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Research Note

Parasitological examination of northern elephant seal (*Mirounga angustirostris*) pups for presence of hookworms (*Uncinaria* spp.) on San Miguel Island, California

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Summary

Necropsy and extensive parasitological examination of dead northern elephant seal (NES) pups was done on San Miguel Island, California, in February, 2015. The main interest in the current study was to determine if hookworms were present in NESs on San Miguel Island where two hookworm species of the genus *Uncinaria* are known to be present – *Uncinaria lyonsi* in California sea lions and *Uncinaria lucasi* in northern fur seals. Hookworms were not detected in any of the NESs examined: stomachs or intestines of 16 pups, blubber of 13 pups and blubber of one bull. The results obtained in the present study of NESs on San Miguel Island plus similar finding on Año Nuevo State Reserve and The Marine Mammal Center provide strong indication that NES are not appropriate hosts for *Uncinaria* spp. Hookworm free-living third stage larvae, developed from eggs of California sea lions and northern fur seals, were recovered from sand. It seems that at this time, further search for hookworms in NESs would be nonproductive.
Keywords: northern elephant seals; hookworms; *Uncinaria*; San Miguel Island; California

Introduction

There are 34 species of pinnipeds (Suborder Pinnipedia Illiger, 1811) assigned to three families of the mammalian order Carnivora Bowdich, 1821: Otariidae Gray, 1825, Phocidae Gray, 1821 and Odobenidae Allen, 1880 (Jefferson *et al*. 1993; Higdon *et al*. 2007). Hookworms from the genus *Uncinaria* Frölich, 1789 have been reported in 15 pinniped species – 12 otariids (six species of fur seals, and six species of sea lions) and three phocids (Lyons *et al*., 2011; Nadler *et al*., 2013).

Four species of hookworms have been described in pinnipeds – *Uncinaria lucasi* Stiles, 1901, a parasite of northern fur seals (NFSs) (*Callorhinus ursinus*) and Steller sea lions (*Eumetopias jubatus*); *Uncinaria hamiltoni* Baylis, 1933, a parasite of South American sea lions (*Otaria flavescens*) and South American fur seals (*Arctocephalus australis*); *Uncinaria sanguinis* Marcus, Higgins, Šlapeta, Gray 2014, a parasite of Australian sea lions (*Neophoca cinerea*) and, probably, Australian fur seals (*Arctocephalus pusillus*) and New Zealand fur seals (*Arctocephalus forsteri*) (Marcus *et al*., 2014); *Uncinaria lyonsi* Kuzmina and Kuzmin 2015, a parasite of California sea lions (CSLs) (*Zalophus californianus*). In addition, three species from the genus *Uncinaria* were found and determined from nucleotide sequence data in the New Zealand sea lion (*Phocarctos hookeri*), southern elephant seal (*Mirounga leonina*) and Mediterranean monk seal (*Monachus monachus*) (Castinel *et al*., 2006; Nadler *et al*., 2013; Ramos *et al*., 2013). Hookworms (*Uncinaria* spp.) have also been reported from Juan Fernandez fur seal pups (*Arctocephalus philippii*) (Sepúlveda, 1998) and Guadaloupe fur seals (*Arctocephalus townsendi*) (Lyons *et al*., personal communication).

From the family Phocidae (earless or true seals), hookworms were found only in the southern elephant seal, ringed seal (*Phoca hispi-*

da) and Mediterranean monk seal (Lyons *et al*., 2011; Nadler *et al*., 2013). Despite the reference that hookworm eggs were detected in feces of a northern elephant seal (NES) (*Mirounga angustirostris*) pup at The Marine Mammal Center (TMMC), Sausalito, California (Dailey, 2001), this information is still questionable. Extensive parasitological examination of NESs for hookworms (*Uncinaria* spp.) has been carried out at the Año Nuevo State Reserve in central California in 2012 (Lyons *et al*., 2012). However, hookworm eggs, larvae or adults were not found in NES pups or larvae in the environment.

San Miguel Island, California, is the sixth largest of the eight California's Channel Islands, located across the Santa Barbara Channel in the Pacific Ocean, California. Currently, six species of pinnipeds may occur on San Miguel Island: California sea lions, northern elephant seals, northern fur seals, harbor seals *(Phoca vitulina*), and Steller sea lions. CSL, harbor seals, NES and NFS have viable breeding populations there (DeLong & Melin, 2002). The population of NFSs was reestablished on San Miguel Island in the 1950s, and has since increased exponentially. It has become the largest breeding population for the species, approximately 83 % of the population, numbering some 50,000 animals (Stewart and DeLong, 1993; Le Boeuf *et al*., 2011).

Two species of hookworms (*U. lyonsi* and *U. lucasi)* have been found in CSLs and NFSs, respectively, at San Miguel Island (Lyons *et al*., 1997, 2000; Kuzmina and Kuzmin, 2015). The primary objective of our work was to perform parasitological examination of dead NES pups on San Miguel Island for presence of hookworms from the genus *Uncinaria*. The reason for examining NES pups for hookworms was that these parasites are known to be present in CSLs and NFSs on the Island. Since all three pinniped species have a sympatric relationship on some rookeries it was decided to find out if hookworms that parasitized CSLs and NFSs there could be a source of infection in NES pups.

Materials and Methods

Our research was performed in February 7 – 15, 2015, at three NES rookeries on San Miguel Island (34°2'N and 120°23'W): West Cove, Adams Cove and Judith Cove. The breeding season of NESs on the Island begins in early December with the arrival of the adult males, and reaches a peak during the period from about January 26 to February 2 which defined the period of our studies.

Basic necropsies were done on 18 recently dead NES pups according to standard procedure (Spraker, 1985). Two of these pups were neonates (stillbirth) which had never been nursed; 15 pups were "black-coats" (of one to eight weeks old), and one "silver-coat" (of eight to ten weeks old). Detailed parasitological techniques for examining the stomach and intestines were as published previously (Lyons, 1963; Lyons *et al*. 2001, 2005). Samples of blubber (about 50 – 100 g each) were collected from 13 of the dead NES pups and from one dead NES bull which was estimated to be 6 – 7 years old. Blubber was cut into small pieces and placed in a Baermann funnel apparatus containing fresh water for 10 – 12 hours, and examined at about eight hours later under a dissecting and a compound microscope for presence of hookworm larvae (L_3). Sand samples were examined for free-living third stage hookworm larvae (known to be present there in the fall and winter) produced from hookworm eggs passed in feces of CSL and NFS pups (Lyons *et al*., 2000).

Results and Discussion

Parasitological examination of dead NESs did not reveal hookworms in the stomach or intestines of 16 pups and blubber of 13 pups, even though hookworm free-living larvae (FLL_3) were found in rookery soil (Table 1). Also hookworm larvae were not recovered from blubber of a dead NES bull. These negative results mirror that from NESs on the Año Nuevo State Reserve (Lyons *et al*., 2012). As no other pinniped species were present on the NES rookeries on Año Nuevo State Reserve, additional studies on rookeries inhabited by other pinniped species infected by hookworms such as on San Miguel Island, were necessary. Moreover, blubber samples obtained from ten NES pups that died at The Marine Mammal Center (TMMC), Sausalito, California, in 2013 – 2015 were negative for hookworm parasitic L_3 (E.T. Lyons, personal communication).

Taking into account the presence of at least two *Uncinaria* species on the Island (*U. lyonsi* and *U. lucasi*), identified by different molecular and morphological characteristics of the adult hookworms, CSL and NFS free living third stage larvae were present in the soil/sand of rookeries. This allowed NESs, because of the sympatric relationship with the aforementioned pinnipeds, to get infected by these parasitic nematodes; however this did not happen.

Hookworms in the genus *Uncinaria* have been found in several species of pinnipeds from different regions of the world (Lyons *et al*., 2011; Nadler *et al*., 2013; Dailey, 2001), that lead to the interest in trying to find if these parasites are present in NESs. However, despite extensive studies of NES pups and the environment performed in 2012 on the Año Nuevo State Reserve (Lyons *et al*., 2012), in examination of NES pups from TMMC and in the present study on San Miguel Island, evidence of *Uncinaria* parasitizing this species of pinnipeds has not been found.

Regarding why hookworms do not seem to parasitize NESs, there can be numerous speculations, none of which are provable at this time. Of particular interest is the assumption that these parasites are strictly specific to this species of pinnipeds, and they may have disappeared due to a dramatic decrease of the NES population. It should be mentioned that the population of NESs declined to near extinction – the effective population size in 1884 may have been as low as 20 elephant seals due to their harvest for oil for lamps and other uses (Bartholomew & Hubbs, 1960; Le Boeuf *et al*., 2011). Possibly during those times the NESs were infected with hookworms but the pool of worms become too low to keep infections "recycled" because of so few animals. This is what seems

Table 1. Summary of data on search for hookworms (*Uncinaria* spp) in or associated with northern elephant seals (*Mirounga angustirostris*) (NES) on San Miguel Island, California (February, 2015)

Type of samples examined	Number of samples	Results of examination No. positive / No. of specimens
Dead NES pups – stomach and intestines	18	negative
Dead NES pups – blubber	13	negative
Dead NES bull – blubber	1	negative
Samples of sand *	31	8 (26 %) / 1 – 6 FLL_3 each

*Plant/soil free living nematodes were found in all sand samples

to be happening in NFSs on the Pribilof Islands, Alaska, where hookworm prevalence was up to 100 % in the 1950 – 60s when the population of NFSs was high (Lyons & Olsen, 1962; Lyons, 1963). However, currently on these islands, hookworms are almost extinct from the parasite fauna of NFSs which have had a tremendous decrease in number the last several decades (DeLong, 2007; Lyons *et al*., 2011, 2014).

It is of interest that in contrast to NES, hookworms have been found in southern elephant seals (Johnston & Mawson, 1945; Ramos *et al*., 2013). Southern elephant seals were exploited but the population apparently was not reduced to numbers as low as NESs.

The specificity of hookworms to their pinniped hosts has repeatedly been suggested (George-Nascimento *et al*., 1992; Nadler *et al*., 2013; Ramos *et al*., 2013). Twelve species of *Uncinaria* which significantly differ both morphologically and according to molecular studies were described in pinnipeds (Castinel *et al*., 2006; Nadler *et al*., 2013; Ramos *et al*., 2013). The fact that two species of Otariidae (CSL and NFS) inhabit and breed on the same areas on San Miguel Island in the same season and have two different species of hookworms – *U. lucasi* and *U. lyonsi* which differ in biology and morphology (Lyons *et al*., 1997, 2000; Lyons & DeLong, 2005) adds support to this hypothesis. Free-living third stage hookworm larvae of one or both of these two hookworm species repeatedly have been found, including in the present study, in the sand on the rookeries on the Island (Lyons *et al*., 1997, 2000), but as mentioned already, cases of NES infection by these nematodes have not been reported.

In conclusion, the research reported here, showing absence of hookworms in NESs on San Miguel Island, plus similar finding at the Año Nuevo State Reserve and TMMC, provides strong indication that NESs now are not appropriate hosts for these nematodes. It is still unknown why NESs are not infected with hookworms, whereas their close relative, the southern elephant seal, clearly is a host for these nematodes. It seems that, at this time, further search for hookworms in NES would be nonproductive.

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