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## Short Communication

### First pathological report of parasitic gastric ulceration in Humboldt penguin (*Spheniscus humboldti*) along the coast of south-central Chile

Francisco Yáñez<sup>1,2</sup>, Ítalo Fernández<sup>2,3</sup>, Víctor Campos V.<sup>2,4</sup>, Miguel Mansilla<sup>1</sup>, Ariel Valenzuela<sup>2</sup>, Hernán González<sup>1</sup>, Cristhian Rodríguez<sup>1</sup>, Meyling Rivas<sup>2</sup>, Katherine Alveal<sup>2</sup> & Ciro Oyarzún<sup>2</sup>

<sup>1</sup>Centro de Anatomía Patológica Veterinaria (CAPAVET), Concepción, Chile

<sup>2</sup>Laboratorio de Piscicultura y Patología Acuática, Facultad de Ciencias Naturales y Oceanográficas  
Universidad de Concepción, Concepción, Chile

<sup>3</sup>Laboratorio de Parasitología, Departamento de Microbiología, Facultad de Ciencias Biológicas  
Universidad de Concepción, Concepción, Chile

<sup>4</sup>Laboratorio de Microbiología ambiental, Departamento de Microbiología, Facultad de Ciencias Biológicas  
Universidad de Concepción, Concepción, Chile

**ABSTRACT.** This report offers a macroscopic and histological description of ulcerative gastric lesions in Humboldt penguins associated with the presence of *Contracaecum pelagicum*. Parasites of this genus have been isolated from different species of marine mammals and birds, including penguins, demonstrating its capacity to generate ulcerative lesions in the gastrointestinal tracts of many hosts. A bibliographic review revealed no publications on gastric ulceration by nematodes in Humboldt penguins. Studies carried out in Chile concerning gastrointestinal parasitism by nematodes of the *Contracaecum* genus in *S. humboldti* only established the parasite's taxonomy. This is the first pathological description of a parasitic ulcerative manifestation in the stomach of *S. humboldti* in the world. The Humboldt penguin is a vulnerable species. Therefore, this study is relevant as it offers a better understanding of the diseases that affect *S. humboldti* and facilitates the development of strategies intended to recover the population.

**Keywords:** *Spheniscus humboldti*, Humboldt penguin, ulcer, stomach, *Contracaecum*, Chile.

### Primera descripción patológica de úlceras gástricas parasitarias en el pingüino de Humboldt (*Spheniscus humboldti*) en la costa centro-sur de Chile

**RESUMEN.** El presente trabajo ofrece una descripción macroscópica e histológica de lesiones gástricas de tipo ulcerativas en el pingüino de Humboldt asociadas a la presencia de *Contracaecum pelagicum*. Parásitos del género *Contracaecum* han sido aislados de diferentes especies de mamíferos y aves marinas, incluyendo pingüinos, en gran parte del mundo, demostrando una gran capacidad de generar lesiones ulcerativas en un número importante de hospederos. La revisión bibliográfica reveló la ausencia de publicaciones relativas a cuadros ulcerativos gástricos causados por nemátodos en el pingüino de Humboldt. Los estudios realizados en Chile concernientes a parasitismo gastrointestinal por nemátodos del género *Contracaecum* en *S. humboldti* sólo han abordado aspectos taxonómicos. Esta publicación constituye la primera descripción patológica de un cuadro de ulceraciones gástricas parasitarias en el pingüino de Humboldt. *S. humboldti* está catalogado como especie vulnerable por lo que este estudio es relevante en cuanto a incrementar el entendimiento de las patologías que afectan a la especie facilitando el desarrollo de estrategias tendientes a la recuperación de la población.

**Palabras clave:** *Spheniscus humboldti*, pingüino de Humboldt, úlcera, estómago, *Contracaecum*, Chile.

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Corresponding author: Francisco Yáñez (capavet@gmail.com)

Humboldt penguins (*Spheniscus humboldti*) are distributed along the Pacific coast of South America in Chile and Peru. They live in colonies, travel and hunt

in groups, and return each year to the same nesting site where they develop neighborly relationships (Wallace *et al.*, 1999; Cranfield, 2003). *S. humboldti* fed predo-

minantly on pelagic fish prey species, krill and squid. Consequently, this species participates in a parasitic life cycle, mostly concerning nematodes, of the family *Anisakidae*.

Specifically, the genus *Contracaecum* has been described in piscivorous birds, particularly different penguin species (Pazos *et al.*, 2003; Fredes *et al.*, 2006; Garbin *et al.*, 2007). Several authors, including Portes-Santos (1984), Grabda (1991) and Fagerholm & Overstreet (2008), have studied the taxonomy of this parasite.

The life cycle of the genus *Contracaecum* is heteroxenous, and adult stages are located in the proventriculus of seabirds, which serve as definitive hosts (Melhorn *et al.*, 1991). Gravid female parasites release eggs that are excreted into the water with bird feces. Third-stage larvae emerge and are ingested by crustaceans, which act as intermediate hosts. Fish may act as paratenic hosts. Thus, if fish consume infected invertebrates, the larvae does not continue to develop but remain encapsulated inside the intestinal wall, mesentery, liver, and other internal organs of the host. When fish are consumed by seabirds, fourth-stage larvae develops and eventually reach the adult stage (Fagerholm & Overstreet, 2008).

Many publications deal with gastric ulceration by *Contracaecum* in mammals and birds such as penguins, but no studies have described these symptoms in Humboldt penguins. Thus, the present work is the first description of gastric ulceration by *C. pelagicum* in *S. humboldti*.

Given the vulnerable condition of the Humboldt penguin (CONAMA, 2008), it is important to improve our knowledge of this disease. Therefore, prevalence and incidence studies are required to determine the real impact of parasitic ulcers on this population. Such knowledge would allow us to define adequate preventive or therapeutic strategies that would reduce mortality and strengthen the species recovery.

### Description and discussion of cases

Three moribund Humboldt penguins were captured by the National Fishing Service (SERNAPESCA) along the shore of Talcahuano, in the Biobío Region, Chile (36°43'S, 73°07'W). A clinical evaluation of the birds showed them to be inattentive to surrounding stimuli, highly undernourished, weak, and suffering digestive alteration (diarrhea). Emergency clinical procedures were applied: penguins were rehydrated orally with glucosaline serum (30 mL kg<sup>-1</sup> +2 g HCl) and an oral vitamin B and E complex was administered (400 U.I.). In keeping with the indications of Wallace & Walsh (2005), an intramuscular antibiotic (Enrofloxacin 10

mg kg<sup>-1</sup>) and an oral antifungal (Itraconazole 10 mg kg<sup>-1</sup>) were administered. Despite these emergency measures, penguins died three hours after treatment. Postmortem evaluations were performed immediately.

### Necropsy

The necropsy was done according to the protocol presented by Work (2000). A severe gastrointestinal parasitic condition was found. *Anisakidae* worms were present in the middle third of the esophagus, increasing their number towards the glandular portion of the stomach. Parasites observed in the esophageal tract had probably been regurgitated since no lesions were found in the esophageal mucosa. The stomachs of the birds showed severe blood congestion, as evidenced by the plethoric condition of the blood vessels superficially arranged on the greater curvature of the organ.

Multiple ulcerations with yellow-whitish borders and a caseous aspect were located exclusively in the glandular portion of the gastric mucosa (Fig. 1a). Nematodes of different sizes were found in most of the observed lesions in the Humboldt penguins. The affected areas varied from 0.6 to 2.3 cm in diameter.

In total, 23 specimens of nematode parasites were collected; one female, four males, and two juveniles from the first penguin; two female, five males, and two juveniles from the second penguin; and three females and four males from the third penguin. When examined under light microscopy, all were found to belong to the genus *Contracaecum*.

Studies describing gastric ulcers caused by *Contracaecum* have reported similar macroscopic lesions in the gastric mucosa of seabirds such as the white pelican (*Pelecanus erythrorhynchus*) and marine mammals such as the northern sea lion (*Eumetopias jubata*; Liu & Edward, 1971), South American sea lion (*Otaria byronia*; Cattán *et al.*, 1976), and the sea bear (*Callorhinus ursinus*; Spraker *et al.*, 2003). Although the distribution of the lesions differed, the authors agreed that most worms were found free in the stomach contents.

In penguins, gastric ulcerative processes caused by *Contracaecum* sp. have been described in the little penguin (*Eudyptula minor*; Obendorf & McColl, 1980) and the Magellanic penguin (*Spheniscus magellanicus*; Garbin *et al.*, 2007). Penguins are fundamentally ichthyophagous and, therefore, they acquire the parasite through the ingestion of infected fish. Ulcerations in the esophageal and intestinal mucosa caused by *Anisakidae* are less frequent in *S. magellanicus* (Mann, 1992; Garbin *et al.*, 2007).

## Histopathology

The injured tissue was extracted, fixed in 10% buffered formalin, and sent to the Veterinary Anatomic Pathology Center (CAPAVET), Concepcion, Chile for histological analysis. Samples were embedded in paraffin. Sections of 5 µm were stained with hematoxylin and eosin.

The histoarchitecture of the gastric mucosa was severely altered by a granulomatous reaction. The central area consisted of an acellular hyaline substance and presented calcium mineralization foci that replaced the mucosal glandular tissue and, in many cases, extended to the muscular tunica (Fig. 1b).

The origin of the hyaline substance is uncertain. However, Liu & Edward (1971) performed histochemical studies of ulcers caused by *Contracaecum osculatum* in the stomachs of northern sea lions and white pelicans, concluding that this substance was essentially made up of parasite secretions and cellular fragments from the host.

The inflammatory infiltration showed a great amount of neutrophils (heterophils), lymphocytes, plasma cells, bordered by multinucleated giant cells (foreign-body-type), and epithelioid cells aggregates. The injured area was surrounded by a severe desmoplastic reaction (Fig. 1c).

Our histological observations agreed with those described by Liu & Edward (1971), Obendorf & McColl (1980), and Garbin *et al.* (2007) for seabirds and by Cattán *et al.* (1976) and Spraker *et al.* (2003) for marine mammals.

## Electron microscopy of worms

The collected parasites were stored in alcohol at 70% for taxonomic purposes and dehydrated in ascending grades of ethanol and finally rinsed in 100% ethanol, several times for 10-15 min according to Anderson (1951). Individual specimens were at first dehydrated by the critical point drying method, mounted in metal stubs, gold-palladium coated and examined in scanning electron microscope (JEOL JSM-6380LV) belonging to the Electron Microscopy Laboratory of the University of Concepción, Chile.

The worm *Contracaecum pelagicum* was identified by the spatial distribution of tail papillae and the presence of bifurcated interlabia (Fig. 1d) according to the descriptions of Johnston & Mawson (1942), Portes-Santos (1984), and Fagerholm & Overstreet (2008). This identification agreed with the diagnostic characteristics established by Garbin *et al.* (2007).

*C. pelagicum* was described for the first time in the black-browed albatross (*Diomedea melanophrys*) in Australia. It was subsequently redescribed on the

Uruguayan coasts. Portes-Santos (1984), Fagerholm *et al.* (1996), and Garbin *et al.* (2007) identified this species in *Sphenicus magellanicus* along the coasts of Brazil, South Africa, and Argentina. Silva *et al.* (2005) reported the occurrence of *C. pelagicum* in the brown booby (*Sula leucogaster*, Sulidae, Pelecaniformes) off São Paulo, Brazil.

In Chile, studies of gastrointestinal parasitosis caused by nematodes of the *Contracaecum* genus have been carried out by Mann (1992) and González-Acuña *et al.* (2008). However, these works were primarily taxonomic studies of the parasite specimens rather than studies of the tissular alterations they caused in the hosts.

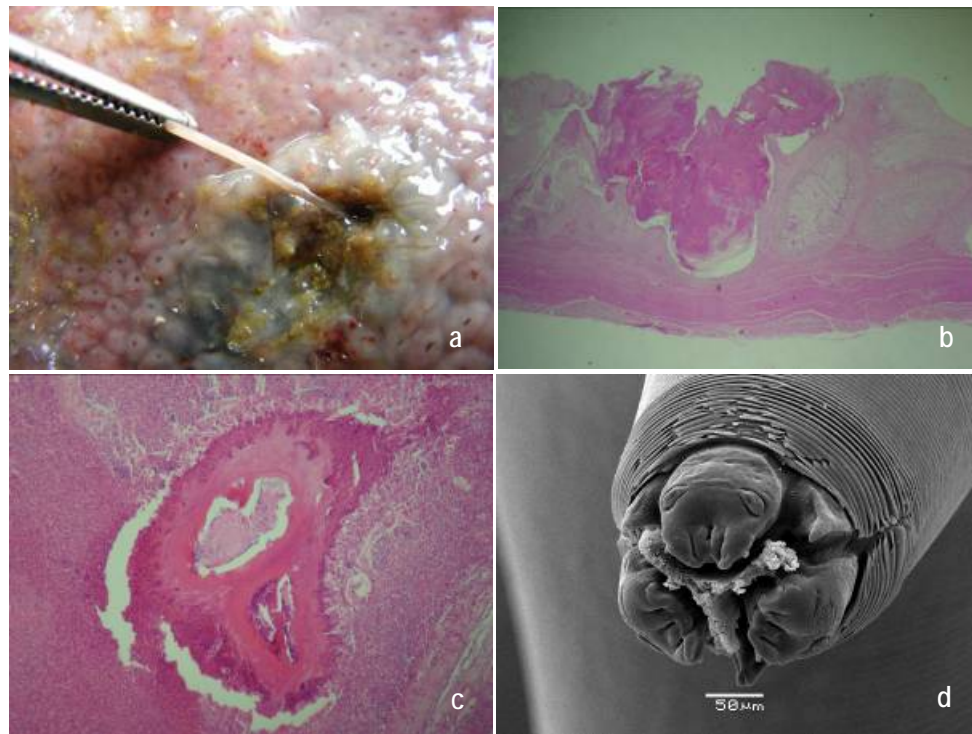
In general, few publications have described the pathological alterations caused by the genus *Contracaecum* in marine birds. To date, no studies dealing with ulcers caused by this parasite in the Humboldt penguin have been published.

Tissular alterations by *Contracaecum pelagicum* should be common in the Humboldt penguin population since these birds are part of the life cycle of this parasite. *S. humboldti* acts as a definitive host when they ingest infected fishes. Herling *et al.* (2005) examined and compared the diet of the Humboldt penguin in northern and southern of Chile. In this study, fish were the dominant prey item, mainly anchovy (*Engraulis rigens*) and Inca scad (*Trachurus murphyi*). Both species have been catalogued as intermediate hosts and transmitters of *Contracaecum* to the penguins (Fagerholm & Overstreet, 2008). Therefore, this worm could play an important role in increasing the vulnerability of Humboldt penguins.

Previously described pathological alterations are enough to generate abdominal pain, general discomfort, reduction of hunting performance, and an insufficient rate of feeding, amongst others, in living penguins. These instances have to be considered debilitating factors and promoters of immunosuppression, hence, facilitators for infectious and non-infectious diseases in population.

For present cases, it is important to mention that the ulcerative lesions were not the cause of death, but the extreme undernourishment and dehydration determined the multisystem organ failure. It is likely that *Contracaecum* becomes a primary etiological agent causative of death if it pierce the stomach wall and induce peritonitis.

We think it's relevant to highlight the great number of questions generated while making this report and once it was finished. For example, how many Humboldt penguins die yearly by this parasitosis as primary disease? How many Humboldt penguins



**Figure 1.** Macroscopic and histological lesions in the gastric glandular portion of the stomach of *S. humboldti* and electron micrograph of *Contracaecum pelagicum*. a) Note ulcerations with yellow-whitish borders and *Contracaecum pelagicum* strongly adhered to the damaged tissue, b) replacement of glandular epithelium of the stomach by hyaline and amorphous tissue; H-E stain, 20x, c) severe desmoplastic reaction surrounded by chronic inflammatory exudate and foreign-body giant cells observed in the histological view; H-E stain, 100x, d) proximal end of *Contracaecum pelagicum* (scanning electron microscopy). Detail of taxonomical features: three bifurcated interlabia and striations.

**Figura 1.** Vista macroscópica e histológica de lesiones ubicadas en porción glandular de estómago de *S. humboldti* y microscopía electrónica de *Contracaecum pelagicum*. a) Note ulceración con bordes blanquecino-amarillento de aspecto caseoso en cuyo centro se advierte *Contracaecum pelagicum* fuertemente adherido a tejido alterado, b) reemplazo de epitelio glandular estomacal por sustancia hialina amorfa; tinción H-E, 20x, c) severa reacción desmoplásica delimitada por exudado inflamatorio crónico con presencia de células gigantes tipo cuerpo extraño tinción H-E, 100x, d) vista de porción proximal de *Contracaecum pelagicum* (microscopía electrónica de barrido). Detalle de características de importancia taxonómicas: tres interlabias bifurcadas y estriaciones.

becomes "easy preys" for its natural predators due to weakness induced by this parasitosis? Are there important variations in the number of penguins affected by this disease throughout the year? seasonal dependence?. Does this parasitosis significantly affect the reproductive rate of *S. humboldti*?

We strongly believe that the effect of *Contracaecum* in Humboldt penguins is an important factor that would impede the success of any enterprise for protecting these birds. Thus, research related to the incidence and prevalence of this parasite and the real impact of gastric ulceration on the penguin population is necessary in order to apply environmental and therapeutic strategies leading to the recovery of the species.

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