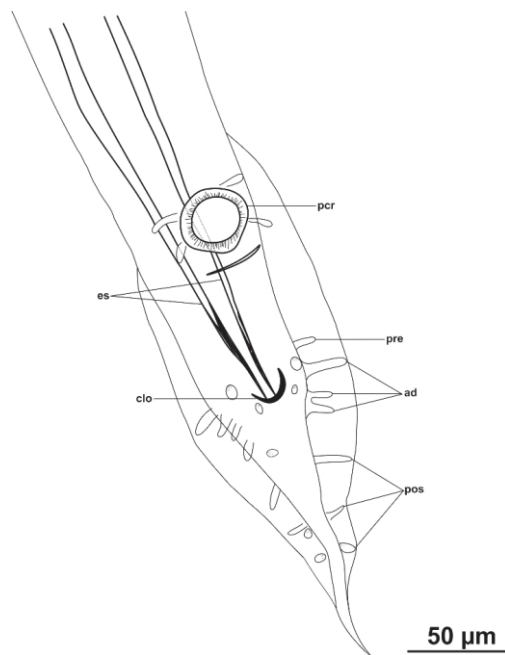


Figure 2. *Heterakis gallinarum*, male. Posterior end, ventral view. pcr: precloacal chitinous-rimmed sucker; es: spicules (terminal portion); pre: precloacal papillae; ad: adcloacal papillae; pos: postcloacal papillae; clo: cloaca.

Female: (N=3) Length 10.17 mm (9.31–10.92) by 392.62 (325.88–452) wide (Fig. 3, 4A). Pharynx 49.62 (34.92–73.54) (Fig. 3) long. Nerve ring and excretory pore located (Fig. 3) 212.42 (151.57–287.51) and 489.30 (419.31–530.50) from anterior end, respectively. Esophagus (Fig. 3, 4A) 770.74 (704.42–854.10) long and robust bulb 263.90 (232.24–290.55) long by 237.59 (222.98–261.48) wide (Fig. 3, 4A). Simple vulvar

opening 4.14 mm (1.99–5.64) from anterior end (Fig. 4A, 4B). Muscular and robust ovijector, divided in two, larger 741.27 (947.01–535.54) long and smaller 279.85 (133.90–425.80) long (Fig. 4B). Ellipsoidal, unembryonated, but different stages are observed, 101.17 (36.51–145.80) long by 59.25 (22.85–83.43) wide. Few pigmented eggs. Slender tail 0.9 mm (0.44–1.19) long.

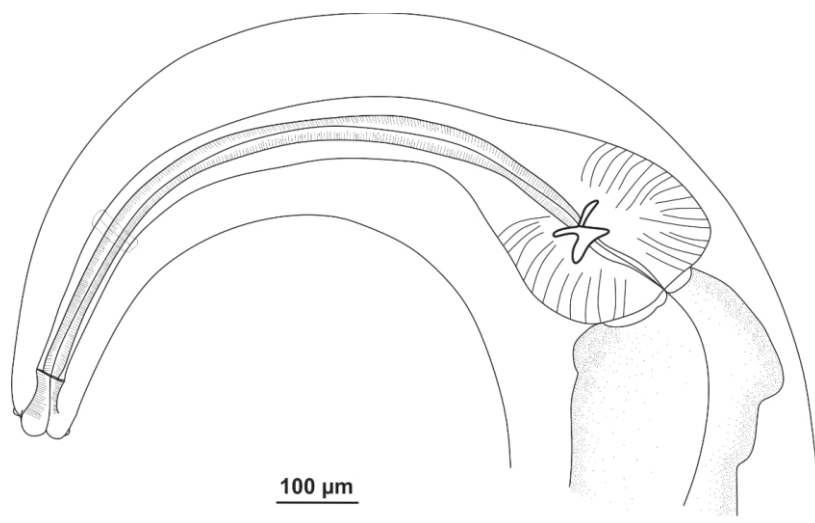


Figure 3. *Heterakis gallinarum*, female anterior portion, ventral view.



Figure 4. *Heterakis gallinarum*, female. **A.** Vulvar opening (arrow) and ovijectors **B.** Vulva and ovijector: detail.

DISCUSSION

In this study, the identification of the parasite was performed by morphological comparison, considering that molecular analysis using mitochondrial sequences that could assist in the process of identifying the species and building a *Heterakis* phylogeny is not well established (Wang *et al.*, 2016; Cupo & Beckstead, 2019).

In the current paper, the specimens analyzed, present the general characteristics described for the species, such as bulb length and position, presence and positioning of the precloacal sucker, spicules size, and caudal papillae projection in caudal alae.

The comparison of spicule structures and lengths, the position and number of caudal papillae, the relative length of sucker in relation to the cloacal aperture, and the length of the tail in the male are suitable taxonomic characters to differentiate species within the genus *Heterakis* (Iglis, 1967). Under this criterion, the species found differs from *H. ibanezi* (Freitas *et al.*, 1969) by the arrangement of the papillae, which presents 2 pairs of adcloacal papillae and 6 postcloacal papillae, while the specimen analyzed by this study displays twice as many papillae. Besides, *H. nattereri* (Travassos,

1923) has a greater amount of postcloacal papillae (8 pairs) and a shorter distance from the sucker to the anus (42 µm x 689.27 µm).

Another taxonomic characteristic observed in *Heterakis* which can be used while identifying species is the s-shaped curvature of the larger spicule at the end of the cloaca (Madsen, 1949, 1950), as observed in *H. gallinarum* (Fig. 1), but not found in *Heterakis isolonche* (Ramos *et al.*, 1953) nor *Heterakis dispar* (Cupo & Beckstead, 2019). The species also show differences in spicule sizes, about 0.99 / 2.20 mm in *H. isolonche* and 0.55 / 0.73 mm in *H. dispar*, compared to the 1,410 / 0.625 mm of *H. gallinarum*.

Furthermore, it was found that the females had eggs at different stages of development, but no larvae. These findings corroborate by Saunders *et al.* (2000) that *H. gallinarum* eliminates non-embryonic eggs in the external environment, where the larvae development occurs.

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