



Mechanisms of hookworm (*Uncinaria sp.*) clearance in South American fur seals (*Arctocephalus australis*)



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Introduction

Marine mammal hookworms are highly pathogenic and have been found in most otariid seal populations^{1,3,4,5,6,9}. This is true of the South American fur seal (SAFS) population on Guafo Island, Southern Chile where it is believed that transmission of infective stages of this parasite of neonates is through the colostrum^{2,3,4,6}.



Hookworms spend the adult stage of their life cycle in the small intestine of these neonates before they are expelled approximately 1-3 months after infection^{6,11}.

This early but acute infection leads to adverse health affects on the pups associated with hookworm infection such as retardation of growth, anemia, hemorrhagic enteritis, secondary opportunistic infections and death in up to 50% of pups^{8,9}.



Objective

This research aims to understand how South American fur seal (*Arctocephalus australis*) pups eliminate or expel hookworms (*Uncinaria sp.*).

Materials and Methods



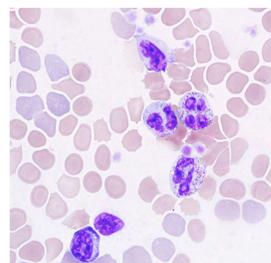
← Blood and fecal samples from SAFS were collected from 2013 to 2015 at Guafo Island, Chile



In 2014 and 2015, a group of pups were treated with Ivermectin to create a (control group) →

Cell Mediated Response

- Differential leukocyte counting was performed according to standard methods at University of Georgia⁹.
- Groups were divided into severe, mild and control based on their parasitic burden and then compared.



Humoral Response

- To know if the SAFS produced an antibody that allowed them to successfully expel the parasite, we used Immunohistochemistry on sections of worms taken from necropsies to identify if pups produce antibodies and if so where in the worm the antibodies were attacking.
- Serum from a SAFS pup that successfully expelled the worms was incubated with hookworm sections.
- Biotinylated protein A was used to label the fur seal antibodies and DAB was used as a chromagen to visualize SAFS antibodies in anatomical parts of the hookworms.

Results

Cell Mediated Immune Response

The number of basophils were higher in the mild and severe groups but the mild group had the highest number among those infected (glm, family=quasipoisson, group: $p=3.39 \times 10^{-2}$, $df=10$), as well as the number of lymphocytes (mild group: $p=1.19 \times 10^{-2}$, severe group: $p=2.09 \times 10^{-2}$, $df=178$). Eosinophils were highest in the groups with infection, but the severe group had the most (severe group: $p=4.34 \times 10^{-8}$, mild group: $p=2.95 \times 10^{-3}$, $df=171$).

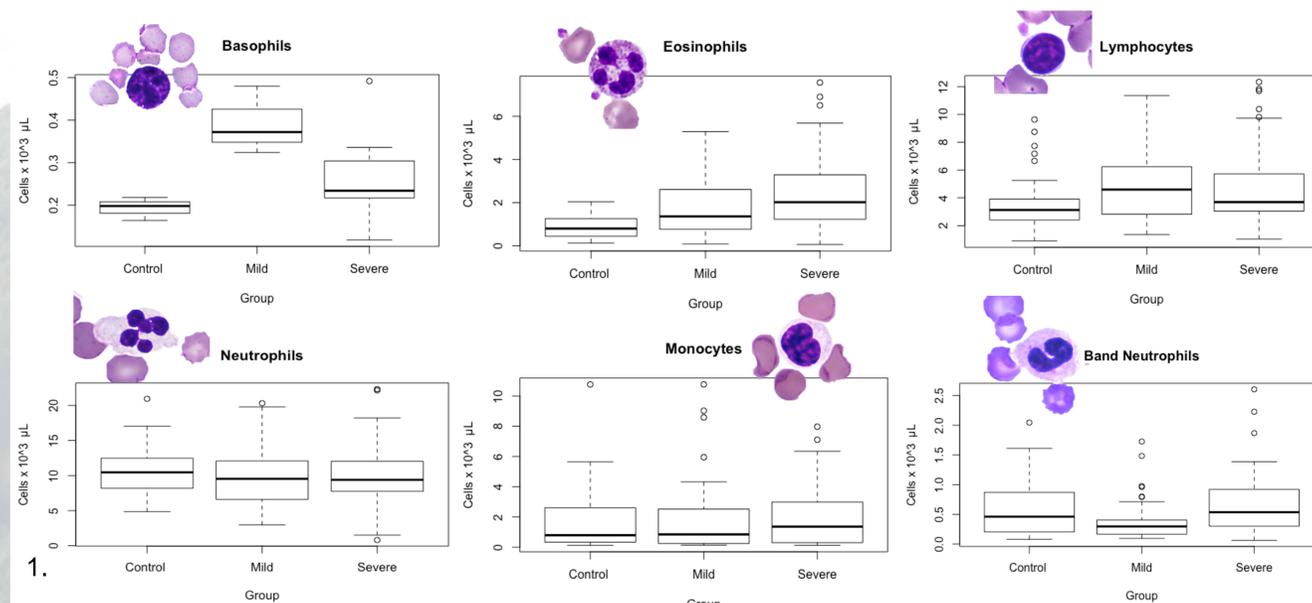
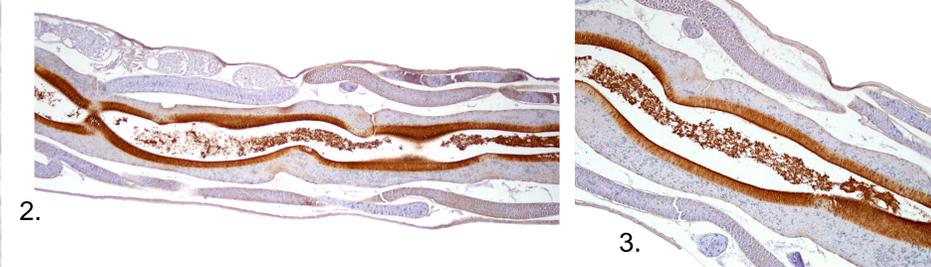


Figure 1. Differences in number of leukocytes between groups severe, mild and control (Ivermectin treated) groups.

Humoral Immune Response

Using serum from a pup who had successfully expelled the worms, we found evidence that SAFS antibodies attach to portions of the gastrointestinal tract of the hookworms and believe that this is a mechanism by which the fur seals are able to expel the parasite.

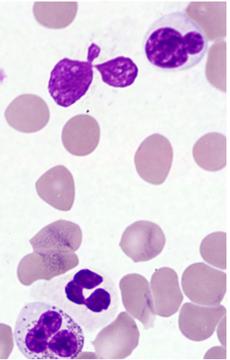


The brown stain is an indicator of SAFS antibodies attacking the intestinal track of the hookworms.

Figure 2 & 3. Hookworm longitudinal section.

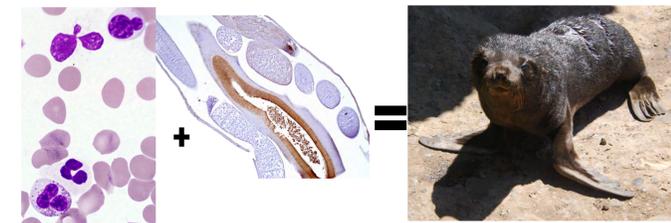
Discussion

The number of eosinophils is proportional to the severity of the hookworm infection, which as been seen in other mammalian systems¹². This data suggests that the increase in eosinophils is a response to the parasitic burden. Further research should be conducted to determine if the increase in eosinophils is enough to expel the parasites alone.



The increase in lymphocytes and basophils in the mild groups is interesting, this could be due to basophils and lymphocytes being protectors from a severe infection, therefore defining the parasitic burden.

SAFS are able to produce antibodies that bind to the gastrointestinal track of the worm. It is possible that the SAFS antibodies are able to hinder the digestive enzymes of the worm preventing proper digestion resulting in death and expulsion of the parasite.



We believe that a combination of these mechanisms allow the SAFS pups to successfully expel the hookworms.



More research needs to be conducted regarding what threshold of antibodies the fur seals need in order to successfully expel the hookworms and the roles of eosinophils, basophils and lymphocytes.

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