Helminth and respiratory mite lesions in Pinnipeds from Punta San Juan, Peru

Mauricio Seguel1*, Karla Calderón2,5, Kathleen Colegrove3, Michael Adkesson4, Susana Cárdenas-Alayza5 and Enrique Paredes6

1Department of Pathology, College of Veterinary Medicine, University of Georgia, 501 DW Brooks, Athens, GA, 30602, USA; 2Universidad Tecnológica del Perú. Lima, Peru; 3Zoological Pathology Program, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Brookfield, IL, 60513, USA; 4Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513, USA; 5Centro para Sostenibilidad Ambiental, Universidad Peruana Cayetano Heredia. Av. Armandáriz 445, Lima 18, Perú; 6Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Isla Teja s/n, 5090000, Valdivia, Chile

Abstract
The tissues and parasites collected from Peruvian fur seals (Arctocephalus australis) and South American sea lions (Otaria byronia) found dead at Punta San Juan, Peru were examined. The respiratory mite, Orthohalarachne attenuata infected 3 out of 32 examined fur seals and 3 out of 8 examined sea lions, however caused moderate to severe lymphohistiocytic pharyngitis only in fur seals. Hookworms, Uncinaria sp, infected 6 of the 32 examined fur seals causing variable degrees of hemorrhagic and eosinophilic enteritis. This parasite caused the death of 2 of these pups. In fur seals and sea lions, Corynosoma australe and Contracaecum osculatum were not associated with significant tissue alterations in the intestine and stomach respectively. Respiratory mites and hookworms have the potential to cause disease and mortality among fur seals, while parasitic infections do not impact significantly the health of sea lions at Punta San Juan, Peru.

Keywords
Hookworm, mite, Orthohalarachne spp., Peruvian fur seal, South American sea lion, Uncinaria sp.

Introduction
Marine Mammals are top predators of aquatic ecosystems and their health is highly influenced by the conditions of the marine environment (Gulland and Hall 2007). Therefore, the study of marine mammals’ health is important from the ecological and one health perspectives. Among the agents of disease described in marine mammals, a large number correspond to metazoan parasites, many of them of zoonotic significance (Sepulveda et al. 2015). In the case of otariids (eared seals), one of the most important parasitic diseases described is hookworm infection, however the effect of these nematodes is highly variable between populations (Seguel and Gottdenker 2017). For other helminth and arthropod parasites information on the effect of these infections in the host is very limited since most studies only survey epidemiological aspects but few investigate the potential role of these parasites as agents of disease or mortality. In the Southern hemisphere, information is even more limited given the lower number of researchers and very large marine ecosystems. In the Peruvian coast, little is known regarding the diversity and potential impact of helminths and arthropod parasites on marine mammal health, despite being one of the areas of the world with the highest diversity of marine vertebrates (Bakun and Week 2008; Gómez-Puerta and Gonzáles-Viera 2015; Gutierrez et al. 2016). Here, we characterize the tissue damage of helminth and arthropod parasites on Peruvian fur seals (PFS, Arctocephalus australis) and South American sea lions (SASL, Otaria byronia) from Punta San Juan, Peru, highlighting the effect of the most pathogenic species identified.
Fig. 1. Nasopharyngeal mite (*Orthohalarachne attenuata*) and hookworm (*Uncinaria sp*) lesions in Peruvian fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) from Punta San Juan, Peru. 

A. – Mites attached to the nasopharyngeal mucosa of a subadult Peruvian fur seal (Arrow). Inset: Morphological details of *O. attenuata*. 

B. – Photomicrograph of a section of the tissue showed in A. Respiratory mites penetrate deep into the mucosa and submucosa and are surrounded by a dense inflammatory infiltrate (asterisk). Hematoxylin and eosin (H&E). Scale bar = 500 µm. Inset, detail of inflammatory infiltrate surrounding rests of mites exoskeleton (arrow head). Scale bar = 100 µm. 

C. – Photomicrograph of the nasopharynx of a South American sea lion parasitized with *O. attenuata*. There is mild erosion and ulceration of the epithelium and scant inflammatory infiltrate and fibrosis in the submucosa (arrow). H&E. Scale bar = 100 µm. 

D. – Hookworm infection in a Peruvian fur seal pup. A large female hookworm is deeply embedded in the small intestine mucosa. In adjacent areas there are several hemorrhagic hookworm attachment sites (arrow head). Scale bar = 1 cm. Inset: Detail of *Uncinaria sp*. eggs within a female’s uterus. 

E. – Photomicrograph of the small intestine showed in D. Intestinal villi are irregular and there is marked goblet cell hyperplasia. Deep in the mucosa are hookworm feeding tracks (arrow) and inflammatory infiltrate extent into the submucosa (asterisk). 

H&E. Scale bar = 100 µm. 

F. – Higher magnification of a hookworm feeding track with numerous bacteria colonies (asterisk) surrounded by macrophages. H&E. Scale bar = 10 µm. 

G) Gram stain of hookworm feeding track showed in G. There are rare gram-negative bacilli (arrow head) and occasional chains of gram-positive cocci. Scale bar = 5 µm.
Table I. Lesions caused by helminth and arthropod parasites in Peruvian fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria flavescens*) at Punta San Juan, Peru

<table>
<thead>
<tr>
<th>Case No</th>
<th>Species</th>
<th>Age class</th>
<th>Sex</th>
<th>Body condition</th>
<th>Parasite species</th>
<th>Burden</th>
<th>Parasite associated lesions</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Subadult</td>
<td>Female</td>
<td>Fair</td>
<td>Orthohalarachne attenuata</td>
<td>78</td>
<td>Marked, diffuse, hyperplastic, lymphoplasmacytic and histiocytic pharyngitis with pharyngeal glands hyperplasia and ectasia</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>2.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Subadult</td>
<td>Female</td>
<td>Fair</td>
<td>Orthohalarachne attenuata + Corynosoma australis</td>
<td>38 (Oa) 56 (Ca)</td>
<td>Moderate hyperplastic lymphoplasmacytic pharyngitis. Diffuse moderate edema.</td>
<td>Trauma</td>
</tr>
<tr>
<td>3.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Pup</td>
<td>Male</td>
<td>Good</td>
<td>Uncinaria sp</td>
<td>101</td>
<td>Moderate to marked, diffuse, lymphoplasmacytic and eosinophilic enteritis with goblet cell hyperplasia and hookworm feeding tracks.</td>
<td>Trauma</td>
</tr>
<tr>
<td>4.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Pup</td>
<td>Female</td>
<td>Poor</td>
<td>Uncinaria sp</td>
<td>21</td>
<td>Moderate to marked, diffuse, lymphoplasmacytic and eosinophilic enteritis with goblet cell hyperplasia and deep hookworm feeding tracks.</td>
<td>Starvation</td>
</tr>
<tr>
<td>5.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Pup</td>
<td>Male</td>
<td>Poor</td>
<td>Uncinaria sp</td>
<td>121</td>
<td>Marked, diffuse, lymphoplasmacytic and histiocytic enteritis with goblet cell hyperplasia, deep hookworm feeding tracks and coc-cobacilli colonies.</td>
<td>HEB</td>
</tr>
<tr>
<td>6.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Pup</td>
<td>Female</td>
<td>Poor</td>
<td>Uncinaria sp</td>
<td>60</td>
<td>Moderate, diffuse, lymphoplasmacytic and histiocytic enteritis with goblet cell hyperplasia, deep hookworm feeding tracks and coc-cobacilli colonies.</td>
<td>Starvation</td>
</tr>
<tr>
<td>7.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Pup</td>
<td>Male</td>
<td>Good</td>
<td>Uncinaria sp</td>
<td>118</td>
<td>Marked, diffuse, lymphoplasmacytic and histiocytic enteritis with goblet cell hyperplasia, deep hookworm feeding tracks and coc-cobacilli colonies.</td>
<td>HEB</td>
</tr>
<tr>
<td>8.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Pup</td>
<td>Male</td>
<td>Good</td>
<td>Uncinaria sp</td>
<td>90</td>
<td>Moderate, lymphoplasmacytic and eosinophilic enteritis with goblet cell hyperplasia and hookworm feeding tracks.</td>
<td>Trauma</td>
</tr>
<tr>
<td>9.</td>
<td>Fur seal (<em>A australis</em>)</td>
<td>Pup</td>
<td>Female</td>
<td>Fair</td>
<td>Orthohalarachne attenuata</td>
<td>41</td>
<td>Mild purulent pharyngitis</td>
<td>Trauma</td>
</tr>
<tr>
<td>10.</td>
<td>Sea lion (<em>O flavescens</em>)</td>
<td>Adult</td>
<td>Female</td>
<td>Good</td>
<td>Orthohalarachne attenuata</td>
<td>61</td>
<td>Minimal diffuse hyperplastic and lymphoplasmacytic pharyngitis with mild multifocal stroma elastosis.</td>
<td>Trauma</td>
</tr>
<tr>
<td>11.</td>
<td>Sea lion (<em>O flavescens</em>)</td>
<td>Adult</td>
<td>Male</td>
<td>Good</td>
<td>Corynosoma australis</td>
<td>300</td>
<td>Diffuse moderate edema.</td>
<td>Unknown</td>
</tr>
<tr>
<td>12.</td>
<td>Sea lion (<em>O flavescens</em>)</td>
<td>Adult</td>
<td>Male</td>
<td>Good</td>
<td>Corynosoma australis</td>
<td>210</td>
<td>Mild, diffuse, lymphoplasmacytic colitis.</td>
<td>Trauma</td>
</tr>
<tr>
<td>13.</td>
<td>Sea lion (<em>O flavescens</em>)</td>
<td>Adult</td>
<td>Male</td>
<td>Good</td>
<td>Orthohalarachne attenuata + Contrac aecum osculatum</td>
<td>31 (Oa) 80 (Co)</td>
<td>Minimal diffuse hyperplastic and lymphoplasmacytic pharyngitis with mild multifocal stroma elastosis (Oa)</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Materials and Methods

In the austral summer of 2014, SASL (n = 8) and PFS (n = 32) were found dead at Punta San Juan, a site of the System of Islands, Islets and Guano Concentration Areas Protected Area (15°22’S, 75°11.5’W). Necropsies were performed in the field following a standard protocol (Dierauf 1994). During necropsies all helminth and arthropod parasites were collected recording the specific anatomic location. Sections of lung, nasopharynx, tonsil, bronchial lymph nodes, liver, kidney, small and large intestines, adrenal gland, spleen and all tissues from where parasites were collected or where parasites were still embedded in the tissue were placed in 10% buffered formalin and routinely processed for histopathology. The cause of death in each animal was determined based on gross and histologic findings following published diagnostic criteria for otariids (Seguel et al. 2011). All metazoan parasites identified at necropsy were placed in 70% ethanol, cleared with lactophenol (helminths) or potassium hydroxide (mites) mounted in glass slides with Canada balsam, measured and photographed for identification using standard parasitology keys (Banks 1910; Baylis 1933, Doestchman 1944; 1947, Lichtenfels 1980; Willmott and Chabaud 2009; Van Cleave 1945). Voucher specimens were placed in the collection of the laboratory of Fauna Silvestre y Zoonosis, Universidad Nacional Mayor de San Marcos, Lima, Peru.

Results

Thirty-two PFS, including 18 male, 14 female, and 24 pups, 8 juvenile/subadults were examined. The cause of death in these animals included blunt trauma to the head (3PFS and 4 SSL) and chest (1 PFS and 1 SSL), starvation (2 PFS), hookworm (Uncinaria sp.) enteritis and bacteremia (HEB) (2 PFS), bronchopneumonia (1 PFS) and in 1 PFS and 1 SSL the cause of death could not be determined (Table I). Nine PFS (28%) had helminth and/or arthropod parasites. In the case of SASL 6 out of the 8 (75%) animals examined (7 males, 1 female, 1 pup, 7 subadults) had metazoan parasites. The demographic information, parasite species, burden, most significant lesions and most likely cause of death of the animals with parasitic infections are summarized in Table I. In PFS, respiratory mites (Orthohalarachne attenuata) were deeply embedded in the nasopharyngeal mucosa and caused moderate to marked lymphoplasmacytic and histiocytic pharyngitis with epithelial and pharyngeal mucous gland hyperplasia (Fig. 1A and 1B). One PFS had occasional small areas of necrosis deep in the mucosa associated with rests of mites and small colonies of Gram-positive cocci. This animal died as consequence of moderate and diffuse histiocytic and neutrophilic bronchopneumonia with presence of small numbers of macrophages and neutrophils in alveolar septae. In SASLs, mite attachment was more superficial and associated with occasional epithelial erosions, mild mucosa hyperplasia, mild fibrosis and infiltration of rare lymphocytes and plasma cells (Fig. 1C). Hookworms (Uncinaria sp.), were the most common parasite in PFS pups (Table I) (Fig. 1D). In three animals between 60 and 118, 1–2 cm in length hookworms admixed with abundant hemmorrhagic intestinal content occupied the distal jejunum and ileum. In these animals, skeletal muscles and mucus membranes were moderately pale and blood moderately aqueous. Histologically, these animals had moderate to marked lymphoplasmacytic and histiocytic enteritis with goblet cell hyperplasia and numerous hookworm feeding tracks in the submucosa (Fig. 1E). Feeding tracks usually contained colonies of gram-negative cocobacilli and gram-positive cocci (Fig. 1F and 1G). Two of these pups were considered to have died due to "hookworm enteritis and bacteremia", while the other pup died most likely due to starvation and hookworm lesions. In three pups, hookworms caused moderate to marked lymphoplasmacytic and eosinophilic enteritis with marked goblet cell hyperplasia. In these pups, hookworm feeding tracks were confined to the mucosa and bacterial translocation to the submucosa was not observed. Additionally, one of these pups had occasional coccidian merozoites within apical enterocytes. Moderate numbers (46 to 300 specimens) of the acanthocephalan Corynosoma australe were found in in the small intestine of one subadult PFS and five adult SASL. There were no major tissue changes associated with the presence of this parasite, besides mild to moderate edema at the site of attachment. The nematode Contracaecum osculatum was found in moderate numbers (80 specimens) in the stomach of one adult SASL, and no associated lesions were observed.

Discussion

The number of PFS with parasites (28%) is low if compared with other populations of fur seals, where nematode or mite infection can affect up to 100% of the population (Kim et al. 1980; Seguel et al. 2017). The number of sampled SASL is...
low to evaluate the overall prevalence of parasitic infections in this population but our preliminary data suggests it could be higher than in fur seals. The reason of the relative low prevalence of parasitic infection in PFS could be related to the age class of the sampled animals which were mostly pups. Parasitic infections in this fur seal age class are limited to hookworms and respiratory mites because they only feed on their mother’s milk. Therefore, only parasites transmitted through the milk (hookworms) or through direct contact (mites) can infect these animals, which contrast with SASL which were infected mostly by parasite transmitted through fish. Additionally, these parasitic infections frequency tend to decrease with low host density. PFS are in lower numbers compared to their historic abundance at Punta San Juan, Peru (Cárdenas-Alayza and Oliveira 2016), being one of the factors that could be associated with low prevalence of parasites in this population.

There was a marked difference between SASL and PFS in the severity of the inflammatory reaction against respiratory mites. PFS tended to have more inflammation and tissue damage associated to these parasites. This difference could be related to the deeper location of mites within the PFS tissues or correspond to animal age and/or temporal variation in the infection process. Lesions observed in fur seal pups may be more recent infections in relatively naıve animals compared to those noted in SASL, which were mostly subadult and adult animals. The effect of parasite burden is probably less significant, as both species had similar parasite loads. The role of O. attenuata in causing disease in pinnipeds is not clear, as reports of significant health effects are rare and related to extremely heavy infections (Dunlap and Piperlij 1976; Kim et al. 1980). However, in this report, one PFS had significant lesions associated with secondary invasion of bacteria, which could have contributed to the development of bronchopneumonia and eventual death.

The lesions caused by Uncinaria sp. in PFS are identical to those described in South American fur seals at the Chilean Patagonia infected with the same Uncinaria sp. (Seguel et al. 2017). In otariid pups, hookworms feed deep into the submucosa generating tracks that are infected by enteric bacteria, with the subsequent translocation of bacteria or toxins into the bloodstream (Seguel et al. 2017). In this study, although blood cultures were not performed, we called the cause of death “hookworm enteritis with bacteremia” since this term describes the aforementioned syndrome (Spraker et al. 2007; Seguel et al. 2017). Although the overall prevalence of this parasite was low (6/32), its presence and the level of tissue damage caused suggest that could become a significant pathogen if the environmental conditions for its transmission change (e.g., increase in host density). Hookworms are the most significant parasitic disease of otariids and in some populations can kill more than 30% of pups born each year (Seguel and Gottdenker 2017). The pathogenic potential of these nematodes is highlighted by this study since animals dying due to hookworm enteritis syndrome had less than 150 nematodes in the intestine, a burden considered low if compared to other fur seal populations with median burdens of ~300 nematodes per pup (Seguel et al. 2017, Seguel and Gottdenker 2017). However, this low burden was enough to cause substantial tissue damage, and associated to secondary bacterial infection, kill some pups.

The lack of significant lesions associated with C. australis infection, despite a moderate parasite burden in several animals, suggests SASL and PFS have developed tolerance to this parasite. For C. osculatum, the observed case had a low burden, which probably accounted for the lack of associated lesions.

The role of metazoan parasites as agents of disease in SASLs is not clear, as the lack of significant lesions in our study could be associated with a small sample size and over-representation of adult males. However, O. attenuata and Uncinaria sp. have the potential to cause disease and mortality in PFS. In the last century, the PFS population suffered a dramatic decline and continues to face significant conservation challenges due to reduced genetic diversity, isolation, small population size and vulnerability to environmental change (Cárdenas-Alayza et al. 2016). Therefore, further evaluation of the population effect, transmission dynamics and factors limiting/favoring respiratory mites and hookworm infection in PFS is warranted.

Acknowledgements. Mauricio Seguel was supported by a Morris Animal Foundation fellowship (Grant N D16ZO-413). Sample collection at Punta San Juan - RNSIIPG was authorized by research permit RJ No.09-2013-SERNANP-RNSIIPG, and funded by Chicago Zoological Society’s Chicago Board of Trade Endangered Species Fund. We gratefully acknowledge support from SERNANP and facility access by Agrorural at Punta San Juan.

References


Baylis H.A. 1933. A new species of the nematode genus Uncinaria from a sea-lion, with some observations on related species. Parasitology, 25, 308–316


Download Date | 10/28/18 4:42 AM
seal (*Callorhinus ursinus*). *Journal of Wildlife Disease*, 12, 42–44.


Received: October 13, 2017

Revised: May 2, 2018

Accepted for publication: July 3, 2018


