Short Communication

Gastrointestinal helminths of wild Humboldt penguins *Spheniscus humboldti* (Meyen, 1834) from the south-central coast of Chile

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ABSTRACT. The study of parasitism in penguins is relevant because it offers information on the biology and ecology of this host. This study reports the parasitism of 156 specimens of *Spheniscus humboldti* rescued from beaches of the south-central coast of Chile, between January 2006 and June 2015, describing their potential forms of transmission and pathogenicity. The parasitic infection was determined by coproparasitic methods in live specimens and by necropsy in dead penguins. The gastrointestinal helminths *Tetrabothrius eudyptidis* (Lönnberg, 1896), *Cardiocephaloides physalis* (Lutz, 1926), *Contracaecum pelagicum* (Johnston & Mawson, 1942) and *Cosmocephalus obvelatus* (Creplin, 1825) were identified by both methods. Parasitism was observed in 29.57% of live specimens and 51.76% of necropsied birds. All the parasites identified corresponded to genera already informed in *S. humboldti*. The richness of the *S. humboldti* parasitic fauna was lower than that reported for other species of penguins. The results demonstrate the participation of *S. humboldti* as a definitive host in the life cycles of the parasites reported in this study. Being *S. humboldti* a vulnerable species, it is necessary to continue with parasitological studies in this penguin to clarify the role of parasites in the health of these birds.

Keywords: *Spheniscus humboldti*; parasites; life cycles; pathology; Chile

The Humboldt penguin *Spheniscus humboldti* (Meyen, 1834) is an endemic oceanic seabird from the west coast of South America. It is distributed from Foca Island (5°12'S, 81°12'W), in northern Perú to Isla Metalqui (42°12'S; 74°10'W) on the west coast of Chiloé Island, Chile (De la Puente *et al*., 2013). *S. humboldti* lives mostly coastal islands and nest in a variety of sites including caves burrows, under vegetation and rocks. The International Union for the Conservation of Nature (IUCN) classifies this species as vulnerable since it has undergone extreme population size fluctuations at major colonies and there is probably an on-going, underlying rapid decline in numbers (BirdLife International, 2017).

Since the available information regarding species parasitizing *S. humboldti* derives mainly from the inci-dental capture or analysis of dead specimens of this penguin, its parasites have been poorly studied until now (Mann, 1992; González-Acuña *et al*., 2008; Yáñez *et al*., 2012). In this sense, because it provides information of the host such as diet, migratory routes, feeding areas (Gardner & Campbell, 1992; Marcogliese & Price, 1997), the study of parasitism in *S. humboldti* is relevant. Furthermore, it provides information regarding the role played by parasites in infectious processes in these animals.

From January 2006 to June 2015, the National Fisheries Service of Chile (SERNAPESCA), rescued a total of 156 specimens of *S. humboldti* from various places on the south-central Chile (36°37'S-38°20'S) and sent them to the Veterinary Clinical Hospital of San Sebastian University, Concepción. Of this total, 102

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arrived alive to the veterinary clinic. Whether if they arrived alive or dead, *S. humboldti* specimens were identified considering the morphological characteristics indicated by Goodall *et al.* (1951). Morphometric characters of each specimen were recorded according to Zavalaga & Paredes (1997) (Table 1).

The health condition of the alive birds was evaluated through clinical examinations which, in most cases, revealed general weakening, malnutrition, persistent diarrhea and lack of attention to environmental stimuli, requiring the hospitalization and the application of emergency clinical procedures in many of them. Despite the efforts, 31 specimens died during the treatment. Animals which satisfactorily recovered their health (71) were released on the south-central Chile.

A coproparasitic analysis was carried out on 71 live specimens to determine the gastrointestinal parasitic fauna. On the floor of the cage of each bird, polyethylene was placed to collect fresh stool samples, and the fecal matter placed in 50 mL plastic bottles containing 10 mL of PAF preservative (Phenol-Alcohol-Formalin) and processed using the Burrows sedimentation technique (Muñoz *et al.*, 1984). Three microscopic preparations of each sample were studied using a Zeiss Stemi DRC microscope and 100X and 400X magnifications to search for helminths and protozoa, respectively.

Necropsy was performed to the 85 dead birds according to the protocol of Work (2000). This procedure revealed the presence of numerous parasite specimens in the gastrointestinal tract, adhered to the mucosa or free in the intestinal lumen. The intestinal content was extracted using saline solution and sieved using a 0.50 mm sieve. Material retained by the sieves was examined utilizing a stereoscopic microscope (Zeiss Stemi DRC) to separate the parasites, which were fixed in 70% ethanol. Nematodes were cleared in lactophenol or in 25% glycerine alcohol. Previously to staining, cestodes and trematodes were discolored, dehydrated and diaphanized and then stained using Harris' hematoxylin and mounted in Canada balsam. The taxonomic identification of each parasite was made at the Parasitology Laboratory (Faculty of Biological Sciences, University of Concepción, Chile) using optical microscopy (Motic, BA 310). Parasites were identified according to Anderson & Wong (1981), Hoberg (1994), Fagerholm & Overstreet (2008), and Diaz *et al.* (2010). Prevalence in alive and dead specimens and the mean intensity in dead specimens were calculated according to Bush *et al.* (1997).

Of the total penguins analyzed by coproparasitic methods, 29.57% were positive to some parasitism, being protozoan elements absent. In the group of penguins subjected to necropsy, 51.76% were positive for some parasitism. The difference in prevalence between the techniques used (Table 2) becomes explained when considering that necropsy, being a procedure that contemplates the *in extenso* revision of the digestive tract, allows finding all the parasites present. However, the diagnostic approach through coproparasitic methods has the advantage to be carried out on live specimens, allowing investigating the parasitic condition of threatened fauna. In both, live and dead birds, the presence of adults and larvae of third and fourth stage of the nematode *Contracaecum pelagicum* (Johnston & Mawson, 1942), adult stages of the nematode *Cosmocephalus obvelatus* (Creplin, 1825), complete specimens and gravid proglottids (fragments) of the tapeworm *Tetrabothrius eudyptidis* (Lönnberg, 1896), and adult and juvenile specimens of the trematode *Cardiocephaloides physalis* (Lutz, 1926), were identified.

None of the identified species has been reported as zoonotic. Besides, larvae of the third stage of *Anisakis* Type I (Berland, 1961) were isolated from the stomach in some birds, but not included as results of this report because, since they are not attached to the tissue of the host, they could correspond to parasitism of the prey (see below). The prevalence, mean intensity of infection and anatomical location of the parasites found in specimens subjected to necropsy are detailed in Table 3. The majority of infections considering live and dead birds (57%) were monospecific, while the rest of the penguins were parasitized by at least two species, with the combination of *C. pelagicum* and *T. eudyptidis* being the most frequent. The type material and other reference specimens are deposited in the Museum of Parasitology of the Universidad de Concepción (MPUDEC), with the registration number PH1-12.

All the parasites identified in the analyzed specimens corresponded to genera already informed in birds of aquatic environments of Chile, including *S. humboldti* (Torres *et al.*, 2000; Hinojosa & González, 2005). However, the number of species and prevalence of the parasitofauna of *S. humboldti* reported in this work was different from what has been previously described for this species (Mann, 1992; González-Acuña *et al.*, 2008), perhaps reflecting the particular ontogeny of the specimens analyzed (Table 3). The richness of the *S. humboldti* parasitic fauna was lower than that reported for other species of penguins (Brandão *et al.*, 2014), perhaps caused by the reduced feeding spectrum of the Humboldt penguin, mainly piscivorous with a reduced diversity of prey-items. Considering that all the parasites isolated in this study could be transmitted to the host by the consumption of infected preys, the quantitative and qualitative varia-

Table 1. Morphometric data of 156 Humboldt penguins (*Spheniscus humboldti*) from south-central Chile. n: number of specimens, SD: standard deviation, x̄: average. Body mass is in kilograms, other measurements are in cm. Range is given in parentheses.

Variable	Pooled (n = 156) x̄ ± SD	Males (n = 62) x̄ ± SD	Females (n = 94) x̄ ± SD
Body mass	3.00 ± 0.7	2.95 ± 0.73 (1.20 - 4.70)	3.02 ± 0.67 (2.90 - 5.10)
Length of the head	12.90 ± 0.87	13.34 ± 0.68 (11.62 - 15.05)	12.67 ± 0.88 (11.56 - 15.00)
Width of the head	4.87 ± 0.30	5.02 ± 0.24 (4.55 - 5.39)	4.77 ± 0.30 (4.32 - 5.38)
Bill length	6.14 ± 0.30	6.39 ± 0.32 (5.70 - 6.98)	5.98 ± 0.13 (5.70 - 6.45)
Bill depth	2.07 ± 0.45	2.13 ± 0.46 (1.26 - 3.02)	2.03 ± 0.45 (1.19 - 2.95)
Flipper length	15.32 ± 0.63	15.58 ± 0.40 (13.90 - 16.90)	15.15 ± 0.70 (13.70 - 16.88)

Table 2. Prevalence (P) of infection by helminth gastrointestinal parasites in 156 penguins from south-central Chile diagnosed by Burrows test and/or necropsy. n: number of penguins analyzed.

Parasitic diagnosis test	n	Results Positive	Results Negative	P (%)
Burrows	71	21	50	29.57
Necropsy	85	44	41	51.76
Total	156	65	91	41.66

tions of these parasitic taxa would represent the composition of their diet (Campbell *et al*., 1980). Their diet is mainly composed of fishes such as anchovy (*Engraulis ringens*), Araucanian herring (*Strangomera bentincki*) and silverside (*Odontesthes regia*) although the common hake (*Merluccius gayi*), Inca scad (*Trachurus murphyi*), garfish (*Scomberesox saurus scombroides*), South American pilchard (*Sardinops sagax*) and various crustaceans and cephalopods have been reported as well (Herling *et al*., 2005). Besides, characteristics of the host (phylogeny and ecology, among others) and the type of relationship it establishes with the parasite could also determine the structure and quantitative characteristics of the parasitic fauna (George-Nascimento, 1987; Rohde, 2005).

Although the majority of the life cycles of the parasites identified in this study are unknown, it is recognized that they are heteroxene with the participation of *S. humboldti* as definitive host and various species of fish and invertebrates as intermediate and/or paratenic hosts. In this way, in the life cycle of *Contracaecum*, penguins release the eggs of the parasite with their feces into the sea from where larvae of the third stage hatch and are eventually ingested by crustaceans, probably copepods, which could serve as intermediaries. Fish, when ingesting infected invertebrates, become infected and, thus, as intermediate/ paratenic hosts in whose tissues larvae are encapsulated. When penguins and other seabirds consume fish, the third larval stage develops and generates the fourth larval stage which, subsequently, advances into the adult stage (Bartlett, 1996; Fagerholm & Overstreet, 2008). Also, infective *Contracaecum* larvae have recently been found in *E. ringens* (George-Nascimento & Moscoso, 2013), the primary prey of the Humboldt penguin's diet (Herling *et al*., 2005). Azuma *et al.*, (1988) indicated that piscivorous birds and penguins might become infected when they ingest *Cosmocephalus* infected fish acting as intermediate hosts of this parasite (Kim *et al*., 2015). The life cycle of *Cardiocephaloides physalis* is unknown although it could be similar to the congeneric parasite *C. longicollis* where gastropods and fish (Sparidae and Scombresocidae) participate as intermediate hosts, with the gulls *Larus argentatus* and *Larus ridibundus* being the definitive hosts (Prevot & Bartoli, 1981; Abdel-Aal *et al*., 2004). Also, the probable role of the Argentine anchoveta *Engraulis anchoita* as the intermediate host of *C. physalis* has been proposed because the metacercaria of this parasite has been described in specimens captured in waters of Argentina and Uruguay (Timi *et al*., 1999; Horne *et al.,* 2011). Although it is recognized that *Tetrabothrius* is one of the most frequently found genera of parasites in birds, its life cycle is mostly unknown, with the participation of teleost fish or cephalopod mollusks as the second intermediate and/or paratenic host (Hoberg, 1994).

Table 3. Parasites, including those reported in this study, so far listed in necropsied *Spheniscus humboldti* from Chilean waters. (n) Number of parasites; (P) Prevalence of infection; (MI) Mean intensity of infection

Parasitic taxa	n	P (%)	MI	Area of collection (latitude)	Reference
Nematoda					
Cosmocephalus oblevatus	1	3.33	1.00	Central (32°31'S-32°82'S)	Mann (1992)
(Creplin, 1825)	48	8.24	6.86	South-central (36°37'S-38°20'S)	Present study
Contracaecum pelagicum	29	40.00	2.41	Central (32°31'S-32°82'S)	Mann (1992)
(Johnston & Mawson, 1942)	30	100	10.00	South-central (33°35'S-36°44'S)	González-Acuña *et al*. (2008)
	477	42.35	13.25	South-central (36°37'S-38°20'S)	Present study
Cestoda					
Tetrabothrius eudyptidis	13	16.60	2.60	Central (32°31'S-32°82'S)	Mann (1992)
(Lönnberg, 1896)	12	33.33	12.00	South-central (33°35'S-36°44'S)	González-Acuña *et al*. (2008)
	118	24.70	5.62	South-central (36°37'S-38°20'S)	Present study
Trematoda					
Cardiocephaloides physalis	43	30.00	4.70	Central (32°31'S-32°82'S)	Mann (1992)
(Lutz, 1926)	361	22.35	19.00	South-central (36°37'S-38°20'S)	Present study

The main causes of rescue of penguins considered in this study were entanglement in fishing nets, severe injuries and oiling events. This fact might be linked to the finding that the weight of the penguins analyzed in the present study was lower than that previously reported for other *S. humboldti* populations (Zavalaga & Paredes, 1997) or perhaps are the consequence of long term undernourished, parasitized or sick birds. In the light of the results presented, questions can be raised about what is the role of parasites as predisposing factors or determinants of alteration of the health in the eventual hosts. Since most of the hospitalized penguins presented nonspecific parasitic symptoms, it was not possible to establish a correlation between the health of each animal and its parasitic fauna. On the other hand, necropsies revealed emaciation in birds with a high parasitic load. The death of these birds, favored or determined by parasites, could be correlated with heavy parasitic loads in birds which are under stress conditions due to environmental contamination, immunodepressors, anorexia and/or secondary bacterial infections (Fagerholm & Overstreet, 2008).

The pathogenicity of *Contracaecum* could be related to its capacity to cause inflammatory processes and to inflict ulcerative damage on the gastric and intestinal mucosa, which in cases of intense parasitism might cause severe anemia episodes. Likewise, specimens of *Contracaecum*, and to a lesser extent *Cosmocephalus*, could also compete with their host for the same nutrients, causing, in cases of intense parasitism, nutrient deficiencies and malnutrition, as previously mentioned (Fagerholm & Overstreet, 2008; Yáñez *et al*., 2012). On the other hand, in some necropsies performed on penguins heavily parasitized by *C. physalis*, we observed severe damage to the intestinal mucosa, coinciding with reports in other bird hosts (Baer, 1969). It has been pointed out that *C. physalis* could affect intestinal digestion and absorption of nutrients, which could cause the death of *Spheniscus demersus* chicks in southern Africa (Randall & Bray, 1983; Horne *et al*., 2011). Finally, in penguins heavily parasitized by *T. eudyptidis* we did not find evidence of intestinal obstruction, although we verified that these specimens were emaciated, an observation in agreement with that of Housse (1945) who reported that high loads of the *T. lutzi* congeneric species caused the thinning and death of magellanic penguins (*S. magellanicus)*, also inhabitants of the coast of Chile.

Being *S. humboldti* a vulnerable species, we consider that it is necessary to continue parasitological studies in this penguin to clarify the role of parasites in the health of this bird.

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