The Dynamics of Hookworm Disease in Northern Fur Seals

by
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Northern fur seals (*Callorhinus ursinus*) breed on the Pribilof Islands and Bogoslof Island in the eastern Bering Sea, the Commander Islands in the western Bering Sea, the Kurile Islands in the western North Pacific, Robben Island in the western Okhotsk Sea, and Southeast Farallon and San Miguel Islands off California in the eastern Pacific. Hookworm disease has been recognized in northern fur seals since 1896 when F. A. Lucas described the worm from the intestine of a 3-month-old dead fur seal pup in the Pribilof Islands. The fur seal hookworm was subsequently described as *Uncinaria lucasi* by C.W. Stiles in 1901. From the time of their description until the early 1980s, hookworms were highly prevalent in fur seal pups and were responsible for a substantial amount of pup mortality in the Pribilof Islands.

Hookworms are intestinal parasites that suck blood, and if the intensity of the infection (number of worms/individual seal) is great enough, pups develop anemia that leads to mortality. Currently, there is virtually no mortality associated with hookworms in Pribilof Island fur seal pups, although hookworm disease continues to cause pup mortality in several rookeries on Bering and Medney Islands in the Commander Islands. Hookworm disease is not a significant cause of mortality among fur seals on the Kurile Islands or Robben Island apparently because of a marked difference in rookery habitat on those islands. However, on San Miguel Island in the California Channel Islands, hookworm disease has become a major source of pup mortality. This article provides an overview of the biology of hookworms, discusses the change in hookworm disease prevalence in the Pribilof Islands, and reviews a recent experiment in which fur seal pups were treated with drugs to control the disease on San Miguel Island.

Biology

Hookworms infect a wide variety and number of vertebrates including man and pinnipeds. A generalized life cycle is as follows. Infective larvae live in soil, penetrate the skin of an appendage, and migrate into the circulatory system. They are transported via the blood to the lungs where they exit the blood and enter the bronchi of the lungs. There they are coughed up through the trachea into the throat and swallowed. This is referred to as the pulmonary route of infection and historically had been considered the only route of hookworm infection. In the small and large intestine the worms attach to the wall of the intestine and suck blood, mature, and lay eggs, which pass out with the feces and are deposited in soil. The eggs hatch and release infective larvae that repeat the infection process. It has been known for many years that in fur seals and sea lions only pups died of hookworm infections, but it was not understood why. This became the source of an interesting case study in which a young researcher made an exciting discovery.

Historical Background

In 1951, a renowned parasitologist named O. Wilford Olsen from Colorado State University began studying hookworm disease in northern fur seals on St. Paul Island in the Pribilof Islands. His research, lasting several years, focused on how pups became infected. His findings indicated that infective larvae overwintered in the soil but seemed to disappear from the soil at about the time fur seal pup births began in June, with the larvae not reoccurring in the soil until sometime in August. The vexing question for Dr. Olsen was, Where were larvae coming from that infected newborn pups?

Figure 1. Reproductive territories of northern fur seals on Adams Cove beach, San Miguel Island, July 2006.

He was unable to discover how the infectious process occurred.

A decade after initiating his study, Olsen brought the doctoral graduate student Gene Lyons to St. Paul Island. Lyons spent the summer of 1960 there conducting research on hookworms and exhausted the possibility that hookworm infections were occurring prenatally. Lyons knew that transplacental infection of some parasitic worms was known to occur, and this became a logical avenue of investigation for him. During a second field season on St. Paul, Lyons had his day of discovery on 4 July 1961. He found hookworm larvae in ingested milk from a pup's stomach; remarkably the animal was only 2 hours old. Lyons subsequently discovered that larvae moved from the blubber in the pregnant female into the mammary tissue sometime prior to birthing and were ingested upon the pup's first feeding. This was the first case in parasitology where transmammary transmission of a parasite had been demonstrated.

Gene Lyons was excited with the discovery but also was aware that his work would face extreme scrutiny. He spent the following year conducting field research addressing the final details of the infectious process. I met Gene Lyons in 1962 while he was working on St. Paul Island, the same year that marked my introduction to fur seals and marine mammal research. I came to St. Paul Island as an undergraduate student working as a biological aid for the summer. Lyon's dedication, meticulous investigative habits, and raw stamina for his work were astounding. He was frequently still in the laboratory at 10:00 pm, already having worked 14 hours, and he maintained that schedule 7 days a week. Lyons completed his doctoral program in 1963 and accepted a position as a research parasitiologist in the Department of Veterinary Sciences at the University of Kentucky.

During the 80 years since their discovery until the late 1970s, hookworms killed thousands of fur seal pups on St. Paul Island in the Pribilofs. In 1957 up to 56% of 1,727 pups examined at necropsy by Professor Leo Doyle had died of hookworm infections, and as late as 1979, 18% of 204 pups necropsied by Dr. Mark Keyes had died of hookworm infections. Then, from 1986 to 2006, Dr. Terry Spraker found only 0.6% of 2,735 fur seal pups examined at necropsy had hookworm infections. Because the intensity of infections was very low in all but one case, hookworms were not classified as the cause of death. (The exception was a pup that had hookworm infections and was anemic at the time of death.) Clearly, some change occurred in the late 1970s or early '80s that caused fur seals in the Pribilof Islands to become nearly free of hookworm infections, but what exactly that change was remains a mystery today.

In a recent review, Gene Lyons and coauthors suggest that the decline in prevalence of hookworm infections corresponds to a decline in the fur seal population on the Pribilof Islands, raising the possibility that the prevalence of infection is a density-dependent response. The authors also point out that most pups there are currently born on rookeries which have rocky substrate which is not good hookworm habitat. As the number of breeding fur seals has declined on all rookeries in the Pribilof Islands their distribution has become restricted to rocky habitat. (Recall that infective larvae live in soil and apparently are not found on cobble or bedrock beaches.) An alternative hypothesis is that Pribilof Island fur seals have, after a long period of exposure, evolved a population immune response to hookworms. It is not entirely clear how this might work, but the leading hypothesis has to do with the major histocompatability complex (MHC) genes that control immune function. If there are MHC haplotypes which confer survival advantages to fur seal pups infected with hookworms, they would be selected for and emerge in animals that survive hookworm infections. Then, gradually, the MHC haplotypes which generate resistance to hookworm would become dominant in the population, thereby providing resistance to infection by the worms.

In Russia, during the 1980s and 1990s hookworm disease was a dominant cause of mortality on single rookeries on Bering and Medney Islands in the Commander Islands. The disease remains an important cause of pup mortality there today, despite the fact that large numbers of pups were treated with antiparasitic drugs during the 1990s. Although I have heard oral reports of some of this work, I have not been successful in finding the relevant papers or having them translated; consequently, I do not know the details of these studies. However, it was reported that following treatment of several thousand pups there the mortality rate of pups declined for several years but then increased to more than 35% annually on those rookeries. Normal fur seal pup mortality rates range from 8% to 15%. It is noteworthy that each of the Commander Island rookeries where hookworm disease is prevalent are on beaches with sandy and cobble substrates. Hookworm disease became apparent in dead pups on San Miguel Island in the California Channel

Islands in the early 1990s. San Miguel Island has a breeding population of approximately 8,000-10,000 northern fur seals, and the population has been increasing, producing about 2,500 pups annually (Fig. 1). By 1996 our investigations showed hookworm disease was associated with most cases of pup mortality there, and by 2004 the level of pup mortality had increased gradually to 50% of all pups less than 2 months old. The pathology of hookworm disease at San Miguel Island appears different from what has been described in the Pribilof Islands. Most pups die of chronic or acute anemia in the Pribilofs. On San Miguel Island, pups with hookworm infections die of bacterial infections in the peritoneal cavity or major organs. The bacteria appear to be inoculated into the capillaries of the small intestines by the hookworms and once in the circulatory system they move around the body and settle in major organs where the bacteria create lesions which ultimately cause death. There is also a situation among some diseased pups where the hookworms penetrate through the small intestine and live in the peritoneal cavity. The hookworms apparently transport pathogenic bacteria into the peritoneal cavity which are responsible for peritonitis and death.

In Adams Cove on San Miguel Island, fur seals compete with California sea lions (*Zalophus californianus*) for rookery space. Although the reproductive territories of the two species are separate on the sandy habitat, pups of both species use the same space during much of the breeding season and into the late summer and autumn months. California sea lions have hookworms of a different species (*Uncinaria hamiltoni*), which also cause disease and mortality. We now know that cross infections of sea lion worms in fur seals occur, but it is not yet clear whether cross infections contribute significantly to fur seal pup mortality from hookworm disease. Because hookworm disease is currently the major cause of fur seal pup mortality, accounting for 50% or more of fur seal pup deaths in Adams Cove each pupping season since 2004 , we decided to conduct an experiment in which young pups would be treated with an antiparasitic drug.

Ivermectin is a broad spectrum antiparasitic drug widely used for controlling arthropods and nematodes in domestic animals. Ivermectin, administered twice, 10 days apart, has been used to control nematodes in captive pinnipeds. The efficacy of a single dose of ivermectin was evaluated in a field setting with a small number (n = 4) of northern fur seal pups on St. Paul Island in 1983. This field trial resulted in almost complete clearance of hookworm infections in treated pups.

San Miguel Study

We conducted a study to evaluate the efficacy of a single treatment of ivermectin for clearing hookworm infections in northern fur seal pups on a population scale at Adams Cove on San Miguel Island. The goal was to assess whether pups receiving the ivermectin treatment had increased growth and survival rates compared to control animals that received a saline placebo. We expected the treatment would likely be effective in controlling infection, but we were uncertain whether a single treatment would be sufficient to improve survival. An observed increase in survival for treated pups would constitute more evidence that hookworm infection has been the primary cause of increased pup mortality and is a necessary precursor to the bacterial infections which are the proximate cause of death.

Pups were sampled from 8 to 10 July and from 18 to 20 July 2006 on the sandy beach habitat of Adams Cove to obtain samples of pups born throughout the pupping season. A group of 300 northern fur seal pups were captured and treated either with ivermectin or a placebo of saline. Ivermectin was administered to 151 pups, and 149 pups received a saline placebo. Pups were treated when they were about 2 weeks old. A mobile blind was used to approach pup aggregations within reproductive territories, which generally consisted of pups whose mothers were feeding at sea. Pups associated with females were not captured so that actively suckling pups were not disturbed. Individual pups were captured with a "noose pole" consisting of a noose fabricated from 4-mm plastic coated cable and a telescoping paint roller pole which extended to 5 m in length (Fig. 2). Pups were returned to the same reproductive territory after treatment.

Morphometric measurements were taken of each pup in the study sample. Pups were tagged with individually numbered pink Dalton Jumbo Rototags® with unique numbers; each pup was double-tagged along the trailing edge of the fore-flipper in the axilla just distal of the hair line. Pups were alternately assigned to treatment and control groups to randomize assignment while controlling for any temporal or spatial pattern in pup selection throughout the capture process. Pups were injected subcutaneously in the lumbar region of the dorsum either with ivermectin (at a dose rate of 200 μg/kg) or 0.9 %

Figure 2. National Marine Mammal Laboratory biologist Tony Orr (right) with visiting biologist Greg Orr adjusts a noose in preparation for capture of fur seal pups on San Miguel Island.

saline (volume dose equal to that of ivermectin for a pup of the same weight). The ivermectin was prepared in a 1 mg/ml suspension of propylene glycol from more concentrated large animal ivermectin at the College of Veterinary Medicine, Colorado State University. Dosages ranged from 0.8 to 1.8 ml and were administered with a disposable syringe and a 21 ga × 2.5 cm needle.

A fecal sample was collected from each animal with a small animal fecal loop that can collect up to approximately 1 ml. The fecal samples were placed in 15 ml centrifuge tubes containing 9.5 ml of seawater. The fecal samples were fixed by adding sufficient formaldehyde to create a 5% formalin solution. The quantity of fecal material obtained was scored numerically from 0 to 3, with 0 being an empty loop and a 3 being a loop filled with feces. Although samples rated as 0 occasionally were positive for hookworm eggs, they were regarded as null samples containing neither fecal material nor hookworm eggs. Only samples that scored 1 to 3 were used in calculations for prevalence of infections. In the laboratory, the samples were centrifuged and the fluid was decanted. Saturated sugar solution was added to each test tube that was centrifuged again with a cover slip covering the top of the test tube. Cover slips were examined at a magnification of 100 under a compound microscope. The presence or absence of hookworm eggs was recorded for each sample. All fecal samples were coded so that the examiner did not know which were from treated or control pups.

Mortality surveys were conducted every 3-5 days following the treatment periods until mid-August when the mortality rate began to decline. Surveys were continued until early December at less frequent intervals. Tag numbers of observed dead pups were recorded during each survey. On 10 and 11 August, 30 tagged fur seal pups from each group were recaptured and a fecal sample was collected to check for hookworm eggs. In late September, about 2.5 months following treatment, a sample of treated and control pups was recaptured to assess rates of growth between the two groups. Length, girth, and weight were recorded. Daily growth rates (kg/day) were used to standardize growth data for varying capture and recapture dates.

Results

In total, 50 control pups and 10 ivermectin-treated pups were found dead after treatment (Fig. 3). No mortality was observed after our 27 September 2006 survey. The observed mortality rates of 6.6% (treatment) and 33.6% (control) were significantly different (Fisher's exact test; $P < 0.00001$). The average growth rate of mass (kg/day) for pups treated with ivermectin was 0.057 (SE = 0.004). Growth rate for control pups was only 0.038 (SE = 0.003) kg/day, which was significantly less than for pups treated with ivermectin ($T = 3.17$, $df = 49$, $P = 0.0026$).

Eleven fecal samples from treated pups and 9 from control pups were rated as 0s at the time of sampling and were not used in the calculation of prevalence of infection. The prevalence of hookworm eggs in fecal samples was identical (24%) for control and treated pups at the time of treatment in July. By mid-August, only 6% of the sample of pups from the ivermectin treatment group had hookworm eggs in their fecal samples, while the sample of control pups showed a significantly higher prevalence of 67% (Fig. 4)(Fisher's exact test: $P < 0.0001$), demonstrating the effectiveness of ivermectin in clearing the hookworm infections. By late September, nearly all of the pups (control and treated) were clear of infection. Fecal samples from 30 control pups were examined in both July and August. The presence of eggs in the fecal samples was as follows: 10 of 30 were positive and 20 of 30 were negative for eggs in July. Of the 20 which were negative in July, 15 contained eggs in August (Fig. 4). A total of 25 pups were shedding eggs in either July or August. We calculate a prevalence of infection from these data as 83%.

Discussion

To date, we do not yet have adequate data on the prevalence of hookworm infections in northern fur seals at San Miguel Island. Virtually all of the dead pups handled at necropsy over the past several years had hookworm infections**.** However, this is a biased sample because of the lack of hookworm egg data on fecal samples from live pups during the same periods when dead pups were examined. Fecal samples taken from a random sample of live pups handled in other studies for tagging in late September or early October have consistently been negative. This indicates that infections have cleared from virtually all pups by early October when the youngest pup would be about 2 months old. Hookworm eggs were found in a surprisingly small proportion of the pups (24%) in our 2006 study at the time of treatment in late July. This almost certainly resulted because the infections were still prepatent. The observed prevalence of infections among control pups in early August was 66%, a substantial increase from when the pups were first sampled in July. The 83% estimate of prevalence of infection in control pups constructed by examining the presence of hookworm eggs in fecal samples from individual pups sampled in both July and August is probably closer to representative for the population. But this estimate is based on a relatively small sample of 30 pups. Additional fecal sampling of pups, all greater than 2 weeks old but less than 2 months old, will be required to establish prevalence of infection for this fur seal pup population.

The efficacy of ivermectin for treating hookworm disease in pups is clear from the data on prevalence of eggs in the feces of northern fur seal pups following treatment. Only 2 of 34 pups treated with ivermectin and resampled 1 month later were shedding eggs. The ivermectin treatment of northern fur seal pups caused a dramatic increase in the growth and subsequent survival of pups when compared to the control group. This appears to have resulted from treatment of pups when they were quite young (about 2 weeks old) before the infections of worms had become severe enough to initiate changes leading to death from hookworm disease. Two pups that were treated with ivermectin died shortly after treatment (one within 16 hours and one within 2 days) and were examined at necropsy. Both pups had small focal hemorrhages in the wall of the small intestine where adult hookworms had been attached,

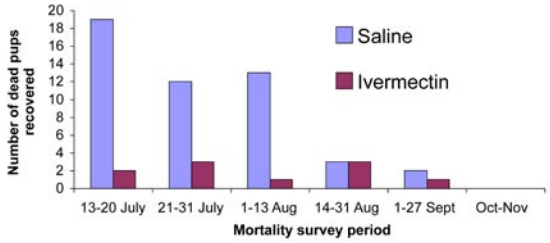

Figure 3. Mortality of tagged northern fur seal pups following administration of treatment with ivermectin or saline, San Miguel Island, 2006.

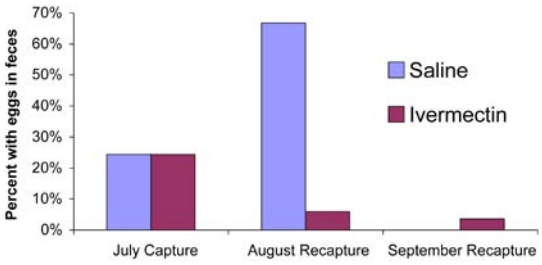

Figure 4. Prevalence of eggs in tagged northern fur seal pups at time of ivermectin or saline treatment and at subsequent recaptures, San Miguel Island, 2006.

but neither pup had any hookworms in the small or large intestines either attached or in the contents of the gut. These observations indicate that the adult hookworms were killed and eliminated from the gut of one of the pups in less than 16 hours following treatment with ivermectin.

We view the experimental treatment of northern fur seal pups with a single dose of ivermectin as successful in controlling hookworm disease. The protocols we applied on fur seal pups at San Miguel Island could be used in the future should conditions warrant such intervention as a conservation measure to increase survival of pups.

There still remain significant unknowns about the dynamics of hookworm disease in northern fur seals. Genetic analysis of MHC genes in fur seal pups from the Pribilof Islands needs to be undertaken. Monitoring the trend of hookworm disease-induced mortality at San Miguel Island will be important over the next decade. Further study of the pathology of northern fur seal pups which die of hookworm disease may allow interpretation of whether the mortality is caused by fur seal hookworms or by cross infection of hookworms from sea lions.