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IDENTIFICATION OF PARASITES AND GROSS FINDINGS IN MAGELLANIC PENGUINS IN ESPÍRITO SANTO, BRAZIL

(*Identificação de parasitos e achados macroscópicos de necropsia em pinguins-de-Magalhães no Espírito Santo, Brasil*)

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ABSTRACT: Penguins are marine birds extremely well adapted to life in the sea but it is not rare to find lifeless individuals along the coast and deaths are caused by factors relating both to human influence on the environment and to specific diseases The aim of the present study was to identify parasite species found in Magellanic penguins on the coast of Espírito Santo, Brazil and describe the main gross findings. Five birds presented ectoparasites. All the birds presented nematodes. Four individuals presented trematodes. All the penguins presented a body state score of 2. Two of the individuals examined presented rupture of the esophagus caused by a foreign object and one individual presented a foreign object in the stomach. In the respiratory system, the lesions found were lung congestion, fibrin in the air sacs, caseous content inside the trachea, lung edema and pneumonia. Magellanic penguins on the coast of the state of Espírito Santo are susceptible to parasitoses. Helminths of the species Contracaecum pelagicum and Cardiocephaloides physaliswere identified, along with the ectoparasite Austrogoniodes bifasciatus. The main macroscopic lesions found were located in the respiratory system and gastrointestinal tract.
Keywords: ectoparasite; foreign body; helminth; respiratory lesions

RESUMO: Pinguins são aves marinhas extremamente bem adaptadas à vida no mar, mas não é raro encontrar indivíduos mortos ao longo da costa e as mortes são causadas por fatores relacionados tanto à interferências humanas no meio ambiente quanto por doenças específicas. Objetivou-se com este estudo identificar espécies de parasitos encontradas em pinguins-de-Magalhães na costa do Espírito Santo, Brasil, e descrever os principais achados macroscópicos de necropsia. Cinco aves apresentaram ectoparasitos. Todas as aves tinham nematodeos. Quatro indivíduos tinham trematodeos. Todos os pinguins estavam com escore de condição corporal 2. Dois dos indivíduos examinados apresentavam ruptura de esôfago por corpo estranho e um indivíduo apresentou corpo estranho no estômago. No sistema respiratório, as lesões encontradas foram congestão pulmonar, fibrina nos sacos aéreos, conteúdo caseoso na traqueia, edema pulmonar e pneumonia. Pinguins-de-Magalhães na costa do estado do Espírito Santo são suscetíveis a parasitoses. Helmintos das espécies Contracaecum pelagicum e Cardiocephaloides physalis foram identificados, bem como ectoparasitos da espécie Austrogoniodes bifasciatus. As principais lesões macroscópicas encontradas estavam localizadas no sistema respiratório e no trato gastrointestinal.
Palavras-chave: corpo estranho; ectoparasito; helminto; lesões respiratórias.

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INTRODUCTION

Penguins are marine birds that are extremely well adapted to life in the sea. Many species are migratory, covering long distances in search of food and reproduction localities. The species most commonly seen on the Brazilian coast is the Magellanic penguin (Spheniscus magellanicus) (Cubas et al., 2007). This species reproduces in Argentina, Chile and the Falkland Islands and starts its migration towards the Brazilian coast searching for food on the continental shelf in states from Rio Grande do Sul until São Paulo, and vagrant animals can be found along the coastline of Rio de Janeiro and Espírito Santo states (Vanstreels et al., 2017).

In Brazil, it is not rare to find stranded individuals (Vanstreels et al., 2017), in most cases requiring human intervention and the mean rehabilitation period of these debilitated animals is one to two months (Coraiola et al., 2014). Penguins are often found dead along the coast and deaths are caused by factors relating both to human influence on the environment and to specific diseases (Duignan, 2001; Mäder et al., 2010).

Among the factors that contribute towards weakening these penguins, parasitosescan be highlighted. Parasites have a variety of impacts on their hosts, such as increased energy demands, altered behavior, reduced fecundity and growth, and even mortality. Basically, all organs and tissues can be damaged by parasitic organisms and damages have different effects to the host health depending on the severity of the damages (Marcogliese, 2004; Rezende et al., 2013). Identification of parasite species is important in order to define host biology and health, because parasites are indicators of the places that these animals have visited during their migration and they canindicate seasonal changes in diet,and also because these informations can be useful to understand host phylogeny (Brandão et al., 2014).

Studies on penguins in Brazil can be justified by the large numbers of these birds that are found dead or alive on the Brazilian coast (Mäder et al., 2010) and Brazilian researchers have prioritized and favored studies on Magellanic penguins. Thus, surveys on the main diseases, causes of death and geographical distribution of these individuals have been conducted (Cubas et al., 2007).

Considering the importance of correctly diagnosing the main diseases that occur in Magellanic penguins, as well as the importance of knowing the etiology of these diseases, the aim of the present study was to identify parasite species found in Magellanic penguins on the coast of Espírito Santo, Brazil and describe the main gross findings both relating and not relating to parasitism.

MATERIAL AND METHODS

Sixteen Magellanic penguins that died at the Instituto de Pesquisa e Reabilitação de AnimaisMarinhos (IPRAM), in Cariacica, Espírito Santo, Brazil, were sent to the Department of Animal Pathology of the Veterinary Hospital (HOVET), Universidade Federal do Espírito Santo (UFES). These animals were donated by IPRAM for taxidermy so that they could be included in the zoological collection of the Natural History Museum of the South of Espírito Santo. The corpses were transported in a frozen state from IPRAM to HOVET (under IBAMA permit no. 045/12-NUFAU/DIPRAM/IBAMA/ES, IBAMA 5176002, procedural no. 02009.001225/10-17), where necropsy was carried out, followed by referral to the museum. The necropsies were performed using the standard pathology laboratory technique of UFES.

The helminths found were fixed in formaldehyde and ectoparasites were

fixed in alcohol. In both cases, specimens were sent to the parasitology laboratory for species to be identified. The helminthswere clarified in acetic acid P.A. and were mounted in balsam. They were classified as proposed by Vicente et al. (1995) and Niewiadomska (2005). The ectoparasites were identified as described by Brum and Becker (2002). Micrographs of some morphological characteristics were obtained using an optical microscope and a camera.

RESULTS

Upon external examination, five birds presented ectoparasites classified as Austrogoniodes bifasciatus and all the penguins that were necropsied presented a body state score of 2 (low fat).

All the birds presented helminths that were identified as nematodes of the species Contracaecum pelagicum, and these were located at sites from the oropharynx to the intestine. In addition to roundworms, four individuals also presented trematodes of the species Cardiocephaloides physalis in the intestine, thus resulting in prevalence of 25% in this study population (Table 1).

Two of the individuals examined presented rupture of the esophagus caused by a foreign object (fishhook), and one individual presented a foreign object (fishhook) in the stomach. In one of the cases, presence of ulcers was observed over the whole length of the esophagus. Other lesions found in the gastrointestinal tract included lymphoid tissue hyperplasia, ulcers, hyperplasia, hyperkeratosis and mucosal nodules. Hemorrhagic enteritis was seen in two corpses.

In the liver, congestion, pseudomelanosis and whitish stains were observed. The spleen presented congestion, rarefaction of lymphoid tissue and pseudomelanosis. The kidneys showed fibrotic areas, congestion, pallor (degeneration), adhered capsule, yellowish secretion on the pelvis and pseudomelanosis. The heart presented fibrin in the pericardial sac, congestion and impregnation of hemoglobin.

Table 1 - Quantification and identification of parasites found during necropsies on each individual of Spheniscus magellanicus.

Bird registration number	Number of specimens of *Contracaecum pelagicum*	Number of specimens of *Cardiocephaloides physalis*
N32/12	8	0
N33/12	2	0
N34/12	54	0
N35/12	2	0
N36/12	24	3
N37/12	76	0
N38/12	19	0
N39/12	16	0
N40/12	57	14
N41/12	1	+*
N42/12	17	0
N43/12	7	2
N44/12	+*	0
N45/12	2	0
N46/12	16	0
N47/12	4	0

* Parasites were found but it was not possible to quantify.

In the respiratory system, the lesions found were lung congestion, fibrin in the air sacs, caseous content inside the trachea and lung edema; in addition, pneumonia was detected in three birds.

DISCUSSION

Five birds presented ectoparasites classified as Austrogoniodes bifasciatus. Brum and Becker (Brum and Becker, 2002) also identified this louse species in Magellanic penguins found in Rio Grande do Sul, while Rodrigues-Guimarães et al. (2009), studying a specimen found on the coast of the state of Rio de Janeiro, counted 275 ectoparasites. Even though ectoparasite quantification was not performed in the

present study, identification of this species on the coast of the state of Espírito Santo provides information about the epidemiology and geographical distribution of parasitoses along the entire Brazilian coast, and this is important for better understanding of factors that contribute towards weakness in these birds.

All the birds presented helminths of the species Contracaecum pelagicum. Ederli et al. (2009) also found specimens of this species in one penguin that was found dead in Espírito Santo, while in the state of Rio de Janeiro, two different species in the genus Contracaecum were found (Campos et al., 2013). This species is considered to be the one most frequently seen during necropsy, but data involving live penguins are still scarce (Campos, 2011). Recently, Borges et al. (2014) published the first genetic characterization of C. pelagicum found in S. magellanicus in Brazil and this data is an important tool for rapid species' identification in future studies.

The life cycle of Contracaecum spp. involves fish that act as intermediate, paratenic or meta-paratenic hosts when they ingest copepods (Acartia tonsa) infected with the L3 larvae or parasite eggs. After aquatic birds have eaten infected fish, they too become infected with the L3 larvae and subsequently develop the adult parasite (Garbin et al., 2013). Even specimens of Contracaecum spp. taken from penguins undergoing decomposition still have viable eggs, and this allows a greater dispersion rate for the eggs (Barquete, 2006). In this way, the parasite can infect a larger number of hosts, because egg dissemination continues for a few days after host death. This could be a possible explanation for the high prevalence found in the present study.

Four individuals presented trematodes of the species Cardiocephaloides physalis, thus resulting in prevalence of 25% in this study population. Similar data have been reported in a study on the coast of the state of São Paulo, which found prevalence of 22.4% (Rezende et al., 2013). The fish Engraulis anchoita is the main component in the diet of Magellanic penguins during their breeding season and this fish species is an intermediate host in the life cycle of C. physalis. The low prevalence found in the present study can be correlated with the time of migration, because there is evidence that the parasite is absent from E. anchoita in northern Argentina during autumn but is prevalent during spring (Rezende et al., 2013). Therefore, the low number of infected penguins could also be explained by ingestion of non-infected fish during migration and specific researches are needed to provide these data.

Another possible explanation for this phenomenon relates to dietary changes. Silva (2013) concluded from stomach content analysis that Magellanic penguins found in Brazilian coast consume large quantities of Argonauta nodosa and that this cephalopod species becomes the main component of the diet during migration towards the Brazilian coast. In this way, changes to the main source of food during migration can influence the quantity and variety of parasites infecting Magellanic penguins. There is not enough data about infection rates in intermediate hosts in Brazilian seas, the periods of parasitosis transmission in this country and the real role of the Brazilian coast in disease dissemination. Hence, further studies on this subject are needed in order to attempt to reach better understanding about illnesses that attack these birds and the causes of their deaths along the Brazilian coast.

Some other research has suggested that occurrences of endoparasitism seem to be more related to the region inhabited by the birds than to the season of the

year. While in Magellanic penguins the presence of endoparasites is a common finding, their presence is only infrequently seen in Little penguins (Eudyptula minor), which inhabit the coast of New Zealand (Hocken, 2000). Hocken (2000) and Hocken (2005) evaluated Little penguins and Yellow-eyed penguins (Megadyptes antipodes) that died in New Zealand, and found that merely because the two populations occupied different regions of the coast, one presented considerable endoparasitism whereas the other was not parasitized. Considering that parasites can be found in penguins along the entire Brazilian coastline, it can be suggested that the Brazilian coast, as a whole, offers favorable conditions for the development of endoparasites and infections in these birds. However, considering that the Magellanic penguins found in the Brazilian coast are migratory birds, the possibility that these animals arrived on this coast already containing parasites also exists, i.e. that they became infected in foreign seas in the southern part of South America.

Endoparasitesare commonly found in necropsies on penguins and they may endanger individuals' survival and thus contribute towards population decline. Initially, parasitism creates a nonspecific state and its signs are usually apathy, anorexia and edema. However, during situations such as intense stress, starvation and immunosuppression, the damage may be so severe that it can impede the success of conservation efforts. This condition also generates a scenario of debility that leaves these birds weakened and more susceptible to predator attacks and concomitant diseases (Campos et al., 2013; Campos, 2011; Duignan, 2001; Yáñez et al., 2012).

All the penguins that were necropsied presented a body state score of 2. This low body score is an important debilitating factor among these birds, and starvation is assumed to be one of the main causes of death (Hocken, 2000; Hocken, 2005). Recent studies have indicated that the fishing industry remove so many fish from the sea that it is responsible for a decline in the food available for penguin populations (Furness, 2003; Trathan et al., 2014). Furthermore, climatic and animal health factors may aggravate the situation of cachexia and inanition (Campos et al., 2013; Carvalho et al.,2012; Duignan, 2001). In the present study, the data suggest that cachexia may be a result from parasitic infection. However, the possibility that these birds may be having difficulty in finding food during migration should be considered, caused by climatic factors, fish population decline due to predatory fishing or environmental pollution.

Two of the individuals examined presented rupture of the esophagus caused by a foreign object (fishhook), and one individual presented a foreign object (fishhook) in the stomach. Foreign objects in the gastrointestinal tract have been reported in the literature and represent an important type of human interference in the survival of marine species (Carvalho et al., 2013).The interactions between the fishing industry and the environment are harmful for penguins, because large quantities of marine debris are left behind and become a threat to these birds (Trathan et al., 2014). Also, human residues that contaminates the seas are eaten by penguins and a study developed in the Brazilian coast found out that the prevalence of solid residues in the stomach of these birds reaches numbers close to 50% (Silva, 2013).

In the present study, ulcerative lesions in the esophagus and stomach were associated with mechanical trauma of the mucosa caused by foreign objects. Differently, according to Yáñez et al. (2012), ulcers with a yellow border and

caseous appearance that are present in the glandular stomach are related to intense parasitism by Contracaecum spp. Similarly, Duignan (2001) and Campos et al. (2013) correlated these parasites with gastric ulceration leading to hemorrhage, which may contribute towards the debility of these birds.

Cardiac congestion was also described by Carvalho et al. (2012) in some necropsied specimens of Magellanic penguins. Furthermore, lesions arising from autolysis were frequently found, such as pseudomelanosis and impregnation of hemoglobin, which may be associated with the long time that elapsed between death and necropsy among these birds, along with the freezing process to which the corpses were subjected.

In the respiratory system, the lesions found were lung congestion, fibrin in the air sacs, caseous content inside the trachea, lung edema and pneumonia. Lesions in the airs sacs can be caused both by viral and bacterial infections. Kincaid et al. (1988) reported that the presence of whitish exudate in thoracic air sacs might be a consequence of infection by herpesvirus, while Osório et al. (2017) reported that sistemic infection caused by Escherichia coli can cause air sacs opacity. Hocken (2000) correlated the caseous content in air sacs with chronic fungal infections, and exudative tracheobronchitis with acute infection. Lung edema is considered a common finding in cases of bird malaria due to Plasmodium spp. (Fix et al., 1988), though this is not an exclusive characteristic of this disease. Besides, avian malaria usually causes liver and spleen enlargement and hydropericardium in penguins (Vanstreels et al., 2016) and those findings were not present in our study. It should be emphasized that Campos et al. (2015) found that endoparasites occur at higher frequency in birds infected with Plasmodium spp., thus showing that immunosuppression caused by this protozoon could predispose towards parasitism, and this association of factors culminates in the birds' death.

Lesions in the respiratory tract are frequent occurences, and some authors have identified these as the most common natural cause of death among penguins (Hocken, 2005). However, it can be seen that the existence of alterations in organs of the respiratory system may have various causes, and therefore, evaluations of greater specificity, such as serological, microbiological and histopathological evaluations, are required in order to reach a definitive diagnosis.

CONCLUSION

Magellanic penguins on the coast of the state of Espírito Santo are susceptible to parasitoses. Helminths of the species Contracaecum pelagicum and Cardiocephaloides physaliswere identified, along with the ectoparasite Austrogoniodes bifasciatus. The main macroscopic lesions found were located in the respiratory system and gastrointestinal tract, with or without associated parasitism.

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