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## LARGE SCALE MORTALITY OF NESTLING ARDEIDS CAUSED BY NEMATODE INFECTION<sup>1</sup>

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**Abstract:** During the summer of 1976, an epornitic of verminous peritonitis caused by *Eustrongylides ignotus* resulted in large scale mortality of young herons and egrets on Pea Patch Island, Delaware. Mortality was highest (84%) in snowy egret nestlings (*Egretta thula*) and less severe in great egrets (*Casmerodius albus*), Louisiana herons (*Hydranassa tricolor*), little blue herons (*Florida caerulea*), and black crowned night herons (*Nycticorax nycticorax*). Most deaths occurred within the first 4 weeks after hatching. Migration of *E. ignotus* resulted in multiple perforations of the visceral organs, escape of intestinal contents into the body cavity and subsequent bacterial peritonitis. Killifish (*Fundulus heteroclitus*) served as the source of infective larvae.

### INTRODUCTION AND HISTORY

Mortality of piscivorous birds caused by nematodes of the genus *Eustrongylides* has occurred in epornitic proportions in red-breasted mergansers (*Mergus serrator*),<sup>7</sup> and in individual great blue herons (*Ardea herodias*),<sup>8</sup> and great egrets (*Casmerodius albus*).<sup>6</sup> Infective larvae of *Eustrongylides* occur in the connective tissue of fishes and, following ingestion by avian hosts, may undergo extensive visceral migration with subsequent host tissue damage. Adult worms reside in nodules in the proventriculus of birds.<sup>14</sup> This report describes unusually high mortality due to parasitism by *Eustrongylides ignotus* in snowy egrets (*Egretta thula*) and other ardeid nestlings on Pea Patch Island, New Castle County, Delaware, during the late spring and early summer of 1976.

Pea Patch Island (lat. 39° 35', long. 74° 34') is located in the Delaware River

16 km south of Wilmington, Delaware. Dense stands of giant reeds (*Phragmites communis*) cover more than half the approximately 125 ha island. High ground in the central and northern sections of the island supports a variety of grasses, forbs, shrubs, and mixed hardwoods.<sup>15</sup>

A heronry became established on the northern end of the island in the early 1960's,<sup>3</sup> and approximately 2,000 pairs of wading birds nested there annually between 1969-1973. This number increased to 8,000 pairs in 1974, and has remained at this level through the 1976 nesting season.<sup>16</sup> Eight species of Ardeidae and one species of Threskiornithidae nested in the heronry in 1976 (Table 1).

Extensive mortality of nestling snowy egrets was observed from mid-May through early July, 1976. Losses of Louisiana herons also were considered

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abnormally high, while mortality of nestlings of the remaining species was comparable to that observed in 1975. An investigation into the cause of this mortality was initiated in June and continued throughout the summer.

#### METHODS

One hundred and forty snowy egret and five Louisiana heron (*Hydranassa tricolor*) nests (15.5% and 12.5% of the total number of nests for each species) were labeled with consecutively-numbered aluminum tags during incubation. Sibling young in each nest were marked with different color food dyes at hatching to facilitate subsequent aging and identification. Dye marks were renewed at 4-5 day intervals, and nests were examined daily until the oldest sibling reached 10-12 days of age. Thereafter, observations continued from nearby blinds to avoid disturbances to the birds. Data recorded at each nest included clutch size, hatching success, growth rates, fledging success (young reaching at least 28 days of age), and mortality.

Eighty-three ardeids, including all nestlings from 14 of the 140 snowy egret nests, were collected between 5-30 June

1976 for necropsy examinations. Adult snowy egrets, Louisiana herons, and little blue herons (*Florida caerulea*) were collected in paired samples with their young. Birds were placed on ice and necropsied within 6 hours after collection, or were frozen and examined at later dates.

At necropsy, birds were examined for gross lesions, and parasites were removed, counted, and preserved in 5% formalin. Tissue samples of major organs and gross lesions were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 6  $\mu$ m, and stained with hematoxylin and eosin.

Bacteriologic examinations were made of abdominal cavities of 19 snowy egret nestlings which had verminous peritonitis and of 9 uninfected cattle egrets (*Bubulcus ibis*). All of these birds had been preserved by freezing. Their abdominal cavities were exposed aseptically, and viscera were swabbed with sterile cotton-tipped applicators. Samples were inoculated into thioglycolate broth and streaked on blood agar plates. Subsequent identifications of isolates were by standard bacteriologic procedures.

Between 28-30 June 1976, small fish were collected by seine and dip net from

TABLE 1. Numbers of nesting pairs of waders in the Pea Patch Island heronry 1968-1976.

Species	1968 <sup>a</sup>	1973 <sup>a</sup>	1974 <sup>a</sup>	1975 <sup>a</sup>	1976 <sup>b</sup>
Great blue heron	40	8	100	20	4
American egret	400	200	600	175	175
B1-crowned night heron	300	150	500	400	400
Y1-crowned night heron	—	—	—	20	40
Little blue heron	200	50	700	200	400
Louisiana heron	—	4	200	20	40
Snowy egret	100	100	900	250	900
Cattle egret	450	600	3,500	4,500	4,500
Glossy ibis	450	400	1,500	1,500	600
	1,940	1,512	8,000	7,085	7,059

<sup>a</sup>Wiese 1976

<sup>b</sup>Wiese 1977

13 locations in the vicinity of Pea Patch Island. Fish were placed in plastic bags, labeled, and transported to a field laboratory where they were examined for *Eustrongylides* larvae by opening the abdominal cavity and teasing apart the viscera. Seventy-four preserved fish which had been collected in the vicinity of the island between 1968 and 1973 also were examined. These fish were dissected in a similar manner to the fresh fish.

### RESULTS

Our sample of 126 snowy egret nests contained 433 eggs, of which 327 (75.5%) hatched. Two (0.6%) of these nestlings were killed by predators, and 276 (84.4%) died of parasitism prior to 4 weeks of age. Only 49 (15%) nestlings fledged. In our sample of 5 Louisiana heron nests all 14 nestlings also died prior to reaching 4 weeks of age. Most deaths in both species occurred during the first 2 weeks after hatching, and first and second-hatched siblings had similar mortality rates.

Affected nestlings became progressively weakened and showed abdominal swell-

ing. Indurated, tract-like lesions were present on the abdominal wall and caused elevation of the overlying skin. Ulceration of the skin covering these tracts was encountered occasionally, and large, red nematodes, identified as *E. ignotus*, often protruded from these wounds. All 149 moribund or dead snowy egret nestlings examined had some of these features on external inspection. On one occasion, an entangled mass of *Eustrongylides* was found on the ground beneath a nest.

Individuals of all species examined, except cattle egrets, were infected with *E. ignotus* (Table 2). Birds found dead or in moribund condition included 43 snowy egret nestlings, 2 fledgling great egrets, 2 nestling Louisiana herons, and one fledgling black-crowned night heron (*Nycticorax nycticorax*). The remainder of the birds examined appeared healthy when collected. Infections were frequent and severe in young snowy egrets, great egrets and Louisiana herons but less common in little blue herons and black-crowned night herons. Mature parasites producing eggs were recovered from two snowy egrets, one great egret and one

TABLE 2. Occurrence of *Eustrongylides ignotus* in 83 ardeids from Pea Patch Island, Delaware.

Species	Age <sup>a</sup>	Number Examined	Number Infected	Percent Infected	x Worms Infection	Range	Parasite <sup>b</sup> Stage
Snowy egret	N	43	41	95%	6.3	1-14	L, G
Snowy egret	F	5	4	80%	6.5	3-10	L, G
Snowy egret	A	5	0 <sup>c</sup>	0	0	0	—
American egret	F	2	2	100%	10.5	1-20	L, G
Louisiana heron	N	2	2	100%	6.5	2-11	L
Louisiana heron	A	2	0	0	0	0	—
Little blue heron	N	3	0	0	0	0	—
Little blue heron	F	2	1	50%	1	1	L
Little blue heron	A	2	0	0	0	0	—
Black-crowned night heron	F	4	2	50%	1.5	1-2	L, G
Cattle egret	N	13	0	0	0	0	—

<sup>a</sup>—N=Nestling, F=Fledgling, and A=Adult.

<sup>b</sup>—L=Larvae and G=Gravid parasite (producing eggs).

<sup>c</sup>—No viable worms present but all 5 birds had resolving lesions.

black-crowned night heron. Live *E. ignotus* were not recovered from adult birds of any species; however, all adult snowy egrets examined had evidence of previous infection.

All infected birds had varying degrees of verminous peritonitis due to migration of *E. ignotus* in abdominal viscera. In recently acquired infections (i.e., in young nestlings), larvae were found actively penetrating the ventriculus and esophagus or free in the abdominal cavity. Nematode-induced perforations in the ventricular wall resulted in hemorrhages and leaking of stomach contents into the abdominal cavity. In the abdominal cavity, parasites penetrated all visceral organs. Parasite migration resulted in hemorrhage, followed by necrosis of damaged tissue, and finally by an inflammatory response by the host. Frequency of parasite-induced lesions were: ventriculus 100%, intestine 100%, liver 84%, kidneys 19%, abdominal musculature 16%, gall bladder 6%, and skeletal structures 3%.

In more chronic infections (i.e., in large nestlings and fledglings), nematodes were found in abdominal organs and frequently were encased in tough fibrotic tracts of connective tissue. Many heavily encapsulated nematodes were dead and degenerating. Massive adhesions of abdominal viscera were present in response to parasitic migration, gastrointestinal contents, and secondary bacterial infections. Caseous airsacculitis and thick-walled abscesses up to 2 cm in diameter were observed. Infected fledglings that seemed healthy prior to necropsy had well organized fibrotic capsules surrounding lesions. Successful resolution of lesions was noted in all adult snowy egrets. Lesions in these birds consisted of small, black fibrous plaques or tracts on the serosa of the ventriculus.

As with gross lesions, histopathologic findings included inflammatory responses from acute to resolved processes. Recent migration of larvae into various abdominal organs resulted in hemorrhages, severe localized congestion, and necrosis of traumatized tissue. As the duration of infection increased, larvae were surround-

ed by a zone of caseous exudate and heterophil infiltration. Inflammatory changes progressed to include wide zones of highly vascularized fibrotic connective tissue with foreign body giant cells lining the interior border. The central core of these lesions contained amorphous eosinophilic material with live or dead nematodes, cuticle, eggs, intestinal contents, necrotic debris and bacteria. Lesions generally were consistent with those described by Locke.<sup>8</sup> Resolving lesions contained minimal amounts of these materials in the central core, and surrounding tissues appeared normal.

Bacteriologic examinations revealed a greater prevalence and diversity of microorganisms in the abdominal cavities of snowy egret nestlings as compared with cattle egret nestlings. The frequency of occurrence of bacteria in snowy egrets was as follows: *Escherichia coli* (74%), *Aeromonas* sp. (37%), *Streptococcus* (26%), *Corynebacterium* sp. (21%), *Bacillus* sp. (5%), *Proteus* sp. (5%), *Klebsiella* sp. (5%), and *Staphylococcus* sp. (5%). The frequency of occurrence of bacteria in cattle egrets was as follows: *Streptococcus* sp. (44%), *E. coli* (11%), and *Aeromonas* sp. (11%). Bacteria were not isolated from one (5%) snowy egret and four (44%) cattle egrets.

*E. ignotus* larvae were found in killifish (*Fundulus heteroclitus*) obtained from the stomachs of adult snowy egrets. Examination of 11 species of fish from 13 nearby feeding areas revealed larvae in 91 of 244 (41%) killifish and 1 of 22 (6%) brown bullheads (*Ictalurus nebulosus*). Killifish harbored an average of 1.9 nematodes per infected fish (range 1-7). All infected killifish were >6 cm in length, and the infected bullhead was 24 cm long. Larvae were not found in 276 *Brevoortia tyrannus*, 62 *Leiostomus xanthurus*, 48 *Menidia beryllina*, 37 *Lepomis gibbosus*, 21 *Morone americana*, 10 *Pomatomus saltatrix*, 9 *Cyprinus carpio*, 7 *Notemigonus crysoleucas* or 5 *Poxomis nigromaculatus*.

Eleven of 74 (15%) preserved killifish collected in the vicinity of Pea Patch Island from 1968 through 1973 contained *E. ignotus* larvae. The average number of larvae per infected fish was 2 (range 1-9).

## DISCUSSION

Verminous peritonitis due to *E. ignotus* was the most significant mortality factor in young snowy egrets and accounted for substantial mortality among other piscivorous ardeids. Most losses occurred prior to fledging when infected fish were fed to the young by their parents. Sick fledglings also were observed, and it is likely that some birds which fledged and departed the heronry eventually died from *Eustrongylides*-induced lesions acquired as nestlings. This assumption is supported by the fact that mortality due to verminous peritonitis has been reported in subadult and adult great blue herons<sup>1,6</sup> and great egrets.<sup>6</sup>

Some birds evidently survive infections since lesions frequently were regressing in older nestlings. Although birds from 6 weeks to 1 year of age were not examined, almost complete resolution of lesions attributed to *E. ignotus* had occurred in all adult snowy egrets examined. Lesions were similar to those reported earlier in herons and egrets<sup>1,6</sup> and mergansers,<sup>7</sup> although a broader spectrum of lesions, i.e., acute to completely resolved, were noted in the present outbreak.

Killifish were the principal source of infection as evidenced by the high percentage that harbored infective larvae and the absence of larvae in other species of fish. Killifish were further incriminated since infected fish were found within some birds, and species preying largely on killifish (snowy egrets, great egrets, and Louisiana herons) were most frequently and heavily infected. Species preying predominantly on amphibians (little blue herons) or other species of fish (black-crowned night herons) were only marginally involved. Cattle egrets feeding on terrestrial invertebrates were not infected. In addition, heavy infections in killifish from the middle Atlantic coast have been documented previously.<sup>2,3,11,12,13</sup>

Only large killifish were infected. A possible reason may be that the first intermediate host, presumably an oligochaete,<sup>14</sup> is so large that it is an acceptable prey item only for large killifish. An

alternative may be that the larvae are harmful to smaller killifish. The single infected bullhead may have ingested infected killifish since earlier studies<sup>9,12</sup> have shown that predacious fish may accumulate larvae; however, it would not have contributed to the transmission of the parasite due to its large size.

The position of avian hosts in the epizootiology of *E. ignotus* during the epornitic was unresolved. One possibility, which is supported by the extreme pathogenicity of the parasite to ardeids, is that these birds are not the normal hosts. Numerous other groups of birds known to harbor *Eustrongylides*, viz., Anseriformes, Charadriiformes, Gaviiformes, Podicipediformes, and Pelicaniformes,<sup>8</sup> overwinter or migrate through this vicinity. Conceivably, birds from these orders could have contaminated the marsh with *E. ignotus* eggs; however, infections in birds of these orders reported in the literature have been of low prevalence and intensity.

A more plausible alternative is that birds of the heronry are the primary definitive hosts for *E. ignotus*. The strongest evidence for this contention was that adult helminths and eggs were found in three species of birds using the heronry. Following fledging, young birds apparently contaminate the environment by shedding eggs in the feces.

Although many nestlings died before the parasites began producing eggs, infection of a relatively low percentage of the first intermediate host population probably would ensure completion of the life cycle since biomagnification of parasitism would occur through the food chain. This was evident at the upper trophic level where the prevalence of infection increased from 41% in killifish to 92% in the three principally afflicted avian species. Parent birds apparently are refractory to infection and only temporarily transport the infective larvae. Opportunity for this theoretical mode of transmission has increased in recent years since nesting populations of piscivorous ardeids on the island have expanded.<sup>15</sup>

Since *E. ignotus* apparently has been present in herons and egrets in this region for decades, it is surprising that there has been no previous documentation of a die-off such as reported herein. High mortality of young ardeids attributed to starvation, predation, and climatic conditions has been well documented.<sup>4,10</sup> It now appears that some of these losses could be due to *E. ignotus*. Additional studies are strongly indicated to determine the significance of this parasite as a limiting factor and to clarify the position of various avian hosts in the epizootiology of this disease.

#### ADDENDUM

Studies presently being conducted on Pea Patch Island during the 1977 nesting period have again revealed extensive mortality caused by *E. ignotus*. Although total mortality cannot be ascertained until reproductive activities cease later in the summer, the mortality pattern of piscivorous ardeids, particularly snowy egrets, observed thus far is similar to that of 1976. These observations suggest that verminous peritonitis is a recurrent and important limiting factor for herons and egrets nesting on the island.

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