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UNCINARIASIS IN NORTHERN FUR SEAL AND CALIFORNIA SEA LION PUPS FROM CALIFORNIA

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ABSTRACT: Northern fur seal (*Callorhinus ursinus*) (n = 25) and California sea lion (*Zalophus californianus*) (n = 53) pups, found dead on rookeries on San Miguel Island (California, USA), were examined for adult *Uncinaria* spp. Prevalence of these nematodes was 96% in fur seal pups and 100% in sea lion pups. Mean intensity of *Uncinaria* spp. per infected pup was 643 in fur seals and 1,284 in sea lions. Eggs of *Uncinaria* spp. from dead sea lion pups underwent embryonation in an incubator; development to the free-living third stage larva occurred within the egg. This study provided some specific information on hookworm infections in northern fur seal and California sea lion pups on San Miguel Island. High prevalence rate of *Uncinaria* spp. in both species of pinnipeds was documented and much higher numbers (2X) of hookworms were present in sea lion than fur seal pups.

Key words: California sea lion, *Callorhinus ursinus*, hookworm intensity, northern fur seal, prevalence, pup mortality, *Uncinaria* spp., *Zalophus californianus*.

INTRODUCTION

Hookworms are reported from several species of pinnipeds, and more commonly from otariids (George-Nascimento et al., 1992). There is uncertainty as to the number of species of the genus *Uncinaria* in pinnipeds. Only two species, *Uncinaria lucasi* and *Uncinaria hamiltoni*, have been described (Baylis, 1933, 1947); they have been reported from several pinniped species (George-Nascimento et al., 1992). *Uncinaria lucasi* was first found, although not described, in northern fur seals (*Callorhinus ursinus*) by Stiles (1901). Intermediate types have been reported from various species of pinnipeds, including California sea lions (*Zalophus californianus*) (Dailey and Hill, 1970). George-Nascimento et al. (1992) suggested that all hookworms reported in pinnipeds should be considered as one species, *U. lucasi*. Throughout the present paper, hookworms are called *Uncinaria* spp. because of uncertainty of the species.

Pathogenicity of *Uncinaria* spp. in pinnipeds is poorly understood. No doubt, hookworms may be a major cause of pinniped pup mortality under circumstances of high intensities combined with environmental or nutritional stresses. The main purpose of the present investigation was to obtain data on prevalence and intensity of adult *Uncinaria* spp. in northern fur seal and California sea lion pups found dead on San Miguel Island (California, USA). Also of interest was determining if free-living third stage larvae (L_3) develop entirely within eggs of hookworms from California sea lion pups, as has been reported for hookworms in northern fur seal pups (Lyons, 1963).

MATERIALS AND METHODS

Collection of hookworms was done on San Miguel Island (California, USA; 34°02′N, 120°26′W) from 16 to 25 July 1996. Afterward, examination of adult hookworms and embryonation of hookworm eggs were done at the University of Kentucky (Lexington, Kentucky, USA). This research was conducted under Marine Mammal Protection Act Research Permit No. 837 and 977 issued to the National Marine Mammal Laboratory (Seattle, Washington, USA).

Pups (n = 78) selected for examination appeared to have died recently, based mainly on lack of hair slippage and of bloating. Twenty five (14 male and 11 female) northern fur seal pups were collected from two rookeries (Adams Cove and West Cove) and 53 (24 male and 29 female) California sea lion pups were collected from six rookeries (Cormorant Rock, Northwest Cove, Northwest Point, Pt. Bennett,

South Cove, and West Cove). Hookworm prevalence was recorded for all 78 dead pups and hookworm abundance was calculated for 76. Specimens from two of the dead sea lion pups were not counted but used for an egg embryonation study.

Examination of the dead pups for hookworms was done under harsh field conditions including lack of water from a plumbing system. Therefore, modifications of routine parasitologic necropsies were necessary. Water in plastic containers was carried by backpack to the processing site.

Pup carcasses were placed ventral side up, usually on pieces of driftwood or a sand bank near the rookery where collected; the ventral abdominal wall was opened and the small and large intestines were placed in a plastic bag in a cooler containing a cold ice pack. Body condition of each dead pup was evaluated as poor, fair, or good, based mainly on amount of subcutaneous ventral blubber. The processing of the small intestine was done outside on a wooden table. The mesentery was stripped from the small intestine. Three flushes of the unopened small intestine were made with either fresh water or seawater forced into one end with a plastic catheter-tipped syringe. A 12, 20, or 60 cc syringe was used depending on the diameter of the intestine. After each addition of water, the intestine was "milked" to expel the contents into a 40-mesh sieve (420 μm opening). The intestine was then slit open with bandage scissors and the opened intestine was placed in a 10 L bucket containing approximately 1 L of water; the bucket was then vigorously swirled and, after removing the intestine, the residue poured into the 40-mesh sieve. This procedure was repeated once. The opened intestine which contained attached hookworms was placed in a plastic bag in a small amount of water for 10 to 12 hr. A plastic sprayer apparatus, attached to a container of water, was used to wash the detached hookworms in the sieve. Hookworms were then picked from the sieve with forceps and placed in a small plastic bag. The sprayer was used to flush any remaining parasites and debris from the sieve into the plastic bag. Hookworms were fixed in the bag by adding alcohol to a concentration of about 70%. In the laboratory, whole hookworm specimens were enumerated and separated by sex under a 3× floating lens. Pieces of hookworms were examined at 10× magnification to verify sexes. All specimens were preserved in vials containing alcohol-formalin-glycerin (AFG) fixative (Soulsby, 1982). Representative adult specimens of *Uncinaria* spp. from the northern fur seal pups (USNPC No. 86948, 86949, and 86950) and the California sea lion pups (USNPC No. 86951, 86952, and 86953) were deposited in the U.S. National Parasite Collection (USNPC; Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, U.S.A.).

Statistical analysis of data for determining any significant differences used the (1) Mann-Whitney Rank Sum Test (Snedecor and Cochran, 1989) for the number of male versus female hookworms and the number of specimens in male versus female pups and (2) Kruskal-Wallis Test (Randles and Wolfe, 1979) for number of hookworms present relative to body condition of the pups.

Specimens of hookworms from the small intestine of two dead sea lion pups were collected for an egg embryonation study. These hookworms were washed in water in the 40-mesh sieve, and placed in plastic bags containing a small amount of saline. The specimens were kept in a cooler containing a cold pack or in a refrigerator (4 C) for 7 days before the embryonation study was begun. Several female hookworms were placed in a 50 mm Petri dish in a thin layer of saline and macerated to release their eggs. The top then was replaced on the Petri dish which was incubated at 26 C and 80% humidity. Periodically, the hookworm eggs were examined microscopically for evidence of embryonation. Hatched L_3 were placed in a few drops of saline and fixed in a straight position by adding hot saline to them. This was followed by preservation in a solution of cold AFG. Total length measurements, excluding sheath, were then made of 25 fixed L_3.

RESULTS

Of the 25 northern fur seal pups examined, only one was from West Cove rookery. It was uninfected with *Uncinaria* spp., but all 24 pups from the other rookery (Adams Cove) were infected. Prevalence of hookworms was 96%. The number of specimens in each infected pup varied from 5 to 1,775 (Table 1). The mean intensity of hookworms per infected pup (n = 24) was 643; slightly fewer males ($\bar{x}$ ± SD = 302 ± 306) than females (342 ± 353) were present. Mean intensity of *Uncinaria* spp. was 716 ± 701 for male pups (n = 13) and 557 ± 620 for female pups (n = 11). There was no significant difference in the number of hookworms present in the pups based upon body condition (Table 1) (Kruskal-Wallis Test = 1.362, df = 2, P = 0.546). Two pups had obvious bloody feces; one had 1,649 hook-

TABLE 1. Hookworm intensity and body condition of northern fur seal pups found dead on a rookery (Adams Cove) on San Miguel Island (California, USA) in July 1996.

Body condition	Number examined	Number of hookworms: Range	Number of hookworms: Mean intensity ± SD[a]
Poor	2	160–1,649	905 ± 1,053
Fair	10	5–1,775	651 ± 649
Good	11	8–1,383	510 ± 624
Combined	23[b]	5–1,775	606[c] ± 643

[a] SD = Standard Deviation.
[b] An additional pup had 1,512 hookworms, but its body condition was not recorded.
[c] Mean intensity is 643 when hookworm specimens in above pup are included in calculations.

TABLE 2. Body condition and hookworm intensity in California sea lion pups found dead on six rookeries on San Miguel Island (California, USA) in July 1996.

Body condition	Number examined	Number of hookworms: Range	Number of hookworms: Mean intensity ± SD[a]
Poor	24	14–2,816	811 ± 977
Fair	11	74–4,521	1,359 ± 1,281
Good	16	69–3,841	1,943 ± 1,156
Combined	51	14–4,521	1,284 ± 1,190

[a] SD = Standard Deviation.

worms and the other had an unknown number because its identity was not recorded at necropsy.

All 53 California sea lion pups examined had *Uncinaria* spp. Hookworm numbers varied from 14 to 4,521 per pup (Table 2). About one-half (n = 25) of the pups had over 1,000 *Uncinaria* spp. The mean intensity was 1,284 with slightly fewer males (621 ± 575) than females (663 ± 621) present. Mean intensity of *Uncinaria* spp. was 1,091 ± 1,235 for male pups (n = 23) and 1,443 ± 1,150 for female pups (n = 28). A significant trend (Kruskal-Wallis Test = 8.024, df = 2, P = 0.018) was evident for pups with poorer body condition having the lowest numbers of hookworms present (Table 2). According to rookery, lowest mean intensities of hookworms per pup were on Cormorant Rock and Northwest Point (Table 3); these also were the rookeries with the fewest number of pups examined. Bloody feces were not observed in any pups.

Data from fur seal pups and from sea lion pups indicate there were no significant differences ($P < 0.05$) in the number of male and female hookworms or the number of hookworm individuals in male and female pups.

Embryonation of eggs from hookworms from dead sea lion pups revealed development to the free-living L_3 within the egg. At about 75 hr of incubation, eggs with embryos inside were placed in a drop of saline on a glass microscope slide and a coverslip applied. Manual pressure on the coverslip ruptured the eggs and released L_3, which were within the sheaths of the L_1 and L_2 (Fig. 1). The L_1 sheath separated either during hatching of the L_3 or soon afterward. By a maximum of 88 hr of incubation, numerous L_3, contained within the L_2 sheath, had hatched by themselves. Total length of 25 L_3, excluding the sheath, varied from 457 to 543 μm (512 ± 25 μm).

DISCUSSION

Prevalence of *Uncinaria* spp. was high in both fur seal and sea lion pups with 77 of the 78 pups being infected. The only uninfected pup, a fur seal, was the single pup

TABLE 3. Hookworm intensity in California sea lion pups found dead on six rookeries on San Miguel Island (California, USA) in July 1996.

Rookery	Number examined	Number of hookworms: Range	Number of hookworms: Mean intensity ± SD[a]
Cormorant Rock	1	260	260 (–)
Northwest Cove	20	78–3,841	1,298 ± 1,041
Northwest Point	3	69–2,414	867 ± 1,340
Pt. Bennett	14	14–4,521	1,156 ± 1,406
South Cove	6	260–3,710	1,469 ± 1,452
West Cove	7	82–2,312	1,245 ± 970

[a] SD = Standard Deviation.

examined from West Cove rookery. Other studies on hookworm infections are minimal in California sea lions (Dailey and Hill, 1970) but extensive in northern fur seals (Olsen, 1958; Lyons, 1963; Olsen and Lyons, 1965). The prevalence of hookworms was much higher in fur seal pups on San Miguel Island than, indicated from combined data from several rookeries, in northern fur seal pups on St. Paul Island (Alaska, USA) (Olsen, 1958). However, Olsen (1954) recorded prevalence of 90% in fur seal pups in two specific studies on St. Paul Island. He found higher prevalence and intensity of hookworms, and higher mortality of fur seal pups on sandy versus rocky types of rookeries; it was surmised that survival of hookworm eggs and larvae were better in the sandy soil than in rocky terrain.

Sea lion pups were infected with an average of twice as many hookworms as the fur seal pups. Interpretation of the number of hookworms in sea lion pups relative to rookery is difficult because of the low number of pups examined on some rookeries.

While there was no apparent correlation between number of hookworms and body condition in the fur seal pups, there was for the sea lion pups. This finding in the sea lion pups is similar to reports by Lucas (1899) and Olsen (1958) of finding fewer hookworms in malnourished than in well-fed dead fur seal pups on St. Paul Island. The higher hookworm infections in well-fed pups may simply be due to the pups ingesting milk in adequate amounts for good nutrition, but containing numerous hookworm larvae known to be transmitted through the mammary system of northern fur seals (Lyons, 1963; Olsen and Lyons, 1965). The authors are unaware of proven transmammary transmission of *Uncinaria* spp. for California sea lions, although there is circumstantial indication it may occur (Lyons and Keyes, 1984).

Comparison on location of hookworms and gross observations on the condition of the small intestine, in addition to the cecum and proximal large intestine, of the pups was not a specific goal of the study. However, there was an impression that hookworms in sea lion pups were distributed throughout the small intestine with the possible exception of the proximal three meters; whereas, they appeared to be concentrated in the cecal area in fur seal pups. This observation was based particularly on obvious freshly-dead pups with heavy infections of hookworms, many of which were still attached; also, petechiae seemed more diffuse in sea lion pups. Hookworm distribution and intestinal damage in fur seal pups on San Miguel Island were similar to the condition found by Olsen (1958) on St. Paul Island in fur seal pups in which the areas of greatest tissue damage caused by hookworms were in the distal small intestine, the cecum and the proximal large intestine. The reason for the apparent difference in hookworm location and pathogenesis in the two species of pinnipeds is unknown. There actually may be two distinct *Uncinaria* spp., *U. lucasi* in northern fur seals and a second species in California sea lions. It is not even known whether the effect of hook-

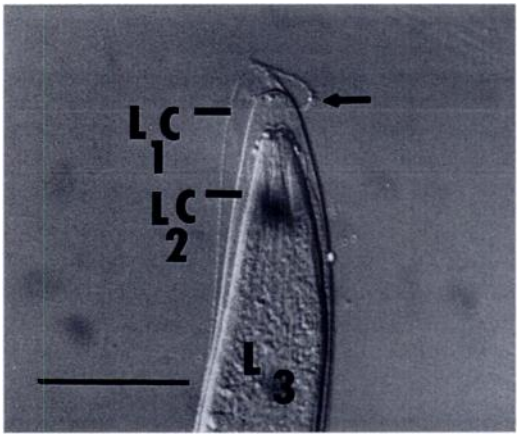

FIGURE 1. Anterior end of a double-sheathed (L_1C and L_2C) free-living third stage *Uncinaria* sp. larva (L_3). The L_3 developed entirely within an egg (then was manually released) collected from a female hookworm found in a dead California sea lion pup. L_1C is the cuticle of the first stage larva. The arrow designates the tip of the folded-over anterior end of L_1C. L_2C is the cuticle of the second stage larva inside L_1C. Scale bar = 30 μm.

worms on these hosts is different. Because the total length of the small intestine in California sea lion pups (n = 3) is about twice (17 to 22 m, 19 ± 3 m) that (9 m) of northern fur seal pups (n = 1) (R. L. DeLong, unpublished data), this may have an influence on the site of the hookworms and numbers of individuals in these hosts.

Embryonation of hookworms from California sea lion pups included development to free-living L_3 within the egg. This is the same as found for hookworms in northern fur seal pups on St. Paul Island (Lyons, 1963; Olsen and Lyons, 1965). The L_3 derived from eggs of hookworms in sea lion pups were about 100 μm shorter than those hatched from eggs in hookworms from fur seal pups on St. Paul Island (Lyons and Keyes, 1978). For the fur seal hookworms on St. Paul Island, hatching of L_3 from eggs was found at 100 hr under room conditions. However, hatching of L_3 from hookworm eggs, first voided in June, is delayed until late August and early September on St. Paul Island rookeries (Lyons, 1963; Olsen and Lyons, 1965). Whether similar delay of hatching of hookworm L_3 occurs on rookeries on San Miguel Island is unknown.

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