http://dx.doi.org/10.1590/1678-4162-12334



Arq. Bras. Med. Vet. Zootec., v.73, n.4, p.885-892, 2021

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Lesions caused by anisakid and capillariid in *Cairina moschata* raised on Marajó island, state of Pará, Brazil

[Lesões por anisakideos e capillariideos em Cairina moschata criados na Ilha de Marajó, estado do Pará, Brasil]

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ABSTRACT

The Muscovy duck is a commercially important bird on the island of Marajó usually raised in a peculiar system that includes supplying fish viscera to the birds under semi-extensive farming conditions. This enables a risk of contamination and losses in the production of these birds, resulting from injuries caused by helminth infections, especially nematodes. The objective of this study was to evaluate the histopathological changes caused by nematodes of the genera: *Eucoleus, Anisakis* and *Contracaecum*. Thirty-three ducks with lesions in the esophagus and ventricle were analyzed. Histopathological exams showed a mild inflammatory infiltrate in the submucosa of the esophagus caused by the fixation of *E. contortus* and third stage larvae of *Anisakis* sp., and we recorded third stage larvae of *Contracaecum* sp. parasitizing the ventricle, this being the first record of this parasite in ducks in Brazil.

Keywords: histopathology, esophagus, birds, parasitism, helminths

RESUMO

O pato doméstico é uma ave amplamente comercializada na Ilha de Marajó, com um peculiar manejo que inclui a oferta de vísceras de peixes aos animais em criações semiextensivas, propiciando, assim, risco de contaminação e perdas na produção dessas aves decorrentes de lesões oriundas de infecções por helmintos, especialmente os nematódeos. Nesse sentido, objetivou-se avaliar as alterações histopatológicas causadas por nematódeos dos gêneros: Eucoleus, Anisakis e Contracaecum. Foram analisados 33 patos, e três exemplares apresentaram lesões no esôfago e no ventrículo. Exames histopatológicos demonstraram discreto infiltrado inflamatório na submucosa do esôfago ocasionado pela fixação de E. contortus e larvas de terceiro estágio de Anisakis sp., bem como foram registradas larvas de terceiro estágio de Contracaecum sp. parasitando o ventrículo, sendo esse o primeiro registro desse parasito em patos no Brasil.

Palavras-chave: histopatologia, esôfago, aves, parasitismo, helmintos

INTRODUCTION

Cairina moschata ducks (Linnaeus, 1758) are bred for the production of eggs and meat for both self-consumption and for sale (Meulen and

Recebido em 9 de fevereiro de 2021

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Dikken, 2003). Muscovy ducks are birds that have filtering habits and are not selective in terms of food, especially when raised extensively, where they absorb enough proteins in the environment, feeding on grass, small fish, crustaceans and insects (Sick, 1997; Meulen and Dikken, 2003). Livelihood creations are common

Aceito em 22 de abril de 2021

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in Brazil, and occur mainly among small producers, commercial houses and open markets, where the hygienic sanitary conditions of this type of creation are not clarified (Souza Almeida *et al.*, 2016). In northern Brazil they are appreciated in the cuisine of Pará and have high commercial value mainly during festive seasons.

Breeding birds under extensive or free-range systems in the environment facilitates the occurrence of endoparasites. That is a point of concern in the different systems of duck farming as it leads to economic losses, and preventive measures are considered the most effective strategy (Rennó et al., 2008). The rate of helminth infection is worrisome, especially since ducks may show unspecific clinical signs of those infections (Cubas, 2007; Rosa and Shivaprasad, 2015). In addition, these helminthiasis are the main diseases that affect birds reared in an extensive regime, causing an increase in the mortality rate, as well as providing the dissemination of a wide variety of parasite species in the environment (Menezes, 1999; Gomes et al., 2009). Among the main changes in the host organism caused by the parasites are spoliation and the inflammatory process resulting from the process of migration, fixation or type of food performed by the parasite, which can vary with the degree of intensity of infection (Menezes et al., 2001; Neves, 2016).

Menezes et al. (2001) described macro and microscopic changes found in several organs, among them Numida meleagris Linnaeus, 1758 esophagus and crop parasitized by Eucoleus perforans, of the macroscopic changes the authors observed that the parasites caused petechiae and congestion, and microscopy showed the parasites inserted deep in the stratified squamous epithelium of the crop, with intense inflammatory reaction and distension of the mucous glands present in the crop's own tunic. These changes by the parasite were considered serious, even with low average intensity of infection. Data on helminths in ducks are scarce and little known, particularly about the spoliative action of the presence of parasites and their relationship with host tissue (Mattos Junior et al., 2008). The investigation of the biota of helminths of Muscovy ducks on the Island of Marajó, can help in the sanitary control and in the productivity of these animals. This study thus

aimed to describe the histopathological aspects of infection and lesions in the tubular digestive system of *Cairina moschata*, caused by *Eucoleus contortus* (Capillariidae), *Anisakis* sp. (Anisakidae) and *Contracaecum* sp. (Anisakidae), in order to contribute to the medical clinic and sanitary management of this species, of this bird, raised free on the Island of Marajó.

MATERIAL AND METHODS

Thirty-three specimens of C. moschata were purchased from rural properties in the municipality of Soure (00° 43' 00" S; 48° 31' 24" W), Marajó Island, State of Pará, under protocols of the Ethics Committee on the use of animals (CEUA) No. 030/2018 and the Federal Rural University of the Amazon (UFRA) No. 23084.014807 / 2018-80. They were necropsied in the laboratory to search for helminths, where each organ was carefully analyzed with the aid of the Leica ES2 stereomicroscope, and three specimens presented nematodes fixed to the esophagus and ventricles, from which fragments were removed and fixed in 10% formalin, and processed according to routine histological techniques 2003). (Tolosa et al., Photomicrographs of the slides were captured and analyzed using a Leica DM2500 microscope with an attached digital camera.

The nematodes collected were fixed in A.F.A (93 parts of 70% ethyl alcohol, 5 parts of formaldehyde and 2 parts of glacial acetic acid), overnight, transferred to a solution containing 70% ethanol. For taxonomic identification, the nematodes specimens were clarified with 20% Aman lactophenol and temporarily mounted between slides and coverslips for observation of morphological characters under a LEICA DM2500 light microscope with an imaging capture system. For the taxonomic classification of nematodes, the works of Vicente et al. (1995). Moravec (1998), De Ley and Blaxter (2002), Felizardo et al. (2009), Gibbons (2010) and Fonseca et al. (2016) were consulted. To determine the ecological indexes of parasitism, these helminths will be analyzed by means of prevalence (%), average intensity of infection (IMI) and average abundance, according to Bush et al. (1997).

RESULTS

The nematodes recovered were found inserted in the esophagus and ventricle of muscovy duck in Marajó Island and morphologically identified as third stage larvae *Anisakis* (Anisakidae) (Figure 1A), and adults from *Eucoleus contortus* (Capillariidae) were also found parasitizing the esophagus with a prevalence of 9.1% and 75.8% respectively. Third stage larvae of *Contracaecum* (Anisakidae) were found inserted in the ventricle in 12.1% of the ducks. The parasitological indices of these nematodes in *Cairina moschata* are shown in Table 1.

Table 1. Parasitological indices of Capillariidae and Anisakidae in *Cairina moschata* (n = 33) from the eastern Amazon (Brazil)

SI	Parameters	Eucoleus contortus	Anisakis sp.	Contracaecum sp.
Esophagus	P (%)	75.8	9.1	3.0
	MI	11.2	95.7	3.0
	MA	85	8.7	0.1
	TNP	281	287	3
Gizzard	P (%)	9.1	9.1	0
	MI	10.7	0.3	0
	MA	1	0.03	0
	TNP	32	1	0
Proventriculus	P (%)	12.1	0	6.1
	MI	17.3	0	3.5
	MA	2.1	0	0.2
	TNP	69	0	7
Ventriculus	P (%)	0	0	12.1
	MI	0	0	2.3
	MA	0	0	0.3
	TNP	0	0	9
Intestine	P (%)	0	3.0	3.0
	MI	0	1.0	2.0
	MA	0	0.03	0.1
	TNP	0	1	2

SI: infection site, P: Prevalence, MI: Mean intensity, MA: Mean abundance, TNP: Total number of parasites.

Histopathological analysis of infection by Anisakis sp. in the esophagus of Cairina that moschata, showed the lesion is predominantly in the mucosa, and able to transpose the muscle of the mucosa and affect the internal muscular layer. The stratified squamous epithelium was found to be intact. However, in the parasite-host relationship, alterations are present in the mucous glands due to the location of the nematodes, which promotes glandular destruction and tissue reaction with accumulations of cellular debris at the tissueparasite interface, bordered by a foreign bodytype granulomatous reaction due to the presence of giant cells (Figure 1B). The lamina propria with prominent and variable infiltration of eosinophils in addition to lymphocytes and staining by Gomori Trichrome did not show fibroplasia associated with inflammation. The connective tissue adjacent to the compromised gland was looser, probably due to tissue edema.

In the cross section of the parasite *Anisakis*, it was possible to observe the epidermal cord in a "Y" shape (Figure 1C).

Histologically, the infection of the ventricle (gizzard) by Contracaecum sp. demonstrates that the nematode is fixed in the mucosa, below the glycoprotein secretion layer, where no tissue reaction due to the presence of the parasite was observed (Figure 1D). In the macroscopic image, we can observe the fixation of the parasite in the ventricle (Figure 1E). Esophageal infection by contortus Eucoleus showed pseudoencapsulation of the parasite in the mucosal pavement epithelium, with mild compressive atrophy of keratinocytes. There was also mild exocytosis due to the presence of eosinophils among the keratinocytes and in the submucosa the inflammatory infiltrate of eosinophils is mild and variable (Figure 1F).

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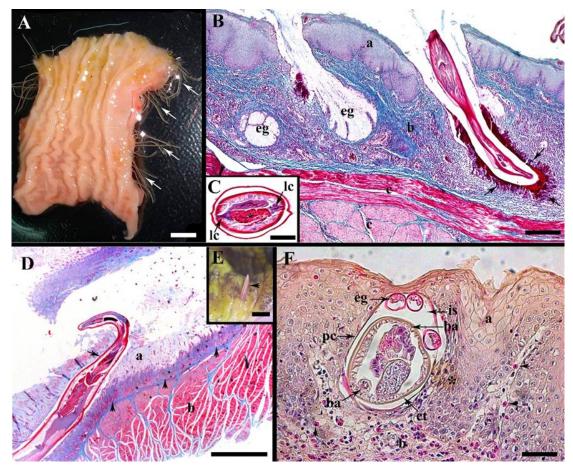


Figure 1. (A) Mesoscopic view of *Anisakis* sp. (arrowhead) inserted in the esophagus of a Muscovy duck on Marajó Island, State of Pará; (B) Photomicrographs of the esophagus parasitized by *Anisakis* sp. larvae (arrows) stained with Gomori Trichrome: a = stratified squamous epithelial tissue, of the esophageal lining, b = subepithelial connective tissue, c = smooth muscle tissue and eg = esophageal gland; (C) Detail of the cross section of an *Anisakis* sp. larva, showing lateral epidermal cord (arrow) and intestine (*). Gomori Trichrome; (D) Photomicrographs of the ventricle parasitized by *Contracaecum* sp. in Muscovy duck on Marajó Island stained with Gomori Trichrome: a = mucous layer of the ventricle, tubular glands of the ventricle (arrowhead) and b = muscular layer of the ventricle, showing the well-developed smooth muscle layer; (E) Mesoscopic view of a *Contracaecum* sp. (arrowhead) inserted in the Muscovy duck on Marajó Island stained with hematoxylin and eosin: a = stratified squamous epithelial tissue of the lining of the esophagus mucosa, b = loose connective tissue of the submucosa with abundant eosinophils (arrowhead), detail of the pseudo-capsule (pc) surrounding the parasite, forming an interstitial space (s) containing eggs (eg), and the nematode with the presence of a thick cuticle (ct) and the presence of bacillary bands (ba), compressive atrophy of keratinocytes (*) of the stratified squamous epithelial tissue of the esophagus. Scale bars: A = 2 cm, B = 200 µm, C = 100 µm, D = 1 mm, E = 2 cm; F: 50 µm.

DISCUSSION

In Brazil, Mattos Junior *et al.* (2008), detected in *Cairina moschata* of Rio de Janeiro a parasitic biota of capillariids nematodes composed of 20% of *Capillaria phasianina* Kotlán, 1914 in esophagus and caecum; 30% of *Capillaria* sp. Pinto and Almeida, 1935 in esophagus, cecum and gallbladder, and 6.6% of *Eucoleus cairinae* (Freitas and Almeida, 1935) Lopez and Neyra,

1947 in esophagus, and histopathological studies of organs injured by parasitism have not yet been published. In our study, the major sites of infection were esophagus with *Eucoleus contortus* and *Anisakis* sp. and the ventricle with *Contracaecum* sp., these sites showed lesions by the parasites, and after histopathological processing it was possible to observe the changes caused by capillary and anisakid nematodes in the tissues. Histopathological changes caused by the presence of Eucoleus contortus, Anisakis sp., Contracaecum sp., are recorded herein for the first time in our research parasitizing the esophagus and ventricle of Muscovy duck in Brazil. These birds raised without confinement, look for food in areas of rivers or lakes, with no selectivity in feeding (Meulen and Dikken, 2003), which may justify the presence of these anisakid and capillary nematodes in birds (Carvalho et al., 2019, 2020). Different studies have been carried out in order to characterize the parasitic fauna of ducks around the world. Among them Bano et al. (2005) recorded the presence of Streptocara incognita Gibson, 1968 in the esophagus of ducks in Italy. In the same host, Muhairwa et al. (2007) recorded E. contortus, E. annulatus (Molin, 1858) and Capillaria anatis (Schrank, 1790) parasitizing the intestinal caecum. Likewise, Yousuf et al. (2009), when assessing the fauna present in the gastrointestinal tract of ducks, registered the presence of Amidostomum anseris and E. contortus.

The presence of E. contortus and third stage larvae of Anisakis sp. inserted in the esophageal tissues of Muscovy ducks induced an inflammatory reaction in the tissue of this organ, as well as the migration of defense cells to this region, especially to the site of larval fixation, caused intense inflammatory infiltrate resulting from foreign body reaction and edema. Railliet and Lucet (1889) described the pathogenic effects by means of hispathological analysis in the gall bladder and esophagus of Galliformes and Anseriformes parasitized by E. contortus, thus demonstrating a major inflammatory process. According to Cram (1936), the Galliformes analyzed in his research were infected with E. contortus eggs and after death revealed necrosis of the esophageal epithelium, connective tissue capsules delicate that surrounded the areas containing the parasites and their eggs, tissue with lymphocytes and large mononuclear leukocytes, with some lesions extended to the submucosa. Pinto et al. (2004) observed lesions by the parasite Eucoleus perforans in the esophagus of Phasianus colchicus, where the parasites were deeply inserted in the stratified squamous epithelium, without inflammatory reaction, there was also thickening and papillary transformation of the mucosa.

Several third-stage larvae of Anisakis sp. were found strongly inserted in the esophagus of Muscovy ducks, causing an intense inflammatory infiltrate, resulting from a foreign body reaction and edema. According to Junqueira (2018), parasites that affect the crop and esophagus are especially harmful, as they can severely damage the mucosa of the affected organ, inducing edema and thickening of the mucous membranes, making eating difficult and even preventing birds from eating food. In cross section of the esophagus of the birds in our study, it was possible to observe the larvae of Anisakis sp. and their identifying structures. Murata et al. (2018) and Lauwers et al. (2017), who recorded the occurrence of anisakiasis in humans, was found as a definition of gender, found the shape of the lateral epidermal cord and the intestinal lumen in "Y" to be determinants for Anisakis spp. Kim et al. (2006) during histopathological analysis in humans recorded a lesion in the submucosa of the stomach that showed severe eosinophilic infiltrations and inflammation.

Anisakids, in their larval and adult forms frequently present important pathological lesions in the alimentary tract and associated organs in their natural host species (Smith, 1999). According to Lymbery and Cheah (2007), the larvae of these nematodes also promote a series of pathological effects in accidental hosts, such as humans. This reinforces the need for attention and care in proper management during the removal and disposal of the viscera of ducks infected with L3 larvae of anisakids, avoiding the migration of these larvae to the musculature of the bird in the postmortem and the possible risks to human health due to ingestion of contaminated meats (Carvalho et al., 2020). In this study, the presence of Contracaecum sp. in the ventricle did not produce injuries in that organ. In contrast, Vicente et al. (1995) and Amato et al. (2006), registered the presence of different larval stages Contracaecum of sp. parasitizing the proventriculus and ventricles of birds of the orders Falconiformes. Accipitriformes, Pelecaniformes, Suliformes, Ciconiiformes, Sphenisciformes, inducing the formation ulcerated eosinophilic granulomas.

Among the parasites that present risks to human health, nematodes of the Anisakidae family can be highlighted. Their intermediate hosts are fish cephalopod molluscs, and small crustaceans

(Adams et al., 1997), all animals that can compose the diet of ducks raised free in the wild. In Brazil, investigations related to helminthinduced diseases have been described in other bird species such as Phasianus colchicus and Meleagris gallopavo by Pinto et al. (2004, 2008). In terms of pathogenesis, when there is a major infection it can be considered extremely harmful to birds (Pizarro et al., 2000). The clinical signs presented by Muscovy ducks can be nonspecific (Rosa and Shivaprasad, 2015), meaning that helminthiasis can lead to an economic problem, as well as to the health of domestic poultry breeding systems (Cubas, 2007). Therefore, preventive health care such as good hygiene, deworming and vaccination of birds can reduce the rate of emergence of various parasites and other diseases (Meulen and Dikken, 2003).

CONCLUSION

The Muscovy duck is the new host of *Contracaecum* sp. in the state of Pará, being important, as there were still no studies on this parasite in this bird. And we also added the description of histopathological changes, such as intense inflammatory reactions in the esophagus caused by *Anisakis* sp. and *Eucoleus contortus*, and the presence of *Contracaecum* sp. in the ventricle, thus contributing to the knowledge of the pathogenicity of these parasites in *Cairina moschata* created and consumed by the island's population.

ACKNOWLEDGMENTS

The authors are grateful the Programa de Pós-Graduação Saúde e Produção Animal na Amazônia (PPG-SPAA), Instituto da Saúde e Produção Animal (ISPA), Universidade Federal Rural da Amazônia (UFRA). This study was part of the master's dissertation of Elaine Lopes de Carvalho, developed for the PPG-SPAA. This study was financed in part by the Coordenação de Aperfeicoamento de Pessoal de Nível Superior (CAPES - BRASIL) - Finance Code 001. Raul Henrique da Silva Pinheiro was supported by a research fellowship from the "Programa de Pós-Graduação em Sociedade, Natureza e Desenvolvimento (PPGSND), Universidade Federal do Oeste do Pará -UFOPA/ CAPES/ BRASIL) - Finance Code 001". Dra. Elane Giese was supported by a researcher fellowship of Conselho Nacional de

Pesquisa e Desenvolvimento Tecnológico (CNPq-Brasil) (#313763/2020-8).

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