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SHORT COMMUNICATION

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Penetration of the small intestine of a California sea lion (*Zalophus californianus*) pup by adult hookworms (*Uncinaria* spp)

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Abstract During a study on the mortality of California sea lion (*Zalophus californianus*) pups born on San Miguel Island, California in 2002, two adult female hookworms (*Uncinaria* spp) were found penetrating the serosal surface of the intestinal wall and protruding into the peritoneal cavity of one pup. Documentation and a description of this unexpected finding and associated lesions are presented here. Also, adult hookworms were found in the peritoneal fluid of two other dead *Z. californianus* pups.

Introduction

Hookworms (*Uncinaria* spp) are common in young otariid pinnipeds, especially those residing on sandy terrain (Lyons et al. 2003). These parasites can cause debilitation and death of pups (Olsen 1958). During a study on causes of mortality of California sea lion (*Zalophus californianus*) pups, 210 animals born in 2002 on San Miguel Island (SMI), California were examined on seven trips from June 2002 to January 2003. Gross examination of one female pup (No. ZC-02-H-175) disclosed an unusual location of hookworms (*Uncinaria* spp).

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Materials and methods

The pup was found dead on North West Cove Rookery on SMI on 12 December 2002. During routine necropsy, its abdominal cavity was opened and the organs examined grossly before various samples were taken. On examination of the exterior of the small intestine, two *Uncinaria* spp were seen attached, about 100 mm apart, to the serosal surface of the mid-jejunum. One end of each parasite extended into the peritoneal cavity. An approximately 3-cm piece of pup small intestine with the two attached hookworms (both gravid females) was preserved in 10% neutral-buffered formalin. One of the hookworms became detached, so further study could not be done with regard to its site of attachment. Thus, observations in this paper are on the second hookworm, which remained attached to the intestinal wall following fixation and transportation from the field. This hookworm was located in an area of the mid-jejunum that had extensive hookworm-related hemorrhages in the wall (Fig. 1A). After fixation of the material in formalin, the hookworm attached to the serosal surface was examined under a dissecting microscope. A 3-mm section of pup small intestine, containing the tail portion of the parasite, was trimmed, embedded in paraffin, and serially sectioned at 5 μm. All slides were stained with hematoxylin and eosin. Approximately 250 sections were cut from this one piece of tissue.

Results and discussion

The pup was in fairly good body condition. Its small intestine had a few red 2-mm to 4-mm plaques that could be visualized through the serosa. Excessive peritoneal fluid, with fibrin tags attached to the serosa of the small intestine, was present within the abdominal cavity.

About two-thirds of the total length of the hookworm protruded from the intestinal wall; and this was the anterior end (Fig. 1B). The tail was threaded through the intestinal wall into the lumen (Fig. 1C).

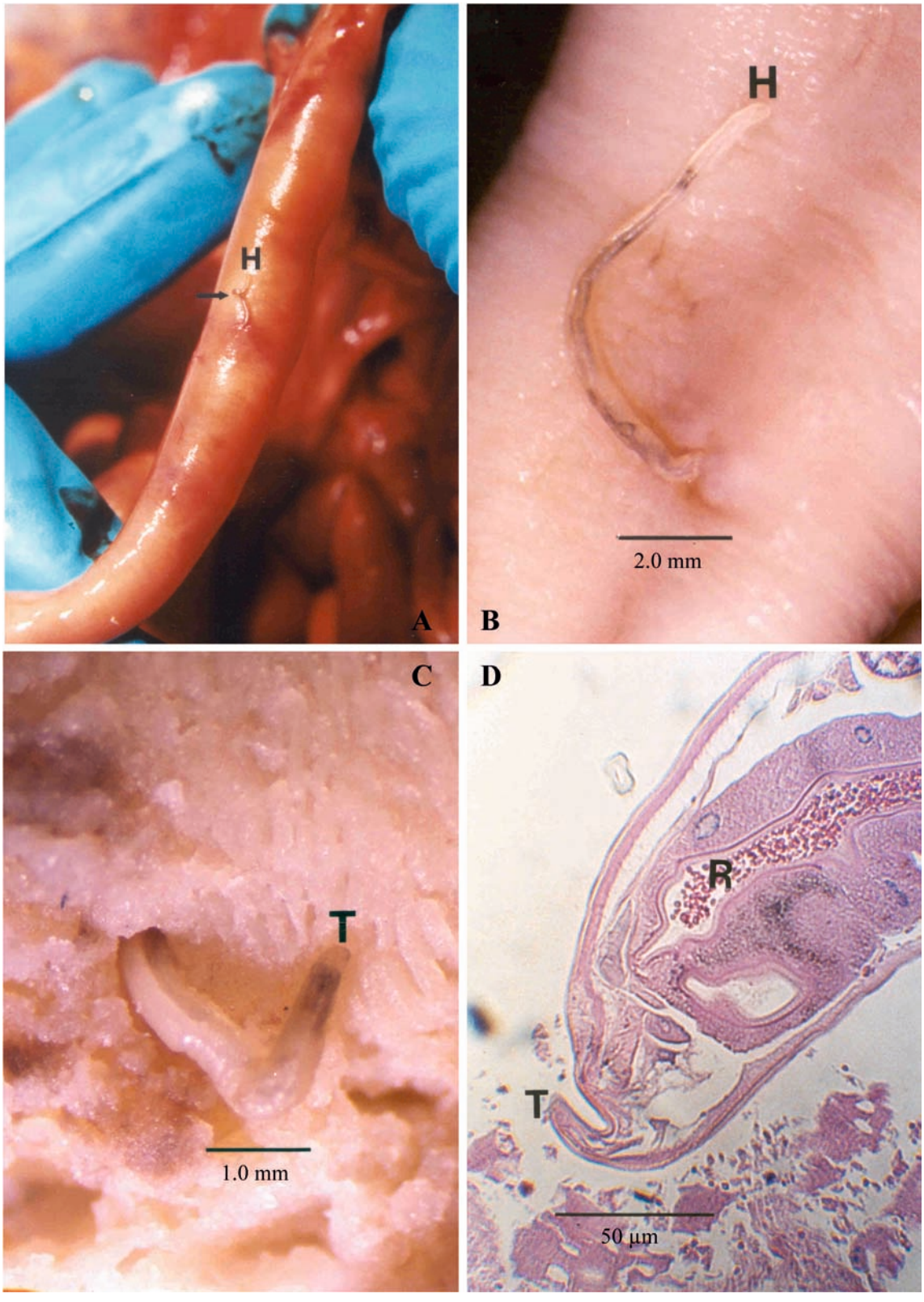

Fig. 1A–D Small intestine of a California sea lion pup (ZC-H-175) with an attached adult female hookworm (*Uncinaria* sp) which penetrated the intestinal wall into the peritoneal cavity. **A** Serosa showing the free head (*H*) portion (*see arrow*). Note hookworm-related hemorrhagic areas in the wall (*light blue areas* are gloved-fingers of the holder). **B** Similar to **A**, but at a higher magnification. **C** Tail (*T*) end in an opening in the mucosal surface (villi evident) and lumen of the small intestine. **D** Histologic sagittal section of the tail. The rectum (*R*) contains what may be red blood cell particles

Portions of the tail of the hookworm were found in 110 sections. The course of this parasite in its journey through the wall of the small intestine appeared to take a somewhat tortuous route. It had penetrated perpendicularly through the mucosa and submucosa (Fig. 2A–C). When it reached the first circular muscle layer, it seemed to head parallel to the muscular layer (Fig. 2C), then coursed straight through, and entered the abdominal cavity. Even though the mucosa had undergone a moderate degree of autolysis, the internal structures of the hookworm appeared to be intact (Fig. 1D). This suggests that the parasite was still alive at the time of death of the pup. There was no evidence of inflammation within the mucosa, but a moderate number of neutrophils, lymphocytes, a few plasma cells, and eosinophils had infiltrated into the submucosa. A moderate amount of hemorrhage was in the submucosa around the worm. This cellular infiltration and hemorrhage were responsible for the red plaques observed on gross examination of the serosa of the small intestine in this pup with hookworm infection (Fig. 1A). There was little to no inflammation within the circular and longitudinal muscle layers. However, a mild infiltration of neutrophils and lymphocytes was present between the two muscle layers. The area of the serosa where the parasite had ruptured the intestinal wall and entered the peritoneal cavity was free of inflammation. A thin layer of bacteria was evident on the outer cuticular surface of the hookworm. In addition to the two hookworms found penetrating the intestinal wall, 166 adult hookworms were recovered from the lumen and from mucosal scrapings of the small intestine of this pup.

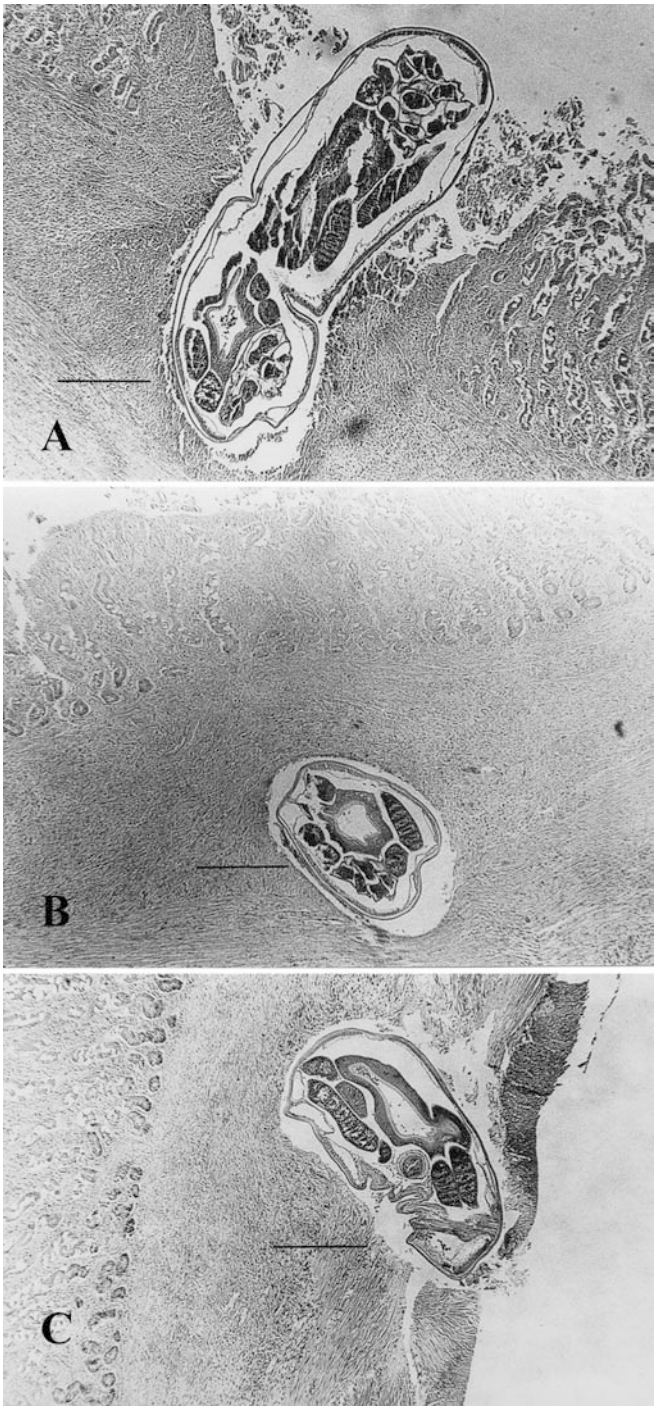

Fig. 2A–C Histologic sections of the opened small intestine and tail end of the hookworm described in Fig. 1. Pieces of the tail are located in the mucosa and submucosa (**A**), submucosa (**B**) and submucosa, muscularis, and serosa (**C**). *Bar* 200 μm

Peritoneal fluid from nine other dead California sea lion pups was examined and adult *Uncinaria* spp were found in two of these pups. Two female hookworms were found in one pup (ZC-H-110) on 26 August 2002 and one female hookworm in another pup (ZC-H-178) on 11 December 2002.

Unexpected was the finding of adult hookworms completely within the peritoneal cavity and an adult hookworm "caught in the act" of penetrating through the intestinal wall of its host. This would easily lead to bacteremia and peritonitis. Of the 130 pups found in this study with the hookworm enteritis/bacteremia complex, 29 had evidence of fibrinous peritonitis. Thus, the present finding indicates the potential life-threatening pathogenicity to California sea lion pups from those adult *Uncinaria* spp which penetrate the intestinal wall, compromising its integrity, during their passage to the peritoneal cavity, predisposing to a bacteremia.

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