

Update on the prevalence of the hookworm, *Uncinaria lucasi*, in northern fur seals (*Callorhinus ursinus*) on St. Paul Island, Alaska, 2011

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Abstract Prevalence of hookworms (*Uncinaria lucasi* Stiles, 1901) was determined in the northern fur seal (*Callorhinus ursinus* Linnaeus, 1758) on St. Paul Island (SPI), Alaska in July and August, 2011. Three of 61 (4.9%) dead pups harbored 1 to 13 adult hookworms each in their intestines. Parasitic larvae (L₃) of hookworms were recovered from the blubber of 4 of 133 (3%) of subadult males (SAMs) examined. One parasitic L₃ was detected from each infected SAM. Adult *U. lucasi* (n=3) were found in the intestine of 1 of 105 SAMs examined (0.95%). This is the first documented finding of adult *U. lucasi* in SAMs of the northern fur seals. Continued low prevalence of hookworms the last several years parallels the tremendous decline in the number of fur seals on SPI over a similar time period.

Introduction

Hookworms were found first in pinnipeds in the late 1800s (Lucas 1899). The host for the initial discovery of these

parasites was the northern fur seal (NFS) (*Callorhinus ursinus* Linnaeus, 1758) on St. Paul Island (SPI), Alaska (Lucas 1899). These hookworms were named *Uncinaria lucasi* Stiles 1901; the species name given was in honor of Lucas, their discoverer (Stiles and Hassall 1899; Stiles 1901). Baylis (1933, 1947) provided more detailed descriptions. Only two hookworm species, *U. lucasi* and *Uncinaria hamiltoni* Baylis 1933, have been described morphologically from pinnipeds. However, molecular differences have been found for specimens of *Uncinaria* spp. from several pinniped hosts (Nadler et al. 2000, 2009). Similar DNA composition was found for *Uncinaria* spp. from *C. ursinus* pups on SPI and Russia in addition to Steller sea lion (*Eumetopias jubatus* Schreber, 1776) pups from Alaska (Nadler et al. 2009). The actual number of species of *Uncinaria* in pinnipeds remains to be clarified. Hookworms have been reported in otariids (five species of fur seals and five species of sea lions) and phocids (two species of elephant seals and the ringed seal) (Lyons et al. 2011a).

Lucas (1899) in his research in 1896 believed that trampling by bulls was the major cause of mortality in fur seal pups on the Pribilof Islands (SPI and St. George Island). However, in summary of his observations in 1897 and 1898, he stated that the majority of deaths of pups were from hookworms. Further, he mentioned that the degree of mortality in pups directly was related to the number of seals and conditions on the rookeries (sandy areas more conducive for hookworm transmission than rocky areas). He reported that fewer pups died from starvation followed by disease and accidents as compared to pups dying from hookworms.

DeLong (2007) summarized the current mortality related to hookworms in NFS pups. He stated that hookworm-related deaths in pups were low on the Pribilof Islands, Kuril Islands, and Robben Island, somewhat higher on the

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Commander Islands (Bering Island and Medny Island), but extremely high on San Miguel Island (SMI), CA.

Current pathology of hookworms in *C. ursinus* pups has been reviewed recently (Spraker and Lander 2010; Lyons et al. 2011a). In fairly well-nourished NFS pups on SPI, the most common lesion is a chronic anemia. Gross lesions may consist of moderate hemorrhagic enteritis with multifocal regions of hemorrhage within a thickened intestinal wall, multifocal small pale areas in the liver, and occasionally a dilated, enlarged heart; blood usually is found in the lumen of the small intestine. NFS pups on SMI have similar lesions to those in NFS pups on SPI. However, in contrast, NFS pups on SMI additionally may have lesions caused by penetration of hookworms through the wall of the small intestine, resulting in peritonitis and bacteremia similar to that described from California sea lion pups (Spraker et al. 2004, 2007; Lyons et al. 2011b). However, one such case of penetration of the intestinal wall was found in 2010 on SPI in a fur seal pup that was anemic and had mild peritonitis and severe hemorrhagic enteritis associated with a heavy burden of hookworms (T.R. Spraker, personal communication).

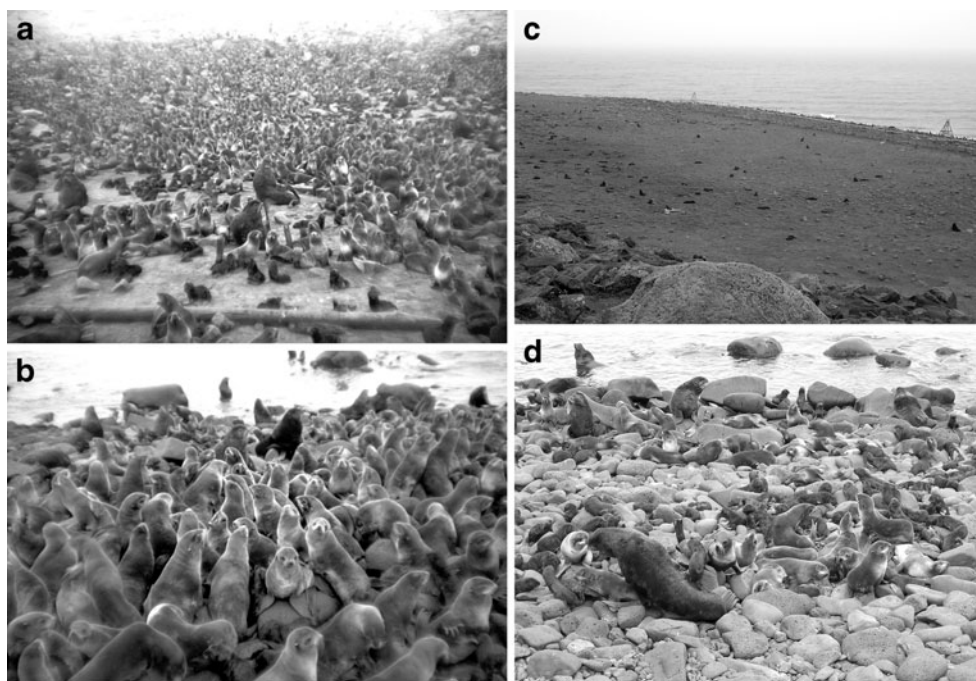
Material and methods

The present hookworm research was conducted on SPI from 10 July to 8 August, 2011. Twice daily, fresh dead pups were collected on Reef Rookery from a catwalk above harems by use of long bamboo poles with hooks or a noose on one end.

The pups ($n=61$) were taken to a biology laboratory and complete necropsies were performed. The cause of death of the pups was related to such factors as starvation, trauma, and perinatal mortality; details will not be reported here. Contents of both the small intestine and large intestine of the pups were examined specifically for hookworms by methods published previously (Lyons et al. 2005).

Subadult male (SAM) fur seals have been harvested each summer since 1985 by the Aleuts for food. The skins with blubber attached were pulled from the carcasses. Approximately 175 to 200 g of blubber were removed with scalpels from the ventral abdominal region of the pelt from 133 SAMs and put in separate plastic bags in the field. In the biology laboratory, 150 g of blubber from individual SAMs was cut up into small pieces and placed in a Baermann funnel system containing warm tap water for a minimum of 6 h. Afterwards, all water in each test tube was examined for hookworm larvae under a dissecting microscope. Any larvae found were identified under a compound microscope using $\times 100$ and $\times 400$ magnifications. Distinguishing characteristics of parasitic hookworm L_3 (Olsen and Lyons 1965) allowed identification of them from free-living soil/plant nematodes which were on the blubber of several samples because of contamination from the ground when skins were removed from SAM carcasses. Examination of gastrointestinal tracts of 105 SAMs, humanely harvested during the annual Aleut subsistence harvest, was performed by classical methods of parasitological dissection (Bowman 1995). Hookworms recovered from the intestines of pups, intestines, and blubber of SAMs were counted and preserved in 70% ethanol for future molecular studies.

Fig. 1 a–d Harems consisting of bulls, cows, and pups on Reef Rookery on St. Paul Island, AK. The harems were on sandy terrain (a) which was quite a favorable habit for hookworm transmission in 1960 because of overcrowding on the rocky terrain (b) by the Bering Sea. In 2011 (d), the harems were mostly only on sparsely populated areas of rocky terrain with almost none on sandy beaches (c)



Results and discussion

Three of 61 (4.9%) dead pups examined were infected with 1 to 13 adult hookworms. Molecular profile of specimens was the same as that found for hookworms from the SPI fur seal herd in previous studies (Nadler et al. 2009). The prevalence was similar to that found in the late 1980s and 1990s (<10%), in 2001 (3%) and in 2007 (6%) in dead pups on SPI (Lyons et al. 2000, 2003; Ionita et al. 2008). These prevalences were in contrast to those of over 90% in dead pups on rookeries on SPI about 50 years ago (Lyons 1963; Olsen and Lyons 1965). Evidence of hookworm disease was not observed during the gross necropsy in these pups. The cause of death in these pups was determined to be starvation. The age of the pups was estimated to be 2.5 to 3 weeks of age by size and shape and degree of eruption of the canine teeth.

Parasitic hookworm *L*₃ were found in blubber of 4 of 133 (3%) SAMs; one larva was recovered from each infected animal. This prevalence of *L*₃ is similar to that detected in blubber of SAMs in 2001 (4%) on SPI. However, the prevalence of larvae in the blubber of SAMs in 2001 and 2011 was dramatically lower than that found for *L*₃ in ventral abdominal blubber about 50 years ago which was 100% on SPI (Lyons 1963; Olsen and Lyons 1965). Adult *U. lucasi* (one male and two females) were found in the intestines of 1 of 105 SAMs (0.95%). This is the first reported finding of adult hookworms in northern fur seals older than pups. Previously, Olsen (1958) did not find adult hookworms in 1,426 adult northern fur seals on SPI.

Several hypotheses are suggested that may explain the tremendous decline of hookworm infections in NFS on SPI. First, the dwindling of the hookworm infections in NFS on SPI parallels the similar huge decrease in the NFS population in the last several decades. The number of pups born on SPI has been reduced from about 300,000 to 400,000 in the 1950s and early 1960s to about 120,000 in the early 2000s according to Melin et al. (2006). Ream (2008) states that the number of pups born on SPI has decreased at an annual rate of 5.2% (SE=0.40) since 1998, which is at the level last observed in about 1916.

It is probable that due to the much lower density of NFS, and therefore pups, there is less chance of recycling of hookworms now. The only source of hookworm eggs on the rookery is from those passed in the feces of pups. This is because the older NFS typically are not infected with adult hookworms (Olsen 1958; Olsen and Lyons 1965) and therefore are not shedding eggs into the environment. Free-living *L*₃ which hatch from the eggs on the rookery do not mature in NFS but only enter tissues, especially blubber. An important factor in the *U. lucasi* life cycle is that the only larval stage of hookworms which develops to adults in pups is the parasitic *L*₃. These larvae primarily are stored in the ventral

abdominal blubber of older age seals and can only escape and pass to newborn pups in the first milk of cows following parturition. There also has been an obvious change in location of harems (Fig. 1a–d) which now (2011) are predominately on rocky areas near the surf (Fig. 1d) and rarely not on sandy beaches (Fig. 1c). This factor reduces hookworm transmission because, years ago, when there was a higher number and density of seals, there was more competition for harem space. At that time (1960), because of so many harems on rocky areas by the surf (Fig. 1b), the harems extended farther inland from the ocean onto a sand/dirt environment (Fig. 1a) and therefore a more favorable microhabitat for hookworm development of eggs and free-living *L*₃. This resulted in more opportunity for hookworm transmission. DeLong (2007) has suggested that lower hookworm infections may be due to the major histocompatibility complex genes in the NFS population, providing the capability for immunity to reduce or prevent hookworm infections.

Transmammary transmission that occurs with *U. lucasi* in NFS is an excellent method of perpetuating the life cycle if there are enough infected animals contributing to this endeavor. This occurs when a pup nurses; it automatically will become infected if parasitic *L*₃ are in the mother's milk. However, when transmammary transmission is the only route of transmission, this limits the possibility of continued propagation of these nematodes. On SPI now, the overall burden of *U. lucasi* in NFS is extremely low and has no effect on the overall population and is not a contributing factor to the general decline in the NFS population.

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