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Bothrigaster variolaris (Trematoda: Cyclocoelidae) Infection in Two Florida Snail Kites (*Rostrhamus sociabilis plumbeus*)

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ABSTRACT: Two Florida snail kites (*Rostrhamus sociabilis plumbeus*, Say) collected in August and December 1993 from Osceola County (28°15′N, 81°17′W) and Glades County (26°45′N, 81°10′W), Florida (USA), respectively, were infected with *Bothrigaster variolaris* (Trematoda: Cyclocoelidae); this parasite has not been reported previously from birds in the United States. One bird, a fledgling female, harbored 270 specimens of *B. variolaris*; the helminths caused airsacculitis and bronchitis, possible contributing factors in the death of this bird. The other bird, a subadult female, was infected with 40 trematodes and had gross and microscopic lesions consistent with trauma as the cause of death.

Key words: Trematodes, pathology, *Rostrhamus sociabilis plumbeus*, snail kite, raptor, *Bothrigaster variolaris*.

The Florida snail kite or everglades kite (*Rostrhamus sociabilis plumbeus*, Say), classified as endangered by the Florida Game and Fresh Water Fish Commission and the U.S. Fish and Wildlife Service (Wood, 1993) ranges from Florida (USA), Cuba, and southeastern Mexico, to southern South America (Sykes, 1984). Although this raptor feeds almost exclusively on the apple snail (*Pomacea paludosa*) (Snyder and Snyder, 1969), alternative food items such as turtles and small aquatic snails are preyed upon during times of drought or scarcity of *P. paludosa* (Sykes and Kale, 1974; Bennetts et al., 1994).

Over the last 15 yr, 17 Florida snail kites have been submitted to the National Wildlife Health Center (National Biological Service, Madison, Wisconsin, USA) for necropsy. Because of the bird's endangered status and often remote location, the opportunity to conduct necropsies and recover endoparasites is limited. Only one report on parasites of *R. sociabilis plumbeus* in the United States is available. Sykes and Forrester (1983) examined fecal samples and blood smears, and collected ectoparasites from nests and nestlings as a part of a study on Florida snail kite ecology. Herein we report the parasitological findings from necropsies performed on two *R. sociabilis plumbeus* and the first report of *Bothrigaster variolaris* (Fuhrmann, 1904) in the United States.

In August 1993, a fledgling female snail kite (Bird A) from the St. Cloud area in Osceola County, Florida (28°15′N, 81°17′W) was found weak and was easily captured. The bird was dehydrated and died in transport to a raptor rehabilitation unit. Upon necropsy, the bird was severely emaciated and 270 trematodes (mean ± SE size = 3.6 ± 0.10 mm × 0.83 ± 0.02 mm, $n = 6$) were counted in, and on, the air sacs. Grossly, the air sacs were opaque; granular tan deposits had accumulated in the folds and angles of the tissues. The esophagus, air sacs, lungs, and small and large intestines were excised and examined using the parasitological methods of Pritchard and Kruse (1982). Live trematodes were fixed in alcohol-formalin-acetic alcohol (AFA) (Pritchard and Kruse, 1982) for 24 to 48 hr and later transferred to 70% ethanol. Dead trematodes were preserved in 70% ethanol. Five trematodes contained ova. Selected trematode specimens were stained in Mayer's acid carmine solution (Pritchard and Kruse, 1982), dehydrated in a series of ethanol, cleared in xylene and mounted in balsam (Pritchard and Kruse, 1982) and identified as *Bothrigaster variolaris* (Fuhrmann, 1904). Thin blood smears were air-dried, fixed in 100% methanol, stained in Giemsa (Sigma Chemical Company, St. Louis, Missouri, USA) and examined for hemoparasites (Pritchard and Kruse, 1982). No protozoal or other helminth parasites were recovered. Samples of lung, air sacs, spleen, heart, kidney, ce-

rebrum, cerebellum, liver, and small and large intestine were fixed in 10% buffered formalin, and embedded in paraffin; 5 μm sections were stained with hematoxylin and eosin for light microscopic examination. The primary microscopic lesions were moderate pyogranulomatous bronchitis and peribronchitis, and bronchiectasis with mild squamous metaplasia of the epithelium near intrabronchial trematodes. Mild granulomatous airsacculitis composed exclusively of large, pigment-laden macrophages also was noted. The pigment was not identified. Sections of lung stained with Grocott's methenamine-silver nitrate (Balows et al., 1991) were negative for fungi. However, a fungus was isolated from lung tissue incubated at 37 C on Sabourared's dextrose agar (Difco Laboratories, Detroit, Michigan, USA) and identified as *Aspergillus* sp. (Emmons et al., 1977). Bacterial cultures of lung at 37 C on 5% sheep blood agar (BAD) and eosin methyline blue (EMB) (Difco Laboratories) yielded *Acinetobacter calcoaceticus* (Rapid Nonfermenter (NFT) Strips, bioMerieux Vitek Inc., Hazelwood, Missouri, USA).

In December 1993, a subadult female (Bird B) from Lake Okeechobee in Glades County, Florida (26°45′N, 81°10′W) was found with injuries consistent with impact trauma and taken to a rehabilitation unit where it died within 24 hr. Upon necropsy, gross lesions included a ruptured left eye, keel fracture, cerebral and mesenteric hemorrhage, and visceral gout. Forty trematodes without ova were removed from the trachea and air sacs (mean ± SE size = 3.4 ± 0.17 mm × 0.90 ± 0.01 mm, $n = 6$). Samples of lung, air sac, small and large intestine, and kidney were excised and examined for parasites. No blood smears were taken. Trematodes were stained, cleared, and identified as *B. variolaris*. No other helminths or protozoa were recovered. Six lice were collected and fixed in 70% ethanol, cleared in 10% KOH, dehydrated in a series of ethanol, cleared in xylene, mounted in balsam (Pritchard and Kruse, 1982) and identified as *Falcolipeurus quadriquttatus* and *Craspedorhynchus obscurus* (Giebel, 1874). Voucher specimens of helminths and lice were deposited in the Harold W. Manter Laboratory (University of Nebraska, Lincoln, Nebraska, USA, HWML Accession Numbers 37969 to 37972). Samples of lung, air sac, cerebrum, cerebellum, liver, kidney, heart, pancreas, spleen, small and large intestine were fixed in 10% formalin and stained. Eyes were fixed in Bouin's solution and prepared for light microscopic examination. Microscopic lesions consistent with subacute trauma included ocular hematoma, focal cerebral hemorrhage and malacia, and multifocal subcapsular hepatic necrosis with hemosiderosis and mild biliary hyperplasia. Nephrosis and visceral gout also were evident but no etiology was determined. The trachea, lung, and air sac contained no distinctive microscopic lesions. Bacterial culture on BAD, EMB as described for Bird A, and on *Salmonella* sp. selective media (Dulcitol Selinite Broth, Xylase-lysine-tergitol 4 Agar, and Brilliant Green Agar; Difco Laboratories) (Balows et al., 1991) were negative.

The life cycle of *Bothrigaster variolaris* is unknown; however, considering the two-host life cycle pattern of cyclocoelids such as *Cyclocoelum mutabile,* (McLaughlin, 1976) snail kites most likely become infected by eating aquatic or amphibious snails which harbor the metacercaria. In addition to infecting the air sac, *B. variolaris* has been recovered from the abdominal cavity (Fuhrmann, 1904) and nasal fossa and sinuses (Perez, 1940, 1955) of snail kites. The snail kite is the only reported definitive host for *B. variolaris*. However, limpkins (*Aramus quarauna pictus*) which have similar food habits to the snail kite and are found in fresh water areas in Florida and southeastern Georgia (USA) (Peterson, 1980; Snyder and Snyder, 1969) harbor an immature cyclocoelid ($\bar{x} = 429$, range = 2 to 3,554, $n = 15$) (Conti et al., 1985). Conti et al. (1985), listed *B. variolaris* as a possible identification for the immature cyclocoelid and stated that

immaturity of the helminths suggested that the limpkin was an abnormal host for this trematode.

The immaturity of birds A and B and the lack of migration between the U.S. and Cuban populations of *R. sociabilis plumbeus* (Beissinger et al., 1983) is evidence that *B. variolaris* is enzootic in the United States. Grossman and Hamlet (1964) reported an increase in "lung flukes" in snail kites subsequent to draining portions of the Everglades. However, the trematodes were not identified nor did they comment on the pathogenicity of these trematodes. Underhill et al. (1994) proposed that *Cyclocoelom mutabile*, which is of the same subfamily as *B. variolaris*, can cause retardation of molting in the knot (*Calidris canutus*) which subsequently may affect breeding performance. The heavy worm burden and reactive tissue changes are evidence that *B. variolaris* contributed to the debilitation and death of Bird A. However, the pathogenecity of this trematode in snail kites has not been fully evaluated.

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